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Using worms to probe basic features of higher eukaryotic chromosomes

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Histone H3K9 methylation is a conserved modification that correlates broadly with gene repression in organisms ranging from fission yeast to man. We have shown that H3K9 methylation is a signal required for localizing heterochromatin at the nuclear envelope, where it is recognized by CEC-4 (C. elegans Chromodomain protein 4), a NE-associated protein with high affinity for all methylated forms of Histone H3K9

In C. elegans, di- and tri-K9 methylation is abundant on repetitive elements (RE), including both transposons and simple repeats, and coats both pseudogenes and silent tissue-specific genes. Using a double mutant that eliminates the two C. elegans histone H3K9 methyltransferases, SET-25 and MET-2, we find that H3K9me is dispensable for development,

although worms become sterile at 25°C. This correlates with extensive DNA damage-driven apoptosis in the germline, although there is no elevation in either mitotic or meiotic chromosome missegregation. Instead, we find that the loss of H3K9methylation leads to the promiscuous and widespread expression of all classes of repetitive elements (DNA and RNA transposons, and simple repeats) in both germline and somatic tissues. The loss of transcriptional silencing correlates with an accumulation of insertions and deletions at repetitive sequences, and renders worms sensitive to replication fork stalling, but not ionizing radiation. RNA-DNA hybrids accumulate in the absence of H3K9me even without exogenous stress. We conclude that a key function of H3K9me is to ensure the stability of a repeat-rich genome, most likely by suppressing the transcription of simple repeats. This is distinct from the role of H3K9me in sequestering chromatin at the nuclear envelope, which contributes to the stability of cell fate decisions.


Tissue-specific analysis of nuclear organization through development of a novel FLP/Frt-based toolkit for spatiotemporal control of gene expression

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The nuclear envelope (NE) plays critical roles in gene expression through controlling access to the nucleus, anchoring of chromosomes at the nuclear periphery and serving as platform for chromatin-interacting proteins, such as histone modifiers and transcription factors. Reflecting the importance of NE functions, several human diseases are attributed to alterations in NE structure. Most notably are the laminopathies, whose name refers to underlying mutations in components of the nuclear lamina and lamina-associated proteins. One example is Emery-Dreifuss muscular dystrophy, which is caused by mutations in the inner nuclear membrane protein emerin or in lamin. We recently found that emerin/EMR-1 is required for correct neuromuscular junction activity in C. elegans. Firstly, EMR-1 associates with genes involved in muscle and neuronal function and deletion of emr-1 causes local changes in nuclear architecture. Secondly, transcriptome analyses revealed that EMR-1 is associated with gene repression, particularly of genes implicated in muscle and nervous system function. Thirdly we demonstrated that emr-1 mutants are sensitive to the cholinesterase inhibitor aldicarb, indicating altered activity at neuromuscular junctions. Although many NE proteins are ubiquitously expressed, laminopathies often affect a single tissue. This has led to the hypothesis that tissue-specific alterations in nuclear organization are responsible for particular clinical manifestations of laminopathies. Specifically, changes in interactions between NE proteins and chromatin are thought to be relevant, but has not been explored in intact organisms due to technical limitations. This triggered us to develop novel tools to dissect the function of EMR-1 in tissue-specific nuclear organization. Using an optimized FLP recombinase, we show specific and efficient activation of dual color reporters in selected tissues. Importantly, our system is based on single-copy FLP and Frt transgenes integrated into the genome by MosSCI to ensure reproducible expression and to facilitate crosses. Until now, the repertoire of cell types amenable to analysis includes striated and nonstriated muscles, intestine, M cell lineage, seam cell lineage, hypodermis, and neurons (pan-neuronal and specific neuronal subtypes). We have implemented our FLP/Frt system to perform tissue-specific DamID, which has provided EMR-1/chromatin interaction profiles for different cell types, but the tool kit can easily be adapted for spatiotemporal control of other transgenes, including fluorescent reporters, dominant alleles or suicide genes.
Probing the mechanisms of C. elegans natural infection by oomycetes

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A number of microbes have evolved pathogenic and parasitic lifestyles targeting a range of eukaryotic hosts including nematodes. One such group is the oomycetes, eukaryotic organisms that are superficially similar to fungi. Oomycetes inhabit a variety of terrestrial and aquatic environments and have evolved saprophytic or parasitic lifestyles, infecting a range of hosts including animals and plants, and causing vast economical damage and disease. Although plant pathogenic oomycetes have been widely studied, research on animal pathogenic oomycetes has been much more limited, largely due to absence of tractable host systems. To redress this, we have recently sampled and currently maintain in culture two biotrophic oomycete species naturally infecting Caenorhabditis elegans. These oomycetes belong to the Myzocytiopsis and Haptoglossa genera and are obligate nematode pathogens. Given the wealth of molecular tools and resources available for C. elegans research, these new systems provide an unprecedented opportunity to establish powerful models to study animal / oomycete interactions. We will describe the establishment of these new pathosystems. We have performed detailed microscopic analysis of the pathogen attachment, entry and growth within C. elegans to show that the two pathogens display related yet distinct infection strategies to colonise and eventually kill the nematode host. We have discovered host specificity for the infection by the Myzocytiopsis isolate and differences in susceptibility among C. elegans wild isolates. We have performed transcriptomic experiments in C. elegans to address pathogen-induced changes of host gene expression. We have found some unique C. elegans responses antagonising the oomycete infection. We have also obtained evidence for host innate immunity pathways, such as the TGF-beta and p38 MAP kinase pathways, involved in conferring resistance to oomycete infection. Our results shed light on previously unknown natural infections of C. elegans and are valuable for comparative studies of nematode defence mechanisms to counter various pathogens.
The SET-2/SET1 histone H3K4 methyltransferase maintains genome stability in the Caenorhabditis elegans germline independently of the DNA damage response.

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Histone H3 Lysine 4 methylation (H3K4me) is deposited by the conserved SET1/MLL family methyltransferases. We previously demonstrated that SET-2, one of the two SET1/MLL family members encoded by the C. elegans genome, is required to promote expression of germline genes and repress somatic genes in the germline. Ectopic expression of somatic genes in germlines lacking SET-2 activity correlates with loss of germline pluripotency and transdifferentiation of germ cells into somatic cell types. I will present recent data showing that in addition to preserving germline identity, SET-2 and H3K4me are also required for genome stability in the C. elegans germline. Specifically, we established that the absence of SET-2 results in (i) a mutator phenotype, (ii) increased sensitivity to DNA damage inducing agents, (iii) an accumulation of DNA double strand breaks (DSBs) and (iv) fragmented chromosomes following exposure to ionizing radiation. Interestingly, genome instability observed in the absence of SET-2 is not associated with defects in the DNA damage response (DDR) pathway, strongly suggesting that SET-2 and H3K4me act downstream of the DDR to control genome stability in the C. elegans germline. Our working model is that the absence of H3K4 methylation in the germline might affect the respective use of conservative homologous recombination and error-prone end-joining pathways to repair DSBs. To test this model, we are now combining molecular analysis of repair footprints at a set of specific loci where we can experimentally induce targeted DSB and genome-wide sequencing approaches.
How the worm completes its skin

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At the end of the first larval molt in C. elegans, ventral epidermal P cells migrate and remodel, relinquishing their epidermal role before taking on neuroectoblast fate. This leaves a ventral ‘hole’ in the architecture of the C. elegans epidermis. Here we describe how this hole is filled by a hitherto-overlooked ventral intercalation event that mirrors the well-described embryonic dorsal epidermal intercalation. Ventral intercalation involves polarised PAK-dependent cytoskeletal remodelling of anterior daughters of asymmetric (seam) stem cell divisions, prior to P cell migration. Conversely, the posterior seam-fated (self-renewing) daughters retain their lateral positions. Thus, for the first time, the major syncytial epidermal organ, hyp7, on the ventral side of the animal is completed. Using this novel system, we show that patterning of adjacent epidermal tissues by pal-1/caudal, acting downstream of Wnt, determines the polarity of apical junction remodelling and thus polarity of migration during intercalation. Finally, we argue that the change of gene expression, structure and function of the P cells during this major epidermal remodelling is indicative of a transdifferentiation event.
PAR polarity in C. elegans zygotes is established by a mechanochemical feedback-patterning motif

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Many conserved morphogenetic processes are orchestrated by a well-controlled interplay between mechanical forces and biochemical regulation. A key example is the early embryonic development of the Caenorhabditis elegans zygote, where large-scale flows of the actomyosin cortex occur simultaneously with the establishment of a polarity pattern in partitioning defective (PAR) proteins. However, how the PAR system interacts with and regulates cortical flow has remained elusive. By combining quantitative fluorescence microscopy, cell biology analysis and a physical theory, we here identify a novel mechanochemical pattern-generating motif, which represents the mechanism that drives the patterning of the PAR polarity proteins in the C. elegans zygote. Using Fluorescence Recovery After Photobleaching (FRAP) and RNA interference (RNAi), we demonstrate that the PAR domains feed back on the mechanics by establishing and maintaining a non-muscle myosin II (NMY-2) - based contractility gradient. To study the consequence of this PAR-mediated feedback on NMY-2, we first measured the dynamics of the PAR and NMY-2 system. Using calibrated, quantitative fluorescence microscopy, we measured the spatiotemporal evolution of the membrane-associated protein concentration of the posterior PAR-2, the anterior PAR-6 and NMY-2 as the mechanical force generator, as well as the cortical flow field. Next we show that these measured dynamics of PAR polarity establishment can be quantitatively recapitulated, using a reaction-diffusion-advection theory for the concentration fields of NMY-2, the posterior PAR-2 and the anterior PAR-6, in combination with a thin-film active-fluids theory for the flow field generated by NMY-2 gradients. Essential for this was the biochemical control of the PAR domains on the NMY-2 binding kinetics, which closes the mechanochemical feedback loop. Remarkably, our physical theory can, for the first time, fully recapitulate the spatiotemporal evolution of all the measured PAR-2, PAR-6 and NMY-2 membrane-concentration fields as well as the actomyosin flow field in the polarization process. We demonstrate that the function of this mechanochemical feedback is to amplify and stabilize cortical flows and thus to promote a rapid transition to the patterned state of the PAR system. We anticipate that this work will open new avenues in our quantitative understanding of the emergence of patterns during the development of an organism.
The Mechanism that Determines the Transgenerational Duration of RNAi in C. elegans

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In C. elegans small RNAs enable transmission of epigenetic responses across multiple generations. Heritable RNAi responses normally last for 3-4 generations, however, the mechanisms that determine the duration of inherited silencing, and allow “forgetting” of the inherited epigenetic effects after a number of generations are completely unknown. We now show that exposure to specific dsRNAs activates a systemic feedback signal, that dictates the transgenerational duration of other ancestral RNAi responses. RNA-seq analysis exposed a change in the production of heritable endogenous small RNAs, which regulate the expression of RNAi factors. Manipulating genes in this feedback pathway was found to change the duration of heritable silencing. We will describe these experiments and present new insights into the function of the specific genes that are involved. Such active control of transgenerational effects could be adaptive, since ancestral responses would be detrimental if the environments of the progeny and the ancestors were different.
Robustness to mechanical deformation during C. elegans embryonic development

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Biological processes such as embryonic development must proceed robustly despite both internal variability and external perturbations. Mechanisms that confer robustness to internal molecular fluctuations have been quite extensively studied. However, the principles that underlie robustness to mechanical perturbation are unclear. In this study we perturbed C. elegans embryos by compression, and compared the development of the embryos to uncompressed embryos. Embryo compression occurs also during the natural life cycle of the animal, specifically when old or starved adult worms retain developing embryos inside their bodies. **Methods** We tracked the nuclei during embryogenesis using RW10029, which expresses histone-GFP fusion proteins. Embryos were compressed for imaging on a microscope slide with 20µm polystyrene beads and imaged on an upright Leica SP5 confocal microscope. Uncompressed embryos were embedded on low-gelling-temperature agarose and imaged on a SPIM microscope. During imaging temperature was controlled at 23-25°C and stacks were taken every minute for 10 hours. To retrieve the nuclei positions over time we employed a segmentation and tracking algorithm based on a model-evolution approach (Krueger, Jelier et al. Dev. Biol. 2015). For the irradiation of selected cells we used a strong IR, normally used for multi-photon imaging connected to the Leica SP5. Non-muscular myosin was observed using a cross between OD70, membranes marked by mCherry::PH, and JJ1473, which expresses NMY-2::GFP, shared by Zhirong Bao. **Results** Systematic single cell analysis reveals that compression predictably constrains cell positioning in the embryo. As a consequence, 90 minutes after the 4-cell stage the relative positions of many cells have been changed causing severe distortions of the embryonic axes. The mechanism that compensates for these distortions is a large-scale movement of cells starting shortly after the 6th division of the AB lineage. During this large-scale rotation the movement of at least 34 cells (39% of cells at this stage of development) is changed compared to the uncompressed embryo. The movements are not homogeneous throughout the embryo, and some lineages move more in the uncompressed embryo. We tested which cells were crucial for these movements by systematically ablating cells at the 8 cell stage. We found the movements were not driven by the concurrent gastrulation movements, instead only the ablation of ABar and specifically its daughter ABarpp stopped the specific movements. Strikingly, the descendants ABarppaa/p of the ABarpp cell show the largest differential movements and a strong reduction of misplacement in the compressed embryo. Looking further we found ABarppap to show a strong accumulation of NMY2 on its apical surface, indicative of force generation, and shows a dramatic shape change during the movements. It develops a basal protrusion towards the left side of the embryo and then physically contacts the ABplappa/p cells. For ABarppap the largest mispositioning in the compressed embryos is relative to ABplappa, followed by ABplappa, and the movements correct this mispositioning. **Conclusion** We propose that the mechanism that underlies this compensatory rotation of cells is the regulative movement of a small number of specific cells to their correct local environment. This mechanism ensures resilience to the major mechanical deformation experienced during the early development of this organism.
Quantitative Analysis of Stochastic Gene Expression Dynamics during the AC/VU Cell Fate Decision

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Cells in developing organisms have to robustly assume the correct fate in order to fulfill their specific function. However, some cell fate decisions are made in a stochastic manner, with cells randomly choosing one cell fate out of a repertoire of different possible ones. It is thought that stochastic cell fate decisions exploit random molecular fluctuations, so-called molecular noise, by using positive feedback loops in the signaling network to convert this noise into discrete cell fates. Yet, how a cell uses such a stochastic process to reliably drive cell fate decisions is an open question. We address this question by novel quantitative approaches, focusing on one of the genetically best-understood stochastic cell fate decisions: the AC/VU decision in C. elegans gonad development. During the AC/VU decision two initially equivalent cells, Z1.ppp and Z4.aaa, interact, so that one cell becomes the anchor cell (AC) and the other cell a ventral uterine precursor cell (VU). It is thought that small stochastic differences in gene expression are amplified by lateral Notch signaling, leading to one cell expressing only the Notch receptor lin-12 (VU) and the other cell only its ligand lag-2 (AC). However, it is currently not known what noise sources drive this decision and how proper cell specification is guaranteed within the given time. To address this, we use two complementary techniques: 1.) single molecule FISH to quantify expression of lag-2 and lin-12 with single mRNA resolution in fixed animals at different stages of the decision process and 2.) a novel timelapse technique to follow expression dynamics of both genes in individual live animals over the first 30 hours of larval development. Surprisingly, our results reveal that lag-2, independently of lin-12, is the main driver of the decision process and exhibits strongly stochastic and Notch-independent expression already in the mother cells Z1.pp and Z4.aa. Our results suggest that the transmission of the resulting variable lag-2 levels from the mother cells to Z1.ppp and Z4.aaa are required for a rapid and robust cell fate decision.
The combined activity of CPB-1\textsuperscript{CPEB} and GLD-3\textsuperscript{Bic-C} opposes FBF\textsuperscript{Pum} to prevent the sperm-to-oocyte switch in C. elegans males

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Gamete formation requires genetic programs that coordinate germ cell differentiation and meiosis with sexual fate choice and maintenance. At the posttranscriptional level these programs are integrated via a dense network of RNA-binding and RNA-modifying proteins. Especially translational regulators, such as PUF, Nanos and cytoplasmic polyadenylation element-binding (CPEB) family proteins were identified in many organisms as molecular nodes of the germ cell fate decision machinery. Caenorhabditis elegans germ cells undergo a programmed sperm-to-oocyte switch in the hermaphrodite to produce -in essence- a self-fertile female animal. This is governed by the sequential action of the RNA-binding protein FOG-1\textsuperscript{CPEB}, which initiates male fate specification, and the FBF\textsuperscript{PUF}/NOS-3\textsuperscript{Nanos} RNA regulatory complex that blocks several male fate-promoting ('fem' and 'fog') genes by preventing their efficient translation, thus limiting sperm production to the final larval stage, prior to adulthood. Previously, we showed that in males the Bicaudal-C protein GLD-3 antagonizes FBF-mediated translational repression: while gld-3 mutant germ cells initiate spermatogenesis during larval stages, adults often fail to maintain their male fate and switch to oogenesis in an fbf-dependent manner, suggesting that GLD-3\textsuperscript{Bic-C} is an important player but not the only one in males to keep the female fate-promoting machinery in check. In search of additional regulators, we identified CPB-1\textsuperscript{CPEB} as a molecular opponent of FBF in sexual fate maintenance. Although cpb-1 hermaphrodites produce -compared to wild type- a similar number of sperm, indicating no crucial role in the sperm-to-oocyte switch fate, we confirmed a previously suggested role in sperm differentiation; all animals are sterile. Importantly, we found that a large fraction of adult males produce oogenic cells after an initial period of spermatogenesis. Our genetic analysis places cpb-1 in the core sex determination pathway upstream of fbf and in parallel to gld-3. Intriguingly, all gld-3; cpb-1 double mutant male gonads produce exclusively oocytes suggesting that CPB-1 works redundantly with GLD-3 to antagonize FBF, which is further corroborated by their expression patterns. To our surprise, detailed structure-function analysis of transgenic rescuing experiments suggested that CPB-1’s RNA recognition motifs are essential for male fate maintenance, rather than a previously mapped FBF-binding region in its amino terminus. Therefore, CPB-1 likely functions in its capacity as an RNA-binding protein to target mRNAs required for maintenance of male germ cell fate. Our findings reveal a novel role for CPEBs and contrast to work in mammals where a transcriptional network is a key regulator of sexual fate maintenance.
A spectrin/PAK/actin-capping network stabilizes cell shapes in a morphogenetic ratchet process during C. elegans elongation

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Embryonic morphogenesis refers to the orderly process of cell shape and cell position changes in the embryo. While it is clearly established that mechanical forces induced by molecular motors play a critical role in driving these changes, proper understanding of how cells respond to such forces is lacking. In particular, the interplay between cell elasticity and the response to forces remains unclear. To address those issues, we are studying elongation of C. elegans embryos, which is initially driven by actomyosin contractility till muscle becomes active at 1.7 fold stage, and then requires muscle activity to reach the 4-fold stage. Our main goal was to understand how the epidermis, an elastic material, maintains its shape through cycles of muscle contractions, while extending only in the anterior-posterior direction.

We previously established that tension resulting from repeated muscle contractions directly influences junction and cytoskeleton remodeling through a mechanotransduction pathway. Previous work has positioned the kinase PAK-1 at the crossroads of hemidesmosome and cytoskeleton remodelling. To better understand how PAK-1 acts, we performed a yeast two-hybrid screen and an RNAi screen in a strong pak-1 mutant background, which identified one gene in common encoding the α-spectrin SPC-1. Using genetic tools and live imaging approaches, we further investigated the role of α-spectrin and its interaction with the kinase PAK-1.

We found that when PAK-1 and SPC-1 are both absent embryos elongate to the 1.5-fold stage and then retract to their initial shape. At the subcellular level, circumferential actin filament bundles become discontinuous at the level of hemidesmosomes acting as a transpeithelial muscle anchoring structure to the cuticle. This retraction phenotype suggests the existence of a yet undescribed locking mechanism during embryonic morphogenesis, whereby the interaction of PAK-1 and SPC-1 ensures shape maintenance both at a cellular and at an embryonic level, through the dynamic remodeling of the actin cytoskeleton. To understand at the molecular level how this locking mechanism might operate, we performed another screen in a spc-1(Ø) background and identified three actin-binding proteins, which when absent with SPC-1 also induce embryos to retract.
Sex- and tissue-specific regulation of RNA interference

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RNA interference (RNAi) is a conserved mechanism modulating gene expression during development of many organisms. While the core RNAi pathway is well known, how different tissues in a living organism respond to exogenous RNAi is less understood. To quantify RNAi efficiency, we measured tissue specific fluorescence decrease upon gfp(RNAi) in C. elegans expressing ubiquitously GFP. We found that RNAi efficiency is sex specific. Hermaphrodites show efficient silencing in both soma and germline. By contrast, only the soma is RNAi sensitive in males, with strong RNAi resistance in the germline. Mutant animals with transformed germline sex revealed RNAi sensitivity of both hermaphrodite spermatocytes and male oocytes. Thus, hermaphroditic germline identity supports exogenous RNAi independently of somatic sex or cell type, suggesting that germline sex regulates germline RNAi. In worms, RNAi proceeds in the following steps: (1) transport into the tissue, (2) cleavage of exogenous trigger, (3) amplification and (4) target silencing. To identify the limiting step in male germline RNAi processing, we analyzed exogenous small RNAs by high throughput sequencing in dissected germlines of both sexes. The results are compatible with a model where amplification of secondary siRNAs is defective in males, while downstream processing is functional. In line with this model, we identified several RNAi genes with lower expression in male germlines that may cause the observed sex-differences in RNAi efficiency.
Cell-focussing, translation of the linear information of DNA into order and form

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Life is order. Therefore, it is a key question, how the linear genetic information of the DNA codes for the plethora of forms of organisms. A worm will produce a worm, a fly will produce a fly and families will pass on their facial features. The classical model suggests that form is created by a series of spatial inductions, which organise tissues, convoyed by growth, migration, and modulation of cell shape. In contrast, using 4D analyses, we showed that in the C. elegans embryo, pattern and form are the result of a cell sorting process involving long-range migrations coordinated by local comparison of cell ‘addresses’, which are intrinsic features of individual cell fates. We termed this novel process for guiding the generation of form ‘cell-focussing’. Revolutionary work in stem cell research has recently shown that eyecups or small brains can develop de novo from stem cells in vitro. Because this opposes the general model of morphogenesis, the existence of a ‘latent intrinsic order principle’ causing dynamic self-patterning is postulated. Cell-focussing embodies these principles and has therefore the potential to explain self-patterning. The creation of cell addresses used to arrange cell position should be an ingenious solution to translate the linear information of DNA into form. In a screen for ts mutants we isolated a mutant in pmm-1 (t3091), strongly reducing N-Glycans, which causes cells to be significantly displaced compared to wild-type. This suggested that glycans may be involved in the generation of the postulated addresses. An analysis of cell behavior, after an interference with glycan synthesis, reveals normal mitosis patterns but an alteration of cell migration. The general movement remains unchanged but cells migrate effectively less far than normal cells, which appears quite enigmatic. To find the cause for this hindrance, we analysed the movement of cells by tracing the cell positions every 35 seconds. It appeared that in normal embryos cells are dancing back and forth all the time and the effective migration appears to be caused by a directional bias of the dancing movement. We propose that this dancing is the basis of migration in the C. elegans embryo. After interference with glycan synthesis, cells migrate effectively only 70% of the distance of wild-type cells, since cells revert their dancing directions 1.6 times more often than normal cells. Do cells err because they are generally hampered to move effectively, or because they have no/less information, where to aim because they lack addresses? The question could be resolved by challenging the cell-focussing process by interfering with the glycans in a glp-1 mutant. The resulting distance map looks like the completely reorganised glp-1 distance map, just slightly blurred. Therefore it is rather improbable that glycans code for cell addresses. However, as also discussed, they are essential for the morphogenesis shaping later the larva. The search must go on.
Mitochondria, neurodegeneration and ageing

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Mitochondria, the main energy hub of the cell, are highly dynamic organelles, playing essential roles in fundamental cellular processes. Mitochondrial function impinges on several signalling pathways modulating cellular metabolism, cell survival and healthspan. Maintenance of mitochondrial function and energy homeostasis requires both generation of newly synthesized and elimination of dysfunctional mitochondria. Impaired mitochondrial function and excessive mitochondrial content are major characteristics of ageing and several human pathophysiological conditions, highlighting the pivotal role of the coordination between mitochondrial biogenesis and mitophagy. However, the cellular and molecular underpinnings of mitochondrial mass homeostasis remain obscure. We found that DCT-1, the C. elegans homolog of mammalian BNIP3 and BNIP3L/NIX, is a key mediator of mitophagy promoting longevity under stress. DCT-1 acts downstream of the PINK-1-PDR-1/Parkin pathway and is ubiquitinated upon mitophagy-inducing conditions to mediate the removal of damaged mitochondria. In neurons, mitophagy eliminates damaged mitochondria transported to the soma of the cell, upon oxidative stress. This process is triggered by intracellular calcium increase and facilitates neuronal survival and ameliorates neurodegeneration under stress. Accumulation of damaged mitochondria triggers SKN-1 activation, which initiates a bipartite retrograde signaling pathway stimulating the coordinated induction of both mitochondrial biogenesis and mitophagy genes. Taken together, our results unravel a homeostatic feedback loop that allows cells to adjust their mitochondrial population in response to environmental and intracellular cues. Age-dependent decline of mitophagy both inhibits removal of dysfunctional or superfluous mitochondria and impairs mitochondrial biogenesis resulting in progressive mitochondrial accretion and consequently, deterioration of cell function.
DAF-16 and HLH-30 cooperate as combinatorial transcription factors to regulate stress responses and aging in C. elegans

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When an organism experiences dire conditions (e.g. stress or nutrient deprivation), dedicated signaling cascades get activated to convey appropriate cellular and organismal responses that ensure the organism’s survival – in particular by rendering it more stress resistant, delaying its aging, and thus promoting its longevity. Central to these responses is the conserved transcription factor DAF-16/FOXO, located downstream of the nutrient-sensing insulin/IGF signaling (IIS) pathway. Under normal conditions, IIS is active, leading to phosphorylation of DAF-16 by AKT kinases and its cytoplasmic sequestration by 14-3-3 proteins – away from its target genes. But under low IIS or also a broad variety of other distress signals, DAF-16 is released from cytoplasmic sequestration and translocates into nucleus to drive expression of stimulus-specific sets of stress resistance and longevity promoting genes. Although DAF-16 has been extensively studied, it remains elusive how one transcription factor alone can integrate such multitude of upstream stimuli and eventually relay them into a customized transcriptional response. Here we show that for many purposes DAF-16/FOXO does not function alone but as a combinatorial transcription factor together with HLH-30/TFEB, a conserved master regulator of autophagy and lysosome biogenesis. Just like DAF-16, also HLH-30 is normally sequestered in the cytoplasm and translocates into the nucleus under similar (although not identical) dire conditions to regulate transcription. Using proteome and genome-scale techniques, we found that DAF-16 and HLH-30 can form a complex and that they co-occupy many target promoters. Consistent with these observations, they co-regulate many target genes in long-lived IIS mutants, germline-deficient animals, under heat, or under oxidative stress, indicating that HLH-30 plays an important role in DAF-16-dependent transcriptional responses. Interestingly though, despite DAF-16 and HLH-30 frequently co-translocating into the nucleus, forming a complex, and co-regulating many target genes, we found that their genetic interaction strongly depends on the upstream stimulus, carefully tuning the physiological outcomes for the animal: i.e. they function in the same pathway when promoting longevity under low IIS or germline-deficiency or when promoting resistance to oxidative stress, but they elicit heat stress responses independently. And while DAF-16 is required for dauer formation, HLH-30 even inhibits this process. We conclude that full integration of nature’s diverse distress signals and their relay into the appropriate responses relies on a synergy between DAF-16 and HLH-30, where they dynamically integrate stimuli convergent on either transcription factor and relay them into an elaborate combinatorial regulation of an extensive panel of target promoters.
The Quality Control Ubiquitin Ligase CHIP Couples Proteostasis and Ageing Through Insulin Receptor Degradation

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Proteostasis relies on cellular quality control networks that balance protein synthesis, folding, and degradation. Impaired proteostasis contributes to aging and protein aggregation diseases. However, mechanistic principles coordinating proteostasis imbalance and longevity are not well understood. Here we report that both processes are strictly interrelated by the quality control ubiquitin ligase CHIP. In Caenorhabditis elegans and Drosophila melanogaster CHIP deficiency stabilizes the DAF-2/insulin receptor, causing reduced lifespan and premature aging. Intriguingly, we identify the insulin receptor as a direct target of CHIP, which mediates receptor monoubiquitylation and endocytic turnover. Upon proteotoxic stress and during aging, however, CHIP engages in the disposal of misfolded proteins, limiting its capacity to degrade the insulin receptor and support longevity. Our study indicates a reciprocal balance between proteostasis and lifespan regulation through chaperone-assisted proteolysis, providing an evolutionarily conserved concept for understanding the impact of proteome imbalance on aging. Protein homeostasis or proteostasis represents the functional balance of the proteome, which is permanently challenged by environmental stress and alterations in physiology. The progressive decline of proteostasis is a hallmark of aging and contributes to protein aggregation diseases. The conserved quality control ubiquitin ligase CHIP interacts both with the chaperone and proteasome systems to support the cellular balance between protein folding and degradation thereby assisting proteostasis maintenance. CHIP mediates the ubiquitylation of nonnative proteins in cooperation with chaperone partners, and prevents an age-dependent pathologic accumulation of protein aggregates. Here we reveal an essential role of CHIP-mediated proteolysis in the regulation of insulin/IGF-1 signaling (IIS), which determines lifespan in metazoan organisms. Our findings establish an evolutionary conserved mechanism that links protein homeostasis to lifespan regulation.
WormBase goes Metabolomics – Curation of metabolites present in the nematode Caenorhabditis elegans

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Metabolomics, the systematic study of metabolites present in a given system, has been an up and coming body of research in the Caenorhabditis elegans field. Extensive research on this soil-dwelling animal provide a solid and rich background on development, aging and longevity on which to explore metabolic profiles, changes and effects. As the community for metabolomics studies grows, the need for database support becomes greater. In order to aid metabolomics research in C. elegans curators at WormBase have expanded a data model to collect metabolite information (www.wormbase.org) and make these data available to the community, in addition, a metabolite nomenclature guide is being developed for naming new metabolites discovered in the worm. At the current stage ~1000 unique metabolites have been added to the molecule class in WormBase, ranging from organic acids, amino acids that occur in all species to secondary worm-specific metabolites likes ascarosides. Metabolite data have been curated from over 45 C. elegans research papers and supplementary materials. Data collected include metabolite names, analysis methods and extraction media. Metabolite names are synced with both MeSH (www.nlm.nih.gov/mesh/MBrowser.html), Kyoto Encyclopedia of Genes and Genomes (KEGG; www.genome.jp/kegg/) and the Chemical Entities of Biological Interest (ChEBI;www.ebi.ac.uk/chebi/) databases. Relevant information for each metabolite is taken from the ChEBI database, this information includes chemical structure representation using SMILES strings and/or InChI code, and molecular formulas together with external database identifiers. Metabolites can be searched using the main search box on the front page of WormBase. Future work will focus on the inclusion of metabolic models similar to those published for other model organisms in the MetaCyc DB collection (www.metacyc.org). We encourage researchers conducting or planning metabolomics experiments using C. elegans to share their data via available data repositories like EBI MetaboLights (www.ebi.ac.uk/metabolights/) or NIH Metabolomics Workbench (www.metabolomicsworkbench.org/) and submit a list of identified metabolites together with unique ChEBI chemical identifiers to WormBase.
Larval crowding accelerates C. elegans development and reduces lifespan

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Environmental conditions experienced during animal development are thought to have sustained impact on maturation and adult lifespan (Ludewig and Schroeder, 2013; Pen et al., 2010, Jung-Hoffmann, 1966). Here we show that in the model organism C. elegans developmental rate and adult lifespan depend on larval population density, and that this effect is mediated by excreted small molecules. By using the time point of first egg laying as a marker for full maturity, we found that wild type hermaphrodites raised under high density conditions developed significantly faster than animals raised in isolation. Furthermore, larval population density, but not population density during adulthood, strongly affected C. elegans lifespan.

Population-density dependent developmental timing (pdde) was dramatically enhanced in fatty acid beta-oxidation mutants that are defective in the production of dauer-inducing ascarosides. In contrast, addition of synthetic ascarosides or steroid hormones that act on the nuclear hormone receptor DAF-12 diminished or abolished pdde by either reducing or accelerating developmental rate. We show that neither ascarosides nor any of the known steroid hormones are required for pdde and that instead another larval population density-dependent signal mediates pdde, in part via the nuclear hormone receptor NHR-8. Taken together, our results demonstrate that C. elegans developmental rate is highly plastic and modulated by several groups of larval small molecules including signals that act via NHR-8. Moreover, our results show that larval imprinting by these population-density dependent small molecules regulates adult lifespan.
Characterization of ribosomal RNA methylations modulating life- and healthspan of Caenorhabditis elegans

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The ribosome has been seen for decades as a static machine that translates mRNAs into proteins. However, over the last few years it became clear that it rather represents a highly dynamic structure that responds to various stimuli by adapting its structure and, as a consequence, its function. Such structurally distinct ribosomes are postulated to be “specialized ribosomes” comprising peculiar functional properties and are thus considered to be engaged in translating specific subsets of cellular messages (Filipovska and Rackham, 2013; Xue and Barna, 2012). Although ribosomal RNA is heavily modified by methylations and pseudouridinylations (Rozenski et al., 1999), the functional roles of such modifications in regulating translation are not understood. We recently reported that lack of a single, conserved C5-methylation at 25S ribosomal RNA residue C2278 alters ribosomal structure and thus translational fidelity in yeast, resulting in a ‘reprogramming’ of the ribosome towards translation of mRNAs involved in cellular stress-response. Importantly, we showed that lack of this methylation by deletion of NSUN5 extends the lifespan and stress resistance of yeast, worms and flies (Schosserer et al., 2015). Interestingly, reduced expression of other ribosomal RNA methyltransferases in addition to nsun-5, such as nol-1 and T07A9.8, were implicated in regulating the lifespan of Caenorhabditis elegans as well (Curran and Ruvkun, 2007). Thus, methylation of ribosomal RNA might represent an important regulator of organismal aging, but the precise molecular mechanisms underlying this lifespan modulation have not been investigated. We will here present a characterization of the aforementioned RNA methyltransferases in C. elegans regarding RNA substrate specificity and effects on translation. Furthermore, we will show that their depletion influences life- and healthspan and demonstrate that similar mechanisms apply to human cells as well.
Ribonuclease-mediated control of body fat

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Over the last century, a continuous increase in obesity and obesity-related diseases such as type-II diabetes and metabolic syndrome has led to a global health crisis. Understanding the molecular mechanisms that underlie the regulatory network of fat metabolism and fat storage is crucial to identify new targets for treatment. So far, transcriptional regulation of fat metabolism has received much attention and key transcription factors involved in lipid metabolism are conserved from nematodes to mammals. However, there has been a growing awareness that lipid metabolism can also be controlled via posttranscriptional mechanisms. Since hibernating animals rely on fat catabolism for survival, we used cold sensitivity of C. elegans to uncover novel factors controlling body fat. One factor identified through this approach is a conserved RNase, REGE-1, related to a key player in the mammalian innate immunity. The examples of posttranscriptional regulation of fat remain few and are limited to specific miRNAs. The miRNAs repress their mRNA targets through translational repression and/or exonucleolytic degradation. By contrast, REGE-1 is an endonuclease, suggesting a possible requirement for the endonucleolytic mRNA degradation in fat regulation. Indeed, we find that REGE-1 controls body fat by degrading mRNA encoding a fat loss-promoting transcription factor, previously implicated in aging. This transcription factor induces many genes functioning in lipid catabolism, possibly explaining how REGE-1 controls body fat. Our findings uncover a novel mechanistic paradigm in fat regulation. Posttranscriptional mechanisms are well suited to a rapid and reversible control of gene expression. Similarly, REGE-1-mediated mRNA degradation might be a way to rapidly re-wire lipid metabolism in response to environmental changes, including change of diet, temperature or exposure to pathogens.
SUMOylation has Key Roles in Coordinating Lifespan and Proteostasis in C. elegans

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The Insulin/Insulin-like growth factor 1 (IGF-1) signaling pathway (IIS) is a prominent aging regulating pathway which is conserved among mammals and nematodes. In Caenorhabditis elegans (C. elegans), the knockdown of daf-2, the sole Insulin/IGF-1 receptor, hyper-activates its downstream transcription factors resulting in lifespan extension, protection from proteotoxicity and elevated stress resistance. Similar outcomes were shown to be achieved by the ablation of the nematode’s germ cells or the knockdown of the Notch receptor encoding gene, glp-1. In this study we asked whether IIS reduction changes protein SUMOylation, a reversible posttranslational modification, to promote these protective functions and whether the aging-regulating functions of the IIS and of the germline intersect. Employing protein pulldown and mass spectrometry we found that CAR-1 (cytokinesis/apoptosis/RNA-binding), an RNA binding protein that functions in the germline of C. elegans, exhibits lower rate of SUMOylation upon IIS reduction. We observed a lifespan reduction of both wildtype worms and of the long lived daf-2 mutant worms after treatment with car-1 RNAi. CAR-1 was previously shown to be a negative regulator of glp-1 expression in the germline. Worms harboring a mutated glp-1 gene are long-lived and exhibit resistance to proteotoxicity. Treating these animals with car-1 RNAi had no effect on their lifespan, indicating that CAR-1 acts upstream of GLP-1 to regulate longevity. Nevertheless proteotoxicity model worms carrying mutated glp-1, showed no difference in proteotoxicity when car-1 was knocked down. Together our discoveries unravel a novel axis of aging regulation by which the IIS modulates signals from the germline by SUMOylation of CAR-1 to mediate longevity and proteostasis.
Discovering cellular pathways that regulate prion-like propagation of aggregation-prone proteins using C. elegans

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In several neurodegenerative diseases, such as Alzheimer’s and Parkinson’s disease (PD), specific proteins form alternative pathogenic conformations that can grow from small oligomeric assemblies to large amyloids. Moreover, pathology is spreading from cell-to-cell in a way reminiscent of prion diseases. There is increasing evidence that misfolded proteins can self-propagate and disseminate between cells to induce the aggregation of like proteins. However, little is known about the exact pathways involved in the cell-to-cell transmission of aggregation-prone proteins. We have tested the transmissibility of several disease-related and model proteins using C. elegans and found that spreading inversely correlates with the aggregation propensity of the proteins. PolyQ40 was highly aggregation-prone and rarely found in neighboring tissues, while α-synuclein, linked to PD, was particularly able to spread from cell-to-cell. Intercellular transmission of α-synuclein was mediated by autophagic uptake from the cytosol into membrane-bound vesicles that allow its exchange between cells and tissues. Spreading of α-synuclein across tissues was age-dependent; it increased during development to early adulthood and persisted during aging. Furthermore, a candidate screen revealed genetic modifiers of α-synuclein spreading. Our results demonstrate that intercellular protein spreading is protein selective and an actively regulated process. A better understanding of these pathways might reveal new therapeutic strategies to combat certain neurodegenerative diseases.
Dose-dependent functions for chromatin modifiers in regulating lifespan.

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*co-first authors Following an RNAi screen targeting chromatin factors, we identified H3K27 modifiers as key regulators of lifespan in C. elegans. Depletion of the jmjd-3.2 and utx-1 H3K27 demethylases, but not jmjd-3.1 or jmjd-3.3, results in significant lifespan and healthspan extension. Furthermore, overexpression of either enzymatically functional or “dead” forms of jmjd-3.2 both extend lifespan, suggesting that, firstly, both up-regulation and down-regulation of jmjd-3.2 has similar lifespan consequences, and secondly, that the enzymatic activity is dispensable for improved longevity in worms. In contrast, while overexpression and depletion of utx-1 also extend lifespan, we show that the enzymatic activity of UTX-1 is absolutely required for lifespan regulation, despite its demethylase-independent role during development (Vandamme et al. 2012). This suggests that jmjd-3.2 and utx-1 may regulate lifespan via different pathways. Epistasis experiments support this hypothesis, as well as suggesting that the insulin signaling pathway is a crucial overall target of regulation for both factors. However, ChIP analysis implies that lifespan extension driven by utx-1 overexpression may involve targets distinct from daf-2, previously associated with UTX-1 function (Jin et al. 2011). It is also striking that heterozygous mutants for some of the chromatin factors we identified in the screen also displayed lifespan extension, suggesting that this is a dominant phenotype. Together with the fact that both up and down-regulation of these factors extend lifespan, this implies that chromatin remodelers promote longevity in a dose-dependent manner. REFERENCES: Jin, C. et al., 2011. Histone demethylase UTX-1 regulates C. elegans life span by targeting the insulin/IGF-1 signaling pathway. Cell metabolism, 14(2), pp.161–172. Vandamme, J. et al., 2012. The C. elegans H3K27 demethylase UTX-1 is essential for normal development, independent of its enzymatic activity. PLoS genetics, 8(5), p.e1002647.
Antagonistic Pleiotropy in the autophagic machinery modulates lifespan conversely over age in *C. elegans*

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In the year 1957 George C. Williams formulated the antagonistic pleiotropy hypothesis of aging. This theory predicts that some genes mediate beneficial effects early in life when natural selection is strong, but are detrimental late in life when natural selection is weak. Accumulation of these harmful effects late in life could explain the aging process in the light of evolution. In an RNAi screen designed to discover novel genes that exhibit antagonistic pleiotropy, we identified the forkhead box (FOX) A transcription factor pha-4 as a top candidate. Inactivation of pha-4 from L1 larval stage and from the first day of adulthood reduced *C. elegans* lifespan. In contrast, post-reproductive inactivation of pha-4 resulted in a significant lifespan extension. As PHA-4 is known to regulate autophagy, we investigated the effects of late-life inactivation of autophagy related genes on lifespan. Post-reproductive inactivation of genes required for the autophagosome nucleation led to a remarkable lifespan increase of more than 60%. Just like pha-4, these genes reduced lifespan when inactivated early in life. Interestingly, post-reproductive inactivation of genes that are involved in later steps of the autophagosome formation had no positive effects on *C. elegans* lifespan. Autophagy is mostly described with its advantageous cytoprotective and longevity-promoting effects. Yet, some studies characterize autophagy as a double-edged sword that, under certain conditions, can be detrimental to the organism. Our data suggests that aging dysregulates autophagy and thereby alters this process from advantageous to harmful. Generally, our findings emphasize that timing needs to be addressed in order to fully understand how genes and gene networks impinge on the aging process.
Nuclear Receptor NHR-49 is a novel regulator of oxidative stress responses

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Cytoprotective mechanisms and lipid metabolism are strongly linked to aging and age-related disorders. Delineating how these processes are regulated is therefore critical. Our work focuses on MDT-15, a subunit of Mediator, an evolutionarily conserved transcriptional coregulator. mdt-15 is required to express genes involved in lipid metabolism and various stress responses and is also essential for the lifespan of wild-type and long-lived worms. To regulate cytoprotective genes, MDT-15 interacts physically and functionally with the detoxification master regulator SKN-1/Nrf. However, not all oxidative stress response genes require skn-1; e.g., the organic peroxide tert-butyl-hydroperoxide (tBOOH) induces a largely SKN-1–independent response. We found that mdt-15 is required for the SKN-1–independent tBOOH response; however, the transcription factors (TFs) that cooperate with MDT-15 in this response remain unknown. To identify these TFs we conducted a candidate RNAi screen for genes required to induce a transcriptional reporter of the flavin-containing monoxygenase fmo-2, a highly tBOOH-responsive gene; candidates tested included TFs that bind MDT-15 (interactome data) or the fmo-2 promoter (modENCODE data). We found that the nuclear hormone receptor nhr-49 was required for fmo-2p::gfp induction by tBOOH. Using qPCR, we confirmed that nhr-49 null mutants fail to induce fmo-2 and several other tBOOH-responsive genes, whereas the induction of SKN-1 target genes by arsenite was not affected in this mutant. Conversely, nhr-49 gain-of-function (gof) mutants showed upregulation of fmo-2 and of other tBOOH-responsive genes in the absence of tBOOH, and mdt-15 was required for this induction. nhr-49 was also required for the induction of fmo-2 and other tBOOH-responsive genes by starvation, and nhr-49 and fmo-2 null mutants were hypersensitive to starvation, demonstrating functional importance of these genes. To delineate upstream regulatory inputs of NHR-49, we depleted various stress-signaling kinases by RNAi and found that depletion of akt-1, akt-2 or sgk-1 strongly upregulates fmo-2p::gfp in wild-type worms but not in nhr-49 null mutants. We propose a model whereby tBOOH and starvation alleviate NHR-49 inhibition by akt-1, akt-2 and sgk-1, thus promoting the expression of cytoprotective programs by an NHR-49:MDT-15 regulatory complex. NHR-49 is a known regulator of lipid metabolism, but our data highlight an interesting new role for NHR-49 as a regulator of oxidative stress responses. Additionally, as NHR-49 has emerged as an important regulator of longevity, e.g. in germline-less glp-1 mutants, our work suggests that NHR-49 may modulate both metabolism and oxidative stress adaptation to confer longevity.
The J-Protein Family of C. elegans – Expression analyses during Aging and Stress

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Proteins have to fold into their specific three-dimensional structure to become fully active. Within the highly crowded environment of cells, protein folding is supported by chaperones such as members of the ATP-dependent Hsp70 family. In addition to de novo protein folding, the Hsp70 family has a broad functional spectrum, as they are e.g. important for protein refolding after stress, protein disaggregation and transport. The different members of the Hsp70 family act in specific complexes with J-proteins. J-proteins are co-chaperones, which bind client proteins and stimulate the ATP hydrolysis activity of their cognate Hsp70. Caenorhabditis elegans encodes for 8 different Hsp70 yet 31 J-proteins. This high diversity within the J-protein family suggests that these co-chaperones play an important role in the specificity and the broad assignment of the Hsp70 machinery. So far, only little is known about their function and specific expression pattern. We can show that four members respond to heat stress with dnj-13 as the most heat-inducible J-protein. The heat shock induction is very transient and rapidly declines with aging, which may explain the arising imbalances in protein homeostasis with the progression of aging. Interestingly, we identified different J-protein expression patterns in neurodegenerative disease models that express e.g. polyQ, α-synuclein or Aβ, which points to specific cellular responses due to the formation of different protein aggregates. For example, only J-proteins that are targeted to the ER were up regulated in the Parkinson’s disease model while in the Huntington’s disease model the JB6/8 homolog, dnj-24 was induced. Depletion of those J-proteins consequently results in increased aggregation of the respective disease-causing protein. Our results show a J-protein signature during various proteotoxic challenges and give insight into the organismal J-protein network in aging and disease.
A neuroligin-like gene and a transthyretin-like gene prevent dopaminergic neurodegeneration and protect from environmental stress

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Dopamine plays a key role in the coordination of movement, emotion and the recognition of reward, and the loss of dopaminergic neurons is a hallmark of Parkinson's Disease. The aetiology of this disease is largely unknown, but exposure to oxidative stress is being considered as a likely cause. We used C. elegans to screen for genes that protect dopaminergic neurons from oxidative stress inflicted by 6-hydroxydopamine (6-OHDA) uptake. In addition to the previously identified tetraspanin tsp-17, we found that mutations in the neuroligin-like gene glit-1 and the transthyretin-related gene ttr-33 highly increase dopaminergic neurodegeneration after 6-OHDA exposure. We wish to understand the mode of action of these genes and hypothesise that they could regulate 6-OHDA uptake, oxidative stress levels or cell death. tsp-17 and glit-1(gt1981) mutants exhibit identical phenotypes in almost all assays including behavioural defects linked to dopamine. Both TSP-17 and GLIT-1 are transmembrane proteins and we think that they might be involved in the regulation of dopamine turnover, impacting on 6-OHDA uptake into the neurons. Interestingly, neuroligin family members have been shown to alter the development and function of synapses. In contrast, ttr-33(gt1983) mutants do not show behavioural defects and we hypothesise that the gene might protect dopaminergic neurons from engulfment. The only characterised C. elegans transthyretin-related protein, TTR-52, was reported to bridge phosphatidylinerine exposed on the surface of apoptotic cells to the phagocytosis machinery. This pathway also seems to be required for axon regeneration. It is not known how dopaminergic neurons die upon exposure to oxidative stress. Yet, we find that neurodegeneration in the ttr-33 mutant is largely suppressed by mutating the phagocytosis gene ced-10. In addition to their role in neurodegeneration, glit-1, tsp-17 and ttr-33 also affect organismal stress protection. Mutant worms are short-lived, hypersensitive to oxidative and osmotic stress but resistant towards ER stress. Intriguingly, the exact opposite phenotypes were reported for mutations in the JNK stress kinase homolog kgb-1, which is also required for axon regeneration. We found that kgb-1 is synthetic lethal with ttr-33. In addition, we are following up potential links with the apoptosis genes ced-3, ced-4 and the calcium-binding chaperone and calreticulin homolog crt-1, all of which were shown to promote neuronal regeneration and to confer organismal stress protection. We found increased neurodegeneration in ced-3 and ced-4 mutants and synthetic lethality between glit-1 and crt-1. Also, we are currently generating reagents to determine where GLIT-1 and TTR-33 act. In summary, we think that tsp-17, glit-1 and ttr-33 – as well as genes reported to be involved in the regeneration of severed axons - protect dopaminergic neurons and play a role in the organismal defence against environmental stress.
DAF-16 interacts with TGF-ß/BMP signaling to promote germline tumor formation in C.elegans

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Activation of the FOXO transcription factor DAF-16 by reduced insulin/IGF signaling (IIS) is considered to be beneficial due to its ability to extend lifespan and enhance stress resistance in C. elegans. Cell-autonomous DAF-16 activity also inhibits cell proliferation in the germline. In contrast, hypodermal DAF-16 may cause a tumorous germline phenotype characterized by hyperproliferation of the stem cells and rupture of the adjacent basement membrane. Here we show that interaction of DAF-16 and transforming growth factor (TGFß)/Bone Morphogenic protein (BMP) signaling pathway causes germline tumor formation. We provide evidence that DAF-16 may directly interact with both R-SMAD proteins SMA-2 and SMA-3 in the nucleus to regulate the expression of several common target genes. Knocking-down each of these target genes in the hypodermis is sufficient to inhibit germline proliferation, indicating a cell-nonautonomously controlled regulation of stem cell proliferation by somatic tissues. We propose the existence of two antagonistic DAF-16/FOXO functions in distinct tissues, a tumor-promoting somatic and a tumor-suppressive germline activity. Whereas germline proliferation under reduced IIS is inhibited by DAF-16 cell-autonomously, activation of hypodermal DAF-16 in the presence of active IIS promotes germline proliferation and eventually leads to tumors formation.
Maternal stress regulates a soma-to-germline insulin-like signalling pathway to control progeny development

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In the late 19th century August Weismann proposed that information about the environment could not pass from somatic cells to germ cells, a hypothesis now known as the Weismann barrier. However, several recent studies have indicated that parental environment can alter progeny physiology. For example, parental exposure of the nematode Caenorhabditis elegans to mild osmotic stress enhances progeny survival during high osmotic stress. It remains unclear if these cases represent the direct effects of stress on the germline and early embryo that in turn result in an adaptation that persists in the progeny or if somatic signals can communicate with the germline to enhance progeny survival. Here we report that C. elegans arrests development in response to high osmotic stress; that this arrest is caused by a decrease in insulin-like signalling; that maternal exposure to mild osmotic stress protects against progeny arrest; and that this protection is a consequence of a decrease in maternal insulin-like signaling to the germline that in turn results in an increase in progeny insulin-like signalling. We conclude that in the mother somatic signalling activates an insulin-like signal transduction pathway in the germline to transduce the signal from maternal osmotic stress to progeny stress-resistance. We speculate that modulation of maternal insulin-like signalling might be responsible for effects of the maternal environment on diseases that involve insulin signalling, including type-2 diabetes.
Serum- and Glucocorticoid-induced Kinase 1 (SGK-1) regulates mitochondrial quality control mechanisms and autophagy to modulate lifespan

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Aging is a biological process characterised by a progressive decline of body homeostasis. Among the different cellular pathways affected by aging, mitochondrial signalling is essential for the maintenance of a proper energy metabolism and cellular health. In that sense, mitochondrial dysfunction triggers the activation of several quality control mechanisms, such as the mitochondrial unfolded protein response (UPRmt) and mitophagy, which are intended to restore mitochondrial homeostasis under stress conditions and to degrade damaged mitochondria, respectively. Although these stress responses have been implicated in the regulation of aging, the underlying molecular mechanisms and their regulation remain largely elusive. We have previously demonstrated that depletion of prohibitin (PHB), a multimeric complex sitting in the inner mitochondrial membrane, shortens lifespan and induces a strong UPRmt in wild type worms. However, loss-of-function mutants of the Insulin/IGF-1 Signalling (IIS) pathway, like Serum- and Glucocorticoid-induced Kinase 1 (SGK-1) mutants, show a longer lifespan and a reduced UPRmt upon PHB depletion compared to wild type. This interaction has been suggested to act, in addition to the IIS pathway, through the mechanistic Target of Rapamycin Complex 2 (mTORC2) pathway, highlighting the implication of SGK-1 in two parallel pathways to regulate longevity and the UPRmt. Moreover, SGK1 has been recently shown to inhibit autophagy in mammals, which can lead to accelerated aging and neurodegenerative diseases. Herein, we show that SGK-1 expression in Caenorhabditis elegans decreases throughout aging. Moreover, inhibition of both the UPRmt and mitophagy by RNAi increases the expression of SGK-1 compared to basal conditions, whereas mitochondrial stress reduces SGK-1 levels and induces autophagy. Interestingly, SGK-1 loss-of-function mutants display a higher accumulation of autophagic vacuoles throughout aging compared to wild type worms, which is enhanced in PHB depleted worms. These data constitute, to our knowledge, the first evidence of SGK-1 regulating the autophagy pathway in C. elegans and underpin a role of SGK-1 in the regulation of the mitochondrial quality control mechanisms, UPRmt and mitophagy, hence affecting lifespan.
Specification of synaptic identity: thinking out of the box at the *C. elegans* neuromuscular junction.

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Synaptic scaffolding molecules control the localization and the abundance of neurotransmitter receptors at the synapse, a key parameter to shape synaptic transfer function. Most characterized synaptic scaffolds are intracellular, yet a growing number of secreted proteins appears to organize the synapse from the outside of the cell. Using a combination of pharmacological and visual genetic screens in *C. elegans* we identified a series of synaptic proteins of unforeseen domain composition that control the identity of postsynaptic domains mainly through extracellular interactions. Specifically, the extracellular matrix protein Ce-Punctin/MADD-4 is differentially secreted by cholinergic and GABAergic motoneurons and dictates the composition of postsynaptic domains by positioning the machineries involved in acetylcholine and GABA receptor clustering. How this synaptic organization can be envisioned from an evolutionary prospective will be discussed.
Sleep and wake in C. elegans are global brain states under tight control of arousal circuits.

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A fundamental question in neuroscience is how global brain states, such as sleep and wakefulness, are efficiently and reversibly generated and maintained. Brain states may be controlled in a top-down manner by dedicated regulatory neural circuits, contrarily they could emerge from a self-organizing mechanism distributed across many neurons, or be a combination of both. In mammals we currently do not have the spatial-temporal resolution to measure brain-wide the activity of single cells and therefore cannot determine how individual neurons across the entire brain are contributing to the emergent properties of global brain states. Nor can we tell how the global brain affects the activity of neurons. For example, does a brain state affect the activity of a subset of crucial neuronal subpopulations or are all neurons in the brain affected by it? Our lab has developed a new approach that is ideally suited to address these fundamental questions: real-time brain-wide imaging with single cell resolution in the nematode C. elegans. Using this technique we have previously demonstrated that brain-wide neuronal activity of awake animals is highly coordinated and dynamical. Transitions in neuronal network activity and their cyclical repetition over time represent the animal’s motor states and their assembly into action sequences respectively. During developmental stages termed lethargus C. elegans exhibits prolonged phases of behavioural quiescence, which share fundamental properties with sleep in other animals. Using behavioural genetics, we have identified that the NPR-1 chemosensory peptidergic arousal circuit allows us to rapidly and robustly switch between behavioural quiescence and wake: during lethargus atmospheric oxygen levels induce sustained arousal, while preferred intermediate oxygen levels permit behavioural quiescence. We have exploited this paradigm to image brain-wide neuronal activity during these state transitions. Unlike the dynamical activity seen during wake which involves ~40% of neurons, we find that quiescence corresponds to sustained decreases in activity of almost all neurons including sensory, inter and motor neurons. However, a subset of GABAergic motor neurons and interneurons maintain activity during quiescence. Computational analysis of neuronal population activity reveals that quiescence is a stable attractor state embedded in the otherwise dynamical and cyclical evolution of brain-wide network activity. Moreover, we observe stereotypic transitions into and out of this stable space (i.e. falling asleep and waking). Furthermore, in lethargic npr-1 animals oxygen stimulation induces a fast transition into a reversal state and maintains higher levels of the cyclical wake activity, while removal of the arousal signal induces decreased wake state probability and cycles. Based on these data we propose a model of sensory arousal operating in a top-down control manner while quiescence during lethargus is a default attractor for neural network activity in the brain.
Sleep-active neuron specification and sleep induction require FLP-11 neuropeptides to systemically induce sleep

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Sleep is induced by sleep-active neurons that are conserved from mammals to C. elegans. These neurons express GABA and neuropeptides, are active at the onset of sleep and actively promote it. However, little is known about how sleep neuron specification is determined and how sleep neurons induce this behavior. In C. elegans, sleep-like behavior occurs during lethargus, preceding the molt (Cassada and Russell 1975, Raizen et al. 2008). It depends on the sleep-active sleep-promoting neuron RIS, which requires the AP2 transcription factor APTF-1 to induce sleep (Turek et al. 2013). Thus, APTF-1 provides a unique starting point to understand sleep neuron specification and sleep induction. We combined transcriptome analysis of aptf-1 mutants with analysis of RIS-expressed genes to find out what determines expression of APTF-1 and how APTF-1 causes RIS to become sleep promoting. We found that the lim box homeodomain transcription factor LIM-6, which broadly specifies GABAergic neuron fate (Hobert et al. 1999), determined the expression of APTF-1 in RIS. Transcriptome analysis of aptf-1 mutants showed that APTF-1 is required for the expression of an FMRFamide-like neuropeptide, FLP-11, in RIS. Deletion of FLP-11 caused substantially reduced sleep behavior during lethargus. Overexpression of FLP-11 caused ectopic sleep behavior, confirming that this peptide is somnogenic. FLP-11 has been shown to activate three receptors, NPR-22, NPR-4, and FRPR-3 (Peyman et al. 2014). All these receptors appeared to decrease the induction of sleep behavior upon FLP-11 overexpression and thus might be involved in the induction of sleep behavior downstream of FLP-11 release by RIS. Thus, we present a mechanism of how RIS becomes sleep inducing: LIM-6 causes APTF-1 expression, which in turn causes expression of the somnogenic peptide FLP-11. At the onset of sleep, RIS is strongly activated and releases FLP-11, which in turn might activate multiple receptors to globally induce sleep.
Neuron-type specific miRNA represses two housekeeping genes to modulate an avoidance behavior

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It has been proposed that very broadly expressed, so-called housekeeping genes, have evolved 3'UTR sequences that avoid regulation by microRNAs. Here, we present mir-791 as a highly restricted neuronal miRNA, expressed exclusively in the CO₂-sensing BAG, AFD and ASE neurons. mir-791 specifically represses two very broadly expressed genes to achieve optimal CO₂ sensory function and behavioral response. In mir-791 mutant animals, generated by CRISPR/Cas9, the three pairs of neurons seem to acquire their basic cell identity, as seen by expression of terminal cell-fate markers. However, these animals display a significant defect in locomotory responses associated with avoidance to high concentration of CO₂. This phenotype is mainly due to the contribution of the BAG neurons, since expression of mir-791 only in the BAG neurons was sufficient to rescue the defect. This observation is also reflected in calcium imaging experiments, where the BAG neurons of mir-791(0) animals show an altered response to CO₂ exposure. We used available target-prediction tools for target identification, which we then validated by removal of all mir-791 binding sites from the top five predicted targets using CRISPR/Cas9. Only the top two, cah-3 and akap-1 are indeed functional targets for mir-791. Removal of the binding sites in the cah-3 3'UTR caused a small but significant decrease in the CO₂ response compared to wild-type, while the akap-1 3'UTR mutant animals display an almost complete phenocopy of the mir-791(0) response. akap-1 is the homolog of human A-kinase anchor protein-1, involved in the generation of functional micro domains of cyclic nucleotide-mediated signaling and thus likely important for the CO₂ response which relies on cGMP as a second messenger. cah-3 encodes for a carbonic anhydrase, known for its ability to interconvert CO₂ and H₂O to HCO₃⁻ and H⁺. In worms, CO₂ is directly sensed by a receptor guanylate cyclase, and the role of carbonic anhydrases in this response was unknown until now. We generated fosmid-based reporters for cah-3 and akap-1 with either the WT 3'UTR or a version where the mir-791 binding sites were mutated, and scored these in wild-type and mir-791(0) backgrounds. This approach showed that CAH-3 as well as AKAP-1 are broadly expressed in the whole worm, including at least 80 % of all neurons. Furthermore, the cah-3 reporter got significantly derepressed in the BAG neurons either upon removal of the mir-791 binding sites or removal of mir-791 itself. This analysis is still ongoing for akap-1. This study has given us insight into the interesting biology behind CO₂ sensing by polymodal neurons and the role miRNAs may play in its physiology and evolution. In addition, it raises the interesting idea that the expression of otherwise broadly expressed, housekeeping genes may have acquired cell-type specific regulation, for example through miRNA-mediated repression, in order to optimize organismal function.
The pharynx as a system to model arrhythmia cardiac diseases using optogenetics

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Hereditary forms of cardiac arrhythmia diseases like catecholaminergic polymorphic ventricular tachycardia (CPVT) and Timothy syndrome (LQT8) are manifested by defects in control of intracellular Ca\textsuperscript{2+} flux and excitation-contraction coupling. Several point mutations in the human Ca\textsuperscript{2+}-activated ryanodine receptor 2 (RyR2) as well as in the SR calcium storage protein CASQ2, were found to cause CPVT by leading to spontaneous Ca\textsuperscript{2+}-release and delayed after depolarization. In LQT8 patients gain-of-function mutations in the L-type voltage gated Ca\textsuperscript{2+}-channel Cav1.2 were detected, leading to delayed channel closure and thus to prolonged contractions. Due to the complexity of excitation-contraction coupling, there is a high probability of disruptive mutations, requiring individual analysis of these mutations and possibly enabling personalized treatment. Accordingly, a simple model and drug screening platform would be beneficial. The process of excitation-extraction coupling and the key players involved in this process, like RyR2, CASQ2, Ca,1.2 and the Na\textsuperscript{+}/Ca\textsuperscript{2+}-exchanger NCX, show high degree of conservation between the mammalian heart and the C. elegans pharynx. Also physiological characteristics of the two muscular pumps, like gap junction coupling, or the conservation of ion channels evoking and shaping the cardiac and pharyngeal action potentials are comparable. Therefore we used the pharynx to establish arrhythmia models. As pharynx pumping strongly depends on the presence of bacterial food and is not particularity regular, unlike the mammalian heart, we installed a synthetic pacemaker system by expressing channelrhodopsin-2 (ChR2(H134R)) in the plasma membrane of pharyngeal muscle cells. In that system we were able to observe stable pumping induction up to 6 Hz by blue light (470 nm) activation, both in electropharyngeograms (EPGs) as well as by video microscopy. For testing the pharynx and its ability to serve as an arrhythmic model, we generated animals carrying orthologous CPVT mutations in the C. elegans ryanodine receptor (UNC-68). We next studied the effects on pharyngeal pumping by a CPVT like mutant in CASQ2 (CSQ-1) and several mutations in Ca,1.2 (EGL-19), including an orthologous LQT8 mutation. In addition, we established a kymograph based optical method to measure pumping events in several animals in parallel. By this approach we detected phenotypes that can be interpreted as worm arrhythmia, for example an inability of the pharynx to follow pacing during prolonged stimulation periods in the CPVT related mutation UNC-68(R4743C). In addition, we could demonstrate arrhythmic events in egl-19 g.o.f. mutations n2368, n582ad952, and ad695, which manifested as prolonged pump duration, particularly in n2368, carrying an LQT8 orthologous mutation, where nearly all animals showed arrhythmia. For egl-19(n582ad952), we found alleviating effects of the dihydropyridine derivative Nemadipine A, indicating allele-specific effects, and establishing the optogenetically paced pharynx as a potential drug-screening platform for specific mutant alleles of arrhythmia-related genes (Schüler, Fischer et al., 2015, Scientific Reports 5:14427).
Identification of neuroprotective molecules using a C. elegans model of Spinal Muscular Atrophy

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Neuronal degeneration underlies a variety of serious pathologies that cause extreme personal discomfort and social costs. Neurodegeneration can result from a variety of insults of genetic or environmental origin. Molecules and pathways that prevent cell death are largely unknown. Uncovering them will help design strategies aimed at preventing neurodegeneration and shed new light on the molecular mechanisms underlying neuron survival. We took advantage of a Spinal Muscular Atrophy (SMA) model, generated using cell-specific RNAi, as a tool to discover natural compounds, synthetic drugs and genes presenting a neuroprotective function. Spinal muscular atrophy is a neuromuscular disorder characterized by the selective degeneration of lower spinal cord motor neurons, which leads to progressive muscle atrophy and death. SMA is caused by mutations of the Survival of Motor Neuron gene, Smn1, and although the genetic bases of SMA have been extensively studied, no effective treatment is available yet. We developed a genetic model which is transgene driven and is based on the expression, under a cell-specific promoter, of sense and antisense RNA molecules corresponding to fragments of a gene of interest. This RNAi strategy enabled us to efficiently reduce the function of smn-1 gene specifically in GABAergic motor neurons. Transgenic strains in which smn-1 is knocked down, present an age-dependent and progressive degeneration of GABAergic motor neurons that results in altered backward movement associated to neuronal cell degeneration and death. Importantly, these animals are viable and fertile allowing us to overcome the lethality problem related to other C.elegans SMA models carrying smn-1 loss of function. Using this genetic model we screened a panel of chemicals, natural compounds and natural extracts and we found a number of conditions that can partially rescue cell death but not the onset of the degenerative process, while others fully protect neuronal integrity and survival. By candidate gene approach and by random chemical mutagenesis we also identified some of the genetic interactors that, when mutated, are able to completely prevent neuronal death. The neuroprotective molecules identified have been tested on different neurodegenerative models (e.g. axon degeneration, huntingtin or α-synuclein overexpression) and we found that a subset of these candidate molecules has a general protective role, while others are specific for our SMA model. By genetic manipulations, drug treatments and detailed phenotypic analysis we successfully identified specific genetic and chemical modifiers of smn-1 function but also generic neuroprotective molecules.
Neuroendocrine Regulation of Forward Locomotion in Caenorhabditis elegans

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Neurons with dedicated secretory properties have yet to be described in C. elegans. Combining serial electron microscopy, molecular genetics, transcriptome profiling, and calcium imaging, we show that RID is a peptidergic neuron that promotes forward locomotion. A Six4/5-family homeobox transcription factor UNC-39 governs lineage-specific neurogenesis to give rise to RID. The RID neuron harbors near-exclusive dense core vesicle clusters, and expresses multiple neuropeptides including FLP-14. RID activity increases during forward movements, and ablating RID reduces sustainability of foraging, a consequence partially recapitulated by removing FLP-14. These results uncover a previously unknown component of the forward motor circuit, and lay the foundation for mechanistic exploration of neuroendocrine development and neuromodulation of animal physiology and behavior.
Sexually dimorphic scaling of synaptic neurotransmitter transport

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We have completed the glutamatergic map of the male C. elegans identifying 9 more neuronal classes (PVV, PCA, HOA, CP1-6, CP7, R2B, R9A, R9B and R7A). Additionally, we have systematically quantified the expression of a fosmid-based reporter of eat-4 in both male and hermaphrodites and we have found dramatic changes in the expression levels, particularly in sex-shared-neurons over time and between sexes that could be reflecting changes in the neurotransmitter usage correlating with their connectivity in the different conditions analyzed.

We focused specifically in the sex-shared sensory neuron PHC, which shows a very dramatic differential expression level of eat-4 between the two sexes. Interestingly, this up-regulation correlates with a massive rewiring of PHC connectivity in the male tail. We dissected the transcriptional regulation that leads to cell-specific dimorphic changes and we found that the sex-determination pathway genes regulate the plethora of changes that occur in PHC in a cell autonomous manner. Interestingly, we identified a member of the conserved family of DMD transcription factors, dmd-3, to be responsible of the up-regulation of eat-4 transcription. But furthermore, dmd-3 is regulating the axon morphology changes and the acquisition of the peptidergic fate specific of the male PHC.

We have further probed the modularity of the cis-regulatory logic of glutamatergic neuron specification. To this end, we have continued our dissection of cis-regulatory elements of the eat-4 locus, defining elements with expression in single neuron types in sex-shared and sex-specific neurons. We found one element expressed specifically in PHC and AIM that recapitulates the dimorphic expression of eat-4 in these two neurons. In a more exhaustive analysis, we identified a combination of elements that precisely integrate the transcriptional control of eat-4 in this two neurons and the sex-specific modulation representing both activation and repression mechanisms.
Pim-related kinases regulate olfactory, but not gustatory sensations in C. elegans

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Background. In C. elegans, there are two homologs for the mammalian pim family genes, prk-1 and prk-2. They encode Pim-related serine/threonine-specific kinases that are 40% identical to murine Pim-1, but have longer C-termini. These proteins are expressed mainly in neurons and in intestinal cells in both larvae and adults. No major phenotypes except for some changes in fat content have been detected in high-throughput RNAi screening experiments when either gene has been targeted, but no one has yet tried to target both of them simultaneously. However, Prk-2 has been shown to be involved in regulation of presynaptic differentiation and neurite branching in C. elegans (Zheng Q et al., Development, 2011). Since we have previously observed pim expression in the olfactory epithelium and other sensory organs of developing mouse embryos (Eichmann et al., Oncogene, 2000), we were interested in testing whether i) Prks are be functional orthologs for Pims and ii) whether Prks can regulate the sensory functions of C. elegans. Results. Using in vitro kinase assays with bacterially produced proteins, we have shown that Prk-2 can phosphorylate several mammalian proteins targeted by Pims. Furthermore, our Pim-selective small molecule inhibitors are able to inhibit also Prk-2 activity. To study the putative roles of Prks in sensory signalling, we have set up C. elegans N2 cultures and have carried out chemotaxis assays using volatile odorants that either attract or repel worms. However, when we incubate synchronized young adults in the presence of a Pim inhibitor, their ability to sense either attractants or repellants is significantly reduced. By contrast, their responses to gustatory cues such as metals are not affected. Experiments are currently ongoing to test whether we can obtain similar results with prk-specific RNA interference. Conclusions. Our preliminary data suggest that Pim-related kinases are indeed functional orthologs of mammalian Pim kinases and that they are actively involved in olfactory, but not gustatory sensations. Next we wish to determine whether Prks regulate also other types of sensory functions and to identify the Prk-regulated signaling molecules involved there.
The Si elegans project • Studying C. elegans behaviour in a virtual environment

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Since the pioneering work of Niebur and Erdős (1991), who replicated C. elegans locomotion in software for the first time, several virtual simulations of the worm have been published (e.g. Bryden and Cohen 2008, Szigeti et al. 2014). The Si elegans project aims at extending these to the simulation of an anatomically accurate model of the nematode in a virtual environment with realistic sensory input from multiple external stimuli. The Si elegans platform will provide a publicly available online toolset where behavioural studies with C. elegans can be executed in a completely virtual, yet physically faithful simulated laboratory environment. It uses a 3D biomechanical model for the virtual embodiment of the nematode based on the currently accepted anatomy of the worm. It considers the cuticle, the body wall muscles and the neurons as described in the WormAtlas (Altun and Hall, 2016). External stimuli including mechano-, thermo-, chemo-, photo- and electro-sensation can be defined by the user. Proprioception models are provided as well. The stimuli, as registered by the sensilla organs of the nematode, are propagated as concrete biophysical representations to the respective neurons for further processing downstream. Each of the 302 neurons is represented by a neuron-specific response model being executed on dedicated hardware based on field-programmable gate arrays (FPGAs). Neurons communicate through an electro-optical hardware connectome. We will present on the Si elegans system architecture and on how the platform can be configured for a specific experiment with user-defined stimuli and neural processing models. We will demonstrate how the platform may help the C. elegans community and neurocomputational scientists in testing hypotheses on neural function and how it will allow to reverse-engineer the principles underlying C. elegans behaviour. The platform will be officially launched for public access in June 2016. For further information, please visit www.si-elegans.eu. Acknowledgements: This project is funded by the EU’s 7th Framework Programme for research, technological development and demonstration under grant agreement no 601215, FET Proactive, call ICT-2011.9.11: Neuro-Bio-Inspired Systems (NBIS). References Niebur, E. and Erdős, P. (1991). Theory of the locomotion of nematodes. Biophysical Journal, 60, 1132–1146 Bryden, A. and Cohen, N. (2008). Neural control of Caenorhabditis elegans forward locomotion: the role of sensory feedback. Biol. Cybern., 98:339–351 Szigeti B., Gleeson P., Vella M., Khayrulin S., Palyanov A., Hokanson J., Currie M., Cantarelli M., Idili G., Larson S. (2014). OpenWorm: an open-science approach to modelling Caenorhabditis elegans. Front. Comput. Neuroscience 2014, 8, 13 Altun, Z.F. and Hall, D.H. (2016). Handbook of C. elegans Anatomy. In WormAtlas.
Neurons, Oxygen and Mighty Worms: A Novel Paradigm for the Exploration of Intrinsic Plasticity in C.elegans.

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To adapt to challenging and changing environmental conditions, all animals dynamically adjust their nervous system function and behaviour for survival. Other than modulating synaptic connections between neurons, neurons can also alter their intrinsic biophysical properties and thus their excitability. This is called intrinsic plasticity and can affect diverse fundamental neuronal processes such as synaptic integration or signal propagation. It has been linked to learning and memory or homeostatic regulation of neuronal excitability in various organisms, however its underlying mechanisms are still poorly understood.

Caenorhabditis elegans is an ideal model to study nervous systems and displays extensive neural plasticity. We found that the well-defined behavioural responses to changes in ambient oxygen levels are highly plastic and represent a novel paradigm for intrinsic plasticity. Animals cultivated at either normoxia (21% O2) or low (7%) oxygen reprogramme the tuning of O2-evoked behavioural states; this plasticity forms directly in the sensory neurons, is activity-dependent and takes several hours to develop, showing hallmarks of long-term memory. To dissect the mechanism underpinning plasticity in the oxygen-sensing circuit, we examined candidate genetic factors, concentrating on the roles of neuromodulation and calcium signalling. We found, amongst other things, that neuromodulation affects the tuning of O2-evoked behavioural states but is not required for the plasticity of the responses. Our results point to O2-evoked plasticity being regulated by a novel mechanism.
Modulation of neural information by neuroglobin alters the preference of Caenorhabditis elegans for oxygen concentrations

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Neuroglobin is one of the globin proteins which reversibly binds to oxygen but it is expressed in a neural circuit. Previous studies of C. elegans neuroglobin, glb-5, suggested that it was involved in modulation of information processing. However, how glb-5 modulates information processing and how the modulation affects behavior are unknown. When the activity of npr-1 (GPCR) is reduced and glb-5 is disrupted, worms prefer narrow range of oxygen concentrations. We found that glb-5 alters worms to prefer broader range of oxygen concentrations. Our in vivo Ca²⁺ imaging experiments indicated that GLB-5 actively transforms a major oxygen sensory neuron (URX) to more accurately encode oxygen concentration levels that are represented by amplitude of its Ca²⁺ responses. Information signal from URX was mainly transmitted to reversal behavior but not worm’s speed. Finally, our computational model showed that the modulated neural encoding by GLB-5 in URX and the affected reversal behavior are sufficient for the alteration of a worm’s preference for oxygen concentrations. Thus, our results demonstrated the information processing mechanism underlying the change in the worm’s preference behavior. Given that the structure and function of GLB-5 are similar to those of vertebrates’ neuroglobin, maybe neuroglobin modulates neural information in the brain of a higher organism as well.
The mechanisms of developmental plasticity: from switch genes and epigenetics to the interplay of organisms and their environment.

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Ever since Darwin, biologists are intrigued about evolution and its underlying mechanisms. We use an interdisciplinary approach that integrates development, ecology and population genetics to unravel the mechanistic changes that give rise to novelty and change. For this, we established *Pristionchus pacificus* as model system and combine laboratory studies building on genetic, genomic and transgenic tools with fieldwork. We integrate evo-devo with population genetics and ecology because insight from population genetics can indicate the contribution of natural variation to the evolution of development. Similarly, insight from ecology can reveal how the environment influences developmental processes.

*P. pacificus* lives in association with scarab beetles. One key feature of its life style is a mouth-form dimorphism that enables predatory feeding. The development of teeth-like denticles of two different forms represents an example of developmental plasticity and we test the hypothesis that developmental plasticity is a facilitator of phenotypic diversification and the evolution of novelty. We identified a regulator of plasticity, *eud-1*, that acts in a developmental switch. Mutations in *eud-1* eliminate one mouth form, whereas over-expression of *eud-1* fixes this form. EUD-1 is a sulfatase that acts dosage-dependently and is sufficient to control the sexual dimorphism and micro- and macroevolutionary variation of feeding forms. I will discuss recent work indicating that *eud-1* is the primary locus of regulation of the mouth dimorphism with a dominant role of chromatin remodeling, the involvement of short open reading frames and encoded micropeptides as well as long non-coding RNAs. Finally, I will describe our most recent work aiming to link the genetic control of plasticity with environmental signals such as those from bacteria that can influence predation behavior at multiple levels.
piRNAs sense environmental perturbations to initiate transgenerational epigenetic responses to stress.

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Piwi-interacting small RNAs (piRNAs) are conserved across metazoans, where their best-known function is in controlling transposable element expression in the germline. In the nematode C. elegans, piRNAs can initiate transgenerational epigenetic silencing of transgenes, which can last independently of the initial piRNA trigger, but whether this applies to endogenous genes is unclear. In order to explore the function of piRNA-mediated epigenetic silencing we investigated how the biogenesis of piRNAs responds to environmental perturbations. The majority of piRNAs in C. elegans are produced from individual transcription units marked by a conserved upstream motif and require PRDE-1 for their biogenesis. Motif independent piRNAs, which do not require PRDE-1, are also found. Both classes bind to the Piwi protein PRG-1 but motif-independent piRNAs are more likely to target innate immune genes whereas motif-dependent piRNAs tend to target transposable elements. Here, using high-throughput small RNA sequencing we show that increased temperature downregulates motif-dependent piRNA biogenesis, which corresponds to desilencing of a reporter for piRNA silencing in the germ line. At the same time, however, motif-independent piRNAs increase in abundance due to increased availability of PRG-1. Through high throughput RNA sequencing to analyse gene expression we show that the alteration in the balance between motif-dependent and motif-independent piRNAs initiates downregulation of innate immune genes, which is maintained transgenerationally after return to a reduced temperature. However, we find that exposure to pathogens suppresses this response such that motif-dependent piRNA biogenesis and motif-dependent piRNA target silencing is maintained. This allows sustained innate immune gene expression at increased temperature and may help to resist infection. Together our results identify the piRNA pathway as a barometer for environmental conditions in C. elegans, and suggest that it may have a function in communicating environmental conditions to the next generation (Figure 1).
A single-molecule view on intraflagellar transport in the chemosensory cilia of living C. elegans

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Intraflagellar transport (IFT) is an intracellular-transport mechanism in the cilia of eukaryotic cells. In C. elegans chemosensory cilia, IFT is driven by the cooperative action of IFT-dynein (driving transport from cilium tip to base) and two kinesin motor proteins, Kinesin-II and OSM-3 (driving transport in the opposite direction). Our goal is to understand what the specific roles of the motors are and how they cooperate. To achieve this, we imaged mutant nematodes expressing fluorescent motors using in vivo single-molecule fluorescence microscopy. Images obtained were analyzed using automated kymograph and single-particle tracking analysis. We find that the role of Kinesin-II, the slower and less processive kinesin, is to load IFT trains, to initiate their transport and to effectively traverse the transition zone, the semi-permeable barrier between cilium and rest of the cell. After crossing the transition zone, Kinesin-II leaves the trains and the faster, more processive kinesin OSM-3 binds and drives transport to the cilium tip. At the tip, IFT-dynein takes over and, on its own, cycles motors and other IFT components to the base. Our results provide unprecedented insight in how cells use a combination of motor proteins to drive complex intracellular transport processes. Furthermore, they show that quantitative fluorescence microscopy with single-molecule sensitivity can be applied successfully to unravel complex molecular processes in living C. elegans.
Cell non-autonomous mechanism of neuronal regeneration in C. elegans

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Very little is known about the genetic pathway of dendritic regeneration following injury. We study C. elegans PVDs neurons characterized by stereotypical arborized dendrites that have a repetitive “menorah” branching pattern to understand plasticity and regeneration of dendritic arbors (1). The PVDs sense strong mechanical stimuli, temperature and posture. We severed PVDs by laser nanosurgery and followed their fate. We demonstrated that: A. Severed dendrites regenerate via fusion. B. Transection of dendrites results in loss of self-avoidance between menorahs. C. The fusogen AFF-1 (2) fuses tertiary branches resulting in menorah-menorah fusion, bypassing the injury site and reestablishing PVD integrity. D. AFF-1 is not detected in the PVD, before or after injury. In contrast, AFF-1 paralog EFF-1 is expressed in the PVD and acts cell-autonomously to retract and restrict dendritic branching (1) E. AFF-1 expression in the PVD is not sufficient to rescue the PVD reconnection post injury in aff-1 mutants. However, AFF-1 expression in the hypodermal seam cells restores the number of fused menorahs to wild type levels. Moreover, AFF-1::GFP in the L4-adult transition is highly expressed in seam cells, vulval rings (VulA, VulD), uterus and anchor cell (2). AFF-1::GFP puncta are often observed in proximity to seam cells and appear to move dynamically. Interestingly, the number of AFF-1::GFP puncta outside the seam cells increased in response to laser –induced injury of PVD dendrites. Heterologous mammalian cells transfected with C. elegans AFF-1 have been shown to express extracellular vesicles decorated with AFF-1 (3). We found that AFF-1 acts non-cell autonomously from the epidermal seam cells to auto-fuse transected dendrites. We propose that extracellular vesicles derived from the seam cells can regenerate severed dendrites. (1) Oren-Suissa M, et al. (2010) Science 328:1285-1288. (2) Sapir A, et al. (2007) Dev Cell 12:683-698. (3) Avinoam O, et al. (2011) Science 332:589-592.
Engineering non-mendelian genetics

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150 years ago, Gregor Mendel described the basic rules of genetics. According to these rules, each parent makes an equal genetic contribution to an offspring in sexually reproducing organisms. The bipolar mitotic spindle controls the equal segregation of paternal and maternal chromosomes during the first cell division. Being able to rewrite the rules of genetic segregation would expand the technological possibilities of biology and biotechnology, which may allow novel synthetic approaches to engineering animals in the future. Here, we manipulated the mitotic spindle during the first cell division of Caenorhabditis elegans to segregate maternal and paternal chromosomes into different cell lineages, resulting in non-mendelian segregation of entire genomes. Non-mendelian genetics was achieved by generating a strain that forms two monopolar spindles instead of one bipolar spindle during the first cell division. Monopolar spindles were generated by up regulating GPR-1 activity to increase microtubule-based cortical force generators to pull apart paternal and maternal pronuclei before nuclear envelope breakdown. Thus, one monopolar spindle was formed from only paternal chromosomes and one monopolar spindle was formed from only maternal chromosomes. The maternal monopolar spindle always formed in the anterior, and the paternal monopolar spindle always formed in the posterior of the zygote. As a consequence, only paternal chromosomes were segregated to P1, and thus to the germline. Thus, this monopolar spindle strain selectively passed on only paternal chromosomes, and thus acted like a “host” after mating: mothers produced F1 offspring with a mixed maternal-paternal genotype, but these hermaphrodites segregated F2 offspring with only paternal chromosomes. This system may be used to study a wide range of phenomena from speciation to epigenetics. Taken together, we found that genetic rules can be rewritten by engineering cell division in an animal to generate non-mendelian segregation of entire genomes. This may be the starting point for a novel, synthetic zoology.
Regulation of spindle positioning through phosphorylation of LIN-5 in asymmetric cell division.

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Asymmetric cell division is required throughout development to generate cells of unequal size, composition and fate. The correct distribution of cell fate determinants depends on the plane of cell cleavage, which is determined by the position of the mitotic spindle. Spindle positioning is mediated by a conserved Galpha–GPR-1/2–LIN-5 complex at the cortex, which interacts through dynein with astral microtubules and thereby promotes the generation of pulling forces. We aim to understand how the developmental regulation of cortical pulling forces determines the plane of cell cleavage and contributes to asymmetric cell division. Previous studies of our lab identified four amino acids in the C-terminal domain of LIN-5 that are phosphorylated by the polarity kinase PKC-3. This phosphorylation leads to reduced pulling forces in the anterior, but additional mechanisms contribute to asymmetric spindle positioning. In recent studies, we identified additional phosphorylations of LIN-5 with critical functions in vivo. Moreover, we defined the LIN-5 C-terminal domain that mediates interaction with GPR-1. Hereto, we used reverse yeast two-hybrid screening to identify single amino acid changes in LIN-5 that prevent GPR-1 binding. All of the identified mutations are confined to a 30 amino-acid domain located upstream of the PKC-3 phosphorylated residues of LIN-5. This GPR-1 binding region contains two additional in vivo phosphorylated LIN-5 residues. We used Cas9/CRISPR-mediated genome editing to substitute these amino acids for non–phosphorylatable alanine or phosphomimetic glutamic acid / aspartic acid. Combined with UV-laser spindle severing experiments, we found that phosphorylation of these residues contributes to GPR-1 binding and cortical pulling forces. In a parallel study, we identified residues in the N-terminus of LIN-5 that are phosphorylated by CDK-1. Again, examination of Cas9/CRISPR-mediated knock-in alleles revealed that these N-terminal residues are critical for LIN-5 function. Mutation of these residues led to severe impairment of cell division. We show this is caused by loss of recruitment of dynein to the cell cortex and, as a consequence, absence of pulling forces. Taken together, our data show that phosphorylation of LIN-5 contributes to the spatiotemporal regulation of the cortical pulling forces that position the mitotic spindle.
Cellular innovations at the origin of pseudogamy in nematodes

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Pseudogamy is a mode of reproduction in which the fertilization of an oocyte by a sperm cell is necessary even tough the male DNA does not contribute to the zygote genome in most embryos. Pseudogamy has been described in several species of nematodes, in particular in the Mesorhabditis genus. In the \textit{M. belari} species, embryos in which the sperm DNA does not decondense give rise to 100\% females, while the contribution of sperm DNA allow the production of rare males in the population (Nigon, 1942).

From recent soil sampling, we have re-isolated Mesorhabditis strains, which had been lost since the 40's. We found 14 pseudogamous strains and 8 regular male/female species. Genetic crosses and morphological description identified 8 independent pseudogamous species. In particular, we identified 3 different strains of \textit{M. belari}. Based on the sequencing of 18S, 28S and ITS, we established a phylogeny of Mesorhabditis species and found that the pseudogamous species share a common ancestor. Thus, the pseudogamous reproductive mode allows speciation.

Combination of cytological observations and SNP genotyping performed in \textit{M. belari} JU2817 showed that a single female can produce two types of oocytes. Oocytes with incomplete meiosis will not utilize the male DNA and produce only females. Interestingly, most meiosis figures show a premature separation of homologs chromosomes during the unique metaphase of meiosis, allowing the maintenance of heterozygosity in the female progenies. In another type of oocytes, the regular two steps of meiosis are seen, followed by the decondensation of the male DNA. These embryos produce only males. These observations suggest that pseudogamy is the combination of three cellular innovations: i) a meiotic drive during male spermatogenesis leading to the production of 100\% of male-sperm, ii) a stochasticity in the execution of female meiosis, iii) a control of the paternal DNA in oocytes, depending on the progression of female meiosis.

We will present our strategies to decipher the mechanisms at the origin of pseudogamy and sex competition in this group of species.
Dynamic cycling of PKC-3 activity between distinct complexes polarizes the C. elegans zygote

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Most metazoan cells are polarized by conserved PAR proteins localized to discrete membrane domains. Regulation of PAR protein distribution is essential for the asymmetric activation of polarization signaling pathways that coordinate many aspects of embryonic development; including asymmetric cell division, cell migration and tissue organization. We aim to decipher the molecular mechanisms involved in asymmetric distribution of PARs during antero-posterior embryonic axis establishment of C. elegans zygotes. To establish polarity it is essential that the PDZ domain proteins PAR-3 & PAR-6, the atypical protein kinase PKC-3 and the small GTPase CDC-42 co-segregate towards the anterior half of the C. elegans zygote. It is proposed that the anterior localization of these proteins is mediated by the cortical acto-myosin flow and the mutual inhibition between these anterior PARs and those that accumulate at the posterior (PAR-1, PAR-2 and LGL-1). However, it is unclear exactly how anterior PARs co-segregate. Analyzing different cell polarity mutants we have dissected a molecular mechanism of anterior PAR protein co-segregation. Our observations indicate that anterior PARs are present in two distinct complexes that localize to a similar anterior cortical domain during polarity establishment. In addition, our results support a model whereby PAR-6/PKC-3 achieve their anterior asymmetric localization by cycling between these co-existent complexes. The different intrinsic stability and molecular properties of these two complexes suggest a robust polarization system where these complexes have acquired specialized functions: (1) PAR-3/PAR-6/PKC-3 complex mediates the acto-myosin dependent anterior segregation of PAR-6/PKC-3 and is sensitive to inhibition by posterior PARs, (2) CDC-42/PAR-6/PKC-3 complex promotes cortical clearance of posterior PARs. We will present the molecular mechanisms responsible for the functional specialization of these co-existing complexes and discuss the polarization advantages it confers.
Differential regulation of centrosome amplification in C. elegans stem cells

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Stability of the genome requires integrity of the centrosomes and the mitotic spindle they organize. The interplay between centrosome function and asymmetric cell division in normal and cancer stem cells is incompletely understood. Structural and numerical centrosome aberrations have been frequently observed in human cancer, but it remains uncertain whether these features are consequences or drivers of cancer development. To date, proof for the latter was only found in flies, where centrosome over-amplification, driven by overexpression of the kinase Plk4, led to tumorigenesis. Here we use seam cells, a stem cell lineage of the C. elegans hypodermis, as a model system to study centrosome amplification in stem cells. We found that overexpression of the Plk4-related kinase ZYG-1 fused to GFP leads to the formation of supernumerary foci that contain the centriolar proteins SAS-4, SAS-5 and SAS-6. These foci function as microtubule organizing centers, as evidenced by the formation of multipolar spindles. Surprisingly, supernumerary centriolar foci were only observed in the interphase preceding the L2 symmetric cell division but not before the following L2 asymmetric cell division. In addition, inhibition of the symmetric division by depletion of the heterochronic gene lin-28, prevented centrosome over-amplification. These findings raise the possibility that symmetric and asymmetric divisions within a stem cell lineage are endowed with different properties to buffer the impact of aberrations in centrosome number.
Orientation of spindles and cell division planes during development ensures that correct cell-cell contacts are established which is vital for proper tissue formation in many species. Although several signaling pathways in oriented cell division have been well characterized such as wnt/frizzled-based anterior-posterior polarity, there is strong evidence for additional signal pathways controlling early anterior-posterior polarity decisions. Recently, we have identified the homolog of the adhesion G protein-coupled receptor latrophilin, LAT-1, as a novel player in oriented cell division in an anterior-posterior direction of specific blastomeres in the early C. elegans embryo. We have combined in vitro and in vivo approaches to identify the signals required for LAT-1 to mediate correct oriented cell division. We identified a classical G protein-cascade based on coupling of the receptor to a $G_s$ protein which leads to elevated intracellular levels of the second messenger cyclic AMP (cAMP). Consistently, in lat-1 null mutant embryos cAMP concentrations are lower than in wild-type embryos. Upon artificially elevating cAMP levels in these mutants defective embryonic cell division plane orientations caused by LAT-1 absence is corrected. Hence, by regulating cAMP levels LAT-1 controls oriented cell division, most likely via the $G_s$ protein homolog GSA-1. These data indicate that G-protein signaling in oriented cell division is not solely GPCR-independent. To transduce this signal LAT-1 is activated by a tethered agonist within its N terminus. Upon activation by this tethered agonist LAT-1 triggers the cAMP-dependent cascade leading to correct embryonic cell division plane orientations. Additionally, we have obtained evidence for an extracellular protein directly involved in LAT-1 activation. In summary, we have identified a novel signalling pathway essential for oriented cell division in the early embryo dependent on the adhesion GPCR LAT-1 which is mediated by controlling cAMP levels.
Posterblitz Abstracts in order of the presentation

P092

A semi-automated EMS screen identifies a postembryonic muscle lineage mutant

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Transdifferentiation is the process by which an already specialized cell is directly converted/reprogrammed into another specialized cell. This naturally not very abundant process is the major focus of our lab. We try to identify and characterize genetic factors that play a role in induced transdifferentiation by mis-expressing transcription factors (TFs). Currently there are several selector genes known that can activate specific cell fates such as hlh-1, the worm homolog of the myogenic bHLH TF MyoD. When mis-expressed, HLH-1 induces muscle fate in early embryonic cells but terminally differentiated cells in older animals are resistant to hlh-1-induced direct reprogramming. In order to identify mechanisms that antagonize selector genes, we apply both forward and reverse genetics. We use transgenic lines allowing ectopic expression of a specific selector gene in combination with the appropriate cell fate reporter. To complement manual microscopic screening, we use a semi-automated high-throughput forward genetics screen combining EMS mutagenesis with the Biosorter system (Union Biometrica). Using this approach we identified a mutant showing additional cells at the posterior end of the pharynx that express a myo-3 reporter. To our surprise, these cells show muscle reporter expression independently of ectopic induction of hlh-1. They appear during the L2 to L3 larval transition, where usually body wall muscle development is already finished. To identify the relevant mutated locus, we used a whole genome sequencing approach and identified a premature STOP in the KASH-domain gene unc-83. We could phenocopy the phenotype using other unc-83 mutant alleles as well as with a mutant allele of unc-84, a SUN-domain containing protein that interacts with UNC-83 to bridge the nuclear lamina with the cytoskeleton. The UNC-83/UNC-84 nuclear envelope bridge was so far not implicated in muscle development. For mutated alleles of these genes, nuclear migration defects of P cells, hyp-7 hypodermal precursors and intestinal polarization defects have been reported – all of which happen during embryonic development. To elucidate the underlying mechanism of the extra body wall muscle cells, we performed lineage ablation studies and we are testing knock downs of cell cycle regulators. This will allow us to identify the mechanism as a missing apoptosis event, an additional cell division or a lineage conversion.
A cell-type-specific small RNA labeling approach uncovers the tissue-specific microRNome in Caenorhabditis elegans

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The implementation of distinct gene expression profiles is essential for accurate animal development and a primary cause for disease. While it is well established that post-transcriptional gene regulation by microRNAs (miRNAs) plays a pivotal role in these processes, uncovering the biological function of small RNAs remains challenging. High-throughput sequencing of miRNAs from individual development stages provided some insights but often lacks the resolution to link miRNA-function to the biology of distinct cell types within complex tissues. Here, we report a chemical small RNA-tagging approach followed by high-throughput sequencing that uncovers tissue- and cell type-specific miRNA profiles in C. elegans. The method relies on cell-specific 3′ terminal methylation of miRNA by selective expression of the small RNA duplex-specific methyltransferase HEN1. Because methylated miRNAs are resistant to chemical ribose oxidation, tissue-specific miRNAs can be recovered by established methylation-specific cloning protocols, followed by high-throughput sequencing. Using this strategy, we report the miRNome in the nervous system, the pharynx and the intestine of the worm, providing insights into miRNA expression at high spatial resolution. Chemical small RNA-tagging overcomes the current challenges in tissue- and cell-type-specific miRNA profiling and provides novel entry points to understanding the regulation of gene expression in the course of animal development.
Posterblitz Abstracts in order of the presentation

P150

A sensitive mass-spectrometry-based method identifies metabolic changes of life history traits in C. elegans

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Abnormal nutrient sensing and nutrient metabolism are a hallmark of the aging process and the development of age-related disease. Although studies in the worm C. elegans have identified various metabolic genes that influence worm development and lifespan, few tools are available to measure the metabolic consequences of such perturbations in life history. We therefore developed and validated a highly sensitive mass spectrometry (MS)-based method for the identification of major metabolite classes in worms. Using (UPLC-)tandem-MS we detected 44 fatty acids (FAs) and 19 amino acids (AAs) in a sample that is equivalent to approximately 500 worms. To confirm the validity of the method, we analyzed the changes in FAs and AAs in worms deficient in mdt-15 and bcat-1 respectively, which encode enzymes important to lipid and branched-chain AA catabolism. As a consequence, mdt-15 RNAi worms accumulate C18:0 FA at the expense of polyunsaturated FAs, while bcat-1 RNAi worms accumulate BCAAs. We then applied this method to analyze the changes in metabolite profiles throughout development and aging. The abundance of FAs was low during the larval stages and followed by an increase in most of FA species, which reached the peak at day 7 of adulthood. Most AAs reached the peak during the later larval stages and decreased in adult phase, except for aspartate and glycine, which remained low in development and early adulthood, and significantly increased in later age. Furthermore, by exposing worms to E. coli strain HT115 or strain OP50 as food, we demonstrated that worm FA and AA composition reflects the diet they eat. Altogether, this MS-based method is a powerful tool to perform worm metabolomics for studies focusing on aging and metabolic challenges.
Insulin signaling links metabolic state to systemic arousal in *C. elegans*

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Neural circuits integrate external and internal signals to inform behavioural decisions. Food search behaviours need to balance energy expenditure during neural processing and motor activity with energy conservation. Ambient oxygen (O₂) is a sensory cue used by worms to instruct food search behaviours. We use O₂ stimulation to characterise the behaviour of worms as they are subjected to starvation of increasing durations. Unlike well-fed worms, 1h fasted worms exhibit enhanced responses to an O₂ concentration downshift. This is suppressed upon 16h starvation; instead, animals are prone to enter a reversible state of sleep-like quiescence, which is further enhanced upon O₂ downshift. The timescale of starvation-controlled behaviours requires insulin signaling as insulin receptor mutants (daf-2) display the behaviour of 16h starved WT worms already upon 1h fasting. This early starvation behaviour requires DAF-2 in the nervous system. Expression of daf-2 only in sensory neurons suppresses quiescence, suggesting that wakefulness in *C. elegans* depends on food availability and insulin signaling acting at the sensory level. To understand the underlying network mechanisms we perform brain-wide single cell resolution Ca²⁺ imaging. While brain-wide network dynamics underlie wakefulness, starvation promotes periods of inactivity among almost all neurons; however, upon arousal the network re-engages in dynamic activity. In summary, we report that starvation systemically affects the brain and promotes quiescence, which likely serves the purpose of energy conservation in a stimuli-free environment. Our results suggest that food availability mediated by elevated insulin signaling serves as arousal cue, thereby preventing quiescence. With lasting starvation, insulin signaling ceases, during which state remaining sensory inputs like high O₂ maintain the aroused state. When O₂ concentrations drop, quiescence occurs. This supports the idea of quiescence as default brain state in the absence of arousing cues.
Using microbial rhodopsins as genetically encoded voltage sensors to analyze neuronal networks in C. elegans

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One goal in optogenetics and neurobiology is developing sensors for membrane voltage. The first generation of such sensors could deliver low signals, which typically were in the few % range for large, often artificial voltage steps (Hughes et al, 2012). Via protein engineering some remarkable improvements for FRET-based or single chromophore sensors were achieved. More recently, rhodopsin based sensors were expressed in cell cultures and neurons, using the dim, but voltage dependent fluorescence of all trans retinal, with low-% absolute signals and requiring extremely intense imaging light. In neurons of small invertebrates like C. elegans, which do not even spike, but produce graded potentials of 10-20 mV, such sensors can hardly detect activities. An enhanced rhodopsin based voltage sensor expressed in C. elegans was introduced by Flytzanis et al., 2014 (Archer1). This protein showed 25-40% changes in fluorescence in AWC amphid neurons, however, it still needs very high excitation light intensities. In combination with fluorescent proteins for electrochromic FRET, up to 40% changes in the signal per 100mV were reported for a rhodopsin sensor (AceQ; Gong et al, 2015). Its utility in C. elegans remains to be tested. We explored an alternative approach to enhance rhodopsin based voltage imaging. The Archaerhodopsin (Arch3) proton pump, which hyperpolarizes membranes, can be used as a voltage sensor (Kralj et al., 2011), especially in its non-pumping mutant variants. To increase the absolute fluorescence of the sensor, we sought for synthetic ATR analogs that would support a higher fluorescence, while still preserving voltage sensitivity. Among eight such analogs tested, we identified two that were incorporated into the protein and showed very intense fluorescence when substituted for ATR in Arch3-derivates, or in Mac, a similar rhodopsin proton pump from fungi, expressed in C. elegans body wall muscle cells. We also observed the changes in the fluorescence signals in the so called electrochromic FRET sensors, consisting a rhodopsin as an acceptor in FRET with a fluorescent protein as donor. We tracked signals caused by spontaneous muscle activities as well as signals caused by optogenetically induced membrane depolarization with the actuator Channelrhodopsin expressed either in cholinergic neurons or body wall muscles. The observed signals increased and decreased reciprocally in dorsal and ventral muscles, as expected for muscle-induced body bending. Besides expression in body wall muscles Arch3 was also expressed in pharynx and pumping was triggered with serotonin. Voltage signals >50%, could be observed during pharynx contraction. A fluorescent voltage reporter would be highly useful to analyze neuronal networks in the generation of behavior, and may even enable closed-loop optogenetics, with a direct readout of optogenetically imposed voltage changes in the neuron of interest.
Animals can be in different states such as sleep and wake and display behaviors that are specific for these states. However, little is known about how behaviors become state-specific. Here we investigated a turning behavior during sleep in C. elegans. Worms usually lie on their side and propel by sinusoidal body movements generated by dorso-ventral muscle contractions. This two-dimensional locomotion is caused by activity in the dorsal and ventral cord and is fairly well understood. Worms also display three-dimensional movements but the neural circuits are much less understood. During larval sleep, worms often turn from one side to the other, and this behavior is almost never found during wake. Neither the neuronal substrates of this flipping behavior are known nor do we understand why this behavior is sleep-specific. We performed a genetic screen for mutants that do not flip during sleep and identified the conserved NK-2 class homeobox protein CEH-24 to be crucially required for flipping. CEH-24 is expressed in sublateral motor neurons, which innervate the four body muscle quadrants. It is required for the formation of sublateral processes and for the expression of acetylcholine, which in turn is required for flip induction. Most important are the SIA neurons, which activate during flipping but also during wake. Optogenetic activation of the SIAs during sleep causes flipping but activation during wake does not. Whether or not SIA activation leads to flipping or not appears to depend on the global activity levels of neurons or muscles. Thus, we show that flipping during sleep is triggered by sublateral neuron activation. Flipping is state-specific because it requires reduced global activity of excitable cells as a permissive signal.
Optogenetic analysis of a peptidergic neuronal network controlling a food related navigation behavior

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Finding a food source or avoiding areas of imminent danger and maintaining this vicinity is a crucial survival strategy. Thus, it is of interest to elucidate how neuronal circuits integrate multiple sensory cues to navigate the environment. We functionally analyzed such a neuronal network, as well as the signaling molecules controlling body posture and locomotion. Using an unbiased optogenetic approach, we investigate the functionally uncharacterized neuropeptidergic AVK interneurons.

Blocking of neuropeptide release using Halorhodopsin (NpHR) cell-specifically expressed in AVK, caused a slowing of locomotion and increased body curvature. In the absence of food, AVK continuously releases the neuropeptide FLP-1, which evokes inhibition in a subset of target motor neurons, maintaining a low body curvature and promoting ‘long-range search’ behavior. Conversely, when FLP-1 release is inhibited, behavior reminiscent of ‘local search’ is imposed. We identified the NPR-6 neuropeptide receptor to be required for these effects. By Ca²⁺ imaging we show, that NPR-6 is needed for FLP-1-mediated inhibition of VC neurons. However, NPR-6 has low affinity for FLP-1, as shown by a cell-based assay, and is not mediating the inhibitory effects of synthetic FLP-1 peptides at the neuromuscular junction, as measured by electrophysiology. We identified other neuropeptides and peptide receptors essential for the behavioral response to AVK inhibition, by mRNA profiling of AVK cells, and by GPCR-neuropeptide affinity assays. We assess the possibility that a cascade of several peptide-signaling molecules mediates AVK’s effect on body curvature. AVK receives extensive synaptic input from the food sensing dopaminergic PDE neurons and FLP-1 release from AVK is increased in cat-2 mutants. Reminiscent of AVK photoinhibition, locomotion was strongly altered by the presence of food and by external application of dopamine. Thus, dopamine may inhibit AVK function. PDE neurons also innervate and modulate the proprioceptive interneuron DVA.

Photoexcitation and -inhibition of this neuron increase and reduce body curvature (contrary to AVK) via the release of excitatory NLP-12 neuropeptides onto motor neurons. Thus, AVK and DVA act in parallel to affect locomotion. Both interneurons are coupled to SMB head motor neurons and eliminating SMB, or gap junctions between AVK and SMB, phenocopied effects of AVK ablation on bending angles. Furthermore, NPR-6 is also required in SMB neurons to mediate AVK inhibition effects. In sum, we identified a neuronal network involving AVK interneurons, which could promote the food-dependent behavioral state switching from ‘local search’ to ‘long-range search’ behavior, via the release of FLP-1 peptides.
P001

Functional studies of circular RNAs in C. elegans

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Circular RNAs (circRNAs) are single-stranded RNAs with covalently linked 5’- and 3’-ends. Recently, thousands of circRNAs have been found to be produced in animals (Memczak et al., 2013, Rybak-Wolf et al., 2015). circRNAs are predominantly generated as a result of a ‘back-splice’ reaction that joins a splice donor site with an upstream splice acceptor site (Ivanov et al. 2105). circRNA expression is often independent of the corresponding linear mRNA suggesting that the process is regulated (Rybak-Wolf et al. 2015). In Drosophila, circRNA expression has been found to strongly increase during aging (Westholm et al. 2015). We investigate the role of circRNAs in the development and aging of C. elegans. For this, we (1) determine and compare circRNAs produced in different developmental stages, age, and tissues of C. elegans, (2) selectively disable production of circRNAs to find its possible function in the development of C. elegans. For C. elegans it is known to be difficult to dissociate the animal into single cells. We will present these ongoing efforts.
A cell-type-specific small RNA labeling approach uncovers the tissue-specific microRNome in Caenorhabditis elegans

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The implementation of distinct gene expression profiles is essential for accurate animal development and a primary cause for disease. While it is well established that post-transcriptional gene regulation by microRNAs (miRNAs) plays a pivotal role in these processes, uncovering the biological function of small RNAs remains challenging. High-throughput sequencing of miRNAs from individual development stages provided some insights but often lacks the resolution to link miRNA-function to the biology of distinct cell types within complex tissues. Here, we report a chemical small RNA-tagging approach followed by high-throughput sequencing that uncovers tissue- and cell type-specific miRNA profiles in C. elegans. The method relies on cell-specific 3′ terminal methylation of miRNA by selective expression of the small RNA duplex-specific methyltransferase HEN1. Because methylated miRNAs are resistant to chemical ribose oxidation, tissue-specific miRNAs can be recovered by established methylation-specific cloning protocols, followed by high-throughput sequencing. Using this strategy, we report the miRNome in the nervous system, the pharynx and the intestine of the worm, providing insights into miRNA expression at high spatial resolution. Chemical small RNA-tagging overcomes the current challenges in tissue- and cell-type-specific miRNA profiling and provides novel entry points to understanding the regulation of gene expression in the course of animal development.
EXPOSURE TO PERCHLORATE AFFECTS KEY LIFE HISTORY PARAMETERS OF THE NEMATODE CAENORHABDITIS ELEGANS

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The perchlorate anion is a powerful oxidizer used in the construction of low order explosives, rockets, missiles, some ammunition, air bags and fireworks. Perchlorate is periodically replaced and disposed of because of its limited shelf life. However, perchlorate salts are very stable and highly soluble and once released into aquatic systems, will dissociate into the corresponding cations and the perchlorate anion. It has been reported that ground and surface waters are contaminated by perchlorate and such contamination can persist for decades. Perchlorate is known to inhibit thyroid hormone synthesis in vertebrates. However, less is known about effects which are independent from the thyroid. Wild type C. elegans worms were exposed to sodium perchlorate added to both NGM agar and food source, OP50 bacteria, at concentrations ranging from 1ng/ml to 5mg/ml. Phenotypic changes including growth, development, reproductive performances and lifespan indices were investigated. Gene expression analysis was also carried out on worms exposed to 1ng/ml and 1mg/ml sodium perchlorate via whole genome microarrays. Perchlorate was found to stimulate growth at low concentrations (1ng/ml) but to induce an arrested development at high concentrations (5mg/ml). Reproductive performance was also decreased in a dose responsive manner in which the total brood size decreased by almost 50% at 3mg/ml and was delayed for 1 day. However, C. elegans life span, pharyngeal pumping and defecation were not affected by sodium perchlorate. Microarrays analysis showed that the primary genes affected in both treatment groups (1ng/ml and 1mg/ml) were genes involved in growth and development, brood size, metabolism and signal transduction. However, treatment with 1mg/ml sodium perchlorate resulted in the upregulation of genes that are of unknown function, which need further investigations. These observations suggest that exposure to perchlorate might trigger a putative horicenic effect at low concentrations but at higher concentrations toxic effects are pronounced. Future experiments will explore target genes involved in the toxicogenomic response of C. elegans to perchlorate via deletion mutants and gene knockouts. Furthermore, the specific pathways where perchlorate confers its physiological effects on C. elegans will be investigated using fluorescently tagged genes.
The *Si elegans* project · An open-access peer-contribution neurocomputational platform for the exploration of neural function and behavioral paradigms in *C. elegans*

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Despite being one of the five best characterized model organisms with all of its 302 neurons and almost its entire connectome precisely mapped, there is only sparse knowledge on how the *Caenorhabditis elegans* nervous system codes for its rich behavioral repertoire. The European *Si elegans* project aims at unravelling *C. elegans*’ nervous system function by its hardware emulation and its biophysically accurate embodiment in a virtual behavioral arena. We present on the final platform, its online access and peer contribution modalities with special focus on the user-friendly neural response model generation and behavioral experiment definition tools. We describe the unique features of the neural representations by custom-designed field-programmable gate array (FPGA) boards synaptically communicating through either a wire-based or an electro-optical connectome. Each of the 302 FPGAs can be configured with a *C. elegans*-specific neural response model. Both novice and expert model definition tools (e.g., LEMS-based, import/export from existing neural libraries) are at the user’s disposition. The hardware nervous system controls the behavior of a virtual representation of the nematode in a virtual arena. The physics-based simulation will allow scientists to test both published and hypothetical behavioral paradigms just as in a real laboratory environment. Users will have access to all biologically relevant variables to study the neural events governing a certain behavior. We furthermore will explain how users can actively contribute to the development of add-on functionality of the platform in a peer-participation approach. For further information, please visit www.si-elegans.eu. Acknowledgements: This project has received funding from the European Union’s Seventh Framework Programme for research, technological development and demonstration under grant agreement no 601215, FET Proactive, call ICT-2011.9.11: Neuro-Bio-Inspired Systems (NBIS).
JMJD-5/KDM8 in the maintenance of genome stability

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Histone post-translational modifications are not only essential for regulation of gene expression but they are also implicated in DNA damage repair, apoptosis and stress responses. The C. elegans germ line is an excellent system to investigate both the dynamic nature of chromatin modifications and the DNA damage checkpoint and repair pathways activated in response to exogenous stresses such as ionizing radiation. Taking advantage of this system, we performed a forward genetic screen and identified histone demethylase mutants showing hypersensitivity to IR. In this study we characterize JMJD-5, the C. elegans homolog of JMJD5/KDM8, a JmjC-containing protein whose catalytic activity is still debated. KDM8 has been shown to be required for mouse embryonic development, cancer growth, hypoxia-induced cell proliferation and for mitotic progression. Here we show that JMJD-5 is a histone demethylase for H3K36me2 and it is required for homologous recombination and genome integrity in C. elegans. Our results indicate that the catalytic activity of JMJD-5 is required for appropriate response to DSBs. Increased level of H3K36me2 in germ cells after loss of jmjd-5 results in aberrantly increased and prolonged retention of RAD-51 at DSBs both in physiological condition and after IR, indicating that correct regulation of the level H3K36me2 is required for proper completion of HR occurring during meiotic recombination and for resolving DSBs generated by IR.
Identification of Conserved MEL-28/ELYS Domains with Essential Roles in Nuclear Assembly and Chromosome Segregation

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Nucleoporins are the constituents of nuclear pore complexes (NPCs) and are essential regulators of nucleocytoplasmic transport, gene expression and genome stability. The nucleoporin MEL-28/ELYS plays a critical role in post-mitotic NPC reassembly through recruitment of the NUP107-160 subcomplex and is required for correct segregation of mitotic chromosomes. MEL-28 has a dynamic behavior: it localizes to nuclear pore complexes and chromatin during interphase and shuttles to kinetochores during cell division. However, it is unknown how MEL-28 localization and activity is regulated. Here we present a systematic functional and structural analysis of MEL-28 in C. elegans early development and human ELYS in cultured cells. We have identified functional domains responsible for NPC and kinetochore localization, chromatin binding, mitotic spindle matrix association and chromosome segregation. Surprisingly, we found that perturbations to MEL-28’s conserved AT-hook domain do not affect MEL-28 localization although they disrupt MEL-28 function and delay cell cycle progression in a DNA damage checkpoint-dependent manner. Our analyses also uncover a novel meiotic role of MEL-28. Together, these results show that MEL-28 has conserved structural domains that are essential for its fundamental roles in NPC assembly and chromosome segregation during meiosis and mitosis. To understand the function of MEL-28 chromatin binding we have used DamID to identify the chromatin regions with which MEL-28 associates. Interestingly, MEL-28 is enriched at transcribed genes and correlates positively with active histone marks, suggesting that it may be involved in regulation of gene expression. We compared the MEL-28 chromatin profile with the profile of another nucleoporin, NPP-22, which is permanently anchored to the nuclear pore complex. Surprisingly, we found that the chromatin association profile of NPP-22 was more similar to the profile of the nuclear lamina protein LMN-1 than to MEL-28’s profile, suggesting that individual NPPs interact with specific chromatin domains. Finally, GO-term analysis reveals that MEL-28-associated genes are related to larval and reproductive development. This suggests that MEL-28 has postembryonic functions that have not yet been studied.
Involvement of DEB-1 in chromosomal dynamics of the gametogenesis

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Meiosis is the key process of sexual reproduction, and it introduces genetic variability in organisms. Detection of new components involved in meiotic division will lead to a better understanding of chromosomal dynamics regulation and overall molecular regulation of this process. Our project is focused on the molecular mechanisms and regulation of chromosomal dynamics in the gametogenesis. Our research points to specific proteins participating in the pairing of homologous chromosome and formation of the synaptonemal complex between them during early meiosis. For identification of these proteins, we use the C. elegans model. Our experimental group discovered new protein DEB-1, previously reported as muscle attachment protein found in dense bodies, involved in meiotic processes. We observed independent role of this protein in the nuclei of early prophase meiocytes. After depletion of DEB-1 in gonad via RNA interference significantly increases the number of males in the progeny and on the contrary decreases brood size. This depletion has also negative effect on stabilization of pairing chromosomes, attachment to cytoskeleton and formation of synaptonemal complex. Taken together, these discrepancies lead to meiosis delay and presence of increased number of chromosome univalents in the proximal gonad. Our project address these aims: • To reveal concrete biological function of DEB-1 in the nuclei of Prophase I meiocytes • Identification of the interacting proteins during pairing process of homologous chromosomes • Identification of functional domains of the DEB-1 responsible for its involvement in the movement mediating complex on the nuclear periphery of the meiocytes

For decipherment of these tasks, we prepared transgenic worms with Flag-tagged DEB-1 for immunoprecipitation of DEB-1 and interacting proteins in the nuclear fraction of the worm lysate. To further confirm direct or indirect connection of DEB-1 to the meiosis specific proteins, we will use yeast two hybrid method. Moreover, we will mutate DEB-1 at different domains to point out the tertiary structure of the active complex during chromosome pairing with DEB-1 contribution. These molecular data will be supported by life-cell imaging, super-resolution microscopy, colocalizing DEB-1 with interacting proteins in leptotene/zygote and pachytene stage, where chromosomes undergo dramatic motions with aim to find its homolog and synapse. Our data should significantly contribute to understanding the molecular mechanisms and regulation of early meiotic prophase in higher eukaryots. This publication is supported by the project „BIOCEV – Biotechnology and Biomedicine Centre of the Academy of Sciences and Charles University“ (CZ.1.05/1.1.00/02.0109) funded by the European Regional Development Fund, by GACR (16-03403S) and UMG/RVO:68378050.
Our goal is to study transcriptional regulation of *C. elegans* in *Dauer* stage using a systems-biology approach. Therefore, we are creating a high-resolution anatomical description from electron microscopy data. Onto this dataset we will map low-resolution live and fixed light microscopy data exploiting the high degree of stereotypicality of *C. elegans* in order to create a computational model of *Dauer exit*. We acquired a transmission electron microscopy (TEM) dataset of an entire *Dauer* *C. elegans* consisting of 25,000 individual images covering 561 sections totalling a size of approximately one terabyte. We are developing an automatic segmentation tool for the extraction of nuclei from this dataset, however, ultimately aim at the creation of a complete ‘Virtual Dauer’. Segmentation of electron microscopy data remains computationally challenging due to the complexity of the specimen, irregularity in structure and intensity, tight clustering, size of the data, and the necessity to characterize biological a priori knowledge in order to achieve precise segmentation results and avoid a labor-intensive process.

We present preliminary results of our segmentation of the TEM dataset. The method utilizes active contours that support global constraints based on biological a priori knowledge. We take into account machine-learning methods, knowledge about spatial organization (e.g. number of nuclei, type of separating tissue), and specific properties of segmented objects (e.g. ratio of euchromatin to heterochromatin, presence of a nucleolus), combined with minimal user input to produce highly accurate results.

Our segmentation method is designed to be flexible in order to adjust to feature extraction from different imaging modalities, labeling techniques, and be applicable to higher-dimensional datasets. We believe it will ultimately allow us to combine any kind of imaging data into our computational model.

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**P008**

**Active Contour based Segmentation of *C. elegans* Electron Microscopy Data**

*Ella Bahry, Mei Sun, Yalin Wang, Eugene Myers, and Stephan Preibisch*
P009

Developmental robustness in C. elegans: from quantification to mechanisms

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Biological systems face continuously perturbations, such as environmental variation in growth conditions or stochastic variation in the abundance of critical macromolecules. Despite this fact, they often manage to operate with remarkable precision and reproducibility. The property of a biological system to produce an invariant output in the presence of such perturbations is called robustness. Organismal development is highly robust and this is critical for the successful transformation of the zygote into a complex multi-cellular individual. While research on developmental robustness has seen a surge over the last years, many studies have remained theoretical or restricted to single cell organisms, so we still lack appropriate experimental systems to elucidate the underlying mechanisms of robustness in multi-cellular animals. We use the stereotypical patterning of C. elegans as a model to study developmental robustness. We focus in particular on seam cell development as a new experimental system to study robustness of epithelial stem cell numbers to stochastic noise. We have conducted the first genetic screen in C. elegans aiming explicitly at identifying genes that restrict phenotypic variance rather than modifying the trait mean. I will discuss the design of this screen and synthesize our first findings offering a proof of concept for cloning and characterising robustness genes. Our ultimate goal is to use this approach in order to derive a developmental framework for phenotypic buffering.
Cells release extracellular vesicles (EVs) that can mediate intercellular communication by the delivery of lipids, proteins and nucleic acids. Therefore, they are able to influence development, immune response and disease. Despite the pleiotropic functions of extracellular vesicles (EVs), little is known about the molecular details of EV release, especially for EVs that form by plasma membrane budding. The outer membrane of EVs contains lipids like phosphatidylethanolamine (PE) that are normally restricted to the inner leaflet of the plasma membrane, suggesting a role for lipid asymmetry in EV release. Previously, we showed that TAT-5 phospholipid flippase activity prevents EV release and maintains PE asymmetry in C. elegans (Wehman et al., 2011, Curr Biol). However, it was unclear how TAT-5 activity is regulated. The yeast homolog of TAT-5 binds to a protein homologous to the large and novel protein PAD-1. Therefore, we hypothesized that PAD-1 could regulate TAT-5 activity and EV release. We examined pad-1 mutants using Transmission Electron Microscopy and a fluorescent membrane reporter that makes EVs visible by light microscopy. We discovered increased membrane labeling and TEM analysis revealed excessive release of EVs, similar to tat-5 mutants. Using a GFP-tagged reporter, we found that PAD-1 localizes to domains on the plasma membrane, where it is likely to interact with TAT-5. In pad-1 mutants, GFP::TAT-5 still localizes to the plasma membrane, suggesting that PAD-1 is not required for TAT-5 localization. To test whether PAD-1 regulates the lipid flipping activity of TAT-5, we stained worms with the PE probe duramycin. PE was exposed on the plasma membrane of pad-1 mutants, similar to tat-5 mutants. Thus, PAD-1 is required for the lipid flipping activity of TAT-5. In summary, we identified PAD-1 as a new regulator for EV release in C. elegans, providing a better understanding of how EVs are released for cell-cell communication. Our study further supports the model that lipid asymmetry regulates plasma membrane budding.
The myogenic transcription factor CeMyoD establishes muscle-specific proteostasis in Caenorhabditis elegans body-wall muscle cells

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Safeguarding the proteome is central to the health of the cell. In multi-cellular organisms, the composition of the proteome, and by extension, protein-folding requirements, varies between cells. In agreement, chaperone network composition differs between tissues. Here, we ask how chaperone expression is regulated in a cell type-specific manner and whether cellular differentiation affects chaperone expression. Our analyses show that chaperones expressed in muscle or required for muscle folding present occupancy sites for the myogenic transcription factor HLH-1 (MyoD) in their promoters. To test this experimentally, we employed HLH-1 myogenic potential to genetically modulate cellular differentiation of Caenorhabditis elegans muscle cells by either ectopic over-expression or down-regulation of HLH-1 and monitoring chaperone expression. We found that modulation of HLH-1 levels affected the expression of muscle chaperones. Moreover, disrupting putative HLH-1 binding sites on ubiquitously expressed CeHsp90 and muscle-enriched hsp-12.2 promoters abolished myogenic-dependent chaperone expression. Reducing HLH-1-dependent function in muscle compromised muscle proteostasis. Moreover, we could show that down-regulation or over-expression of muscle chaperones disrupted embryonic development and muscle folding. Specifically, over-expression of the DNAJB6 homolog DNJ-24, a limb-girdle muscular dystrophy-associated chaperone, disrupted the muscle chaperone network and exposed synthetic motility defects. Thus, cellular differentiation could establish a proteostasis network dedicated to the folding and maintenance of the muscle proteome. We propose that cell-specific expression of chaperones can explain the selective vulnerability that many protein misfolding diseases exhibit even when the misfolded protein is over-expressed.
Microfluidic long term immobilization enabling developmental studies at the subcellular level

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Thanks to its size, fecundity and transparency, Caenorhabditis elegans has become one of the most important model organisms. C. elegans has been used to study processes as diverse as aging and stress, to developmental studies at subcellular level. However, while C. elegans has proven to be immensely useful, progress in many areas of biology has been hindered by the need to immobilize nematodes while studying. Conventionally immobilization is achieved by padding the nematode in between coverslips thereby applying pressure, often improving the immobilization by adding tranquilizing drugs or polystyrene nanoparticles. While an effective immobilization method the large pressure applied as well as the drugs used, negatively affect most processes of interest. This is especially true when studying adult C. elegans as the immobilization not only applies large amounts of pressure and interferes with food supply but also interferes with egg laying, practically stopping all germline activity within minutes and killing the animal within hours. In recent years a number of microfluidic strategies for the immobilization of C. elegans have been published. Strategies vary greatly from straight narrow channels in which the nematodes are trapped, to actively deformed on-chip valves holding the nematode in place, to intricate temperature and flow schemes. However while some of these strategies reach immobilization comparable to the conventional method they suffer from similar drawbacks. The pressure or the geometry employed for immobilization interfere with many processes of interest, making these microfluidic devices unsuited for long term studies. At the same time devices aimed at long term viability, in general fail to deliver good immobilization as the nematodes are only loosely held or simply confined to large chambers without any immobilization. Further many of the published microfluidic methods are unsuitable for adult C. elegans as egg laying and food supply are severely hindered. A combination of both seemingly opposite tasks, immobilization and long term viability, suited for C. elegans in different developmental stages has therefore not been demonstrated yet. Herein we demonstrate a microfluidic immobilization platform capable of immobilizing adult C. elegans for extended periods of time and compatible with subcellular resolution imaging. Viability within the microfluidic device was extensively tested, showing on average survival of 100 hours while immobilized and egg laying rates comparable to worms kept on plate. Simultaneously we demonstrate excellent immobilization, making our microfluidic device perfectly compatible with subcellular resolution imaging over extended periods of time. The performance of the microfluidic platform is demonstrated within the germline. For the first time enabling long term studies of germline processes in vivo. In addition to the immobilization of adult C. elegans, we demonstrate the applicability of our microfluidic device to the immobilization of nematodes in different larval stages, allowing the interference free study of developmental processes in vivo and at subcellular resolution.
P013

Implementation of Cl–-conducting channelrhodopsins ACR1, ACR2 ChloC-XXL as well as ChR2-XXL and ChR2-QUINT as new hyper-sensitive optogenetic tools for inhibition and excitation

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Light-responding rhodopsin cation channels have been used as depolarizing tools in optogenetic research, allowing targeted stimulation of neuron firing. By expressing Channelrhodopsin-2 in excitable cells of *C. elegans* - one of the best-defined systems to study neuronal circuits – it is possible to examine the specific role of single neurons, exploiting the animals’ light-evoked behaviour, electrophysiological or optical signals as readout. However, so far, neuronal silencing has been limited because of a lack of equally efficient hyperpolarizing tools. Commonly used hyperpolarizers, such as the ion-pumps halorhodopsin or achaerhodopsin suffer from a restricted transport capacity, since only one charge is moved per absorbed photon and may lead to formation of undesired abnormal ionic gradients. The recently described natural hyperpolarizing anion channel rhodopsins (ACR1 and ACR2) are promising candidates to fill this gap. Another addition to the ‘zoo’ of rhodopsin-based optogenetic tools are XXL step-function mutants that take advantage of dramatically slowed kinetics, strongly increasing their light sensitivity and overcoming the need for a continuous illumination. Here, we probed the potential of ACR1 and ACR2 in *C. elegans*, as well as of the XXL variants of Cl–-conducting ChR2 (ChloC) and ChR2, by evaluating the animals’ elongation and contraction behaviour. In cholinergic neurons, ACRs evoked a robust body elongation of up to 8 % at a saturating light level, the strongest effect observed so far, already relaxing at a minimum light intensity of around 3 µW/mm². The red-shifted action spectrum of ACR1, peaking at 515 nm, allows to specifically stimulate ACR1 with green light at a low intensity level, allowing ACR1 to be combined with ACR2 or other blue light activated tools in different subsets of neurons within a single circuit. In contrast to ACRs, ChloC-XXL mediated a smaller relaxation of up to 5.5 % that, however, was long-lasting. Animals expressing the depolarizing tool ChR2-XXL showed an exceptionally high light sensitivity, surpassing that of ACRs by 10 times. These worms show sustained body contractions of up to 15 % and relaxed only after around 15 min. Last, we also generated a quintuple mutant of ChR2 (ChR2-QUINT), with extreme light sensitivity, and more robust performance than other step-function opsins. In sum, ACRs expressed in *C. elegans* provide inhibition with superior efficiency, where previously only pumps, and not channels, could be used for hyperpolarization. They enable a more natural hyperpolarization, depending on membrane voltage, unlike the existing rhodopsin pumps. With ChR2’s XXL and QUINT, exceptionally light-sensitive tools for depolarization could be established, that will enable applications when only a very low protein expression can be achieved for a given promoter or neuron of interest.
Characterization of the SET1/MLL complex in C. elegans

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Methylation of histone H3 Lys4 (H3K4me) is associated with active transcription in all species, and is catalyzed by highly conserved multiprotein complexes known as Compass in yeast or SET1/MLL in mammals. Biochemical analysis has shown that the H3K4 HMT activity of SET1/MLL proteins relies on protein-protein interactions within large multisubunit complexes that include three core components: RbBP5, Ash2L, and WDR5. However, it remains unclear how the composition and specificity of these complexes varies between cell types and during development. We have previously shown that SET-2, the sole SET1 homologue in C. elegans, and the core component ASH-2, play different roles in H3K4me during development. We have also shown a physical interaction between SET-2 and WDR-5.1 in embryos, but the full composition of the C. elegans H3K4 HMT complex in vivo is unknown. In order to better understand the regulation of H3K4me, we have decided to develop a proteomic approach to identify SET-2/WDR-5.1 molecular partners. Using a functional WDR-5.1::HA protein to immunoprecipitate the complex from embryonic extracts, we have identified components of the SET-1/MLL complex, additional chromatin remodeling factors, proteosomal subunits and factors which may be involved in the specific targeting of H3K4 methylation. I will present data from proteomic analysis and validation of potential partners.
Poster abstracts in alphabetical order (first author)

P015

Reduced expression of the C. elegans Aryl-Hydrocarbon Receptor (AHR-1) promotes healthy ageing

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Background: The ageing process is concurrently shaped by genetic, environmental and nutritional factors. The Aryl hydrocarbon Receptor (AhR) is an evolutionarily conserved transcription factor of the bHLH/PAS superfamily, which in mammals plays a major role in mediating biological responses to environmental cues (e.g. xenobiotics present in pollution or food). Binding of exogenous or endogenous ligands to AhR and its dimerization to ARNT are required to activate its transcriptional activity. AhR in turn regulates diverse physiological functions from cell growth and proliferation to differentiation and apoptosis, but only little and inconsistent information exist on its role in the ageing process. The C. elegans AhR homolog, AHR-1, can bind human ARNT, but no AHR-1 ligands have been found so far. The main function described for AHR-1 in C. elegans is in the differentiation of diverse types of neurons, which control perception of food, chemicals and oxygen. We have now shown for the first time that the C. elegans AhR has a negative impact on healthy aging (Eckers et al. Sci Rep 2016). Results and Conclusions: We found that C. elegans ahr-1 mutants, fed HT115 bacteria live longer and move better for a longer time. Moreover, their pharyngeal pumping activity declines slower during ageing compared to wild-type animals. While ahr-1 mutants had reduced resistance to UVB they showed higher resistance to heat-stress during the ageing process. Of note, we found a similar protective role against age-related parameters in mammals (data not shown, see Eckers et al. Sci Rep 2016). Interestingly, opposite results on lifespan, movement and heat-stress resistance were observed when animals were fed OP50 bacteria. Our data indicate that (1) reduced AhR expression has beneficial anti-ageing effects, (2) and that these effects are likely dependent on nutritional factors, which remain to be identified. Support: This project is funded by the Jürgen Manchot Foundation.
Control of mRNA decay is a key feature in post-transcriptional regulation of gene expression in eukaryotic cells. mRNA decapping, performed by a well conserved among eukaryotes decapping complex, holds an all important role in this process, as it is the step that renders both normal, silenced and aberrant mRNA molecules susceptible to degradation by 5'-3' exonucleases. Previous work in the nematode *C. elegans* has shown that disruption of decapping activity affects many aspects of organismal development and physiology, leading to short-lived and stress-sensitive animals. Here we focus on the effects of tissue-specific overexpression of the core 5'-3' mRNA degradation components, such as the regulatory subunit of the decapping enzyme DCAP-1 and the 5'-3' exonuclease XRN-1. We show that overexpression of the relative trangenes in selective tissues has different effects in lifespan determination and stress resistance, revealing that alterations in the mRNA decay machinery can act cell non-autonomously to influence the ageing process. By unveiling the endocrine pathways and molecular mechanisms that mediate such organismal responses to tissue-specific changes we provide new links between complex biological processes, like mRNA metabolism, proteostasis maintenance and ageing.
A combined candidate gene and optogenetic approach for the study of cognitive decline in neurodegenerative disorders

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Because of the expected increase in the incidence of several neurodegenerative disorders, there is a pressing need for effective treatments. Since cognitive decline is one of the earliest events in neurodegenerative disorders, studying these early events will provide key insights in molecular processes underlying neurodegeneration and cognitive decline. For this purpose, the involvement of a set of novel neurodegeneration modifying factors in cognitive decline is being investigated. This set of genes has been selected based on a meta-analysis of neurodegeneration-based genetic screens in *C. elegans*, *D. melanogaster*, and *S. cerevisiae*; resulting in a list of common modifier genes all of which have human orthologs. Several of these genes are of special interest as they have never been studied further in relation to neurodegenerative pathology and cognitive decline. We are studying these genes in aging animals while performing associative or non-associative learning tasks, in order to reveal key insights into relationships between cognitive decline and neurodegeneration. Two distinct learning assays, salt conditioned learning and tap habituation, have been optimized for our specific purpose. By doing so; both non-associative and associative learning, which are both impaired in neurodegenerative disorders, can be studied, and general vs. specific effects can be evaluated. A selected set of *C. elegans* mutants for these disease-modifying genes are being compared to wild-type animals and a *C. elegans* model for Alzheimer’s disease relying on these learning paradigms and during different days of adulthood. This approach unveils potential new key players in cognitive decline and neurodegeneration.
Molecular Study of the EGFR/RAS/MAPK pathway in *C. elegans* using a FRET biosensor

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The activation of the RAS/MAPK kinase pathway is involved in essential signaling cascades controlling cell differentiation, growth, and survival in all metazoans. Moreover, aberrant RAS/MAPK signaling has been detected in many cancer types and various developmental diseases. In *C. elegans*, the different functions of the RAS/MAPK pathway are best studied during vulval and germline development. The core pathway components and targets identified in *C. elegans* are strongly conserved and many of them can be functionally replaced with their mammalian orthologs. Natural polymorphisms identified in different *C. elegans* isolates further modify RAS/MAPK signaling strength. For example, our previous studies with polymorphic RAS/MAPK modifiers have suggested that hypoxia attenuates RAS/MAPK signaling in different organs of *C. elegans*. So far, we have used vulval inductions (i.e. the number of differentiated cells) to quantify the output of the RAS/MAPK signaling cascade. The aim of this project is to directly measure MAPK activation in live animals in a spatiotemporal way using a MAPK biosensor. Förster/fluorescence energy transfer (FRET) allows the real-time monitoring of protein conformational changes and protein-protein interactions. A FRET-based MAPK biosensor for in vivo imaging will be created by expressing a CFP and YFP tagged MAPK substrate, which undergoes a conformational change when phosphorylated. The emission ratio of YFP/CFP will be used to quantify MAPK activity. The MAPK FRET sensor construct will be inserted into the *C. elegans* genome under a germline-specific (*pie-1*) or epithelial (*dlg-1*) promoter using Mos-1 transposon-mediated single copy Insertion (MosSCI). We will validate the functionality of the MAPK sensors by testing the FRET signal in different ras pathway mutants and study the effect of hypoxic signaling on the RAS/MAPK pathway by quantifying the FRET signal in animals grown under hypoxia.
A connectomics approach to understanding variation in synaptic connectivity.

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This year marks the 30th anniversary since the official landmark publication of “Mind of the Worm” (MoW) by White et al. (1986), which provided a comprehensive map of synaptic connections in the C. elegans nervous system. We present an analysis of neural connectivity in the L4 and adult nerve ring based on the recent volumetric reconstruction of legacy electron micrographs from MoW. By comparing the spatial adjacency between neurons with the connectivity map, we show that the frequency of synaptic connectivity varies between but not within neuron classes and that network connectivity is largely independent of the amount of physical contact between neurons. Furthermore, we identify two distinct sets of synaptic connections: asymmetric and symmetric connections. Asymmetric connections occur on either the right or left side of the animal and can be predicted by a probabilistic model, suggesting that these connections occur randomly. Symmetric connections occur on both the left and right side of the animal and cannot be predicted by the same probabilistic model, suggesting that these connections are somehow specified. Though seemingly random, asymmetric connections account for ~40% of the connectivity in the nerve ring suggesting that these types of connections likely contribute to nervous system development and/or function. Both symmetric and asymmetric connections are distributed similarly across neurons, suggesting that asymmetric connections likely do not support any specific behavioral function. Compared to symmetric connections, asymmetric connections are generally smaller and occur with lower probability. With this in mind, we suggest a model where asymmetric connections support the development and function of symmetric connections. Our analysis of polyadic synapses provides circumstantial evidence for such a model. Roughly a third of C. elegans synapses are monadic (single post-synaptic partners) while the majority are polyadic (multiple post-synaptic partners for a single presynaptic neuron). Compared to monadic and polyadic synapses with only symmetric post-synaptic partners, we find that polyadic synapses that include both an asymmetric and symmetric post-synaptic partner exhibit significantly less contact between the presynaptic neuron and the symmetric post-synaptic partners. Thus, by pairing asymmetric and symmetric connections, the presynaptic neuron is able to make a symmetric connection while requiring less membrane contact with the symmetric post-synaptic partner. This could be suggestive of a mechanism whereby asymmetric synaptic connections serve as a catalyst for symmetric synaptic connections.
Role of Patched-like proteins in endocytosis in *C. elegans*

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Endocytosis refers to the process by which material is taken up by the cell through plasma membrane invaginations and subsequent vesicle formations. The vesicles fuse with early endosomes and its its fate will be determined by sorting of the cargo into either recycling pathways or intralumenal vesicles. After this endosome maturation step cargoes remaining in the endosomal compartments (i.e. late endosomes) will be degraded in lysosomes. The membrane identity of endosomes is determined by Rab GTPases. Early endosomes are marked by RAB-5, while late endosomes accumulate RAB-7. The switch from RAB-5 to RAB-7, the so-called rab conversion, is meditated by SAND-1/Mon1. A screen to identify genetic interactors of sand-1 revealed the PTCH family member ptc-3. PTCH family proteins act as SSD (sterol-sensing domain) hedgehog (HH) signaling receptors in most metazoans. However, crucial members of the HH signaling pathway are missing in *C. elegans* and hence the role of these signaling receptors remains largely unexplored. Interestingly the PTCH and PTCH-related (PTR) protein families are expanded; the reason for this expansion is still unclear. We are aiming to understand the role to PTC and PTR proteins in the cellular and developmental context. *ptc-3* is an essential gene, and *sand-1* loss-of-function rescued the lethal phenotype. Conversely, *ptc-3(RNAi)* did not alleviate the *sand-1(ok1963)* block in rab conversion. However, *ptc-3(RNAi)* caused late endosomal defects and altered RAB-7 localization indicating a role downstream of *sand-1*. PTR-4 is an essential PTCH-related protein in *C. elegans*. While *ptr-4(RNAi)* also interfered with late endosomal transport and RAB-7 localization, the *unc* phenotype of *ptr-4(RNAi)* was not rescued by *sand-1(\\textit{ok1963})* suggesting different roles of the PTCH and PTCH-related proteins. Consistent with this notion, lipid droplets were enlarged in *ptr-4(RNAi)* animals, while *ptc-3(RNAi)* animals maintained small lipid droplets. We are currently investigating the individual roles of PTC-3 and PTR-4 in trafficking in the late endosomal pathway and in lipid droplet formation.
Poster abstracts in alphabetical order (first author)

P021

Regulation of C. elegans development by CDK-12 and phosphorylation of the RNA Polymerase II CTD

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The C-terminal domain (CTD) of the RNA Polymerase II is composed of tandem repeats of the consensus sequence Y\textsubscript{1}S\textsubscript{2}P\textsubscript{3}Ts\textsubscript{5}P\textsubscript{6}S\textsubscript{7}. The CTD is highly modified during transcription, which plays a major role in the coordination of transcription and mRNA maturation. CDK-12 phosphorylates the S\textsubscript{2} of the CTD, a mark classically associated with elongation, splicing and cleavage/polyadenylation. However, recent studies in yeast, fly and mammalian cell, highlighted a gene-specific requirement for CDK-12. For instance, in fission yeast, the CTD S\textsubscript{2} phosphorylation is only required for the induction of sexual differentiation. In C. elegans, cdk-12 knockdown by RNAi results in a L1 arrest. The embryos hatch in presence of food but are enable to induce the post-L1 development, mimicking a L1 diapause. Homozygous cdk-12 disruption leads to the same terminal phenotype. We have constructed an analogue-sensitive (as) version of CDK-12, cdk-12as, to investigate the role of the CTD S\textsubscript{2} phosphorylation by CDK-12 during the early larval development. In the presence of low dose of the ATP analogue, the cdk-12as strain arrests at the L1 stage with undetectable CTD S\textsubscript{2} phosphorylation. We show that the inhibition is specific, very fast, and can be reversed by washing out the ATP analogue. Transcriptomic analyses by RNA-seq were performed during physiological or ATP analogue-induced L1 arrest, which revealed that the loss of CDK-12 kinase activity only affects a subset of genes strongly enriched for “development related genes”. Deeper analyses indicated that most target genes belong to operons and undergo SL2 trans-splicing. Therefore, our results suggest that the CDK-12 kinase is specifically required for the induction of SL2-trans-spliced development related genes. Preliminary data indicate that the RNA Polymerase II occupancy along the operons is barely affected when CDK-12 is inhibited, suggesting a posttranscriptional requirement, maybe during trans-splicing. Taken together our results show that the phosphorylation of CTD S\textsubscript{2} by CDK-12 is dispensable during embryogenesis but is required to escape L1 arrest and pursue larval development. We are currently investigating the role of RNA Polymerase II CTD S\textsubscript{2} phosphorylation in the integration of environmental cues that occurs at the L1 stage.
Proper mitochondrial transcription fine-tunes germline development in C. elegans

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Mitochondria are semi-autonomous organelles, mainly relying on nuclear gene products for their biogenesis and function. Nevertheless they have retained a remnant prokaryotic genome which encodes a dozen electron transport chain components. Transcription of the mitochondrial genome is conducted by a tripartite complex consisting of a dedicated RNA polymerase (POLRMT) and two auxiliary transcription factors (TFAM and TFB2M). We show that inhibition of mitochondrial transcription in C. elegans causes a profound reduction in the brood size of adult hermaphrodites. Notably, it also results in gonad hyperplasia, although apoptosis is induced and acts as a compensatory mechanism in the gonad syncytium. By contrast, knockdown of rpom-1, the worm homolog of POLRMT in animals with reduced insulin/IGF and TGF-β signaling results in deformed and atrophic gonads, whereas mutants with attenuated protein synthesis are insensitive to RPOM-1 depletion. This indicates a direct interplay between mitochondrial function and conserved signaling cascades converging on the germline. We are currently investigating the molecular circuitry that links mitochondrial transcription with fate decisions such as the transition from mitosis to meiosis in the nematode gonad.
Biomechanical control of cytoplasmic streaming and oocyte growth in the germline syncytium

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The germline of many species, including humans, develops through incomplete cytokinesis mechanism to form cysts, or syncytia, where several nuclei are connected and share a common cytoplasm. Physiologically, this particular organization is thought to favor the equilibration of genes products between haploid gametes, allow synchronization of their cell cycle and enable overall protein transport. A non-exclusive recent hypothesis is that syncytial structures could confer peculiar biomechanical properties to tissues, allowing cells to resist specific mechanical stresses (1). We use the gonads of Caenorhabditis elegans as a model for germline syncytium formation and maintenance and analyze its mechanical properties. Using high-resolution confocal videomicroscopy coupled to laser ablation experiments, we show that the actomyosin-rich syncytium cortex is under longitudinal and circular active tensions. While the tension along the distal to proximal axis of the gonad is constant between each ovulation events, the circular tension in the rachis is higher in the late pachytene region, where oocytes progressively grow. Oocytes growth was previously shown to be dependent on cytoplasmic streaming, a process driven by actomyosin contractility (2). Using a Particle Imaging Velocimetry algorithm, we quantify precisely the velocity of these cytoplasmic flows throughout the gonads of wild-type worms, as well as in conditions where the rachis shape and contractility are affected by RNAi targeting actomyosin regulators. Our results indicate that the cytoplasmic flows are higher in the regions were rachis circular tension is also maximal, suggesting that cortical active tension in the proximal region of the syncytial germline favors cytoplasmic streaming. Overall, the results from this work will provide novel information about the specific biophysical properties of syncytial structures. (1) Amini R, Goupil E, Labella S, Zetka M, Maddox AS, Labbé JC and Chartier NT. C. elegans Anillin proteins regulate intercellular bridge stability and germline syncytial organization (2014). Journal of Cell Biology 206 (1) (2) Wolke U, Jezuit EA and Priess JR. Actin-dependent cytoplasmic streaming in C. elegans oogenesis (2007). Development (134)
P024

Analysis of inter-individual transcriptional variability of stress response genes in *C. elegans* by high-throughput qRT-PCR in single worms

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Across species, it has been noted that lifespan is highly variable among individuals. Understanding the basis for such variability is crucial for personalised medicine where not the average population, but rather the individual is center stage. It is also crucial for the identification of new factors that can have an impact for human ageing and health and that may have been missed by the analysis of population averages. Remarkably, lifespan is highly variable across *C. elegans* individuals, although they are genetically identical and reared in controlled environmental conditions. Individual nematodes will experience lifespans ranging over 20% of the population mean. Both genetic and environmental factors are known to control lifespan. The widespread phenotypic variability, however, may point at additional non-genetic factors influencing ageing. *C. elegans* lifespan is controlled by several genetic pathways, including Insulin-Like Signalling and Target Of Rapamycin. These longevity pathways converge, among other transcriptional targets, on stress response genes and molecular chaperones. Remarkably, inter-individual variability in stress response genes has consequences for the robustness to both genetic and environmental perturbations in Caenorhabditis elegans and correlates with lifespan and mutation penetrance (Rea et al., 2005, Casanueva et al., Science 2011). To monitor inter-individual variability in gene expression, we have established a high throughput quantitative real time PCR assay on single worms using nano-fluidic technology. We also developed an integrative Bayesian statistical method to estimate biological variability from PCR datasets, while accounting for technical variability. This technique allows us to identify highly variable genes throughout ageing. Our aim is to understand the molecular basis of the recorded probabilistic nature of gene expression and to exploit inter-individual variability in gene expression as a method to study gene network architecture in a metazoan model organism.
P025

Involvement of epigenetic factors in pluripotency and metabolism in C. elegans

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A promising therapy for degenerative diseases is the replacement by the injection of differentiated progenitors. Differentiation is a crucial step as insufficiently differentiated cells will lead to the formation of teratomas. Understanding the epigenetic determinants of cell plasticity and the involved signalling pathways regulating cell differentiation are therefore of prime importance for regenerative medicine. Using muscle transdifferentiation ectopically induced by the expression of a single transcription factor, we test cell plasticity in fully differentiated L1 animals. Using this system, we characterized cells which are amenable to muscle progenitors differentiation. Moreover, using a variety of genetic mutants we show that histone 3 lysine 27 methylation deposited by the MES/Polycomb complex is essential to restrict cell fate plasticity upon cell fate challenge. In the absence of this histone mark, animals completely arrest their development upon expression of the transcription factor. We discovered that surprisingly plasticity depends on the metabolic state of the animal: starved animals are insensitive to transcription factor expression, while in the presence of food animals arrest development with very high penetrance. We will present the processes which lead to larval arrest and characterizing the link between metabolism and cell fate plasticity.
P026

A microfluidic-based platform for automated longitudinal monitoring of protein aggregation in neurodegenerative disease C. elegans models

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The growing incidence of neurodegenerative diseases (NDs) increasingly demands a comprehensive understanding of the molecular phenomena related to neurodegeneration, as premise towards a new class of treatments for these diseases. Cell-based systems can be used for some of these studies, but the investigation of complex phenotypes in which several tissues and pathways are involved usually requires long-term \textit{in vivo} analyses. The nematode \textit{Caenorhabditis elegans} represents a convenient model organism for many of these tests, primarily because of its short life cycle, ease of genetic manipulation and relatively high amount of conserved mechanisms between \textit{C. elegans} and humans. In this framework, automated systems and methodologies for accurate time-resolved studies on \textit{C. elegans} are becoming precious tools in the field. Here, we present a microfluidic-based platform for \textit{C. elegans} research, enabling: (i) automated worm isolation and maintenance, with active control of the main culture parameters, including temperature; (ii) reversible worm immobilization and long-term high-resolution imaging. By means of our device, we attain accurate monitoring of the growth rate and the development of worms at single-organism resolution, starting from a desired larval stage (typically L1 or L4). Moreover, we use our platform for \textit{in vivo} analyses of the long-term dynamics of protein aggregation phenomena in \textit{C. elegans} models for NDs. In particular, we employ an amyotrophic lateral sclerosis (ALS) \textit{C. elegans} model to monitor the expression of mutated human superoxide dismutase 1 - Yellow Fluorescent Protein (SOD1-YFP) fusion proteins in the body wall muscles of worms, for individual worms, over several days. By combining reversible worm immobilization and on-chip high-resolution imaging, our device allows precisely localizing the protein expression within each worm's tissues, as well as following the evolution of individual protein aggregates over consecutive days at sub-cellular level. Similarly, we demonstrate the suitability of our system for protein aggregation monitoring in a \textit{C. elegans} Huntington disease (HD) model. Finally, to test the potential of our platform for high-content pharmacological screening, we employ it to investigate the effects of a doxycycline treatment on the ALS model. In this study we show that, while the average number of SOD-1 aggregates per worm proves to be unaffected by the treatment, a reduced growth rate of the average aggregate size can be observed in the treated worms. Ref: Cornaglia, M. et al., \textit{Mol Neurodegener} 2016, 11.
Coupling of mitochondrial metabolism and mRNA turnover modulates cellular energy homeostasis during aging in *C. elegans*

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Cellular homeostasis is critically influenced by the mitochondrial metabolic state. Mitochondrial number and functionality largely determine intracellular ATP, Ca\(^{2+}\) and ROS levels, which in turn regulate cellular redox state, and may initiate signaling cascades involved in cell death, aging and disease. Various stressors affect mitochondrial function, thereby altering cellular metabolism. Cellular metabolic state is also modulated by the fraction of mRNAs undergoing translation, storage or degradation. Recent studies indicate that tight control of mRNA turnover mechanisms is needed for normal cell physiology through aging, highlighting their high regulatory capacity. The importance of these mechanisms is further supported by their evolutionary conservation from lower to higher eukaryotes. Emerging findings indicating that mitochondrial metabolism is altered in response to cytoplasmic stress, prompted us to examine the possible functional crosstalk between the two pathways. We are currently investigating this interplay *in vivo*. Our findings indicate that the two mechanisms are coupled and regulate energy metabolism, stress resistance and longevity in *C. elegans*. Elucidation of this complex regulatory association is important towards understanding how alterations of mitochondrial function influence healthspan and aging.
Mechanism of left-right symmetry breaking during early development of C. elegans

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Body plan of most of the organisms is left-right (L-R) symmetric on the exterior but highly asymmetric in arrangement of the internal organs. This asymmetry is established during early stages of embryonic development and is crucial for a proper cell fate determination and organ development. The anterior-posterior (A-P) and the dorsal-ventral (D-V) symmetries need to be broken prior to establishing the L-R body axis. For example, the entry of a sperm in a C. elegans oocyte breaks the A-P symmetry and the point of entry becomes the posterior part before the first cell division in the embryo. Then, the asymmetric division of posterior cell (P1) results in breaking the D-V symmetry as location of the daughter cells- AB and EMS, determine the dorsal and ventral side, respectively. The event that breaks the L-R symmetry at 4 to 6 cell transition in C. elegans embryos has been previously recognized to be a directionally consistent and simultaneous skew or rotation of the daughter cells of AB. Recently, it was shown by Naganathan et. al (2014) that the skew is executed by chiral actomyosin flows in the cortex of the embryonic cells. However, it is still unknown what sets the definite direction of the skew and how. In our current work, we investigate the relationship between the chirality of cortical flows and the skewing of cells. We report on the observation of an asymmetric distribution of cortical components on the dorsal and ventral surfaces of the embryo, which may be sufficient to generate the asymmetry required for a non-zero torque that skews the cells. This supports the predictions of our theoretical model and sets the direction for a full mechanistic description of the event. We also explore the role of cell-cell interactions at the cell junctions in determining the extent of the skew.
Different mechanical optimizations of the mitotic spindle sustain a conserved asymmetric cell division in 42 different nematode species

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The cell is a level of biological organization that has been poorly explored from an evolutionary perspective because basic cell functions (e.g. cell division) show remarkable conservation across phyla. Thus, an essential question remains: to what extent cellular mechanisms evolve without altering the basic function they sustain? We have developed the asymmetric cell division of nematode embryos as a study system. The first embryonic division of the nematode C. elegans gives rise to two daughter cells of asymmetric size and fate, due to the asymmetric positioning of the mitotic spindle. This initial event is crucial to embryogenesis and conserved in all nematode species of the Secernentea class. We quantified spindle positioning in the one-cell embryo of 42 closely related nematode species of this class, and compared it to the well-known pattern observed in C. elegans embryos. We first identified a conserved property of scaling of spindle size with embryo size across the phylogeny (Farhadifar & al. Curr. Biol. 2015). However, we found that significantly different combinations of spindle movements ultimately lead to an asymmetric displacement of the spindle and established that even between virtually identical phenotypes, mechanical optimization of the spindle differs. By analyzing the covariation of parameters associated with spindle movement over the course of evolution, we could next revealed important properties of spindle mechanics. Overall, we uncovered that an essential cellular function (asymmetric cell division) is maintained over the course of evolution while the underlying mechanisms that sustain it (asymmetric spindle positioning) change rapidly.
PAQR-2 is Specifically Required in Response to Membrane Stressors

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\textit{C. elegans} PAQR-2, a homolog of the mammalian adiponectin receptors \cite{1,2} has been previously described as an important regulator of fatty acid metabolism \cite{3}. The mutant exhibit membrane fluidity defects that result in three easily observable phenotypes, namely a cold adaptation defect, a withered tail tip and a defect in glucose tolerance. In order to better understand the specificity of PAQR-2, we have challenged wild type and \textit{paqr}-2 mutant worms by cultivation under various stress conditions: osmotic stress, oxidative stress, chemicals (DMSO, benzyl alcohol), drugs (Fluvastatin, FCCP), cold and glucose. We found that \textit{paqr}-2 mutant is specifically sensitive to those stressors that impair membrane homeostasis. Based on our preliminary observations, we tentatively conclude that the sensitivity of the \textit{paqr}-2 mutant to certain stressors is not an effect of a general sickness but rather associated with membrane homeostasis. 

unc-63 is a new gene involved in dopaminergic function

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Dopamine (DA) is a neurotransmitter that plays important roles in animals, regulating movement, behavior and cognition. Excess or deficiency of DA have been associated with a number of neurological diseases, including Parkinson’s disease and drug addiction. The molecular mechanisms responsible for these disorders have not been identified yet, nor the physiological regulation of DA release has been completely elucidated. We are interested in identifying new players which play a role in regulating DAergic system function. To this aim, we investigated the role of the unc-63 gene, encoding a cholinergic nicotinic receptor, in DAergic neurons. unc-63 is expressed in muscles and in unidentified neurons, and its depletion causes a strong uncoordinated phenotype and a partial resistance to the lethal effects of the nicotinic agonist 1,1-dimethyl-4-phenylpiperazinium (DMPP) (Ruaud, Dev. 2006). Here, we demonstrated that unc-63 is expressed in DAergic neurons. To elucidate the specific role of this gene within this context, we silenced unc-63 only in DAergic neurons. By exposing these animals to DMPP, we obtained partial resistance to the lethal effects of DMPP, which was similar to that observed in unc-63 loss of function mutants. This result was confirmed by specific expression of unc-63 in DAergic neurons, demonstrating that unc-63 is necessary and sufficient to mediate the toxic effect of DMPP in DAergic neurons. Then we demonstrated that expression of unc-63 in DAergic neurons is able to positively regulate dopamine release by means of biochemical, behavioral and morphometric approaches. Since we identified a de novo heterozygous missense change in the Alpha 6 CHolinergic Nicotinic Receptor (CHRNA6) in a patient with early-onset Parkinsonism, we used our model to establish whether this mutation impairs AChR function in vivo. Expression of mutant unc-63, the C.elegans homolog of the mammalian CHRNA6, demonstrated a loss-of-function role of the mutation in AChR expressed in muscles, while it dominantly impaired neuronal AChR, revealing a cell-context specific function of the mutant subunit. Our data strongly support for a new role played by unc-63 gene in the regulation of the DAergic system and, for the first time, we show the contribution of aberrant nAChR function in Parkinson’s disease.
A spatially resolved RNA map for the Caenorhabditis elegans germline

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The determination of the spatial pattern of gene expression is an important step in understanding gene function during development of an organism. A key question is how spatial distribution of RNAs shape cell proliferation and differentiation and their transition zone during development. We address this question using the C. elegans germline as model system as it emerged as a powerful in vivo model tissue to investigate the underlying gene expression mechanisms regulating cell proliferation and differentiation (Nousch et al. 2015). The basic molecular architecture of C. elegans germline is similar to that of higher organisms. However, despite many studies, the spatially resolved molecular makeup of the germline is, besides expression patterns of a few regulators, largely unknown. To investigate the spatial distribution of RNAs we applied a new, cryo-cut based method (Junker et al. Cell 2014) that allows spatially resolved transcriptomics at the scale of single cells. We extracted the germline of C. elegans and dissected it into at least 15 different sections. After RNA extraction from the individual sections, in vitro transcription for linear amplification of cDNA and high-throughput sequencing we are than able to identify candidate genes expressed in any specific pattern in an unbiased way. We will report on this ongoing work and how we use this approach to determine genes and molecular mechanisms important for proliferation and differentiation during germline development.
P033

GATA transcription factor as a likely key regulator of the Caenorhabditis elegans innate immune response against gut pathogens

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Comprehensive information has been collected over the last years on the molecular components of invertebrate immunity and the involved signaling processes, especially for the main invertebrate model species, Drosophila melanogaster and Caenorhabditis elegans. Yet, the exact regulation of general and specific defenses is still not well understood. In the current study, we take advantage of a recently established database, WormExp, which combines all available gene expression studies for C. elegans, in order to explore commonalities and differences in the regulation of nematode immune defense against a large variety of pathogens versus food microbes. We identified significant overlaps in the transcriptional response towards microbes, especially pathogenic bacteria. We also found that the GATA motif is overrepresented in many microbe-induced gene sets and in targets of other previously identified regulators of worm immunity. Moreover, the activated targets of one of the known C. elegans GATA transcription factors, ELT-2, are significantly enriched in the gene sets, which are differentially regulated by gut-infecting pathogens. These findings strongly suggest that GATA transcription factors and particularly ELT-2 play a central role in regulating the C. elegans immune response against gut pathogens. More specific responses to distinct pathogens may be mediated by additional transcription factors, either acting alone or jointly with GATA transcription factors. Taken together, our analysis of the worm’s transcriptional response to microbes provides a new perspective on the C. elegans immune system, which we propose to be coordinated in the gut by the GATA transcription factor ELT-2.
Deciphering the role of microRNAs in the DNA damage response of *Caenorhabditis elegans*

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The integrity of DNA is constantly challenged by a variety of endogenous and exogenous genotoxic attacks. In order to cope with DNA damage and thus ensure faithful transmittance of intact DNA from one generation to another, evolution provided cells with DNA damage response (DDR) and repair mechanisms capable of sensing and removing genomic lesions. A comprehensive understanding of these DDR pathways is crucial for the design of therapeutic approaches targeting the deleterious consequences of impaired DDRs. MicroRNAs (miRNAs) are small, non-translated RNA species of 20-25 nt length that associate with specific protein complexes in order to block translation of endogenously expressed mRNAs, thus regulating various biological processes in the cell. In mammals, miRNAs have been demonstrated to directly affect the expression of various DDR genes. In this study, we exploit the nematode model organism *Caenorhabditis elegans* to decipher the role of miRNAs and other non-coding RNA molecules in the DDR upon UV-irradiation. The work involves deep sequencing of small RNAs and full transcriptome analyses in response to UV-induced DNA damage. Accordingly, we are testing deletion mutants of selected miRNA candidates as well as their most interesting target genes for DNA-repair deficiencies. Using this approach, we hope to identify novel factors and/or signalling pathways important in the regulation of successful DNA damage recognition and repair.
C. elegans flavin-containing monooxygenase-4 is essential for osmoregulation in hypotonic stress

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Soil-dwelling nematodes such as C. elegans will be exposed to a range of osmotic environments from hypertonic conditions, for example during dry weather, to sudden hypotonic exposure after rainfall. A permeable cuticle and pseudocoelomic cavity under hydrostatic pressure complicates the osmoregulatory challenge. To ensure survival nematodes combine osmotic avoidance behaviour with robust osmoregulatory machinery to tightly regulate water and ionic balance. Studies in C. elegans have revealed some of the systems engaged during hypertonic conditions but less is known about specific osmoregulatory measures employed when faced with sudden hypotonic exposure. Flavin-containing monooxygenases (FMOs) comprise a large, evolutionarily conserved family of NADPH-, FAD- and O2-dependent enzymes active at heteroatom centres, particularly S and N, in a range of structurally diverse compounds. The C. elegans genome contains five distinct fmo genes – fmo-1 to -5. Strains homozygous for inactivating deletions in fmo-4 exhibit dramatic hypoosmotic shock hypersensitivity – worms are unable to prevent overwhelming water influx and swell rapidly becoming immobile, stiff and rod-like before exploding due to high internal hydrostatic pressure. The phenotype was absent in strains lacking fmos-1, -2, -3 or -5, was phenocopied via RNAi targeting fmo-4 but not other fmos and was rescued completely by extrachromosomal fmo-4 thus confirming hypoosmotic sensitivity was due solely to loss of fmo-4. As well as the hypodermis fmo-4 is expressed prominently in duct and pore cells but is excluded from the excretory cell. These results indicate FMO-4 plays a crucial osmoregulatory role by promoting rapid clearance of excess body water that enters during hypotonicity, perhaps by establishing an osmotic gradient from excretory cell to duct and pore cells. FMO-4 has a C-terminal extension containing sequence and predicted structural characteristics conserved in all other nematode FMO-4s. Interestingly, mammalian FMO4 (the numbering is coincidental) also has an extended C-terminus with features intriguingly similar to those in the FMO-4 extension. We suggest the presence in both FMO-4 and FMO4 of C-terminal extensions containing very similar sequence and structural features represents evidence that the corresponding genes evolved from a common ancient ancestor. Although human FMO4 was unable to rescue the hypoosmotic shock sensitivity, suggesting divergence of catalytic activity, human FMO4 mRNA is abundant in the kidney – an appropriate site of expression if mammalian FMO4 has, or once had, a role in osmotic homeostasis. Thus FMO-4, by attenuating water ingress and/or promoting efficient water efflux, plays a key osmoregulatory role in C. elegans during acute hypotonic exposure. The underlying FMO-4 substrate-product activity presumed to underlie this function has yet to be identified.
Tissue-specific activity of pathway components regulating *C. elegans* embryonic epidermal contraction

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Morphogenetic regulation of *C. elegans* embryonic elongation utilises non-uniform epidermal cell-shape changes. Smooth muscle-like contractions govern this process such that an ovoid embryo elongates to a tubular shape upon hatching. Contraction occurs predominantly in the lateral epidermal cells, which maintain a mesh-like network of actin in the early stages of elongation. Dorsoventral epidermal cells, by contrast, have their actin organised into parallel bundles, and have limited contractile activity. Elongation is regulated by parallel pathways, with LET-502/Rho-binding kinase and MEL-11/myosin phosphatase in one branch, and FEM-2/PP2c phosphatase and PAK-1/p21-activated kinase in the other. Though it is known that LET-502 and MEL-11 are expressed ubiquitously throughout the epidermis of the embryo, it is yet to be determined in which cells the genes are necessary or sufficient. LET-502 activity is regulated by RHGF-2/Rho GEF (Guanine exchange factor) and RGA-2/Rho GAP (GTPase activating protein). Both RHGF-2 and RGA-2 are ubiquitously expressed throughout the embryonic epidermis, but RHGF-2 is sufficient in the lateral (contractile) cells, while RGA-2 is sufficient in the dorsoventral (passive) cells. Evidence from our lab also suggests that FEM-2 is sufficient in dorsoventral cells. I am interested in determining whether LET-502, MEL-11, and PAK-1 are sufficient and/or necessary in lateral, dorsoventral, or both cell types. I will be expressing these genes using cell-specific promoters and eliminating them with cell-specific RNAi. Answering these questions will help broaden our understanding of the roles of actinomyosin dynamics in developmental and morphological events.
P037

**Spatially resolved transcriptomics: generating a detailed gene expression map of C. elegans**

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Even though the genome-sequence of C. elegans has been determined over a decade ago, we still have only limited information on when and where the approximately 20,000 genes that are encoded in the genome are expressed. Detailed insight into the temporal and spatial expression patterns of all genes would be a major step towards understanding developmental mechanisms and physiological processes such as tissue-homeostasis and aging. Two well-established methods for quantitative analysis of gene expression are RNA sequencing (RNA-seq) and single molecule mRNA fluorescent in situ hybridization (smFISH). RNA-seq is ideal for transcriptome-wide analysis of gene expression, but does not provide information on where genes are expressed. Such spatial information can be obtained with smFISH, but this method is restricted to the analysis of only a few genes at a time. To determine gene expression patterns at a whole-genome level, RNA-seq therefore needs to be combined with a method that provides positional information. Such a method was recently developed for zebrafish (Junker et al., Cell 2013). In this approach, which is referred to as RNA tomography (abbreviated as tomo-seq), the embryo is cryo-sectioned along the three major body axes and a detailed three-dimensional atlas of gene expression is generated by performing RNA-seq on each of these sections. We have adapted the tomo-seq method for C. elegans. By cryo-sectioning a young adult animal from the tip of the nose to the tail (which typically takes about 40 slices of 20 micrometers thickness) and subjecting each of these slices to RNA-seq, we have generated a detailed map of gene expression along the anteroposterior body axis. Clustering analysis of the expression data reveals gene expression profiles that are unique to major anatomical structures such as the pharynx, nerve ring, gonads, spermatheca, vulva and the tail. Furthermore, the method is sensitive enough to detect genes that are known to be expressed in specific cells such as, for example, the amphid neurons, the excretory cell and the anchor cell. In addition to providing a gene expression resource to the C. elegans community, we are using the tomo-seq method to explore long-range transcriptional effects of morphogens and to address questions on the evolutionary conservation of gene expression patterns.
A PR-SET lysine methyltransferase orchestrates spermatocyte gene expression to promote fertility

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Optimal sperm production is important for reproductive fitness. During sperm production the genome has to make the proteins for sperm formation and be prepared for packaging into the sperm. However, little knowledge exists of the transcriptional processes involved in sperm production. Lysine methyltransferases (KMTs) are an important class of chromatin regulators that catalyze the methylation of lysines in histone tails to affect transcriptional states. We are investigating the fertility of mutants defective in germline-expressed C. elegans KMTs. We found that set-17, a PRDF1-RIZ (PR) SET-domain containing KMT, promotes fertility by driving the expression of sperm-specific genes in spermatocytes. Strikingly, SET-17 localizes to distinct nuclear territories in spermatocytes. set-17-regulated genes occur in clusters in the genome. While the set-17 PR-SET-domain is orthologous to the PR-SET domains of mammalian PRDM7 and PRDM9, set-17 lacks their Zn-finger DNA-binding domains. We therefore hypothesized that a DNA-binding co-factor recruits set-17 to target genes. Interestingly, a previously defined motif for the GATA Zn-finger transcription factor elt-1 is enriched upstream of set-17-regulated genes. Indeed, we find that elt-1 functions in the same pathway as set-17 to promote fertility and sperm-specific gene expression. We propose that elt-1 GATA recruits set-17 PR-SET to DNA in spermatocytes. The interaction of a PR-SET KMT and a Zn-finger transcription factor provides a basis for the hypothesized emergence of PRDM KMTs through gene fusion. In short, we have established that PR-SET KMTs can drive gene expression and that they are regulators of transcription in spermatocytes.
A streamlined protocol to facilitate CRISPR/Cas9 genome engineering using a highly efficient sgRNA and an easily selectable phenotype

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Current CRISPR/Cas9 genome engineering strategies enable the directed modification of the entire C. elegans genome to introduce point mutations, generate knock-out mutants and insert epitope or fluorescent tags. Three practical aspects can complicate CRISPR/Cas9 experiments. First, the efficiency of any given sgRNA (single-guide RNA) cannot be reliably predicted. Second, the detection of animals in which the genome has been modified, can be very challenging or time consuming in the absence of a clearly visible or selectable phenotype. Third, the sgRNA target site must be “inactivated” following CRISPR/Cas9-directed editing to avoid further double-strand break events. This almost always requires the introduction of silent mutations (e.g. in the Protospacer Adjacent Motif, PAM). We describe here a strategy that attempts to address and circumvent these complications. First, we used CRISPR/Cas9 editing to transplant the protospacer and PAM sequence recognized by a highly efficient C. elegans sgRNA into five different loci. We chose an sgRNA targeting the dpy-10 gene (Arribere et al., 2014) because (i) it generates genome edits at comparatively high frequency and because (ii) F1 progeny carrying genome edits can be easily identified based on dominant phenotypes (Rol or DpyRol) caused by the dpy-10(cn64) mutation. Indeed, we found that the transplanted protospacer (aka. d10 site) was cleaved in 30 to 50% of Dpy-10 F1 progeny, rendering targeted loci highly susceptible to homology directed genome engineering. Next, to engineer each d10 locus, we coinjected (i) a Cas9 expression vector, (ii) the specific repair template containing the desired modifications (flanked by long homology arm of 1.5 to 2 kb), and (iii) a single sgRNA targeting the dpy-10 gene and the transplanted d10 site. We reasoned that each P0 producing Dpy-10 F1 progeny should also generate animals with the desired modification by co-conversion of both loci. By focusing exclusively on Dpy-10 F1 worms we could drastically reduce the number of animals to be tested by PCR, visual inspection or phenotypic analysis. Indeed, we were able to reliably engineer the five different target loci and rapidly introduce multiple fluorescent genes or epitope tags in as little as 6 days (38 independent lines in total). Gene edits were identified in 2 to 18 % of screened F1 worms, and in 50 to 83 % of injected P0 worms yielding more than five Dpy-10 F1s. In addition, since worms were cloned at the F1 generation, we were able to isolate multiple independent lines from a single injected P0. This strategy is also suitable to generate large and precise deletion starting from the same d10-engineered strain. Finally, by using a transplanted sgRNA site, our strategy allows for perfectly tailored and scarless genome modifications that do not introduce mutations in endogenous PAM sites or otherwise disrupt the genome sequence around the targeted region.
P040

The jack of all trades, GSK-3, meets the mitochondrial prohibitins

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Impaired mitochondrial function and the insulin/IGF-like signalling (IIS) pathway are known to affect lifespan across phyla. Prohibitins (PHBs) are conserved mitochondrial proteins comprising two subunits, PHB-1 and PHB-2, forming a large ring-like structure in the inner mitochondrial membrane. PHBs show a striking interaction with IIS in aging regulation. PHB depletion shortens the lifespan of wild type worms, whilst it dramatically extends the lifespan of the IIS receptor daf-2 mutants. Additionally, PHB depletion reduces vital Nile Red staining in wild type and daf-2 mutants in an age-specific manner¹. To better comprehend the function of PHBs in aging regulation, we exploited the Nile Red phenotype by performing a kinase RNAi screen. We looked for genetic pathways mediating the metabolic responses in phb-2 and phb-2; daf-2 mutants. We identified Glycogen Synthase Kinase – 3 (GSK-3), as a strong candidate suppressing the reduced Nile Red staining phenotype in both the aforementioned genetic backgrounds. We investigated the role of GSK-3 in lifespan regulation and its interaction with mitochondrial PHBs and IIS. Depletion of GSK-3 shortens the lifespan of wild type worms, but does not affect the lifespan of phb-2 mutants. Ultimately, lack of PHB is beneficial for the longevity of GSK-3 deficient worms. Interestingly, the long-lived status of the daf-2 mutant is strongly suppressed upon gsk-3 RNAi, whereas PHB deficiency significantly increases the lifespan of daf-2 mutants on gsk-3 RNAi. GSK-3 is a known key regulator of insulin-dependent glycogen synthesis in mammals². For a better understanding of the metabolic alterations caused by GSK-3 depletion in the different genetic backgrounds, we are examining glycogen and triglyceride content. Further experiments will help delineate the role of GSK-3 and its interaction with IIS and mitochondrial function in the regulation of aging and metabolism in C. elegans.

Identification of novel regulators of the two-pore domain potassium channel EGL-23 in *Caenorhabditis elegans*

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Two-pore domain potassium channels (K2P) compose a family of conserved potassium-selective ion channels responsible for the establishment and maintenance of the electrical membrane potential of metazoan cells. Despite their fundamental role, little is known about the cellular processes that control K2P channel function *in vivo*. In particular, we know only of few factors that directly control the number, the activity and the localization of K2Ps at the cell surface. Thus, we have performed two genetic screens targeting the EGL-23 channel in order to identify new regulators of K2P channels in *C. elegans*. We first used CRISPR/Cas9 genome engineering to generate an *egl-23::SL2::RFP* knockin strain. EGL-23 is expressed in VM2 vulval muscle, somatointestinal, and anal depressor smooth muscles. EGL-23 is also expressed in VC4-VC5 motorneurons and in unidentified head and tail neurons. Finally, EGL-23 expression is observed in the HMC (head mesodermal cell). *n601* gain-of-functions mutants of *egl-23* are egg-laying and defecation defective (Trent *et al.*, 1983). These phenotypes are consistent with expression of a hyperpolarizing EGL-23 channel in VM2 muscles and VC4-VC5 neurons for the egg-laying defect and in somatointestinal, and anal depressor smooth muscles for the defecation phenotype. We screened 20 000 haploid genomes mutagenized with EMS and isolated 28 intragenic and 4 extragenic suppressor mutants which were able to lay eggs again. This suppressor screen based on the *n601* phenotype has shown that egg-laying can be restored mainly by mutation of *egl-23*. To try to circumvent this bias, we performed a screen based on direct visualization of the channel in vivo by using a translational fusion strain (*egl-23::TagRFP-T*). Using EMS mutagenesis, we have isolated only 1 mutation in *egl-23* and 3 extragenic mutations among 1060 mutagenized haploid genomes screened. The 4 mutants suppressing the egg-laying phenotype define two complementation groups. The first is composed by mutants, which show a loss of EGL-23-TagRFP-T expression in VM2 muscles but not in VC4 and VC5 neurons. The mutant of the second group shows a general reduction in fluorescence. Using whole genome sequencing we have identified a list of candidate genes, which we are currently validating. Given the significant gene conservation between *C. elegans* and vertebrates, our studies may provide new leads to understand the cellular pathways that control K2P function in other organisms.
Role of VHL-1 in extracellular matrix formation in *C. elegans*

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Germline mutations of the von-Hippel-Lindau (VHL) gene lead to an autosomal dominant multi-tumour syndrome, which is characterized by the formation of hemangioblastomas, pheochromocytomas and renal cell carcinoma (RCC). Even more importantly, mutations in this gene are found in more than 80% of all sporadic clear-cell RCCs — the most common malignant renal tumour — making it the most prominent renal tumour suppressor gene. Tumour formation upon loss of the von-Hippel-Lindau protein (pVHL) is partly explained by highly increased hypoxia-inducible factor (HIF) signalling, which is normally repressed by the role of pVHL as part of an E3-ubiquitin ligase. However, pVHL fulfils other functions such as promotion of ciliogenesis, which are less well understood. Interestingly, pVHL has also been linked to the regulation of extracellular matrix (ECM) formation, a process that has a strong impact on the tumour microenvironment influencing tumour growth, vascularization and metastasis. How pVHL exerts this function on a molecular level is incompletely understood. We thus set out to examine this role of pVHL using *Caenorhabditis elegans* (*C. elegans*) as a model. We found that *vhl-1(ok161)* mutants show a *Dpy* (dumpy) phenotype which is a classical readout of defects in ECM formation. This phenotype depends at least partly on the degradation of HIF-1. Introduction of a VHL-1::GFP fusion protein was able to rescue the Dpy phenotype in *vhl-1(ok161)* mutant L1 larvae. Using RNA sequencing we identified a row of genes linked to ECM formation that are dysregulated in *vhl-1(ok161)* mutant worms. Interestingly, collagen genes are also upregulated in other long-lived mutant strains. Further we found that *vhl-1(ok161)* mutant worms have a more permeable ECM. We will now investigate the genes that mediate both the *Dpy* phenotype as well as ECM permeability using an RNAi screen. These findings can now be the basis to a more detailed molecular characterization that is crucial to the development of potential therapeutic strategies targeting ECM changes in pVHL-associated disease. We show for the first time that *C. elegans* is a valuable model for studying the role of pVHL in ECM formation. Using this model will greatly facilitate the search for the molecular mechanism behind this phenomenon.
C. elegans metallothioneins: new insights into isoform specific differences

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Metals are either essential or non-essential elements. Although many proteins require a metal as a cofactor, high concentrations of (even essential) metals can cause damage to an organism. Metallothioneins (MTs) are thought to play an important role in protecting the organism against metal toxicity. Metallothioneins are metal binding proteins of low molecular weight, they lack (or are low in) aromatic amino acids and contain a high proportion of cysteines. Each cysteine residue contains a thiol group which facilitate the binding of divalent heavy metals. The C. elegans genome contains two isoforms encoding for MT-1 and MT-2. Intriguingly, MT-1 has 15 additional amino acids (three of which are metal-binding residues) in the C-terminal region. Both isoforms are expressed in the gut in worms exposed to heavy metals. However MT-1 is also constitutively expressed in the lower bulb of the pharynx. The objective of this study was to assess how an exposure to cadmium and/or zinc modulates the expression of metallothioneins in C. elegans. Nematodes were exposed (acutely or chronically) to single metals (Zn and Cd) or combined doses of both metals. To analyse the response, different assays were applied. The toxicity of the metals towards the organism was evaluated via life span, growth and brood size assays. The transcriptional response was assessed by means of qPCR. X-ray fluorescence imaging (SLAC-Institute Stanford Linear Accelerator Centre/ National Accelerator Laboratory, Menlo Park, USA) allowed the visualisation of heavy metal accumulation in the nematodes body at 5 micron resolution. All assays were performed with wild type (N2) and metallothionein deletion mutants (mtl-1(tm1770), mtl-2(gk125) and the mtl-1(tm1770);mtl-2(gk125) double mutant). Taken together, the results provided new insights into metallothionein isoform specific differences in metal handling.
P044

Reduced insulin/IGF-1-signalling implicates extracellular matrix remodelling in longevity

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Interventions that delay ageing mobilize mechanisms that protect and repair cellular components, but it is unknown how these interventions might slow the functional decline of extracellular matrices, which are also damaged during ageing. Reduced insulin/IGF-1 signalling (rIIS) extends lifespan across the evolutionary spectrum. Here we show that rIIS can promote C. elegans longevity through a program that requires the Nrf (NF-E2-related factor) orthologue SKN-1 acting in parallel to DAF-16. SKN-1 is inhibited by IIS and has been broadly implicated in longevity. When IIS is decreased, SKN-1 most prominently increases expression of collagens and other extracellular matrix genes. Diverse genetic, nutritional, and pharmacological pro-longevity interventions delay an age-related decline in collagen expression. These collagens mediate adulthood extracellular matrix remodelling, and are needed for ageing to be delayed by interventions. The importance of collagen production in diverse anti-ageing interventions implies that extracellular matrix remodelling is a generally essential signature of longevity assurance, and that agents promoting extracellular matrix youthfulness may have systemic benefit.
After extrusion, the second polar body is internalized via receptor-mediated phagocytosis in C. elegans embryos

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Polar bodies are commonly used for preimplantation genetic diagnosis in human embryos. During egg development, polar bodies are extruded to expel the extra DNA made during Meiosis and to form a haploid oocyte. In contrast to the well-studied mechanisms of polar body extrusion, their fate after extrusion is unknown in any system. We studied the fate of polar bodies using time-lapse imaging of fluorescent reporter strains and a panel of C. elegans mutants. We found that the first polar body becomes trapped between eggshell layers and persists until hatching. In contrast, the second polar body is internalized in a stereotyped manner by one of the anterior AB daughter cells around the 4-cell stage. Prior to internalization, actin and the phagocytic receptor CED-1 are enriched around the second polar body, consistent with formation of a phagocytic cup. CED-1 persists around the phagosome after internalization and is required for polar body internalization. Other CED-10-dependent engulfment pathway proteins, such as CED-2, are also required, indicating that the polar body is internalized via receptor-mediated phagocytosis. As part of a candidate screen, we also discovered that the second polar body was rarely internalized in PI3K-deficient vps-34 mutants or in rab-5 RNAi-treated embryos. The phagocytic receptor CED-1 is known to be localized to the plasma membrane via retromer-mediated recycling, which has been shown to require PI3K function. Furthermore, PI3K localization is known to depend on the small GTPase Rab5. Therefore, we examined CED-1 localization and discovered that CED-1 is no longer found around the second polar body in vps-34 mutants and in rab-5-depleted embryos, explaining the observed defects in polar body internalization. Thus, embryos internalize the second polar body via receptor-mediated phagocytosis, showing that the polar body signals to neighboring cells. This observation raises the possibility that polar bodies have a function after extrusion, which could indicate that polar body removal for genetic testing may not be non-invasive.
The NAD⁺-SIRT1-PGC1α nuclear to mitochondrial signaling axis as a pathogenic denominator in age-related diseases

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Aging is the most important risk factor for neurodegenerative diseases and progressive mitochondrial dysfunction with consequent gradual accumulation of damage to cellular macromolecules, including DNA, is a central premise in theories of aging and neurodegeneration. Mitochondrial dysfunction is prevalent in neurodegenerative diseases, and is undoubtedly the best candidate for age-related risk mediation. We have identified a molecular mechanism by which imbalanced DNA repair and DNA damage response signaling as result of deficiency in the DNA double strand break repair protein ATM-1, leads to mitochondrial dysfunction via suppression of the NAD⁺-SIRT1-PGCα axis. Importantly, we have demonstrated that restoration of the NAD⁺/SIR-2.1 activity through NAD⁺ supplementation or SIR-2.1 activation rescues mitochondrial morphology and aging phenotypes of atm-1.
**Poster abstracts in alphabetical order (first author)**

**P047**

**Crosstalk between protein disaggregation and degradation machinery upon aging and disease**

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The maintenance of the proteome is critical for the cellular and organismal physiology. Upon aging, a decline in protein quality control is observed and the accumulation of protein aggregates can lead to several neurodegenerative disorders. The cell employs a proteostasis network that is composed of molecular chaperones and proteases to respond to any proteotoxic challenges and prevent, or reverse protein misfolding and aggregation. We study the crosstalk between protein aggregate clearance strategies. Protein aggregates can be eliminated by either proteolytic means such as autophagy or the ubiquitin-proteasome system (UPS) or disaggregation and subsequent refolding by the disaggregate complex HSP110-70-40. Our research aims to understand the regulation and communication between the different clearance options and its changes with the progression of aging and upon proteotoxic challenges such as neurodegeneration. To unravel this crosstalk, we investigated how autophagy responds to perturbations in the chaperone disaggregase complex and vice versa. We found that autophagy flux is altered by depletion of dnj-12 and dnj-13, J-proteins from the disaggregase complex. The loss of disaggregation capacity leads to accumulation of autophagosomal structures and an increase of LGG-1, an essential autophagy protein. Protein degradation rates are known to slow down in the progression of aging and disease. We discovered that autophagy responds when the disaggregation complex is compromised in a Huntington’s disease model where a polyglutamine (Q40) stretch is expressed in the body wall muscle of C. elegans. The mRNA levels of crucial autophagy genes like unc-51, lgg-1 or sqst-1 are increased mainly upon knockdown of dnj-12 and dnj-13 genes. Inhibition of autophagy with wortmannin and bafilomycinA1 in the polyQ model led to down-regulation of chaperone genes from the hsp-70 family, such as hsp-1, c12c8.1 or f44Ee5.4. Although the underlying mechanism is unknown, we detected that upon proteotoxic stress the impairment of autophagy decreases the mRNA levels of specific chaperones. The ex vivo and in vivo UPS activity will also be addressed upon modulation of autophagy and protein disaggregation. It is expected that UPS activity changes in response to proteostasis imbalances and proteotoxic stress. The obtained data indicate an interaction between disaggregation and degradation processes, which was not yet described and opens a different perspective of the regulatory nodes of the proteostasis network.
We use the C-lineage as a model to address the cellular and molecular mechanisms that generate left-right (L/R) asymmetric neurogenesis. This lineage is largely L/R bilaterally symmetric, with Ca and Cp giving rise to bilaterally symmetric pairs of hypodermal and body wall muscle cells (Sulston et al. 1983). However, on the left side of the lineage only, two unilateral glutamatergic neurons known as DVC and PVR are produced. We have previously shown that the proneural gene *hlh-14/ac-sc* is expressed asymmetrically in PVR and the mother of DVC (Caapa) and is required for this L/R asymmetric neurogenesis event (Poole et al. 2011). To identify the mechanisms that regulate asymmetric *hlh-14* expression we have performed a series of laser ablation experiments in combination with two different screens. We find that ablation of all early blastomeres with the exception of C does not affect the production DVC, suggesting that asymmetric neurogenesis in the C-lineage does not require a late asymmetry signal from outside the C-lineage. Through a GFP-based forward genetic screen for the loss of both DVC and PVR and a 4D-lineage based screen of a collection temperature sensitive embryonic lethal mutants isolated and maintained by our collaborator Ralf Schnabel (TU Braunschweig), we have generated a collection of asymmetric neurogenesis defective (and) mutants. We find through mapping, complementation testing and rescue that two of the mutants are alleles of *let-19/mdt-13*, part of the Mediator complex, a conserved transcriptional regulator of asymmetric cell fate decisions (Yoda et al. 2005). We have also identified a possible *hlh-2/daughterless* allele, a bHLH transcription factor that heterodimerises with many other bHLH transcription factors, including proneural genes such as *hlh-14*, to activate transcription (Grove et al. 2009). We find that in *let-19* mutants the asymmetric expression of *hlh-14* is lost, the mother of DVC divides early, and DVC and PVR acquire a hypodermal fate. In *hlh-2* mutants, a similar set of phenotypes is observed. Temporally, we find that *let-19*, likely acts from the birth of Caa to the birth of Caapa. Intriguingly, it is at the Caa-stage that we first observe *hlh-2* expression and it is maintained in the Caapa lineage until the birth of DVC. Together our data suggest that *let-19/mdt-13* and *hlh-2/daughterless* regulate asymmetric neurogenesis in the C-lineage through the asymmetric regulation of *hlh-14* expression. It is tempting to speculate that they may collaborate in this role, as interpreters of an as yet unidentified asymmetrically segregated cell fate determinant.
Aging is a progressive decline of physiological functions and stress resistance, associated with increased incidence of various diseases. Interventions that enhance antioxidant defenses or repair cellular components are often used to delay the development of diseases and promote healthy aging. Among these strategies, herbal polysaccharides have shown protective effects against diverse age-related stresses and modulate physiological homeostasis. In the present study, we isolated polysaccharides from Panax notoginseng, a medicinal Chinese herbal, by hot water-extraction and alcohol-precipitation method. Polysaccharides were further purified using ion exchange chromatography and gel exclusion chromatography. Heat shock assay and lifespan assay were used to screen bioactive polysaccharide factions. We showed that in C. elegans, polysaccharides demonstrated significantly enhanced survival rate at 35 °C and lifespan extension was increased by 21%. Polysaccharide treatment also increased the activity of enzymatic antioxidant systems such as CAT and SOD. In order to investigate underlay molecular mechanism of protective effective of purified polysaccharide faction, we carried out RNA-Seq of C. elegans treated with polysaccharide faction for 48 h. Preliminary data analysis suggests that polysaccharide faction significantly affect C. elegans lipid metabolic processes, and lipid modifications such as glycosylation. Using Nile red assay as a first indication for animal fat content we observed that polysaccharide faction significantly decreased lipid content of C. elegans. According to above results, we preliminary hypothesize that polysaccharide faction may prolong C. elegans lifespan by affecting lipid metabolism. Additional work is currently underway to pin down molecular mechanisms of P. notoginseng polysaccharide fractions extension of lifespan.
Has C. elegans revealed a potential neural focus for a role of the ryanodine receptor in human disorders and aging?

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Single point mutations in the gene encoding the ryanodine receptor RyR1 are implicated in human muscle disorders, where myocyte calcium regulation is disrupted, including malignant hyperthermia (MH), exertional heat illness (EHI), central core disease (CCD) and late onset axial myopathy (LOAM). Some of these mutations have also been associated with premature ageing-related phenotypes in mouse models. Caenorhabditis elegans strains were generated by introducing single amino acid changes into the C. elegans version of the ryanodine receptor, UNC-68, precisely equivalent to those found in these human myopathic conditions. These C. elegans model strains were challenged with the pharmacological agents used in the in vitro contracture test (IVCT), the primary method of diagnosing susceptibility to MH. MH is triggered by inhalation anaesthetics, such as halothane, and the IVCT measures the contractile response of patients’ muscle biopsies to both halothane and caffeine. The C. elegans model strains showed alterations in locomotory response when challenged with increasing concentrations of caffeine and halothane. Notably, it was only the LOAM associated variant strains for which increased locomotion induced by progressively higher caffeine concentrations was seen and enhanced with age. On the other hand, all the model strains displayed shortened lifespan, with a median life-span of 14 to 16 days into adulthood, compared to the 23 to 25 days for the wild type control strains. These findings support the hypothesis that failure to maintain calcium ion balance may contribute to aging. Although, an MH episode appears to involve the opening of sensitised skeletal muscle RyR1 channels by inhalational anaesthetics, the mechanism of caffeine action in the IVCT is not known. C. elegans caffeine resistant strains had been isolated previously with mutations in osm-3 and che-3, genes required specifically for chemosensory nerve cell function. Therefore, the role of these genes in the differential response to caffeine attributed to the unc-68 variants was tested by RNAi knockdown. Both the stimulation of locomotion at low concentrations of caffeine and the inhibition at high concentrations was either lost or reduced for all variant strains. Furthermore, the progressive stimulation of locomotion in response to increasing concentrations of caffeine in old adults of the LOAM variant strain was also eliminated with knockdown of these genes. The focus of influence of the UNC-68 amino acid changes to the caffeine response may actually be in these chemosensory nerve cells. Alternatively, the chemosensory nerve cells may only be the primary site of caffeine action, with changes in downstream consequences dependent on the modified UNC-68 in other cells, such as muscle cells. While RyR1 is considered predominantly a skeletal muscle isoform and other human isoforms are expressed in other cell types, UNC-68 is the only C. elegans ryanodine isoform and likely to be the key intracellular Ca2+ channel in all cells, but particularly excitable cells. The observed involvement of nerve cells in the C. elegans response to caffeine may relate to rare medical symptoms concerning the central nervous system that have been associated with ryanodine receptor variants.
Poster abstracts in alphabetical order (first author)

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A role for the adhesion-GPCR LAT-1 in C. elegans fertility

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Reproduction is one of the most important mechanisms of organisms to preserve their species. Therefore, development of intact germ cells is indispensable. The signalling pathways underlying these processes are highly complex and tightly controlled. However, these mechanisms are still insufficiently understood. Recently, we revealed that the Latrophilin homolog lat-1 has so far unknown functions in fertility. LAT-1 is one of the most conserved family members of adhesion G protein-coupled receptors and was so far only implicated in neuronal and developmental processes. A C. elegans strain lacking lat-1 displays a reduced number of laid eggs and an increase in unfertilised oocytes. First functional analyses using imaging as well as biochemical techniques showed that lat-1 null mutants have less sperm than wild-type individuals. These results are consistent with the observation that the number of laid eggs is decreased in lat-1-deficient nematodes. Mating analyses of wild-type and lat-1 mutant individuals suggest that LAT-1 function is not only essential for development of sperm number but potentially also involved in processes controlling oocyte numbers or fertilisation. Elevated oocyte numbers were only observed in a homozygous mating between a lat-1 male and lat-1 female but not when either of them was wild-type. Interestingly, we found no LAT-1 in germ cells such as mature sperm or oocytes using a transcriptional fluorescent lat-1 reporter. However, strong lat-1 expression was detected in the somatic gonad such as the gonadal sheath cells and the spermatheca. Taken together, we describe a novel player in fertility of C. elegans essential for correct number of sperm as well as involved in processes regulating oocyte numbers or fertilisation. Due to the expression of lat-1 in the surrounding somatic tissue, we suggest an indirect function of LAT-1 on germ cells.
Asymmetric cell division is a key mechanism to generate different cell types during embryonic development, tissue morphogenesis, and tissue homeostasis. In intrinsic asymmetric cell division, the asymmetry arises during mitosis of the mother cell. The resulting daughter cells can be different in both size and content. To achieve this, the mother cell distributes cellular content along the defined polarity axis and positions the mitotic spindle such that cell division results in two cells with the desired size and content. The *C. elegans* zygote is a classical model of an intrinsically asymmetric dividing cell. Its asymmetry is regulated by Anterior-Posterior PAR-polarity. The mitotic spindle is positioned by cortical pulling forces that act on the astral microtubules. These microtubules are anchored to the cortex via the motor protein dynein, which associates with the conserved trimeric force generator complex (Gα,GPR-1/2,LIN-5). Microtubule dynamics, dynein, and the force generator components are all essential to generate cortical pulling forces. Depletion of any of these factors abolishes force generation and therefore spindle movements. Our aim is to understand how cortical pulling forces on astral microtubules are regulated in order to position the mitotic spindle correctly. Biochemical and in vitro work have shown that Gα-GDP is the functional state to constitute the force generator, in contrast to Gα-GTP in canonical G-protein signaling. The nucleotide state of Gα is regulated by RIC-8 (GEF) and RGS-7 (GAP), which are paradoxically both essential and required to generate cortical pulling forces. We therefore hypothesize that the force generator needs to be dynamic to generate force and that this is accomplished through a dynamic nucleotide state turnover of Gα. To test this hypothesis, we block Gα in either the GDP- or GTP-bound form and perform UV-laser spindle severing experiments to quantify cortical forces. We also assess the dynamics of the force generator with TIRF/FRAP using endogenous tags of force generator components. To study the process of this non-canonical G-protein signaling during mitosis in more detail, we want to use light-controlled heterodimerization to control the localization of force generator components and regulators. This combined approach with a variety of novel techniques may provide more detailed insights into the highly conserved mechanism of mitotic spindle positioning.
Cell focussing, the dance of cells and glycans in C. elegans embryogenesis

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Using 4D-microscopic and bioinformatics analyses Schnabel et al. (2006) proposed that pattern formation in the C. elegans embryo is not directed by mitoses, but a result of a sorting process involving far ranging cell migrations. The process, termed "cell focussing", is governed by local cell-cell interactions, where "cell addresses", which are part of their identity, are compared. When cell identities are altered extensively for example in a glp-1 mutant embryo, cells sort extensively in a spectacular new pattern corresponding to the new identities. A mutant in pmm-1 (t3091), strongly reducing N-Glycans, isolated in a screen for ts mutants affecting cell focussing causing cells to be significantly displaced compared to wild-type. This could be taken as a hint that glycans may be involved in the generation of the postulated addresses. The aim of this study is to elucidate whether glycans, which are widely spread among the cell surface indeed encode the addresses. Embryos in which N-glycosylation is disrupted strongly, either using a mutant or after tag-335 RNAi treatment show Delta/Notch and Wnt specific phenotypes. This could be observed similarly in a knockout mutant of the single t-synthase homolog, which prevents the formation of core 1 O-glycans and thus most of O-glycosylation. This indicates that both N- and O-glycosylation are necessary for the proper function of these signalling pathways. In lesser affected tag-335 RNAi embryos, which execute overall normal cell fates, cells occupy aberrant cell positions at the premorphogenetic stage and fail to initiate morphogenesis. An analysis of cell behavior in three tag-335 RNAi embryos reveals normal mitosis patterns but an alteration of cell migration. The general movement remains unchanged but cells migrate effectively less far than normal cells, which appeared quite enigmatic. To find the cause for this hindrance, we analysed the movement of cells by tracing the cell positions every 35 seconds. It appeared that in normal embryos cells are dancing back and forth all the time and the effective migration appears to be caused by a directional bias of the dancing movement. We propose that this dancing is the basis of migration in the C. elegans embryo. After interference with glycan synthesis, cells migrate effectively only 70% of the distance of wild-type cells, since cells revert their dancing directions 1.6 times more often than normal cells. Do cells revert their dancing directions because they are generally hampered to move effectively, or because they have no/less information, where to aim because they lack addresses? The question could be resolved by challenging the cell-focussing process by interfering with the glycans in a glp-1 mutant. The resulting distance map looks like the glp-1 map, only slightly blurred. We have to conclude that it is rather improbable that glycans code for cell addresses.
P054

Intensive measurement of metabolite profiles similar in dietetic and genetic longevity models

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Objective and methods: There is a complex network from endogenous and exogenous factors influencing the process of aging. We investigated the simple but complex model organism Caenorhabditis elegans and a very complex measurement of metabolic profiles (GC-TOF-MS, UPLC-FT-ICR-MS). As the exogenous model we employed the wild type worm N2 with a dietetic regimen. The restricted worms were fed 1\*10^8 e.coli (long-lived), the control worms 0.5\*10^10 and the overfed worms 1\*10^11 e.coli (short-lived). The endogenous genetic models were the dauer-constitutive mutant daf-2 (long-lived) and the dauer-defective mutant daf-16 (short-lived), both involved in the insulin-like signaling pathway of Caenorhabditis elegans.

Results: With the investigated GC-TOF-MS and UPLC-FT-ICR-MS measurements, we were able to detect in total 745 metabolites from which were 462 polar and 283 lipophilic compounds. To investigate the separation between groups we performed a principal component analysis. Separation between investigated long- and short-lived groups was clearly performed. In total 30 polar and 36 lipophilic compounds are increased and 37 polar and 46 lipophilic metabolites are decreased in the long-lived models. Protein degradation seems to be increased and oxidative stress decreased in those worms. Also metabolites involved in neuromodulatory processes are affected and in the long-lived models triglycerides are increased. Conclusion: Until now those data are more descriptive than functional. There are a lot of metabolites not even known by name but clearly important for the separation of longevity groups. Out of this work important longevity processes are connected to oxidative stress, protein degradation, neuromodulatory processes or DNA- and RNA-synthesis.
Germline organization and histone modifications in the human parasitic nematode *Brugia malayi*, and implication of the *Wolbachia* mutualistic endosymbiont

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Animals are engaged in symbiotic interactions with microorganisms, with outcomes ranging from parasitism to mutualism. Understanding the intimate functioning of these interactions is of a huge interest because many pests and vectors live in multipartite associations with virus and bacteria and their control strategy poses significant challenges for the medical communities. The mutualism of *Wolbachia* bacteria with parasitic filarial nematodes, like *Brugia malayi*, a causing agent of Elephantiasis, has triggered a resurgence of interest for this endosymbiont. Filariasis are diseases extremely debilitating in humans, and are lethal in cats and dogs. From eradication of neglected tropical diseases to marketable veterinary drugs, interests converge toward *Wolbachia*. The search for antibiotic compounds more potent against *Wolbachia* is therefore actively ongoing. However, the lack of knowledge on *Wolbachia*-host interactions is abysmal and the essential role of *Wolbachia* to the worm’s survival and fertility is still poorly understood. The success of this symbiosis relies on the maternal transmission of *Wolbachia* through the female germline and the dependency of the nematodes on *Wolbachia* to complete their life cycle. Indeed, the depletion of *Wolbachia* from filarial nematodes by antibiotic treatment leads ultimately to apoptosis of developing embryos, resulting in female sterility. In *C. elegans*, the dynamic of histone modifications in during germline development is well described and the disturbance of these marks can lead to profound effects on gametogenesis and impair the fertility. Many bacteria species can hijack the heterochromatin machinery to manipulate host phenotypes. But this mechanism has not been explored in *Wolbachia*-host interactions, even though this ability of *Wolbachia* to interfere with host chromatin has been suggested. We are currently characterizing i) the germline and the early embryonic development of *B. malayi* and the associated histone modifications, and ii) the consequences of *Wolbachia* depletion on the correct germline programing. We analyzed a large panel of histone marks on whole mount ovaries and embryos from wild-type and *Wolbachia*-depleted *B. malayi* females. The dynamics of these marks has also been characterized in the male germline, naturally lacking *Wolbachia*. The comparison between *B. malayi* and *C. elegans* revealed an overall high degree of conservation in the germline development and programing strategies. We will however report differences between these species, and some histone pattern defects associated with cellular phenotypes caused by *Wolbachia* depletion.
Computational inference of the patterns of synaptic connections for locomotor interneurons in C. Elegans.

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The command interneuron circuit for C. Elegans locomotion has been known for a long time. However, synaptic polarities of synapses of interneurons, and thus, the circuit functioning is largely unknown. We computationally inferred the likely polarities of all synapses in the circuit and afferent current input pattern projecting on the circuit. We constructed a theoretical interneuron model, in which neural activities and calcium concentrations are represented by a set of differential equations. The parameterisation of the model was carried out by comparison of the results against the experimental data: the times worms spend in forward and backward motion, after ablation of selected combinations of interneurones[1]. The parameter optimisation was carried out using genetic algorithm approach implemented in the Parallel Computation in Java library, and performed on a massively parallel computational architecture[2]. The optimal solution is when either all or almost all of the synapses are inhibitory, which suggests that the main mode of the locomotory circuit operation is by inhibition. The optimised model allows also for studying average levels of synaptic and gap-junction current flows between interneurones and calcium concentration in the circuit cells.

2. Górski Ł., Rakowski F., Bała P. Parallel differential evolution in the PGAS programming model implemented with PCJ Java library, submitted.
P057

Functional Analysis of Regulatory Elements in Caenorhabditis elegans using CRISPR/Cas9

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Post-transcriptional regulation is directed by regulatory elements in the untranslated regions of mRNAs. Previously, we have identified RNA elements which are implicated in regulating mRNA abundance during the oocyte to embryo transition in Caenorhabditis elegans. To determine the function of these RNA regulatory elements, we have chosen candidate targets and have used CRISPR/Cas9 based genome editing to delete these elements. Aside from studying known elements we are developing an approach to perform mutational scanning targeted to a given locus for unbiased interrogation of regulatory regions. Followed by high-throughput phenotyping and sequencing this could be used to determine regions important for a biological process of interest and might allow identification of new regulatory elements.
Cellular and molecular mechanisms of response to the chemotherapeutic drug cisplatin

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Cisplatin is one of the most efficient chemotherapeutic agents against diverse types of solid tumors. However, clinical oncologists have two main difficulties in the prescription of cisplatin and its derivatives: (i) intrinsic or acquired resistance of tumor cells and (ii) side effects produced by citotoxicity in normal cells. Since the action of cisplatin within the cell is quite unspecific, we are using C. elegans to identify genes and pathways involved in the cellular response to this drug. Transcriptomic analyses hinted that cisplatin exposure triggers a stress response in which the conserved transcription factors DAF-16/FOXO and SKN-1/Nrf2 are key regulators. We corroborated the importance of the cellular redox balance in the onset of cisplatin resistance by demonstrating that glutathione-S-transferase cdr-1 and the thioredoxin reductase trxr-1 regulate cisplatin effects. Editing the genome by CRISPR/Cas9, we pointed that the cytotoxic action of TRXR-1 in presence of cisplatin relies on a single selenocysteine amino acid. In addition, we have observed that spermatogenesis is more sensitive to cisplatin that oogenesis, and that inhibition of apoptosis does not confer cisplatin protection to animals. Concurrently, we have identified genomic regions associated to cisplatin resistance in patients-derived orthotopic models (human tumors implanted in mice) and have used C. elegans to narrow which of the genes in these regions may be involved in the cellular response to cisplatin. In summary, this comprehensive study of the animal response to cisplatin settles C. elegans as a preclinical model to investigate molecules that can reduce cisplatin resistance or side effects.
Massive C. elegans whole-genome sequencing profiling mutational signatures of carcinogens and DNA repair deficiency

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Mutation is associated with developmental and hereditary disorders, ageing and cancer. While we understand some mutational processes operative in human disease, most remain mysterious. We developed C. elegans whole genome sequencing to investigate genetic and environmental contributions to mutational signatures. In an initial study, we analysed 183 worm populations across 17 DNA repair-deficient backgrounds, propagated for 20 generations or exposed to carcinogens (Meier et al., 2014). The baseline mutation rate in C. elegans was ~1/genome/generation, stable across wild-type and many DNA repair deficiencies. Telomere erosion led to complex chromosomal rearrangements initiated by breakage-fusion-bridge cycles and completed by simultaneously acquired, localized clusters of breakpoints, a process akin to chromotripsis. It has been reported that such genome rearrangements occur in lymphoblastic leukemia. Aflatoxin-B1 induced substitutions of guanines in a GpC context, a mutation profile enhanced in the absence of nucleotide excision repair and consistent with signatures observed in aflatoxin-induced liver cancers. Cisplatin and mechlorethamine, DNA crosslinking agents, caused dose- and genotype-dependent signatures among indels, substitutions and rearrangements. Strikingly, both agents induced clustered rearrangements resembling ‘chromoanaysynthesis’, a replication-based mutational signature seen in constitutional genomic disorders, suggesting interstrand crosslinks may play a pathogenic role in such events. Cisplatin mutagenicity was most pronounced in xpf-1 mutants, suggesting that XPF critically protects cells against platinum chemotherapy. Thus, experimental model systems combined with genome sequencing can recapture and mechanistically explain mutational signatures associated with human disease. To date we have sequenced more than 2000 genomes and are currently analyzing their mutation profiles. We will present our progress on the consequence of DNA repair defects on genome stability. Defects in mismatch repair (MMR) exhibit the highest mutation frequency among all DNA repair mutant backgrounds tested in our dataset with mainly single base substitutions and 1 base indels. Hypermutation is observed when defects in Polε replicative polymerase are combined with MMR deficiency, similar to recent observations in yeast and cancers from children with inherited biallelic MMR deficiency (Shlien et al., 2015, Kunkel 2014). Moreover, we will discuss our findings on mutation types and rates following exposure of wild type and ~50 treatment with a variety of DNA damaging agents. These include ionizing radiation, methylating and ethylating agents, UV, DNA crosslinkers and several bulky DNA adducts. Our results provide a mechanistic understanding how the various lesions are repaired in vivo and how DNA repair mechanism both prevent, but occasionally also contribute to mutagenesis.
Proteome analysis in C. elegans at the single worm level

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Proteomics studies typically analyze proteins using extracts prepared from tens of thousands to millions of cells. The resulting measurements therefore correspond to average values across the cell population and this can therefore mask considerable variation in protein expression levels between individual cells and/or organisms. Our understanding of biological regulatory mechanisms can thus be hampered without knowing whether averaged values across the population are either representative of more uniform responses, or obscuring stochastic variation in response levels at the individual cell/organism level. Here, we describe the development of a ‘micro-proteomics’ workflow for the analysis of C. elegans, a eukaryote composed of 959 somatic cells and ~1,500 germ cells. We report measuring the worm proteome at a single organism level to a depth of ~3,000 proteins. This detects proteins across a wide dynamic range of expression levels (> 6 orders of magnitude), including many chromatin-associated factors involved in chromosome structure and gene regulation. We apply the micro-proteomics workflow to measure the global proteome response to heat-shock in individual nematodes. This shows variation between individual animals in the magnitude of the global proteome response following heat-shock, including variable induction of heat-shock proteins. Strikingly, although our sample size is relatively small (10 individuals), we find that the animals can be grouped into 2 classes based on their response to heat stress. Whether this reflects molecular differences in the response of the two groups to heat stress remains to be explored. C. elegans provides an ideal system to study the stochastic variation in an isogenic background. For instance, when worms age, there is a ~10 days timeframe between the first and the last worm to succumb to death. We are now extending the use of micro-proteomics to the investigation of stochastic variation between isogenic worm populations leading to different aging patterns (class A, B and C worms). All data described in this study are available online via the Encyclopedia of Proteome Dynamics (http://www.peptracker.com/epd), an open access, searchable database resource.
Developmental and circadian timing: two sides of the same coin?

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Postembryonic development of the nematode Caenorhabditis elegans is structured into four larval stages (L1-L4) interrupted by short molts (M1-M4). Larval stages are characterized by locomotor activity, food uptake, and growth. Molts are defined by a quiescent phase, called lethargus, formation of a new cuticle and shedding of the old cuticle at the end of the molt. Molts occur at very regular intervals (every 8-10 hours at 22 °C). Timing of molts and larval stages is regulated by heterochronic genes such as lin-42. lin-42 is a PAS-domain containing transcription factor and the C. elegans homologue to the mammalian per-2, a part of the transcription-translation feedback loop that controls circadian gene expression. This raises the question whether the circadian system and developmental timing are controlled by similar mechanisms. The circadian system is a temporal program that coordinates endogenous circa 24-hour oscillations in behavior, physiology and gene expression. This allows organisms to adapt to and anticipate daily changes in environmental conditions such as food availability. A developmental timer might have a similar role in adapting developmental progress to the current environment. To compare regulation of circadian and developmental timing we used a recently developed luminometry assay for precise measurement of developmental timing¹. Chemical compounds such as lithium chloride, deuterium and kinase inhibitors that are known to modulate circadian timing were tested for their effect on development. Several of these compounds alter developmental timing suggesting a partially shared molecular mechanism between circadian and C. elegans developmental timing. 1. Olmedo, M., Geibel, M., Artal-Sanz, M. & Merrow, M. A High-Throughput Method for the Analysis of Larval Developmental Phenotypes in Caenorhabditis elegans. Genetics 201, 443–448 (2015).
A novel function for the MAP kinase SMA-5 in intestinal tube stability

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Intermediate filaments are major components of the metazoan cytoskeleton. They are expressed in cell type- and function-specific combinations. Mechanisms of intermediate filament assembly into complex networks and their isotype-specific functions are largely unknown. \textit{Caenorhabditis elegans} provides an excellent model system to study intermediate filament organization and function in vivo. Its intestinal intermediate filaments localize exclusively to the endotube, a circumferential sheet just below the actin-based terminal web. It is anchored to the \textit{Caenorhabditis elegans} apical junction. A genetic screen for defects in the organization of intermediate filaments identified \textit{sma-5(kc1)}, a mutation in the catalytic domain of the MAP kinase 7 ortholog. In \textit{sma-5(kc1)} mutants pockets of lumen penetrate the cytoplasm of the intestinal cells. These membrane hernias increase over time without affecting junctional integrity and epithelial polarity. A more pronounced phenotype was observed in the deletion allele \textit{sma-5(n678)}. Ultrastructural analyses revealed that the luminal pockets include the complete subapical cytoskeleton, and most notably, local thickenings and gaps in the endotube. Increased intermediate filament phosphorylation was detected by 2D-gel electrophoresis. Together, the phenotypes suggest that loss of SMA-5 function leads to reduced intestinal tube stability due to altered intermediate filament network phosphorylation.
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An alternative hypoxia response mechanism, independent of HIF-1, involves mitochondrial metabolic adaptation in *C. elegans*

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Hypoxia has been implicated in the pathophysiology of several common and devastating disorders including stroke, ischemic heart disease and cancer. Survival under hypoxia at the cellular, tissue, and organismal level, requires activation of various hypoxia-responsive genes, involved in mitochondrial function, glucose metabolism, glycolysis, autophagy, the unfolded protein response (UPR) and apoptosis. Although, hypoxia-inducible factor-1 (HIF-1) is an essential transcription factor coordinating many of these transcriptional responses to hypoxia, it is becoming apparent that HIF-1-independent hypoxia-responses can also occur. The complex regulatory network activated upon hypoxia, independently of HIF-1, is not fully understood. We uncovered an alternative hypoxia response mechanism independent of HIF-1 which requires mitochondrial metabolic adaptation. Specifically, we found that expression of T09A5.7/TRIAP-1, the *C. elegans* homolog of the mammalian TRIAP1/p53CSV, is associated with hypoxia and its activity is HIF-1-independent. Importantly, TRIAP-1 promotes organismal survival under conditions of prolonged hypoxia in the absence of HIF-1. In addition, TRIAP-1 mediates mitochondrial metabolic adaptation upon hypoxia. We further demonstrated that TRIAP-1 regulates various stress response pathways including autophagy, UPR and the intrinsic apoptotic pathway. Last, we showed that TRIAP-1 can regulate *C. elegans* lifespan when oxygen is abundant. Based on these findings, we propose a novel role of this gene in organismal adaptation to hypoxia independent of HIF-1. Tumor cells are often challenged by extreme oxygen deprivation. It is therefore essential for tumor survival to acquire hypoxia adaptation in response to low oxygen concentration. TRIAP-1 has been associated with various types of cancers and stress responses. Our findings suggest a novel, HIF-1-independent, role for TRIAP-1 in hypoxia adaptation, which could modulate hypoxia-induced resistance to anticancer drugs.
P064

Lichenan extent lifespan via DAF-16/FoxO transcription factor and attenuate oxidative stress in Caenorhabditis elegans

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Lichenan is a complex polysaccharide present in Cetriana islandica which uses longtime in folk medicine. Most studies illustrate the antioxidant, antimicrobial, anti-inflammatory and anti-proliferative activities of phenolic substances from C. islandica. Lichenan has some beneficial effects described; although on its aging-related effects yet not to be studied. This present study is conducted to find whether the lifespan extended using Lichenan in C.elegans. Age Synchronized wild type L4 worms was fed E.coli (OP50) on agar plate at 20°C with 100µM, 200 µM, 500µM, 1000µM Lichenan and 0.2% DMSO solvent control. lifespan analysis adults were transferred to fresh NGM every other day with Lichenan and scored until death. Oxidative stress assay was conducted with wild type worm at L4 Larvae pre-treated with lichenan at respective concentration and shifted to juglone (500µM) plates and allowed 5 hrs at RT and scored survival. Sod-3 and Daf-16 expression was conducted by GFP-tagged strains zls356 [daf-16p::daf-16a/b::GFP + rol-6] and muls84 [(pAD76) sod-3p::GFP + rol-6] and treated with 500 µM and 1000µM at 20°C in liquid medium and fluorescence level was measured by ImageJ 1.46. Statistical analyse was performed by One-way ANOVA, a p>0.05 was considered as significant. There was no significant difference on median lifespan at 100 or 200 µM although 500 and 1000µM have 12% and 20% increased, maximum lifespan lengthened by an average of 25%. Oxidative stress resistance observed 69% and 76% on treated with 500 and 1000 µM and sod-3 expression was significantly increased to 109%. Our present observation proving Lichenan extent lifespan through diminish the Insulin like growth factor-1(IGF-1) downstream regulators and enhance stress resistance.
Muscle Ageing and Gene Expression Balance in *Caenorhabditis elegans*

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The age-related deterioration of skeletal muscle, sarcopenia, affects mobility in later life. The ryanodine receptor (RyR1) is the sarcoplasmic reticulum calcium release channel required for skeletal muscle contraction in humans. Leaking RyR1 channels, and consequential calcium ion imbalance, have been connected to the declining function of aged muscle. Suboptimal levels of proteins which influence RyR1 activity could contribute to this calcium leakage in old age. Gene expression levels may not be optimal in old age. As an animal ages and begins to fulfil its reproductive potential the force of natural selection declines. Because of this, gene function late in life is not under strong selective pressure. Therefore, the relative amounts of proteins required stoichiometrically to regulate the RyR1 channel, may become unbalanced as we age. Assessing gene expression levels with age may reveal biologically significant changes contributing to calcium ion imbalance. *C. elegans* makes a good model for ageing studies. Assessment of the expression levels of the *C. elegans* RYR1 orthologue, *unc-68*, and related genes involved in calcium homeostasis may reveal genes with expression levels that deviate significantly with age. Assessment of the transcriptome can reveal age-related variation in gene expression and prior transcriptome data was analysed with the above considerations. Transcript levels across the lifespan were compared for 25 *C. elegans* genes seen as relevant to calcium homeostasis. Data from five microarray studies was examined, but comparison between the studies was difficult. RNA-seq has an infinite range and an absolute output, facilitating cross study comparison. The RNA-seq output for the selected genes was found to be mutually consistent both between and within the two independent studies for which lifespan data were freely available, when analysed through an online pipeline, Galaxy. *C. elegans* genes required for calcium ion balance in muscle cells with significant changes of expression level with age were identified. Such genes are to be manipulated in future muscle ageing studies. We thank the *C. elegans* researchers who made their transcriptome data freely available for our analysis.
Long-term time-lapse microscopy of C. elegans post-embryonic development

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We present a microscopy technique that enables long-term time-lapse microscopy at single-cell resolution in moving and feeding C. elegans larvae. Time-lapse microscopy of C. elegans post-embryonic development is challenging, as larvae are highly motile. Moreover, immobilization, e.g. by anesthetizing drugs, generally leads to developmental arrest. Instead, we confine larval movement to microchambers that contain bacteria as food, and use fast image acquisition and image analysis to follow the dynamics of cells inside individual larvae, as they move within each microchamber. This allows us to perform fluorescence microscopy of 10-20 animals in parallel, with ~10 minute time resolution and over the full ~48 hours of post-embryonic development. We demonstrate the power of our approach by i) following all cell divisions in the seam cell lineages, ii) measuring the kinetics of the full migration of the distal tip cells during gonadogenesis and iii) quantifying the oscillatory expression dynamics of the molting cycle genes mlt-10 and wrt-2 over all four larval stages. We show that the dynamical information captured by our approach can provide new insights into the mechanisms that control these processes. We believe that our approach could make time-lapse microscopy a routine tool to study C. elegans post-embryonic development, with the potential to significantly increase our understanding of lineage control, morphogenesis and the dynamic regulation of gene expression.
Identifying age-dependent heterologous seeds for amyloid-β aggregation

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Aging is the most important risk factor for neurodegenerative diseases associated with pathological protein aggregation such as Alzheimer's Disease (AD). Previously we identified in C. elegans several hundred proteins that consistently become more insoluble during normal aging and in the absence of disease aggregating proteins. Although aging is an important player, it remains unknown which changes are relevant for disease initiation. In recent years, the role of misfolded protein seeds in the initiation and spread of hallmark aggregates has become a central area of investigation. Our hypothesis is that some proteins in the aggregating proteome build heterologous seeds with age and can drive the pathological aggregation of β-amyloid protein (Aβ) in Alzheimer's disease. To this end we examined detergent-insoluble proteins from wild-type C. elegans and aged wildtype mice. We found that only aggregated proteins from aged individuals can seed Aβ aggregation in vitro. In transgenic models, injection of brain extracts containing Aβ seeds is sufficient to instigate the formation and spreading of Aβ plaques. We could show that detergent-insoluble proteins from aged mouse brains alone are able to seed Aβ plaque formation in vivo in mice expressing human APP containing the Swedish mutation. In conclusion our preliminary results highlight that physiological protein aggregation with age might indeed constitute a heterologous seed for disease-associated protein aggregation. Identifying the key seed forming proteins would have a major impact in advancing our understanding of aging and its influence on pathophysiology.
Mechanisms of hydrogen sulfide tolerance in the nematode C. elegans

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The gas hydrogen sulfide is a double-edged sword. On the one hand, hydrogen sulfide regulates diverse physiological processes, such as blood circulation. On the other hand, high physiological levels of hydrogen sulfide are toxic, and may underlie the development of diseases such as colorectal cancer and ulcerative colitis. However, how cells tolerate high levels of hydrogen sulfide is not completely understood. Here, we describe a new method to screen for mutations that confer tolerance to hydrogen sulfide toxicity in the nematode C. elegans. We identify mutations in genes encoding for thioredoxin, mitochondrial, and collagen proteins that confer tolerance against hydrogen sulfide toxicity. Combined inhibition of mitochondrial activity and thioredoxin function synergistically increases the survival of worms in high hydrogen sulfide levels. In addition, we found that hydrogen sulfide toxicity is mediated by reactive oxygen species, and that both antioxidants and sublethal levels of paraquat protect against hydrogen sulfide toxicity. Intriguingly, the transcription factor HIF-1 is important for the hydrogen sulfide tolerance of both mitochondrial, thioredoxin, and collagen mutants, suggesting that HIF-1 is a key regulator of both adaptation to low oxygen and high hydrogen sulfide levels. Finally, we show that hydrogen sulfide resistance varies among wild C. elegans and other nematode species, suggesting that tolerance to hydrogen sulfide was naturally selected in certain habitats.
Identification of novel regulators of GABAergic synaptogenesis in the nematode *Caenorhabditis elegans*.

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In the central nervous system, the inhibitory system plays a key role in neuronal network excitability. Defects in the excitatory versus inhibitory balance could lead to neuropathies. To identify novel genes and mechanisms involved in the formation and the regulation of inhibitory synapses, we use the inhibitory GABAergic neuromuscular junction of the nematode *Caenorhabditis elegans* as a genetically tractable model. At these synapses, fast neurotransmission is ensured by type A ionotropic GABA receptors (GABA\(_A\)R), which form post-synaptic clusters in front of GABA release sites. Specifically, we performed an EMS genetic screen based on the direct visualization of fluorescently tagged GABA\(_A\)R in vivo in a knock-in strain. We identified 35 mutants with abnormal GABA\(_A\)R localization among the 1,728 mutagenized haploid genomes isolated. We characterized and categorized a first set of 13 mutants that displayed strong GABA\(_A\)R mislocalization. We used a novel whole genome sequencing strategy to simultaneously map and identify mutations responsible for the GABA\(_A\)R localization defect without any prior time-consuming genetic mapping. We are currently validating some of the potential mutations and reproducing the procedure for a second set of mutants to enlarge our candidate genes list.
Modulation of ageing characteristics with an anti-ageing compound

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Ageing and age-related disorders pose huge social and economic challenges to our society as the world’s population ages. Investigating the cellular processes anti-ageing compounds interact with can identify genes and pathways involved in the general ageing process. The macrolide lactone RDC5 extends lifespan in yeast and C. elegans, and ameliorates neurodegeneration and age-related weight gain in rodents. The anti-ageing mechanism of RDC5 is unknown, although it is known to bind members of the FKBP (FK506-binding protein) family or proteins. As the general mechanisms of ageing are well conserved between C. elegans and mammals, the drug-sensitive C. elegans strain bus-5(br19) has been used to understand how RDC5 acts on ageing and age-related conditions. RDC5 treatment was shown to inhibit neither E. coli OP50 growth nor bus-5(br19) pharyngeal pumping, indicating RDC5 does not extend C. elegans lifespan by inducing dietary restriction. RDC5-treated bus-5(br19) had an increased rate of thrashing at days 1 and 3 of adulthood, suggesting an extension of healthspan as well as lifespan. A transcriptome analysis of RDC5-treated worms allowed the identification of transcripts whose levels change and potential pathways by which RDC5 manifests its effect. To explore this and to identify potential targets of RDC5, bus-5(br19) worms were co-treated with RDC5 and a variety of small molecule inhibitors. The cellular functions required for RDC5 to extend C. elegans lifespan and healthspan will be presented.
Oscillatory transcription drives rhythmic mRNA accumulation during C. elegans larval development

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Oscillation is a fundamental characteristic of many biological processes. Rhythmic processes such as the cell cycle or the circadian rhythm can both drive and result from oscillatory gene expression. Our group recently observed extensive oscillations of thousands of mRNAs during Caenorhabditis elegans larval development, characterized by an 8-hour period and high-fold changes. To shed light on the mechanism leading to transcript oscillation, we drove the expression of a destabilized fluorophore from promoters of oscillating genes. Quantification of reporter mRNA revealed sufficiency of the promoters to direct oscillations. In fact, promoters fully recapitulated phase and amplitude of the endogenous transcript of different genes tested. Rhythmic abundance of reporter protein levels was also observed by microscopy, revealing expression in various tissues, but particularly the epidermis. Mutational analysis uncovered a conserved element in one particular promoter that is necessary and sufficient to induce tissue specific oscillation in the epidermis. This indicated that oscillatory expression of a single gene may occur with distinct phases across different tissues. We conclude that the observed oscillation is mostly and perhaps exclusively regulated transcriptionally. Moreover, differences in expression phases implicate the operation of tissue-specific transcriptional regulation. However, the detection of transcript oscillation on whole animal RNA samples reveals transcriptional regulation activities to be coordinated, yielding stable phase differences. We are currently dissecting the function of a transcription factor that we have identified as a potential driver of oscillations.
CHP-1 regulates Wnt and EGFR signaling pathways during C. elegans vulval development

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EGFR/RAS/MAPK signaling is essential for animal development and is misregulated in various human epithelial cancers. The subcellular distribution of the EGFR profoundly affects its activation by different growth factor ligands as well as the type of downstream signaling pathway used. However, the mechanisms regulating EGFR localization in polarized epithelial cells remain poorly understood. The C. elegans hermaphrodite vulva is a well-established system to study EGFR signaling and localization in vivo. The EGFR/RAS/MAPK signaling pathway, in concert with the Notch and the Wnt signaling pathways, forms a complex and highly interconnected signaling network, resulting in the specification of an invariant cell fate pattern among the six vulval precursor cells (VPCs). C. elegans let-23 encodes the single member of the EGFR family and it is localized on the basolateral membrane of the VPCs. Towards the end of the L2 larval stage, LET-23 receives the inductive LIN-3 EGF signal from the anchor cell and activates the RAS/MAPK pathway, thereby inducing a primary (1°) cell fate in the central VPC (P6.p). Lateral LIN-12 NOTCH signaling determines the secondary (2°) cell fate in the adjacent VPCs (P5.p and P7.p). Moreover, hyperactivation of the Wnt signaling pathway can compensate for the absence of RAS/MAPK signaling, suggesting that the Wnt pathway plays a partially redundant role during vulval induction. We have performed an in vivo RNAi screen to identify novel regulators of LET-23 localization and signaling in the VPCs using a functional LET-23::GFP reporter. In this screen, we have identified the chp-1 gene, which encodes a conserved CHORD domain protein homologous to human CHORDC1 and ITGB1BP2. A chp-1 loss-of-function mutation leads to the intracellular accumulation of LET-23, while the localization of other membrane receptors such as the Integrins or Notch remained unchanged. Analysis of various cell fate markers indicates that chp-1 promotes EGFR/RAS/MAPK signaling and at the same time suppresses Wnt signaling in the VPCs. Thus, we are investigating how chp-1 coordinates Wnt and EGFR signaling during vulval development. Moreover, we are investigating the molecular mechanism by which CHP-1 controls LET-23 localization. In particular, we are determining, to which sub-cellular compartment LET-23::GFP is mislocalized in chp-1(II) mutants. These results will further help us in understanding the precise regulation of signaling pathway interaction for correct cell fate patterning during organogenesis.
Poster abstracts in alphabetical order (first author)

P073

Post-transcriptional histone modifications maintain cell identity and genome stability in the Caenorhabditis elegans germline

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Germ cells were acknowledged more than one century ago as cells functioning as agents of heridity. This implies that they (i) maintain the ability to differentiate into any somatic tissue after fertilization and (ii) protect their genome against mutation to faithfully transmit genetic information. The role of epigenetic regulation in the establishment and maintenance of germline specific properties is still poorly understood. We are using C. elegans as a model system to study the role of post-translational histone modifications in germline genome stability. We have performed standard assays to test genome stability in mutants with decreased H3K4, H3K9 and H3K36 methylations. We will present results showing that mutants with reduced H3K4 and H3K36 methylation patterns show increased sensitivity to DNA damage inducing agents and a mutator phenotype. These phenotypes are not seen in mutants with reduced H3K9 methylation. These observations suggest that H3K4me and H3K36me are involved in genome stability in the germline of C. elegans. We are now performing additional assays to characterize the mechanisms responsible for the observed genome instability. In addition, we have shown that in germlines lacking H3K4 methylation, misexpression of germline and somatic genes correlates with loss of germline pluripotency and transdifferentiation of germ cells into somatic cell types (Robert, Mercier et al, 2014). Loss of H3K9 methylation is also observed in transdifferentiated gonads, suggesting the existence of complex crosstalks between histone modifications in the regulation of germline homeostasis. The role of the epigenetic landscape on regulation of cell fate and genome stability is being further analyzed in our lab.
Aging is one of the most intriguing and fundamental biological processes, with clear implications for human health. The insulin/IGF-1 signaling (IIS) pathway and mitochondria are associated with aging and age-related pathologies. Among the mitochondrial proteins shown to affect lifespan are prohibitins, PHB-1 and PHB-2, which together form a ring-like macromolecular complex in the mitochondrial inner membrane. Depletion of the PHB complex shows an opposite effect on aging; it shortens the lifespan of wild type worms while it dramatically extends the lifespan of animals with reduced insulin signaling. The accumulation of unfolded proteins within mitochondria triggers a mitochondrial stress response known as the mitochondrial unfolded protein response (UPR\textsuperscript{mt}), which signals to the nucleus to activate the expression of specific mitochondrial chaperones. Interestingly, prohibitin depletion induces the UPR\textsuperscript{mt} in wild type animals while in IIS mutants the induction of the UPR\textsuperscript{mt} upon prohibitin depletion is strongly reduced. Furthermore, some of the previously described components of the UPR\textsuperscript{mt} are not required for the PHB-mediated activation of the UPR\textsuperscript{mt}, suggesting a different mitochondria-to-nucleus signaling mechanism. We are performing high throughput RNAi screens in order to find additional players involved in the regulation of the mitochondrial stress response elicited by PHB depletion in wild type and in IIS mutants, and to elucidate the role of the UPR\textsuperscript{mt} in the modulation of aging. Herein, we present preliminary candidates of the screens using a sub-library based on the OrthoList, Caenorhabditis elegans genes sharing a human orthologous gene. The identification of new players involved in the UPR\textsuperscript{mt} will unveil the molecular pathways regulating mitochondrial quality control mechanisms and could shed light on the mechanisms contributing to the differential effect in aging of PHB depletion in wild type and metabolically compromised animals.
P075

Analysis of neurotoxicity in C. elegans induced by platinating agents commonly used in chemotherapy

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Cancer is the second leading cause of death worldwide and the WHO forecasts a rise in the number of cases by over 60% in the next 20 years. Although effective anticancer therapies are available, they are often accompanied by severe adverse effects. One of the most common dose-limiting side-effects in using platinating agents (e.g. cis- or oxaliplatin) as anti-tumor drugs is chemotherapy induced peripheral neuropathy (CIPN). The pathophysiology of this clinically relevant side-effect is still unknown and neither prophylaxis nor specific treatment is available. Therefore, further research elucidating the underlying molecular mechanisms of CIPN caused by platinating anti-tumor drugs is required as basis for future development of preventive or therapeutic strategies. Platinum compounds kill tumor cells by induction of DNA damage and disruption of redox homeostasis. Since they cannot specifically target tumor cells, other cells in particular nerve cells are also damaged. Here, we use C. elegans to elucidate the underlying mechanisms of platinum-induced neurotoxicity. So far, we proved uptake using inductively coupled plasma mass spectrometry (ICP-MS) whereby we found a dose dependent increase of platinum per individual. Correspondingly we detected also a dose dependent induction of typical platinum-derived DNA 1,2-GpG-intrastrand crosslinks by south-western blotting. The tested concentrations were sufficient to induce robust apoptosis in C. elegans as measured in the germline. Even more important we found that doses having only moderate effects on development, reproduction and musculature, led to strong neurotoxicity as could be demonstrated by impaired pharyngeal pumping and chemotaxis. With regard to redox homeostasis, we measured reactive oxygen species (ROS) after short term treatment with platinating agents, but observed no ROS induction. However, we found sensitization of C. elegans to oxidative stress delivered either by hydrogen peroxide or paraquat after cis- and oxaliplatin treatment. This discrepancy could be resolved by measuring the internal antioxidant capacity of platinating agent-treated C. elegans populations by HPLC-MS, which showed a considerable reduction in the amount of glutathione (GSH) without any increase of glutathione disulfide (GSSG). Consequently the reduction in oxidative stress resistance could be prevented by co- or pre-treatment with N-acetylcysteine, a clinically used antioxidant and cysteine donor. Currently we are using this setup to identify molecular targets via RNAi approaches (whole animal and tissue specific) as well as testing putative neuroprotective compounds to help building pharmacological strategies for the treatment or even prevention of neurotoxic effects by chemotherapeutics.
P076

Using *C. elegans* to study the role of ATRX in regulation of the ALT pathway

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ATRX (Alpha-Thalassemia, mental Retardation, X-linked) is a Snf2-family chromatin remodelling enzyme whose mutation causes ATRX syndrome, a developmental disorder in humans. Interestingly, loss of ATRX expression has also been linked to a subset of human cancers that utilise the alternative lengthening of telomeres (ALT) pathway of telomere maintenance. ATRX acts as a negative regulator of ALT but the exact mechanism of ATRX-mediated ALT suppression remains elusive. In part, this is because loss of ATRX alone is not sufficient to induce ALT in human tissue culture, making it difficult to study this relationship comprehensively. The nematode worm *Caenorhabditis elegans* is an established and tractable genetic model system and has a clear ATRX homolog, *xnp-1*. Here we show that a null *xnp-1(tm678)* mutant has elongated telomeres and displays robust levels of extrachromosomal C-circles, two major phenotypic markers for ALT activity. *xnp-1(tm678)* therefore represents a unique model system for studying ALT and will enable us to assess which of the many functions attributed to ATRX are important for regulating the ALT pathway.
Inhibition of the mevalonate synthesis causes a DAF-16 dependent longevity phenotype in C. elegans

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Statins are broadly used as cholesterol-lowering agents in the clinic since 1989. Their competitive inhibition of the HMG-CoA reductase within the mevalonate pathway causes a depletion of mevalonate a precursor for cholesterol synthesis. Furthermore it is known that mevalonate depletion attenuates prenylation and glycosylation of proteins. Recently, a cohort study showed that a decreased mortality rate in humans between age 78 – 90 correlates with statin treatment, but is independent of cholesterol levels. As C. elegans harbors the mevalonate pathway, but the branch leading to cholesterol synthesis is missing, we believe it is a well-suited model organism to study cholesterol –independent effects of statins on aging-associated phenotypes and the underlying molecular mechanisms. Especially because its lifespan is depends on multiple signaling pathways (e.g. Insulin or mTOR signaling), which are conserved up to humans. Here, we show that treatment of C. elegans with statins decelerate ageing. While the level of age pigments roughly doubled in control animals, there was only a slight increase in the lovastatin group. The use of atorvastatin gave comparable results indicating a more general effect of statins. The reduced accumulation of age pigments could be partly phenocopied using RNAi against the HMG-CoA reductase. The reduced level of age pigments was prognostic for an elevated mean lifespan (about 20%) through statins in C. elegans. A post reproductive treatment with lovastatin, a situation better comparable to clinical use of statins, increased the mean lifespan in C. elegans even further. In addition, we could show a mild reduction of fertility and a developmental delay as well as a marked increase in acute thermal stress resistance mediated by lovastatin. All in all the observed phenotypes point to DAF-16 overactivity. Consequently we found an increased nuclear localization of DAF-16 in the presence of lovastatin and lovastatin completely failed to reduce age pigments in a daf-16-KO mutant background. A candidate based RT-qPCR array brought amongst others JNK-1, a known activator of DAF-16, into play as a possible effector induced by statins. Our current working hypothesis is that statin exposure induces a longevity phenotype in C. elegans, which might be DAF-16 dependent. These findings indicate that a product of the mevalonate pathway somehow influences the activity of JNK-1 which in turn modulates DAF-16 action.
The contribution of intermediate filaments to the mechanical resilience of the *Caenorhabditis elegans* intestine

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Intermediate filaments are essential components of the *C. elegans* endotube, an electron dense structure below the apical plasma membrane. The endotube surrounds the intestinal lumen as a dense sheet in all of the 20 intestinal cells. It is commonly assumed that intermediate filaments have an important mechanical function. Yet, measurements on biomechanical functions of intermediate filaments in living cells have been scarce and were mostly restricted to single cells. Besides encountering a very high degree of cell-to-cell variability, these analyses neglected the tight coupling of epithelial cells to each other and the extracellular matrix within their native tissue context. We therefore decided to use the *C. elegans* intestine as a suitable paradigm to study and quantify the contribution of the intermediate filament system to cell mechanics in a viable epithelial tissue context. We developed methods to prepare and examine viable intestines with a fully intact intermediate filament cytoskeleton. Using a dual micropipette aspiration assay, we measured the mechanical properties of the dissected intestine while monitoring the fluorescence-labelled intermediate filament system (Fig. 1). We detected an enormous mechanical resilience of the dissected intestines withstanding forces up to 1 µN. They exhibited both elastic and plastic properties. Thus, the intermediate filament-rich endotube could be stretched to more than 150% without rupture. On the other hand, stretched endotubes did not fully resume their original length. Investigation of mutant intestines lacking an intact endotube revealed that they are softer than their wildtype counterparts. Taken together, our observations demonstrate the importance of intermediate filaments for tissue mechanics in *C. elegans* and exemplify that the experimental setup allows studying the contribution of specific cytoskeletal components in the native tissue environment.
Poster abstracts in alphabetical order (first author)
Identification of genes affecting synaptic vesicle recycling using a high-throughput all-optical reverse-genetic screen

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The synaptic vesicle (SV) cycle has been investigated for more than 40 years but the precise protein complement contributing to its function is still unclear. Some SV cycle proteins (such as clathrin) are known to participate in the process, however the exact site of action is still debated. Moreover, it is conceivable that additional, not yet identified factors contribute to the cycle and the coupling of exo- and endocytosis. Previous screens or analyses addressing genes involved in synaptic transmission were based on forward or reverse genetic approaches, followed by pharmacological assays (Miller 1996, Sieburth 2005). The expression of the light-gated cation channel Channelrhodopsin-2 (ChR2) in motoneurons of C. elegans has enabled to trigger transmitter release through photostimulation, causing contraction. Using this technique in SV recycling mutants resulted in progressive loss of contraction during ongoing stimulation (Liewald 2008). For screening, these methods are tedious due to low throughput, and attempts to use microfluidic chip systems for automatization (Stirman 2010) were of limited success. Moreover, previous screens could not distinguish between general synaptic transmission defects and defects which affect a specific step in the SV cycle, such as SV recycling. Thus, a new screening method was developed in our lab (Wabnig et al., 2015) based on Channelrhodopsin stimulation of cholinergic motor neurons. Combining this method with Ca²⁺ imaging in body wall muscle cells (using RCaMP as Ca²⁺ sensor (Akerboom 2013)), we could assess triggered Ca²⁺ signals in a time-dependent manner. Thus it was possible to discriminate SV recycling factors from other proteins affecting synaptic transmission. Among some well-known endocytotic retrieval factors (e.g. synaptojanin or endophilin) a typical Ca²⁺ signal pattern was recognized that allowed distinguishing recycling genes from general transmission factors. By RNAi, we tested 106 genes previously identified in aldicarb assays. Around 60 genes were identified as possible recycling factors, based on hierarchical clustering (Wabnig et al., 2015). From these candidates we chose a smaller number of genes for further assays. Experiments in genomic mutants confirmed, that optical analysis allows identifying genes affecting SV recycling.
A new approach to aging research: Death as a tipping point

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Single-mutant screens in the nematode Caenorhabditis elegans have shown to be successful in identifying genes that increase a healthy lifespan in the model organism. However, in spite of the presence of most of these genes in humans, only very few could also be associated with human longevity. One of the underlying reasons is that aging is a multifactorial complex trait influenced by a network of interacting processes. Studying the genetic processes leading to senescence are further complicated by the higher complexity of the human genome. Therefore, it is essential to complement reductionist mutant analyses with more system-oriented approaches based on new paradigms. Considering the complex nature of aging, we propose to use the paradigm of catastrophic regime shifts in complex dynamic systems – a concept taken from ecology – to study and discover gene networks closely linked to aging. Tipping points of collapse in ecological systems are characterized by a sudden and catastrophic shift between two alternative stable states. Before the transition, these systems often appear stable on a wide range of external conditions until a point is reached at which stability can no longer be maintained. At this point, slight changes in the environment lead to a collapse. The theory predicts that early warning signals precede the occurrence of a tipping point. One of the indicators is the slowing down of the recovery rate from transient perturbations as the system approaches a tipping point. In C. elegans, a close link between the resistance to stress perturbations and lifespan has long been established. We introduce the concept of using C. elegans stress response in combination with the paradigm of catastrophic regime shifts to study aging as a complex trait with the ultimate aim of predicting death.
Dissecting the steering neural circuit of *Caernobhabditis elegans* with a focus on the SMB motor neurons

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Many of the behaviours of *Caernobhabditis elegans* are mediated by the worm’s ability to orient itself towards a desired destination. One mechanism of orientation, steering, has been observed in *C. elegans* since the ’70s. However, the neural circuit controlling steering is largely unknown. One class of head motor neurons, the SMB neurons, have previously been postulated to play a role in steering. This conjecture was based on behavioural observations of SMB laser-ablated animals, showing a ‘loopy’ phenotype (Gray et al., 2005, *PNAS*) suggesting a role of the SMBs both in the modulation of amplitude of head swings and in steering. Recent experimental evidence has strengthened SMBs’ potential role in steering (Kocabas et al., 2012, *Nature*). A computational model of steering was also developed on the basis of these data (Izquierdo and Beer, 2013, *PLoS Comput Biol*). We are employing a genetic ablation approach to directly test the role of SMBs in steering. We used part of the *flp-12* gene promoter region to drive expression of GFP uniquely to the SMBs (Kyuhyung Kim, personal communication). The generated transgenic strains confirmed that the promoter used drove GFP expression in a manner consistent with SMBs’ cell body and axon localization. The same promoter region was used to genetically ablate the SMBs by targeted expression of the worm’s caspase encoded by the *ced-3* gene to these neurons specifically. We are currently running behavioural experiments to investigate the reported ‘loopy’ phenotype of SMB ablation and to explore in greater detail the role of SMBs in steering.
Development of large-scale and reproducible double RNAi protocol

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The knockdown of a single gene by RNAi has long been established in \textit{C. elegans} and helped to identify inhibitors of induced cell transdifferentiation (Tursun \textit{et al.}, 2011). A systemic RNAi response in worms is achieved upon feeding worms with bacteria strains which express dsRNA targeting a specific gene. This simplicity allows straight-forward whole-genome screens by feeding worms with 20,000 different bacterial strains. Nevertheless, using the latter for a whole-genome approach would mean to generate 20,000 new plasmids containing both targets on the same construct, which is obviously not feasible. This gets even more impracticable if different combinations of simultaneous knockdowns should be carried out. Currently, two approaches are available. Either two bacteria strains expressing specific dsRNA can be grown together in one well or a new bacterial RNAi plasmid can be generated to carry both RNAi clones of interest. While the first approach suffers from high variability, the second approach yields high reproducibility of results. Nevertheless, using the latter for a whole-genome approach is not feasible. We are developing a protocol utilizing bacterial conjugation mediated by the ‘Fertility Factor’ (F) Episome in order to combine two different RNAi plasmids in a high-throughput manner for large-scale ‘double’ or even ‘triple’ RNAi screens. This requires genetic engineering of bacterial 60 kb F Episome in order to make it suitable for using them in the RNAi bacteria. The objective is to be able to transfer RNAi-plasmids to a large number of bacterial strains that carry already different RNAi clones. Our double RNAi protocol will allow testing for additive effects of knocking down two or three genes simultaneously and thus might help understanding how barriers for the induction of direct cell type conversion in \textit{C. elegans} are formed. Reference Tursun, B., Patel, T., Kratsios, P. & Hobert, O. Direct conversion of \textit{C. elegans} germ cells into specific neuron types. Science 331, 304–8 (2011).
Positive, selective mRNA translational control in the germ line.

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More than other cell types, germ cells and early embryos regulate new protein synthesis by mRNA translational control. The products are largely responsible for new cell fates (differentiation). Control often involves repression at the 3’ untranslated region (3’utr) by a specific RNA binding protein (RBP) and/or changes in mRNA poly(A) length. However, mRNA regulation cannot be fully understood by repression or stabilization alone. Unfortunately, the mechanism of positive translational control remains poorly understood, though it is arguably the most important for getting a new protein made. Translation factors eIF4E and eIF4G work in concert with RBPs to recruit mRNAs to ribosomes. The eIF4 factors are the first to bind mRNAs and catalyze initiation. eIF4E binds the mRNA 7-methyl-GTP cap, and eIF4G scaffolds the cap, poly(A) tail, and eIF2, eIF3, eIF4A to the ribosome. This mechanism is modulated by eIF4E~eIF4G association for cap-dependent initiation. In C. elegans there are five isoforms of eIF4E (IFE-1-5) and two of eIF4G (IFG-1 p170 and p130). The naturally occuring “short” IFG-1 p130 lacks the binding site for eIF4E, but catalyzes cap-independent translation. We’ve shown that the CED-3 caspase cleaves IFG-1 p170 to render translation cap-independent (like p130) during germ cell apoptosis. The result is a shift in mechanism that favors stress and apoptotic mRNAs. Our evidence suggests that cap-dependent and –independent translation naturally co-exist to play balanced physiological roles in germ cell homeostasis. The eIF4E isoforms also have distinct roles in oogenesis and spermatogenesis. Certain IFEs and IFGs are found resident in known oocyte/embryo mRNP like P granules or OMA particles. Our lab described unique functions for IFE-1, IFE-2 and IFE-3, as well as the cap-dependent IFG-1 p170. Mutations in each reduce fertility, but in unique ways. Each alters a different aspect of germ cell differentiation: meiotic recombination, apoptosis, maturation signaling, cytokinesis, or even sperm/oocyte fate. By implication, each IFE-IFG complex must be selective for a subset of mRNAs promoting one function. We used polysome bioinformatics to identify selected mRNAs from ife and ifg mutant worms. Thus, eIF4E and eIF4G exert the positive, selective force for completing mRNA translational control to drive expression and differentiation. Our findings refute a long-held perception that translation factors perform generic housekeeping functions. Rather, evidence suggests that positive mRNA regulation is at the heart of gametogenesis and embryogenesis. (Supported by NSF Grants MCB0321017, 0842475 and a Brody Seed Grant.)

“Circuitry” for Germ Cell Translational Control
The C-type lectin like domain gene C54G4.4 is involved in behavioural immune defences against Bacillus thuringiensis

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C-type lectin-like domain (CTLD) proteins are known to be important in the immune defence of vertebrates, but their involvement in invertebrate immunity is much less understood. The nematode Caenorhabditis elegans has 283 CTLD protein encoding (clec) genes, but experimental evidence for their involvement in C. elegans immune defences is still scarce. To investigate the contribution of clec genes to C. elegans immune defences in more detail, we screened 39 clec mutants for survival after exposure to the Gram-positive bacterium Bacillus thuringiensis (BT). Unexpectedly we found both more susceptible, but also more resistant clec mutants. Increased resistance correlated with increased pathogen avoidance behaviour. We thus asked whether clec genes are not only involved in the physiological, but also in behavioural immune defences against BT. One of the highly resistant clec mutants was C54G4.4(ok2110). We confirmed that C54G4.4(ok2110) mutants show an increased avoidance behaviour in a concentration dependent manner towards BT. Moreover, C54G4.4(ok2110) mutants exhibited prolonged feeding cessation on pathogenic BT. Our results indicate that this increased avoidance behaviour and prolonged feeding cessation significantly contribute to the resistance phenotype of C54G4.4(ok2110) mutants. We conclude that C. elegans clec genes are involved in immune defences against pathogenic BT, but having both negative and positive effects. Moreover, we provide experimental evidence of the involvement of the CTLD encoding gene C54G4.4 in behavioural immune defences against BT.
Two putative selenium binding proteins as modulators of *C. elegans* stress response and life span

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Selenium binding proteins are selenoproteins that do not contain Se in the form of selenocysteine or selenomethionine, but rather as inorganic selenium bound by the protein. The *C. elegans* genome encodes only one selenocysteine-containing selenoprotein, thioredoxin reductase-1. However, at least two ORFs encode putative selenium binding proteins, Y37A1B.5 and R11G10.2. These are 54% and 36% homologous to human selenium binding protein-1 (SELENBP1), respectively, and contain several cysteine residues conserved from nematode to humans, including one cysteine that was hypothesized to bind selenite in SELENBP1. Considering the role of selenium in antioxidant defense and in upholding protein integrity, we hypothesized that Y37A1B.5 and R11G10.2 modulate the response of *C. elegans* to oxidative stress. We targeted these two genes separately by feeding the nematodes with the respective RNAi bacterial clones. Life-long knock-down of either of the genes significantly increased mean life span by at least 10% compared to control nematodes. DAF-16 is a key player in *C. elegans* stress response and aging. Moreover, its human orthologs, the FOXO proteins, are involved in the regulation of selenium homeostasis. Therefore, we tested whether DAF-16 is involved in the observed effects of Y37A1B.5 or R11G10.2 depletion on *C. elegans* life span. Feeding DAF-16-deficient mutant nematodes with RNAi against Y37A1B.5 or R11G10.2 significantly increase life span of the mutants to an extent similar to that found in wild type worms exposed to RNAi targeting either of the putative selenium binding proteins. This suggests that DAF-16 is not involved in life span extension elicited by knock-down of Y37A1B.5 or R11G10.2. Should Y37A1B.5 or R11G10.2 indeed bind inorganic selenium (which still requires experimental proof), a major question is, to what end? Two alternate potential roles include (i) the extraction of selenium from the environment to ensure *C. elegans* selenium supply or (ii) the protective selenium sequestration in order to prevent toxicity. In order to decide on the likelihood of either, we exposed wild-type nematodes as well as Y37A1B.5- or R11G10.2-deficient worms to different concentrations of selenite. Selenite appeared to be toxic, shortening *C. elegans* mean life span by 9% and 25% if grown in the presence of 1 µM or 200 µM selenite, respectively. Both Y37A1B.5 and R11G10.2 deficiencies partly rescued selenite-induced life span reduction, suggesting that neither of these putative selenium binding proteins protect against selenium toxicity. The mode of action of Y37A1B.5 and R11G10.2 in modulating *C. elegans* life span remains to be further investigated.
Poster abstracts in alphabetical order (first author)

P086

A combined binary interaction and phenotypic map of C. elegans cell polarity proteins

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The ability to polarize is a fundamental cellular property, required for processes such as cell migration and asymmetric cell division, and for the specification of functionally distinct domains. Several key determinants of cell polarity have been identified, including the Par, Crumbs, and Scribble groups of cortical polarity regulators. However, we know comparatively little of the mechanisms through which cortical polarity is integrated with cellular events such as cytoskeletal rearrangement, organization of a polarized trafficking machinery, and functional specialization of membrane domains. A full understanding of polarity establishment will require a comprehensive knowledge of the proteins involved in this process and the molecular interactions between them. Here, we study the network of physical interactions that underlies polarity establishment in the nematode Caenorhabditis elegans using a combination of large-scale yeast two-hybrid screens and phenotypic profiling. Using a fragment-based yeast two-hybrid strategy, we identified 439 interactions between 296 proteins, as well as the protein regions that mediate these interactions. Phenotypic profiling of the network resulted in the identification of 100 physically interacting protein pairs for which RNAi-mediated depletion caused a defect in the same polarity-related process. We demonstrate the predictive capabilities of the network by showing that the physical interaction between the RhoGAP PAC-1 and PAR-6 is required for radial polarization of the C. elegans embryo. Our network represents a valuable resource of candidate interactions that can be used to further our insight into cell polarization.
The role of the metazoan disaggregase network in prion-like propagation of α-synuclein

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The protein misfolding disease Parkinson’s disease is characterized by the selective loss of dopaminergic neurons in the substantia nigra and the presence of Lewy bodies, neuronal cytoplasmic inclusions consisting mainly of aggregated α-synuclein. α-synuclein aggregates spread via a mechanism similar to prion propagation, key to which is seeded polymerization, the induced misfolding of α-synuclein by an already aberrantly folded species. In yeast, prion propagation depends on the activity of the heat shock protein (Hsp)104/Hsp70 bichaperone system. The disaggregation activity of this machine results in the fragmentation of large prion aggregates into smaller seeds that more efficiently template conversion of new monomers. This activity is crucial for the maintenance of prion aggregates in yeast and also allows their stable inheritance to daughter cells. There is no Hsp104 homolog in metazoans. However, it was recently shown that the constitutive Hsc70, together with an Hsp40 and an Hsp110-family nucleotide exchange factor, was able to efficiently re-solubilize pre-formed α-synuclein fibers in vitro. This project aims to decipher whether the metazoan disaggregate system promotes the propagation of α-synuclein aggregates, analogous to the role of Hsp104 in yeast prion maintenance. For this, we will modulate the levels of the crucial member of the disaggregate system, Hsp110, and test the effect on dissociation and spreading of α-synuclein aggregates in C. elegans by live-cell imaging. To circumvent toxicity correlated to the knockout of essential Hsp110 in worms, we are generating strains expressing hairpin constructs that target Hsp110 for RNA interference in a tissue-specific manner and will judge the impact of tissue-specific Hsp110 knockdown on the age-dependent formation of YFP-tagged α-synuclein aggregates. Moreover, we are employing CRISPR/Cas9-mediated genome editing to tag endogenous Hsp110 to visualize potential co-localization with α-synuclein aggregates. In summary, this project will help to elucidate the role of the metazoan disaggregate network in α-synuclein aggregation and spreading.
Concerning the molecular control of development basal but not derived nematodes share many similarities with outgroups

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Nematodes constitute a speciose phylum. They occupy essentially all ecological niches and show very diverse life histories while sharing a highly conserved Bauplan. Development and its genetic control have been analyzed in detail in C. elegans. Studies on a variety of other nematodes revealed that unexpected variations exist with respect to the cellular patterns of early development. We have started to determine to what extent this diversity is reflected on the molecular level. Here we present some findings from a multispecies cross-phylum genome and transcriptome comparison including data from basal Enoplean nematodes and a nematomorph. These data in conjunction with proteomes from more distantly related outgroups allow us to appraise the gene content of ancestral nematodes and the pattern of gene gain and loss in this phylum. It appears that in comparison to C. elegans basal nematodes possess a developmental toolkit much more similar to arthropod and deuterostome outgroups. Nevertheless, structural and functional peculiarities of nematodes are imprinted in the backbone of the ancestral gene content as well. Our data are in accordance with the view that Developmental System Drift rewired Gene Regulatory Networks active during development and expression patterns of single genes not only between distantly but also between closely related nematode genera. In summary, our findings unravel a large hidden diversity in the genetic control of development among nematodes making it attractive to extend the number of species for a detailed comparative molecular analysis.
Cellular Decision Making During Development: Quantitative Analysis of the P3.p Stochastic Cell Fate Decision

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During development, cells must integrate signals and cues from their environment to obtain their proper cell fate. While most cells follow a highly regulated process leading to a single predetermined fate, there are examples of cellular decision making in which a cell may adopt one of many potential fates in a stochastic manner. In *C. elegans*, one example is the P3.p cell, which will either acquire a vulval precursor cell (VPC) fate, or a hypodermal fate, characterized by fusion of the P3.p to the hyp7 hypodermal cell. The stochastic cell fate decision is regulated by many genes, including the *Wnt* signaling pathway, *lin-39/Hox*, and *eff-1*, a protein required for cell fusion. The current model suggests that variations in a *Wnt* ligand gradient originating in the posterior of the worm provides an initial source of noise and variation that biases the P3.p cell into one of the two fates. However, how the cell interprets this *Wnt* gradient, and how the initial source of noise is amplified and stabilized into a binary decision of one fate is unclear. To understand the stochastic cell fate decision of the P3.p cell, we will use a recently developed time-lapse microscope to quantify the gene expression and protein dynamics of components of the *Wnt* signaling pathway during the cell fate decision. In addition, we will perform single molecule FISH to quantify mRNA levels in these genes. Overall, our quantitative analysis of gene activity and expression will provide an understanding of how cells receive complex information and then transform that information into a binary outcome.
P090

Interfacing ER stress and lipid metabolism with ageing via the adiponectin receptor PAQR-1 in C. elegans

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The metabolic and endocrine functions of adipose tissue as well as the ability of organisms to cope with cellular stress have a direct impact on physiological ageing and the etiology of various diseases such as obesity-related pathologies, neurodegenerative conditions and cancer. The endocrine effects of adipose tissue are mediated by secreted adipose-derived hormones, known as adipokines (e.g. adiponectin), which modulate metabolic processes and influence related maladies. Although a large number of molecules and signaling pathways associate ageing with proteotoxic stress and cellular metabolism, our understanding of how these pathways interconnect to coordinate organismal physiology remains limited. We used the experimental versatility of C. elegans to dissect the mechanisms linking adiponectin signalling pathways and endoplasmic reticulum (ER) proteotoxic stress responses that individually or synergistically affect longevity. We show that PAQR-1 deficient animals respond to ER stress, by rapidly activating the canonical endoplasmic reticulum unfolded protein response (UPRER) pathway. These animals also exhibit induced survival under ER stress or during impaired insulin/IGF-1 signalling. Interestingly, a complex interplay of stress response transcription factors regulates paqr-1(tm3262)-dependent survival and proper induction of ER stress responses. Further, we show that PAQR-1 is involved in lipid droplet turnover in a process that involves UPRER, autophagy and tight regulation of ATGL-1 lipase. Overall, our data demonstrate a link between adiponectin-driven pathways, the UPRER and selective autophagy, with a direct impact in healthspan and lifespan. Elucidation of the molecular specifics that underlie such mechanisms will facilitate the development of strategies for the treatment of relevant age-associated conditions.
Orientation of spindles and cell division planes during development ensures that correct cell-cell contacts are established which is vital for proper tissue formation in many species. Although several signaling pathways in oriented cell division have been well characterized such as wnt/frizzled-based anterior-posterior polarity, there is strong evidence for additional signal pathways controlling early anterior-posterior polarity decisions. Recently, we have identified the homolog of the adhesion G protein-coupled receptor latrophilin, LAT-1, as a novel player in oriented cell division in an anterior-posterior direction of specific blastomeres in the early C. elegans embryo. We have combined in vitro and in vivo approaches to identify the signals required for LAT-1 to mediate correct oriented cell division. We identified a classical G protein-cascade based on coupling of the receptor to a Gs protein which leads to elevated intracellular levels of the second messenger cyclic AMP (cAMP). Consistently, in lat-1 null mutant embryos cAMP concentrations are lower than in wild-type embryos. Upon artificially elevating cAMP levels in these mutants defective embryonic cell division plane orientations caused by LAT-1 absence is corrected. Hence, by regulating cAMP levels LAT-1 controls oriented cell division, most likely via the Gs protein homolog GSA-1. These data indicate that G-protein signaling in oriented cell division is not solely GPCR-independent. To transduce this signal LAT-1 is activated by a tethered agonist within its N terminus. Upon activation by this tethered agonist LAT-1 triggers the cAMP-dependent cascade leading to correct embryonic cell division plane orientations. Additionally, we have obtained evidence for an extracellular protein directly involved in LAT-1 activation. In summary, we have identified a novel signalling pathway essential for oriented cell division in the early embryo dependent on the adhesion GPCR LAT-1 which is mediated by controlling cAMP levels.
P092

A semi-automated EMS screen identifies a postembryonic muscle lineage mutant

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Transdifferentiation is the process by which an already specialized cell is directly converted/reprogrammed into another specialized cell. This naturally not very abundant process is the major focus of our lab. We try to identify and characterize genetic factors that play a role in induced transdifferentiation by mis-expressing transcription factors (TFs). Currently there are several selector genes known that can activate specific cell fates such as hlh-1, the worm homolog of the myogenic bHLH TF MyoD. When mis-expressed, HLH-1 induces muscle fate in early embryonic cells but terminally differentiated cells in older animals are resistant to hlh-1-induced direct reprogramming. In order to identify mechanisms that antagonize selector genes, we apply both forward and reverse genetics. We use transgenic lines allowing ectopic expression of a specific selector gene in combination with the appropriate cell fate reporter. To complement manual microscopic screening, we use a semi-automated high-throughput forward genetics screen combining EMS mutagenesis with the Biosorter system (Union Biometrica). Using this approach we identified a mutant showing additional cells at the posterior end of the pharynx that express a myo-3 reporter. To our surprise, these cells show muscle reporter expression independently of ectopic induction of hlh-1. They appear during the L2 to L3 larval transition, where usually body wall muscle development is already finished. To identify the relevant mutated locus, we used a whole genome sequencing approach and identified a premature STOP in the KASH-domain gene unc-83. We could phenocopy the phenotype using other unc-83 mutant alleles as well as with a mutant allele of unc-84, a SUN-domain containing protein that interacts with UNC-83 to bridge the nuclear lamina with the cytoskeleton. The UNC-83/UNC-84 nuclear envelope bridge was so far not implicated in muscle development. For mutated alleles of these genes, nuclear migration defects of P cells, hyp-7 hypodermal precursors and intestinal polarization defects have been reported – all of which happen during embryonic development. To elucidate the underlying mechanism of the extra body wall muscle cells, we performed lineage ablation studies and we are testing knock downs of cell cycle regulators. This will allow us to identify the mechanism as a missing apoptosis event, an additional cell division or a lineage conversion.
P093

Using microbial rhodopsins as genetically encoded voltage sensors to analyze neuronal networks in C. elegans

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One goal in optogenetics and neurobiology is developing sensors for membrane voltage. The first generation of such sensors could deliver low signals, which typically were in the few % range for large, often artificial voltage steps (Hughes et al, 2012). Via protein engineering some remarkable improvements for FRET-based or single chromophore sensors were achieved. More recently, rhodopsin based sensors were expressed in cell cultures and neurons, using the dim, but voltage dependent fluorescence of all trans retinal, with low-% absolute signals and requiring extremely intense imaging light. In neurons of small invertebrates like C. elegans, which do not even spike, but produce graded potentials of 10-20 mV, such sensors can hardly detect activities. An enhanced rhodopsin based voltage sensor expressed in C. elegans was introduced by Flytzanis et al., 2014 (Archer1). This protein showed 25-40% changes in fluorescence in AWC amphid neurons, however, it still needs very high excitation light intensities. In combination with fluorescent proteins for electrochromic FRET, up to 40% changes in the signal per 100mV were reported for a rhodopsin sensor (AceQ; Gong et al, 2015). Its utility in C. elegans remains to be tested. We explored an alternative approach to enhance rhodopsin based voltage imaging. The Archaerhodopsin (Arch3) proton pump, which hyperpolarizes membranes, can be used as a voltage sensor (Kralj et al., 2011), especially in its non-pumping mutant variants. To increase the absolute fluorescence of the sensor, we sought for synthetic ATR analogs that would support a higher fluorescence, while still preserving voltage sensitivity. Among eight such analogs tested, we identified two that were incorporated into the protein and showed very intense fluorescence when substituted for ATR in Arch3-derivates, or in Mac, a similar rhodopsin proton pump from fungi, expressed in C. elegans body wall muscle cells. We also observed the changes in the fluorescence signals in the so called electrochromic FRET sensors, consisting a rhodopsin as an acceptor in FRET with a fluorescent protein as donor. We tracked signals caused by spontaneous muscle activities as well as signals caused by optogenetically induced membrane depolarization with the actuator Channelrhodopsin expressed either in cholinergic neurons or body wall muscles. The observed signals increased and decreased reciprocally in dorsal and ventral muscles, as expected for muscle-induced body bending. Besides expression in body wall muscles Arch3 was also expressed in pharynx and pumping was triggered with serotonin. Voltage signals >50%, could be observed during pharynx contraction. A fluorescent voltage reporter would be highly useful to analyze neuronal networks in the generation of behavior, and may even enable closed-loop optogenetics, with a direct readout of optogenetically imposed voltage changes in the neuron of interest.
Mapping of natural genetic variation in C. elegans dauer formation

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Understanding the composition of complex traits can help resolve a long standing question in evolutionary biology, the inconsistency of intraspecific variation and interspecific divergence, with higher degree of phenotypic difference at the population than species level. The dauer stage of nematodes represents an interesting case study for addressing the evolution of complex traits, since it is a phenotype conserved across nematode species but with a high degree of intraspecific variation. Although several studies have attempted to explain the phenotypic variation in several nematode species, the resolution rarely reaches the level of genes. In order to find out the genetic basis underlying the observed variations in the dauer formation in C. elegans, we constructed 144 recombinant inbred lines of two natural isolates differing substantially in their ability to form dauer, searching for genetic variants that could explain the phenotypic differences between the parental strains. QTL mapping uncovered a single large-effect QTL and further fine mapping narrowed down the QTL region to a target interval of 0.7Mb. The resemblance of F1 progeny to one parent that forms less dauers indicates a complete dominance of the heterozygote, thus providing clues into the modes of genetic interaction of parental. Furthermore, we plan on including whole genome sequenced natural isolates of C. elegans in order to understand the frequency and distribution of the genetic variants and its contributions to the phenotypes of natural isolates. By breaking down the basis of the complex trait, we could identify genetic variants that result in the phenotypic variation at the population level and understand the selection forces acting on them.
P095

Distinct nuclear architecture in the germ line founder cell of the C. elegans embryo

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Cells belonging to the germ lineage segregate physically and molecularly from their somatic neighbors during embryogenesis. Recently, we have found using light and electron microscopy that the chromatin organization in the P4 germ line founder cell of the early C. elegans embryo is distinct from that in the neighboring somatic cells. The ultrastructure of individual chromatin domains does not differ between germ line and somatic cells, pointing to a specific organization mainly at the level of the whole nucleus. This unique organization is characterized by a greater chromatin compaction and an expansion of the interchromatin compartment. Imaging of living embryos expressing fluorescent markers for both chromatin and germ line P granules revealed that the appearance of a distinct chromatin organization in the germ line founder cell occurs approximately 10 min after its birth and coincides with the aggregation of P granules around the nucleus, suggesting a possible link between these two events. These features are now being investigated using STED super-resolution microscopy. Initial results show that the LMN1 lamin protein and RNA polymerase II accumulate in the expanded interchromatin compartment of the P4 blastomere. This accumulation might play a role in shaping the chromatin organization in the germ line founder cell of the C. elegans embryo. The putative link between nuclear architecture and totipotency is discussed.
Investigating stress granule insolubility with age

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A growing number of familial and sporadic forms of neurodegenerative diseases show pathological aggregation of RNA-binding proteins featuring low-complexity domains. In a healthy organism, low-complexity domains allow RNA-binding proteins to promote the assembly of ribonucleoprotein (RNP) granules. Strikingly, new data from in vitro studies reveals that the dynamic nature of RNP granules is halted with time concomitant with fibril formation by RNA-binding proteins (Lin et al, 2015; Molliex et al, 2015; Murakami et al, 2015; Patel et al, 2015). Therefore, the question arises whether the special proprieties of RNP granules put RNA-binding proteins with low complexity domains in general at risk of aggregating in vivo and not just in the context of disease. Our recent work reveals that two key stress granule RNA-binding proteins harboring low-complexity domains, PAB-1 and TIAR-2, aggregate with age forming insoluble puncta in C. elegans in the absence of disease. We found that stress granule protein aggregation is influenced by aggregation of other inherently aggregating proteins and that the formation of stress granules might provide a seed for aggregation. Delaying aging through dietary restriction, mitochondrial inhibition or reducing insulin/IGF-1 signaling prevents RNP granule protein insolubility with age. The activity of the transcription factor HSF-1 is necessary during development to prevent PAB-1 aggregation in wildtype animals and in long-lived animals with reduced insulin/IGF-1 signaling. RNP granules are normally highly dynamic structures yet our results suggest that aging is a sufficient stress to cause the irreversible aggregation of RNP granule proteins, which could impair the function of RNP granules. As stress granule proteins have been identified as minor components in pathological RNA-binding protein aggregates, we predict that our findings will be relevant in the context of disease.
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Cell-to-cell communication modulates the immune response in *Caenorhabditis elegans*

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*C. elegans* is widely used as a model organism to dissect host innate immune responses to bacterial and fungal pathogens. Like other multicellular organisms, *C. elegans* specifically responds to infection by upregulating the expression of defense genes. Increasing evidence suggests that cell-cell communication is essential to generate coordinated protective responses (reviewed in 1). For instance, Kawli and Tan demonstrated that neuroendocrine signals modulate the expression of intestinal defence genes through the *daf-2/daf-16* insulin-signalling pathway (2), whilst neuronally produced TGF-$\beta$ regulates expression of antimicrobial peptides in the epidermis (3). The expression of one insulin-like peptide gene, *ins-11* is induced upon intestinal infection by the bacterial pathogen *Serratia marcescens* and fungal pathogen *Dechmeria coniospora*, which infects epidermis. We have now found that these infections specifically induce *ins-11* expression in intestine or epidermis respectively. This insulin peptide gene therefore behaves differently from many genes involved in the innate immune response, since comparative genomic analyses indicated that half of the genes up-regulated by *D. coniospora* infection are down-regulated upon *S. marcescens* infection (4). We are currently investigating the role of *ins-11* on pathogen-specific and tissue-specific defence gene expression. We aim to take a genetic approach to dissect the mechanisms underlying cross-tissue signalling and to study how a master tissue is capable of sensing perturbations within its own cellular environment and subsequently mediating organism-wide protection on other tissues upon infection. 1. Ewbank and Pujol, Current Opinion in Immunology 38:1-7, 2016 2. Kawli and Tan, Nature Immunology 9: 1415-1424, 2008 3. Zugasti and Ewbank, Nature Immunology 10: 249-256, 2009 4. Engelmann et al., PLoS ONE 6(5): e19055, 2011
Antioxidant activity of liquorice in *C. elegans* – a hormetic action?

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Liquorice (*Glycyrrhiza* spp.) is a well known medicinal plant in European and in Asian medicine. It is best known for its anti-inflammatory activity, but also neuroprotective, anti-adiposity, and anti-cancer effects have been reported. These bioactivities are accompanied by an antioxidant effect. In the present study this antioxidant property and its underlying mechanisms were further studied in the model organism *Caenorhabditis elegans*. A methanolic extract of *G. uralensis* roots and its major compounds glycyrrhizic acid, glycyrrhentic acid, liquiritigenin, and isoliquiritigenin were tested for their ability to reduce oxidative stress in a *C. elegans* strain expressing a *hsp-16.2p::GFP* construct. GFP fluorescence intensity in this strain indicates the stress level, that was most effectively reduced by isoliquiritigenin (26% reduction). This compound could also activate the transcription factor DAF-16, that regulates the expression of stress related genes and lifespan in *C. elegans*. However, in a lifespan assay the same compound rather reduced the median lifespan of wildtype worms by 7.3 days, contrary to the expectations based on the former results. The results indicate that the most active antioxidant compound is isoliquiritigenin, but a long term treatment with this substance shows its toxicity. Many phytochemicals are produced to protect the plants from herbivores, which in return protect themselves against the toxicity by innate defence mechanisms. These are controlled by transcription factors like DAF-16. Therefore, the initial protective activity against oxidative stress via DAF-16 activation may be a result of hormesis.
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The depletion of the mitochondrial prohibitin complex in conditions leading to opposing longevity phenotypes has differential effects in the C. elegans metabolome.

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The mitochondrial prohibitin complex, composed of two proteins, PHB-1 and PHB-2, is a context-dependent modulator of longevity. Specifically, prohibitin deficiency shortens the lifespan of otherwise wild type worms, while it dramatically extends lifespan under compromised metabolic conditions, as is the case of insulin-receptor daf-2(e1370) mutants. This extremely intriguingly phenotype has been linked to alterations in mitochondrial function and in fat metabolism. However, the true function of the mitochondrial prohibitin complex remains elusive. 1H-NMR spectroscopy analysis of the effect of prohibitin depletion on the metabolome of wild type animals and daf-2(e1370) mutants revealed changes in carbohydrate and amino acid metabolism, being the effect stronger in wild type worms than in daf-2 mutants. We now extended the coverage of our metabolic analysis using a mass spectrometry (MS)-based metabolomic approach. The depletion of prohibitin in wild-type animals perturbs a high number of metabolites belonging to different metabolic pathways such as arginine and proline metabolism, pyrimidine metabolism, glyoxylate and dicarboxylate metabolism, glycerophospholipid metabolism and the pentose phosphate pathway. On top of this, it also reveals that prohibitin depletion in either wild type animals or daf-2 mutants leads to a differential effect on different metabolic pathways, namely, on riboflavin metabolism, glycerophospholipid metabolism and glutathione metabolism, among others. Furthermore, several other metabolites, including different lipid species and different types of peptides, are found to have a differential effect upon prohibitin depletion. Our ultimate goal is to pinpoint the metabolic pathways, and more specifically the players, that might be involved in how mitochondrial prohibitin complex affects longevity. We are currently exploring the relevance of identified metabolites in the context of the effect of prohibitin on the C. elegans longevity.
Impaired removal of H3K4 methylation affects cell fate determination and gene transcription

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Methylation of Histone 3 Lysine 4 (H3K4) is largely associated with promoters and enhancers of actively transcribed genes and it is finely regulated during development by the action of histone methyltransferases and demethylases. H3K4me3 demethylases of the KDM5 family have been previously implicated in development, but the contribution of their catalytic activity in cell fate determination and differentiation has not been investigated. Moreover, how the regulation of H3K4me3 level controls developmental processes is not fully established. Here, we show that the H3K4 demethylase RBR-2, the unique member of the KDM5 family in C. elegans, acts cell-autonomously and in a catalytic-dependent manner to control vulva precursor cell fate acquisition, by promoting the LIN-12/Notch pathway. Using genome-wide approaches, we show that RBR-2 reduces the H3K4me3 level at transcription start sites (TSSs) and in regions upstream the TSSs, and acts both as a transcription repressor and activator. The analysis of the lin-11 genetic locus, a direct RBR-2 target gene required for vulva precursor cell fate acquisition, shows that RBR-2 controls the epigenetic signature of the lin-11 vulva-specific enhancer and lin-11 expression, providing in vivo evidences that RBR-2 can positively regulate transcription and cell fate acquisition by controlling enhancer activity.
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Molecular details of tissue-specific alternative splicing by MEC-8

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The regulated expression of splicing factors during development enables the generation of tissue-specific isoforms via the process of alternative splicing. We have investigated the molecular details of pre-mRNA binding by several splicing factor proteins involved in both nematode muscle and neuron biology. We have previously studied the atomic details of RNA-binding by the muscle-specific SUP-12 protein which has led to a series of in vivo splicing assays in live worms using a fluorescent mini-gene two-colour reporter system built on the isoform regulation of the EGL-15 fibroblast growth factor receptor. Using the same approach in neurons, we have now investigated the association of the MEC-8 splicing factor with mec-2 pre-mRNA. The first RNA recognition motif (RRM) domain of MEC-8 is homologous to the RRM domains from Drosophila couch potato and the human protein RBPMS (RNA-binding protein with multiple splicing). This RRM domain dimerizes and we have found that it is specific to a double GCAC RNA motif. We have discovered that the second RRM domain also contributes to RNA-binding. Apart from roles in early development, MEC-8 is integral to worm mechanosensory function via the regulation of the mec-2 gene. Recent crystal structures have revealed the key elements in the formation of the RNA-bound MEC-8 protein, and we have determined the critical elements of the RNA sequences bound by MEC-8 in target pre-mRNA such as mec-2. These and other studies continue to build a molecular-based understanding of tissue-specific alternative splicing which we have coupled with in vivo assays and strategic perturbation of protein-RNA interaction.
An automated phenotype-based microscopy screen to identify pro-longevity interventions acting through mitochondria in C. elegans

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Mitochondria are multifunctional organelles that play a central role in cellular homeostasis. Severe mitochondrial dysfunction leads to life-threatening diseases in humans and accelerates the ageing process. Surprisingly, moderate reduction of mitochondrial function in different species has anti-ageing effects. High-throughput screenings in the nematode Caenorhabditis elegans lead to the identification of several pro-longevity genetic and pharmacological interventions. Large-scale screens, however, are manual, subjective, time consuming and costly. These limitations could be reduced by the identification of automatically quantifiable biomarkers of healthy ageing. In this study we exploit the distinct and reproducible phenotypes described in C. elegans upon different levels of mitochondrial alteration to develop an automated high-content strategy to identify new potential pro-longevity interventions. Utilizing the microscopy platform Cellomics ArrayScan Reader, we optimized a workflow to automatically and reliably quantify the discrete phenotypic readouts associated with different degrees of silencing of mitochondrial respiratory chain regulatory proteins, and validated the approach with mitochondrial-targeting drugs known to extend lifespan in C. elegans. Finally, we reported that a new mitochondrial ATPase modulator matches our screening phenotypic criteria and extends nematode's lifespan thus providing the proof of principle that our strategy could be exploited to identify novel mitochondrial-targeted drugs with pro-longevity activity.
Hypoxia and the hypoxic response pathway negatively regulate EGFR/RAS/MAPK signaling in several tissues

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During vertebrate embryogenesis or in growing tumors, the hypoxic response regulates adaptation to low oxygen concentrations by switching from an aerobic to an anaerobic metabolism (Warburg effect) and by inducing angiogenesis through VEGF secretion. Many studies have focused on the effect of tumor hypoxia on surrounding cells. However, how cancer cells carrying mutations in the oncogenic RAS/MAPK pathway respond to hypoxia is less understood. A deeper insight into the effects of hypoxia on RAS/MAPK signaling might allow us to control the switch from benign to malignant tumors by counteracting cancer cell-autonomous responses to hypoxia. We have previously used a quantitative genetic approach to identify polymorphic modifiers of EGFR/RAS/MAPK signaling in C. elegans Among the genes identified were a calpain homologue (F44F1.1) and the homologue of the VHL binding protein VBP (pfd-3), both of which suppress an egl-9 phenotype. Since vhl-1/VHL and egl-9/EGLN1/2/3 act in the highly conserved hypoxia response pathway in modifying the hypoxia-inducible factor HIF-1 we assume hypoxic signaling modifies EGFR/RAS/MAPK signaling. We have investigated the effect of hypoxia and hypoxic signaling on the RAS/MAPK pathway in several tissues. Hypoxic treatment of let-60 ras(gf) animals significantly reduces RAS/MAPK signaling during vulval induction, duct cell fate determination and during pachytene exit of germ cells, while a If mutation in the hypoxia-inducible factor hif-1 increases RAS/MAPK signaling even under normoxic conditions. On the other hand, a If mutation in the prolyl hydroxylase egl-9, which induces the degradation of HIF-1, reduces EGFR/RAS/MAPK signaling. Interestingly, we have evidence for a HIF-1 independent hypoxic effect on RAS/MAPK signaling, probably via another EGL-9 substrate. Moreover, an EGL-9::GFP transgene shows strong expression in 2° VPCs, whereas the descendants of the 1° VPC only weakly express EGL-9::GFP. Since RAS signaling is downregulated by NOTCH signaling in 2° VPCs, we suspect that EGL-9 interacts with the NOTCH pathway to regulate RAS/MAPK signaling. We are currently investigating the link between the NOTCH pathway and EGL-9.
Enzymes of the transsulfuration pathway in *C. elegans*

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Enzymes of the transsulfuration pathway, namely cystathionine beta-synthase (CBS) and cystathionine gamma-lyase (CTH) are involved in conversion of homocysteine to cysteine. In addition, these enzymes can catalyze the formation of hydrogen sulfide, an important gaseous signaling molecule. *C. elegans* possesses CBS-1 enzyme with confirmed CBS activity and three enzymes with predicted CTH activity. In an ongoing study we have purified and further characterized the following nematode cystathionase enzymes: CTH-1, CTH-2 and CBL-1. Production of aminothiols cysteine and homocysteine was detected by HPLC analysis with fluorescent detection, the thioethers were detected by LC-MS/MS. We observed that only nematode enzyme CTH-2 is able to catalyze α- and γ-elimination of cystathionine to yield cysteine, α-ketobutyrate and ammonia. We have also determined the activity of CTH-2 enzyme in alternative reactions leading to production of hydrogen sulfide and to generation of the thioethers lanthionine and homolanthionine. RNA interference of the *cth-2* gene in *C. elegans* results in elevated levels of cystathionine and decrease levels of cysteine, however we did not observe any effect on the lifespan of *cth-2* iRNA worms. All worm cystathionase orthologs (mainly CTH-1 and CTH-2) were found to catalyze also reverse transsulfuration reactions which use cysteine as the sulfur donor and homoserine or O-sucinylhomoserine to yield cystathionine. In our study, we have shown broad substrate flexibility of nematode cystathionase enzymes and identified the pivotal role of CTH-2 protein in metabolism of cystathionine and other sulfur amino acids in *C. elegans*. This work was supported by the grant LD 14082 from the Ministry of Education of the Czech Republic and by projects PRVOUK P24 from Charles University and 16-30384A from the Czech Health Research Council.
Electrophysiological models of C. elegans neurons for the FP7 Si elegans European Project

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The C. elegans nematode has been widely studied over recent decades, as a consequence of having one of the simplest and well characterized Biological Nervous Systems (BNS). The European Si elegans project offers an open-access framework designed to unravel the C. elegans' nervous system function and behavior via hardware emulation and a virtual behavioral arena. A Virtual Arena provides a realistic sensory input to the Si elegans hardware and allows users to control and visualize the simulation. Field Programmable Gate Arrays (FPGAs) as a high performance computing platform offer the required flexibility to model nervous system function and muscle actuation of the worm on hardware. The Si elegans platform has been designed for continuous improvement by its users, particularly in terms of neuron model fidelity upon availability of new or more detailed knowledge of how C. elegans neurons process information. Although all C. elegans neurons are known and its connectome has been fully mapped, it is difficult to gain a deeper understanding of neural and synaptic properties through electrophysiological recordings: the cell bodies are about 2 um in diameter and protected by a pressurized cuticle. Nonetheless, electrophysiology is the only technique for recording currents, studying voltage transients or single action potentials. Up to now, only a small number of recordings have been obtained in very specific neurons such as AVA, ASER and RMD neurons. These neurons display isopotential behavior and the recordings do not show classical action potentials. As a result, classical mathematical methods to model neuronal responses such as the integrate-and-fire or the Hodgkin-Huxley models are not able to fully replicate the properties of C. elegans neurons. Within the Si elegans project we have designed new mathematical models that replicate the plateau potentials observed in the worm's neurons. Three models are presented that are able to simulate the voltage changes in AVA, ASER and RMD neurons as described in the literature. Such models provide the Si elegans platform with a more realistic base to simulate the behavior of the worm, which can be expanded as new electrophysiological data become available in the future.
The Glyoxylate Cycle is part of the *C. elegans* stress response.

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The Glyoxylate Cycle, an anabolic shunt of the tricarboxylic acid cycle, converts fatty acids into succinate for the synthesis of carbohydrates. When carbohydrate sources are depleted, the glyoxylate cycle can use fatty acids for replenishing the carbohydrate pool through gluconeogenesis. This cycle possesses two key enzymes which are isocitrate lyase (ICL) and malate synthase (MS). In *C. elegans*, ICL and MS activities are restricted to one bifunctional polypeptide. Two genes encode for this polypeptide, *icl-1* and *C08F11.14*. *icl-1* is upregulated in stress resistant worms, such as *daf-2* mutants or dauers. It is also known that *icl-1* acts downstream of *daf-16*, *nhr-49* and probably also *pha-4* and *skn-1*. All of these transcription factors are related with stress responses and longevity. In this study, we show that glyoxylate activity is necessary, at least partially, to respond to several stresses, as *icl-1;C08F11.14* double mutants are less stress resistant than wild type or the single mutants. We also found that both single *icl-1* and *C08F11.14* mutants show a slight increase in longevity not present in the double mutant. These data seem to reinforce the idea that the Glyoxylate Cycle plays an important role on stress responses and longevity pathways on *C. elegans*.
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A novel C. elegans melatonin receptor is required for efficient pathogen clearance

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C. elegans defends itself from infection with pathogenic microbes by activating conserved signalling pathways that play central roles in the host defences of mammals and insects. Although significant advances have been made in understanding how these pathways protect C. elegans the cell surface receptors that activate them during infection remain largely elusive. G-protein coupled receptors (GPCRs) detect the presence of bacterial pathogens and regulate immune responses in worms, flies, mice and humans. Here I present data characterising the role of an orphan GPCR, F59D12.1, in the clearance of Microbacterium nematophilum infections from C. elegans. We have previously shown that EGL-30(Gαq) signalling is required for the C. elegans response to infection with M. nematophilum. F59D12.1 acts upstream of, and in parallel to, this EGL-30(Gαq) signalling pathway in epithelial cells to regulate pathogen clearance. Genetic and pharmacological data identify melatonin as the ligand for F59D12.1. Melatonin synthesis in C. elegans is regulated by infection and melatonin signalling via F59D12.1 is required for efficient clearance of M. nematophilum infections. To our knowledge this is the first identification of a C. elegans melatonin receptor and the first demonstration of a role for melatonin signalling in C. elegans. In mammals pathogen clearance is mediated by macrophages. We find that mammalian macrophages express orthologs of F59D12.1 suggesting that melatonin may be an evolutionarily conserved regulator of host defences. We are currently working to determine whether activation of mammalian melatonin receptors can enhance macrophage phagocytosis to promote pathogen clearance.
Identification of UNC-120/SRF as a determinant of muscle aging in Caenorhabditis elegans

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Aging is accompanied by a progressive loss of muscle mass and function termed sarcopenia. In human, sarcopenia is responsible for a decrease in mobility, leading to a reduction in the quality of life. Several cell autonomous mechanisms have been proposed to be involved in muscle aging including mitochondria default [1], apoptosis [2] and alteration of muscle protein turnover [3]. However, those data have been essentially obtained in models of experimentally induced muscle atrophy. Thus the question of their importance in the context of physiological aging remains unanswered. We are using C. elegans to identify genetic pathways involved in muscle aging. Previous studies have shown that worms exhibit loss of mobility [4], myosin filament disorganization and change in muscle nuclei shape with age [5]. More recently, Liu et al. also reported a decline in motor neurons function with age [6]. We first aimed to further investigate the time course of cellular and molecular changes that take place during muscle cells with age. We showed that muscle aging is firstly characterized by a decrease in the expression of some but not all muscle genes, followed by a change in mitochondria morphology and an impairment of muscular proteostasis. Further genetic, cellular and molecular investigations identified the transcription factor UNC-120/SRF as both a determinant of muscle aging and an effector of the DAF-2/insulin-IGF-1 pathway, which function may be conserved in mammals. 1. Romanello V et al. EMBO J. 2010;29: 1774–1785. 2. Marzetti E et al. Gerontology. 2012;58: 99–106. 3. Masiero E et al. Autophagy. 2010;6: 307–309. 4. Huang C et al. Proc Natl Acad Sci U S A. 2004;101: 8084–8089. 5. Herndon LA et al. Nature. 2002;419: 808–814. 6. Liu J et al. Cell Metab. 2013;18.
The circadian clock and neurodegeneration: temperature cycles and protein aggregate formation in C. elegans

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The Si elegans project - A C. elegans GUI-based neuron and neural network capture, modelling, FPGA hardware implementation and simulation results viewer.

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The European Si elegans project aims to emulate the C. elegans nervous system in accelerated FPGA (Field Programmable Gate Array) hardware, while providing an accessible experiment definition and results review interface to its end-users. The authors present an open-access web-based platform that includes a Graphical User Interface (GUI) for capturing neuron, synapse and neural sub-component models; C. elegans network definition; generation and simulation of LEMS (Low Entropy Model Specification) language models; synthesisable VHDL (Hardware Description Language) generation; FPGA hardware implementation; and review of hardware simulation results. The toolsuite aims to aid neuroscientists in their investigation of the workings of the C. elegans nervous system, by providing a rapid and user-friendly C. elegans model to hardware simulation and results review for hypothesis evaluation. The user-friendly, drag-and-drop GUI allows users to select, extend and execute pre-compiled neuron models, including NeuroML library components [1], and to create or edit custom neuron models, and share these with other platform users. The toolsuite enables construction and population of neuron models into the C. elegans connectome. The platform also auto-builds and implements the FPGA configuration files and metadata for rapid evaluation of C. elegans neuron, neural network and phenotypic behaviour hypotheses on high-performance hardware. The parallel nature of FPGA hardware allows for the concurrent execution of large models comprising multiple components in a fast and efficient manner. User registration provides access for users to create and maintain Si elegans experiments. The LEMS language source can be extracted and downloaded for any graphically constructed neuron or synapse model for further exploration in the jLEMS simulator or for export to NEURON simulations. The Si elegans neuron-model library contains a broad range of parameterisable neuron, synapse and ion-channel models. Prior to executing the experiment on the Si elegans FPGA hardware platform, the user may select the neuron variables to be recorded. On completion of the FPGA hardware neural network simulation, neuron and neural network simulation results are viewable in a graphical results viewer and are available to download for further analysis. For further information and to request an account, please visit [2]. Acknowledgements: This project is funded by the EU’s 7th Framework Programme for research, technological development and demonstration under grant agreement no 601215, FET Proactive, call ICT-2011.9.11: Neuro-Bio-Inspired Systems (NBIS). References: [1] Gleeson, P., Crook, S., Cannon, R., Hines, M., Billings, G., Farinella, M., Morse, T., Davison, A., Ray, S., Bhalla, U. and Barnes, S., “NeuroML: a language for describing data driven models of neurons and networks with a high degree of biological detail”. PLoS Comput Biol, 6(6), 2010. [2] www.si-elegans.eu
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Optogenetic control of cGMP mediated signal transduction

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Cyclic guanosine monophosphate (cGMP) is a widely used 2nd messenger in cellular signaling, acting via protein kinase G (PKG), or cyclic nucleotide gated (CNG) channels. In sensory neurons, cGMP allows for signal modulation and amplification, before depolarization. Manipulating cGMP levels is required to access this signalling and provide insights into signal encoding. We achieve this by implementing two photo-activatable guanylyl cyclases - 1) guanylyl cyclase rhodopsin from Blastocladiella emersonii (BeCyclOp) and 2) a mutated version of Beggiatoa sp. bacterial light-activated adenylyl cyclase (BlaC), with specificity for GTP, termed BlgC or bPGC (Beggiatoa photoactivated guanylyl cyclase). BeCyclOp enabled rapid and precise light-triggered cGMP increases in heterologous cells (Xenopus oocytes) and in C. elegans. BeCyclOp exhibits an unusual 8 transmembrane topology and cytosolic N-terminus. Oocyte experiments revealed a light/dark activity ratio of \approx 5,000 and no cAMP production. Via co-expression of the TAX-2/-4 CNG channel in C. elegans body wall muscle, BeCyclOp photoactivation induced rapid light-driven depolarization and contraction of muscle cells. In C. elegans O\textsubscript{2}/CO\textsubscript{2} sensory BAG neurons, BeCyclOp activation rapidly triggered slowing of locomotion, consistent with the normal sensory function of BAG, and in agreement with previous BAG activation by channelrhodopsin (ChR2). Interestingly, a quick ‘recovery’ of the slowing response was observed both in ChR2 and BeCyclOp stimulation, despite ongoing photostimulation, arguing that this apparent desensitization is neither mediated at the level of cGMP nor the CNG channel, but at the output synapses or in downstream networks. Light activation of bPGC expressed in muscle cells along with TAX-2 and TAX-4 caused a relatively slower and less pronounced contraction as compared to BeCyclOp. We could validate these kinetic and magnitude differences in cGMP production from the two cyclases by directly imaging the cGMP rise, using a genetically encoded cGMP sensor, WincG2. WincG2 (or worm indicator of cGMP) is based on FlincG3, a circularly permutated EGFP fused to cGMP binding domain of PKG. As an outlook, we are expressing the cyclases in a variety of C. elegans sensory neurons that use cGMP as the 2nd messenger and aim to perform behavioural experiments that recapitulate cGMP mediated signal transduction in these sensory neurons using optogenetic activation of the cyclases.
Screen of Naturally Originated Anti-Obesity Drug Leads Using C. elegans as a Versatile Platform

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Serving as crucial components to a human diet, Oligomeric proanthocyanidins (OPCs, degree of polymerization ≤ 5) have been shown to be effective in improving lipid homeostasis in rodents and humans. The effectiveness of OPCs as an anti-obesity treatment has been tested but poorly described due to the vague understanding of their complex composition. An in vivo study using purified OPCs is yet to be elucidated, a shortfall we aimed to address here. Owing to the high-throughput and efficient capacity, Caenorhabditis elegans is well positioned to advance the knowledge of OPCs. This effort has been accelerated enormously by versatile biochemical, biophysical and genetic tools available for nematode study. OPC compounds (drug #1 to drug #8) were purified by a two-dimensional chromatography system (silica liquid chromatography X reversed-phase high performance liquid chromatography). Their structures were confirmed by mass spectrometry, presenting a full range of OPC dimer, trimer, tetramer and their galloylated forms (dimer gallate, trimer gallate and dimer di-gallate). The flat volumetric surface area decreased in nematodes exposed to OPCs, an effect that was most significant with trimeric OPCs and gallates. Lipase was strongly inhibited in vitro accompanied by the reduction of total triglyceride storage capacity in vivo. Lipophilic staining was attenuated in wild type worms and high-fat mutants exposed to OPCs, which was most prominent in the presence of a trimeric OPC scaffold. Although no anorexia mechanism was observed, OPCs increased the pharyngeal pumping and reduced defecation rates of nematodes, possibly due to an energy compensation mechanism. Trimeric proanthocyanidin gallate in particular decreased lipid saturation and enrichment in C. elegans, revealed by spectral interferometric polarization coherent anti-Stokes Raman scattering. The positive regulation of nhr-49, a key regulator of fat metabolism, and the down regulation of fat-5 and acs-2 was revealed by microarray and validated by RT-qPCR at the transcriptional level. This is the first comprehensive in vivo investigation of pure OPCs of various DPs and galloylation. By using the model nematode C. elegans, we provide an account of the composition, physiological benefits, structure-bioactivity relationship and genetic mechanisms of OPCs. Our results suggest that developmental toxicity and appetite suppression were not induced by OPCs, but the mechanism is proposed to be closely linked to triglyceride homeostasis, namely inhibition on lipase, stimulation on β-oxidation and targeting of Δ9 desaturases. The anti-oxidation potency and their regulation over cholesterol and insulin metabolism may also contribute to OPCs role in lipid regulation. OPC gallate and trimer moieties seem to be crucial features in the
regulation of lipid metabolism in nematodes, likely by accelerating their catabolism via an nhr-49/fat-5/acs-2 related pathway.
A major, yet poorly understood theme in the field of proteostasis is that protein quality control mechanisms in multicellular organisms are communicated between different tissues, rather than being confined within cellular borders. For example, in C. elegans, we found that locally reduced expression of the molecular chaperone hsp90 (daf-21) in the intestine signals an induction of the protective HSR in the bodywall muscle. This inter-tissue signalling ensures that a local imbalance of proteostasis is responded by a compensatory activation of chaperone expression in adjacent tissues to confer systemic protection of the entire animal. However, the mechanism of transcellular chaperone signalling is not understood and the identity of transcellular signalling molecules is currently unknown. To determine cellular components that mediate this inter-tissue response, we analysed organism-wide transcriptional changes upon a tissue-specific imbalance of hsp90 chaperone expression, using RNA-seq. We have identified a set of innate immune peptides that are specifically up-regulated in response to a chaperone imbalance in the neurons, the intestine and the bodywall muscle. Our data suggest that these innate immune peptides may function as mediators of transcellular chaperone signalling and are novel regulators of the proteostasis network.
Mechanisms behind temperature dependence of developmental timing

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C. elegans progresses through four larval stages from hatching to adulthood. The larval stages are separated by molts, that entail a series of events including the formation of a new cuticle and escape from the old one, cessation of pharyngeal pumping and behavioral quiescence. Temperature regulates the speed of embryonic and post-embryonic development in many ectothermic organisms. The effect of temperature on embryonic development in C. elegans and Drosophila, and on postembryonic development in Drosophila have been described in detail\textsuperscript{1,2}. However, a quantitative analysis of the entire larval development program at different temperatures has never been shown for C. elegans. We have measured temperature dependence of development between 10.3 °C and 27.5 °C, using a high-throughput method that we recently described\textsuperscript{3}. At lower temperatures, the speed of development increases with temperature in an exponential manner following the Arrhenius equation. At higher temperatures, however, the speed of development deviates from Arrhenius. We have investigated the implication of thermosensory pathways in the control of developmental speed. The AFD thermosensory neuron regulates the heat shock response (HSR) through the activation of the heat shock factor-1 (HSF-1)\textsuperscript{4}. AFD genetic ablation and hsf-1 RNAi knockdown provoked a reduction of the speed of development even at lower temperatures. These results suggest that the AFD thermosensory system contributes to regulation of the speed of development. 1. Powsner, L. The Effects of Temperature on the Durations of the Developmental Stages of Drosophila melanogaster. Physiological Zoology 8, 474–520 (1935). 2. Begasse, M. L., Leaver, M., Vazquez, F., Grill, S. W. & Hyman, A. A. Temperature Dependence of Cell Division Timing Accounts for a Shift in the Thermal Limits of C. elegans and C. briggsae. Cell Reports 10, 647–653 (2015). 3. Olmedo, M., Geibel, M., Artal-Sanz, M. & Merrow, M. A High-Throughput Method for the Analysis of Larval Developmental Phenotypes in Caenorhabditis elegans. Genetics 201, 443–448 (2015). 4. Tatum, M. C. et al. Neuronal Serotonin Release Triggers the Heat Shock Response in C. elegans in the Absence of Temperature Increase. Current Biology 25, 163–174 (2015).
Optogenetic analysis of a peptidergic neuronal network controlling a food related navigation behavior

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Finding a food source or avoiding areas of imminent danger and maintaining this vicinity is a crucial survival strategy. Thus, it is of interest to elucidate how neuronal circuits integrate multiple sensory cues to navigate the environment. We functionally analyzed such a neuronal network, as well as the signaling molecules controlling body posture and locomotion. Using an unbiased optogenetic approach, we investigate the functionally uncharacterized neuropeptidergic AVK interneurons. Blocking of neuropeptide release using Halorhodopsin (NpHR) cell-specifically expressed in AVK, caused a slowing of locomotion and increased body curvature. In the absence of food, AVK continuously releases the neuropeptide FLP-1, which evokes inhibition in a subset of target motor neurons, maintaining a low body curvature and promoting ‘long-range search’ behavior. Conversely, when FLP-1 release is inhibited, behavior reminiscent of ‘local search’ is imposed. We identified the NPR-6 neuropeptide receptor to be required for these effects. By Ca\textsuperscript{2+} imaging we show, that NPR-6 is needed for FLP-1-mediated inhibition of VC neurons. However, NPR-6 has low affinity for FLP-1, as shown by a cell-based assay, and is not mediating the inhibitory effects of synthetic FLP-1 peptides at the neuromuscular junction, as measured by electrophysiology. We identified other neuropeptides and peptide receptors essential for the behavioral response to AVK inhibition, by mRNA profiling of AVK cells, and by GPCR-neuropeptide affinity assays. We assess the possibility that a cascade of several peptide-signaling molecules mediates AVK’s effect on body curvature. AVK receives extensive synaptic input from the food sensing dopaminergic PDE neurons and FLP-1 release from AVK is increased in cat-2 mutants. Reminiscent of AVK photoinhibition, locomotion was strongly altered by the presence of food and by external application of dopamine. Thus, dopamine may inhibit AVK function. PDE neurons also innervate and modulate the proprioceptive interneuron DVA. Photoexcitation and -inhibition of this neuron increase and reduce body curvature (contrary to AVK) via the release of excitatory NLP-12 neuropeptides onto motor neurons. Thus, AVK and DVA act in parallel to affect locomotion. Both interneurons are coupled to SMB head motor neurons and eliminating SMB, or gap junctions between AVK and SMB, phenocopied effects of AVK ablation on bending angles. Furthermore, NPR-6 is also required in SMB neurons to mediate AVK inhibition effects. In sum, we identified a neuronal network involving AVK interneurons, which could promote the food-dependent behavioral state switching from ‘local search’ to ‘long-range search’ behavior, via the release of FLP-1 peptides.
Ectopic fat deposition contributes to ageing-associated pathology in C. elegans

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Ageing is accompanied by impairment of tissue-regenerative capacity as well as age-dependent collapse of lipid homeostasis resulting in spillover of lipids and increased fat accumulation in non-adipose tissues and organs. This is known as ectopic fat and it is implicated in the development of metabolic syndrome leading to a higher risk of age-associated diseases including type II diabetes, hypertension and cardiovascular diseases among others. However, the molecular mechanisms coupling ectopic fat accumulation with ageing remain obscure. We developed Second Harmonic Generation (SHG) and Third Harmonic Generation (THG) nonlinear imaging techniques to visualize and quantify ectopic lipid deposition aiming to delineate the precise connection between ectopic fat accumulation and ageing in C. elegans. Our results suggest that fat gradually accrues with age in non-adipose tissues, such as body wall muscles, pharyngeal muscles, glial and neuronal cells, independently of growing temperature. Interestingly, caloric restriction, the most effective intervention delaying ageing in evolutionary divergent organisms, diminishes ectopic fat levels in an HLH-30/TFEB- and autophagy-dependent manner. Overall, our findings establish nonlinear imaging as a powerful tool for monitoring ectopic lipid droplet deposition in vivo and highlight the pivotal role of HLH-30/TFEB in the maintenance of lipid homeostasis during ageing. Investigating the interplay between ageing and ectopic lipid accumulation will contribute to our understanding of metabolic syndrome pathophysiology and facilitate the development of potential therapeutic interventions.
DNA damage is a major contributing factor in ageing and has been implicated in neurodegeneration. A critical question that emerges is whether intrinsic neuronal stress response pathways engage to protect against DNA damage-triggered neurodegeneration. Moreover, although it is well-established that DNA damage induces apoptosis, the contribution of necrotic cell death to DNA damage-related pathology remains largely elusive. To detect spontaneous necrotic cell death during ageing UV-hypersensitive ercc-1 C. elegans mutants were used, which are defective in the nucleotide excision repair pathway (NER). Besides the classical DNA damage repair pathway, nuclear membrane dynamics play a critical role in sensing and resolving DNA damage. Thus we also also examined the anc-1 mutant which lacks the outer nuclear membrane protein ANC-1. Next, we generated neuronal reporter strains carrying the ercc-1 mutation and observed decreased neuron viability and increased susceptibility to neurodegeneration as animals aged. Given the interplay between ERCC1 and the DNA damage and oxidative stress response pathways, we examined ercc-1 mutant stress sensitivity and responses. We are currently dissecting the crosstalk between DNA damage-induced necrosis and neurodegeneration, aiming to identify evolutionarily conserved molecular mechanisms interfacing these processes.
Agrochemical resistance in natural populations: prevalence, mechanism and evolution

Liisa Parts, Anthony Flemming, Alison Woollard

Resistance to pesticides is a growing problem in the agrochemical sector, just as antibiotic resistance is in medicine, yet little is known about the evolutionary biology of resistance. What biological factors underlie the rate of resistance evolution? How much natural resistance occurs in wild populations, and what is the genetic basis of this? Is resistance likely to evolve at the same rate or with the same likelihood to different pesticides? Such questions are very challenging to address in wild pest populations but knowledge in this area could be invaluable in assessing the relative risk of emerging resistance as new, possibly competing, products undergo development. We are using C. elegans to investigate natural variation in sensitivity to agrochemicals, sampling isolates distributed throughout C. elegans phylogeny. We will present the work we have done towards the optimization of an assay designed to monitor agrochemical resistance and the first results comparing sensitivity of a number of wild isolates of C. elegans to a range of proprietary pesticides.
Mysterious role of clec-4 in C. elegans immunity

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Despite the increasing understanding of Caenorhabditis elegans immunity certain aspects such as pathogen recognition and clearance still need to be determined. The highly diverse C. elegans gene family encoding C-type lectin-like domain proteins (clec genes) has the potential to act in either of these functional groups. As the majority of clec genes is up-regulated by pathogen infection, a role of clec genes in C. elegans immunity has been repeatedly suggested and in some cases even been taken for granted. Their exact immune function, however, is completely unknown. In this study we asked whether pathogen responsive clec genes indeed function in C. elegans immunity. Using WormExp, a recently established database for C. elegans gene expression analysis, we analyzed microbe- and stress-induced expression of clec genes and identified clec-4 as being highly responsive to infection with different pathogens, such as Pseudomonas aeruginosa, Serratia marcescens, and Bacillus thuringiensis (BT), and exposure to several stressors. We thus focused on clec-4 for subsequent functional analyses at the gene and protein level. Contrary to our expectations, clec-4 knock-out had no effect on worm survival on P. aeruginosa, and clec-4(ok2050) mutants were even more resistant to the infection with Serratia spec. and BT18247. Interestingly, clec-4 mutants were more susceptible after infection with another BT strain, BT18679. By looking at the expression of clec-4 paralogs, we identified clec-41, which is mainly co-expressed with clec-4. clec-41 RNAi-treated worms were more susceptible to infection by BT18247 and BT18679. Epistasis analysis revealed a genetic interaction between clec-41 and clec-4 on BT18247. Analysis of transgenic GFP reporter strains showed that both clec genes are expressed mainly in the intestine and in the case of clec-4 also in amphid neurons. We conclude that the two pathogen responsive clec genes, clec-4 and clec-41, function in C. elegans immunity. However, while clec-41 is required for resistance against BT infection, the role of clec-4 is much less clear. clec-4 knock-out caused both a resistance and susceptibility phenotype or did not affect survival at all – depending on the bacterial strain. Thus, the function of clec genes in C. elegans immunity cannot be inferred from their transcriptional up-regulation upon pathogen infection. We are currently further investigating the exact function of CLEC-4 and CLEC-41 in C. elegans immunity on both the gene and protein level.
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Cold induced torpor in Caenorhabditis elegans

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Induced hypothermia is used for the treatment of acute traumatic injuries, as it protects the nervous system, reduces inflammatory response and the chances of cardiac arrest. Despite prevalent clinical use, the molecular rationales behind the beneficial effects of cold remain elusive. To address this problem, we are using Caenorhabditis elegans as a genetically tractable model. C. elegans are, in principle, sensitive to a sudden temperature drop to near-freezing but, after short adaptation at an intermediate temperature, the animals survive at 4 degrees Celsius for several days, without feeding or moving, which we refer to as cold-induced torpor. To identify pathways required for cold survival, we performed transcriptome analysis and ribosomal footprinting, identifying many factors differentially expressed upon cold treatment. Interestingly, we observed a subset of transcripts that became translationally repressed in cold. Several among these transcripts encode fatty acid desaturases (FATs). FATs introduce a double bond in membrane fatty acids resulting in mono- or polyunsaturated fatty acids (PUFAs). The general notion is that fatty acid unsaturation promotes membrane fluidity and is essential for cold tolerance, thus the observation that, in cold, FATs are subject to repression is unexpected. We are interested in elucidating the mechanism underlying the translational repression of FATs, understanding the functional significance of their repression and the potential conservation.
C. elegans natural ecology: Hitch-hiking with slugs, woodlice, and chilopods

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The abilities of very small animals to migrate over long distances are often limited. However, if these animals prefer ephemeral habitats such as decomposing fruits or other rotting plant materials the ability to migrate between locations is essential. The free-living nematodes \textit{Caenorhabditis elegans} and congeneric taxa can be found in these short-lived environments. Animal vectors are likely to be involved in their dispersal, yet this assumption is based on a limited number of studies. Consequently, we asked which invertebrate taxonomic groups represent vectors of \textit{Caenorhabditis} nematodes. We conducted three comprehensive field surveys on potential invertebrate vectors in North German locations containing populations of \textit{C. elegans} and two related species, especially \textit{C. remanei}. \textit{Caenorhabditis} nematodes were commonly found in slugs, isopods, and chilopods, whereas the other examined taxonomic groups did not harbor any \textit{Caenorhabditis} species. Interestingly, slugs contained nematodes in their intestine even if the slug sampling site did not represent suitable substrates for \textit{Caenorhabditis} proliferation. As this observation suggested the ability of \textit{Caenorhabditis} to enter the slug intestine and persist for a certain time period we performed an experimental analysis of worm persistence in slug intestines. Under laboratory conditions \textit{C. elegans} was able to invade slug intestines and was excreted alive with the slug feces. We conclude that slugs, isopods, and chilopods represent potential vectors of \textit{Caenorhabditis} nematodes. The adaptations allowing the nematodes to enter and persist in the unfavorable environment of slug intestines may indicate a first step towards the evolution of a parasitic life-style.
Neuropeptidergic control of learning & memory

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Learning and memory are crucial processes that have been under intense investigation for many decades as they endow behavioural flexibility, enabling animals to adjust their behaviour based on previous experiences and the environment. Neuropeptides, which are involved in a wide variety of physiological functions, also have an emerging role in learning and memory. However, little is known about how neuropeptides modify learning and memory circuits in light of recent experience. One of the main focuses of our lab therefore includes identifying neuropeptide systems involved in both short-term and long-term associative learning in *C. elegans* and gaining a more in-depth understanding of the underlying cellular and molecular mechanisms. To this end we have been testing mutants of evolutionary conserved neuropeptide systems in associative short-term memory and long-term memory assays that mainly rely on the association of food with olfactory or gustatory cues. We found that the same neuropeptide system is involved in both short- and long-term modification of salt chemotaxis in light of recent experience. Whereas worms are normally attracted to low amounts of NaCl, they learn to avoid salt when they are pre-exposed to it in the absence of food. Depending on the training procedure, this behavioural change can be stored in short- or long-term memory. Further investigation into this neuropeptide system will pinpoint the cellular circuit that will allow us to ascertain whether neuropeptide-mediated short- and long-term memory relies on the same set of cells.
Caenorhabditis elegans PAQR-2 and IGLR-2 Protect Against Glucose Toxicity by Modulating Membrane Lipid Composition

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Surprisingly very little is known about the mechanisms responsible for membrane homeostasis in eukaryotes. Using C. elegans, we have discovered the first sensor/regulator of membrane homeostasis in animals: it is composed of two proteins, PAQR-2 and IGLR-2, which are homologs of the mammalian adiponectin receptors and of LRIG-type proteins, respectively. This protein complex is essential for C. elegans to regulate membrane composition when challenged by low temperature or by diets rich in saturated fatty acids, which can be derived from glucose. Mosaic analysis reveals that IGLR-2 and PAQR-2 acts cell non-autonomously in hypodermal and gonad sheath cells to regulate membrane homeostasis systemically. Using FRAP (Fluorescence Recovery After Photobleaching) on living worms, we found that cultivation in the presence of glucose causes a lethal decrease in membrane fluidity in paqr-2 and iglr-2 mutants and that genetic suppressors of this sensitivity act to restore membrane fluidity by promoting fatty acid desaturation. Importantly, the essential roles of PAQR-2 and IGLR-2 in the presence of glucose are completely independent from DAF-2 and DAF-16, the C. elegans homologs of the insulin receptor and its downstream target FoxO, respectively; PAQR-2 and IGLR-2 therefore represent an entirely new pathway essential for survival in the presence of glucose. Using bimolecular fluorescence complementation (BiFC), we also show that PAQR-2 and IGLR-2 interact on plasma membranes and thus may act together as a fluidity sensor that controls membrane lipid composition.
PAQR-2 and IGLR-2 Regulate Membrane Homeostasis
Spatiotemporal dynamics of cortical polarity regulators in C. elegans epithelia

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Epithelia are an essential tissue type that functions both as a barrier and regulator of transport between different compartments of the body and the outside environment. To perform these functions, epithelial cells are polarized along an apical-basal axis and compartmentalized into functionally distinct domains. Defects in polarity interfere with epithelial functions and are linked to cancer and other diseases. Three main protein complexes that coordinate cell polarity have been identified: the apical Crumbs and PAR complexes and the basolateral Scribble complex. However, we still lack a detailed mechanistic understanding of how polarity proteins interact and regulate each other to establish and maintain cell polarity. To fully understand the interplay between polarity regulators, we need a better spatial and temporal insight into the distribution and interdependence of the components involved. This is important both in the initial stages of epithelial polarization (in what order do the different components arrive), and in established epithelia (what components are essential after polarity has formed, what is the interdependence of polarity regulators in established epithelia). Our overall aim is to investigate the spatiotemporal dynamics and the interdependencies of the main cell polarity regulators in C. elegans epithelial cells. To address our hypothesis, that polarity proteins are dynamic during development, it is essential to have a comprehensive understanding of the behavior of these proteins with a precise spatiotemporal resolution. Therefore, we used the CRISPR/CAS9 system to endogenously tag the main polarity regulators with fluorescent proteins and follow, with unparalleled detail and accuracy, their localization in vivo. By using dual combinations, we are able to follow the subcellular localization of the polarity regulators in distinct epithelia during development and know which proteins first arrive at their specific membrane domains or at the junctions that form the apical and lateral domains. This will enable us to understand the sequential roles of each polarity protein in different epithelial tissues during development.
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The NEET homolog CISD-1 is a novel determinant of ageing in C. elegans

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The NEET family of Iron Sulfur Cluster (ISC) binding proteins includes members implicated in various human pathologies ranging from neurodegeneration to cancer and age-related diseases. However, their mode of action remains largely enigmatic. The C. elegans gene cisd-1 (W02B12.15) encodes a functional homolog of the mammalian CISD1 (CDGSH Iron Sulfur Domain 1) and CISD2 (CDGSH Iron Sulfur Domain 2) proteins, based on sequence analysis and the presence of the conserved domain CDGSH for binding to ISCs. CISD-1 is ubiquitously expressed in neuronal, muscle and intestinal cells, and is tethered on the outer mitochondrial membrane. Downregulation of cisd-1 expression by RNAi significantly reduces animal lifespan and induces germline apoptosis, rendering CISD-1 an important determinant of ageing. CISD-1 effects on ageing are mainly mediated by the mitochondria-associated, intrinsic apoptotic pathway. Interestingly, CISD-1 deficiency results in hyperactivation of mitochondrial function, as indicated by ATP production, oxygen consumption and generation of reactive oxygen species. Conclusively, our findings suggest that CISD-1 influences longevity by maintaining mitochondrial activity steady-state levels and by interacting with factors of the intrinsic apoptosis pathway. Since deficiency in NEETs is linked with the neurodegenerative Wolfram syndrome, characterization of the molecular pathways involving CISD-1 could provide new insights towards the development of effective therapeutic strategies.
Novel functions for IκB proteins in a NF-κB free model system

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The nuclear factor-κB (NF-κB) signaling pathway participates in multiple biological processes implying complex and precise regulation; among them, proliferation, differentiation and cell survival. IκB (for Inhibitor of κB) proteins negatively regulate NF-κB by keeping it retained in the cytoplasm. Increasing evidence in mammals indicates that IκB proteins exert specific nuclear roles to control gene transcription in a NF-κB independent manner. C. elegans represents an interesting model to study such functions since there is no NF-κB gene but two IκB homologues, named C33A11.1 (nbid-1) and C04F12.3 (ikb-1). nbid-1 is the closest worm homolog to human IκB. We generated a translational reporter and observed that NBID-1::GFP is present in the nucleus of several cell types, while there is not an evident expression in the cytoplasm. ChIP-seq experiments specifically located the binding site for NBID-1 around the transcriptional start site of many genes, suggesting a role in gene expression regulation. Even when knockdown experiments revealed that neither nbid-1 nor ikb-1 genes are essential for worm viability, RNA-seq experiments exposed that nbid-1 regulates the expression of genes located at specific clusters in the C. elegans genome, similarly to the regulation observed in HOX genes. To date, our results suggest a function in chromatin regulation for IκB proteins in the C. elegans model system. However, further work needs to be addressed both to confirm this hypothesis and to shed some light on the mechanism underneath. Ongoing work includes the generation of strains by CRISPR/Cas9, new transcriptomic analyses and in depth chromatin studies.
Neuron segmentation from electron microscopy images

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We present a method of segmentation of the neurons from C. elegans electron microscopy images. The process of imaging the worms is time consuming and complicated but after the images are acquired they still need to be interpreted, which is no less complicated or time consuming than the previous step. The first step towards interpreting the images is labeling them, that means segmenting known structures within the worm images, for example neurons. The method we developed to segment the neurons is based on Deep Learning. Deep Learning is a powerful machine learning technique which has been thoroughly employed lately due to its superior capacity to recognize patterns in data, in some cases even showing above human performance. This capacity is due to the fact that the algorithm is based on the visual cortex structure of living beings. As the neuron segmentation in images is a pure pattern recognition task, the algorithm shows a good performance.
SUMOylation regulates lifespan and mitochondrial homeostasis through DAF-16/FOXO in *C. elegans*

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The pathways modulating ageing in the nematode are well-characterized and conserved. Amongst these signaling pathways, insulin/IGF-1 has a major role in determining the lifespan of animals, mainly through the DAF-16/FOXO transcription factor and the stress response-related transcription factor SKN-1/NRF2. Interestingly, these two key transcription factors contain putative SUMOylation sites. SUMOylation, the attachment of SUMO (small ubiquitin-related modifier) to a protein, is a posttranslational modification implicated in the regulation of diverse cellular processes, including the DNA damage response, sub-cellular protein localization and protein-protein interactions, among others. Protein SUMOylation levels increase progressively during ageing. However, whether elevated SUMOylation is only an unrelated consequence of the ageing process or it serves a causative, regulatory role in senescent decline is not understood. The *C. elegans* genome contains a single gene encoding SUMO (*smo-1*), rendering the nematode a convenient model in which to genetically dissect the role of SUMOylation in organismal physiology and ageing. Deletion of *smo-1* causes embryonic lethality. Nevertheless, we find that RNAi knockdown of *smo-1* initiated at the L4 stage shortens the lifespan of both wild type and long-lived animals. Notably, knockdown of a SUMO protease gene (*ulp-1*), extends the lifespan of long-lived mutants (*clk-1, daf-2, ife-2*), but not wild type animals. The lifespan changing effect of SUMOylation is tissue-specific. In addition, we observed that manipulation of SUMOylation levels by either knockdown of *smo-1* or *ulp-1* influences the activity of DAF-16 and SKN-1, as well as, stress resistance, energy metabolism and mitochondrial homeostasis, in a genetic background- and age-dependent manner.
Regulation of ERM-1 activity in epithelial membrane morphogenesis

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Dynamic remodeling of specific cortical membrane domains occurs in many polarized cells and is an essential process for animal development and homeostasis. Membrane remodeling requires the reorganization of the cortical actin cytoskeleton and regulated contacts between F-actin and components of the plasma membrane. Proteins of the conserved ERM family interact with PIP₂ and several membrane-associated proteins through the N-terminal FERM domain, and with F-actin via the C-terminal domain. Across animal models ERM activity is associated with formation of dynamic structures such as the cleavage furrow of dividing cells, lumen/microvilli of intestinal epithelia, axonal growth cones in neurons, leading edges of migrating cells, and immunological synapses in B/T cells. Activity of ERM proteins depends on a conformational change that turns an inactive cytoplasmic form, in which an N- to C-terminal intramolecular interaction masks most regulatory sites, into an active membrane- and actin-bound form. In vitro and cell culture studies suggest an activation model dependent on initial binding to PIP₂ followed by phosphorylation of a conserved C-terminal threonine residue. However, in vivo data supporting this model is scarce and contradictory. We use the animal model C. elegans to assess the contribution of the different conserved regulatory sites for ERM function in vivo. C. elegans encodes a single ERM protein, ERM-1, that is essential for viability and formation of tubular epithelia including the intestine, excretory canal, gonad, and vulva. We are using CRISPR/Cas9 to engineer worm strains in which endogenous regulatory sites have been altered, combining them with different epithelial markers and an endogenous erm-1::GFP, followed high-resolution microscopy analysis. We are interested in understanding how each site contributes to conformational activation of ERM-1, ERM-1 dynamics at the plasma membrane, actin organization at the cortex, and development of epithelial tissues. We hope that our research will further the understanding of molecular mechanisms required for ERM protein activation and regulation during tissue morphogenesis.
Exploring the role of Hypoxia Inducible Factor-1 (HIF-1) in the specification and maintenance of ASE neuron cell fate in *Caenorhabditis elegans*

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Understanding how cells and tissues specify and maintain their fate is a crucial step towards the efficient and direct reprogramming of cells into other cell types. In *C. elegans*, the zinc finger-containing transcription factor CHE-1 functions to specify the ASE neuron fate. Ectopic CHE-1 expression in animals during the last stages of development leads to ectopic expression of the ASE neuron marker *gcy-5prom::gfp* in additional head neurons. It has previously been shown that RNAi knockdown of the histone chaperone *lin-53* results in ASE marker expression in the germline and conversion of germ cells into neurons (Tursun *et al.*, 2011 Science). A number of genes that may also function as a ‘barrier’ to the conversion of cell fate have been identified in a whole genome RNAi screen performed in our laboratory by Ena Kolundzic and Martina Hajduskova (unpublished). RNAi knockdown of two candidate ‘barrier’ genes, *vhl-1* and *egl-9*, results in ectopic ASE marker expression in the anterior-most cells of the intestine. The encoded proteins for *vhl-1* and *egl-9*, Von Hippel-Lindau-1 (VHL-1) and Egg-laying defective-9 (EGL-9), respectively, are involved in regulating levels of the transcription factor Hypoxia-Induced Factor-1 (HIF-1) in *C. elegans*. Animals carrying a mutation in *hif-1* do not show ectopic ASE marker expression following *egl-9* RNAi. Next, expressing a HIF-1 variant carrying the P621G mutation, which abolishes VHL-1-mediated degradation of HIF-1, under the control of the *hif-1* promoter is not sufficient to drive ASE marker expression in the intestine. Examination of a *hif-1* transcriptional reporter revealed prominent *hif-1* expression in the anterior-most cells of the intestine. Consistent with a cell autonomous role for HIF-1 in the regulation of ASE marker expression, intestine-specific *egl-9* RNAi lead to ectopic ASE marker expression in the anterior-most cells of the intestine. Interestingly, HIF-1 may function to specify and/or maintain cell fate in a context-dependent manner with a low number of *hif-1* mutants showing ASE marker expression in the germline. In summary, intestinal HIF-1 may function in a cell autonomous manner to regulate the expression of a marker for the ASE neuron fate.
Continuous and automated measurement of food intake

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Caenorhabditis elegans has become a promising model to study food-associated diseases and behaviors, and food intake regulatory mechanisms. Feeding motions can be modulated by the worm’s environment and internal state. Food intake occurs as a consequence of two motions: the contraction of pharynx (pumping) and the isthmus peristalsis. Feeding rate is proportional to the worm pharyngeal pumping, which can be directly observed under a stereoscope. This allows the routinely study of food intake by means of direct quantification of pumps per minute. Alternatively, food intake can be measured indirectly, using fluorescent bacteria. Unfortunately, these methods are short-term and unsuitable for independent measurements of high numbers of animals. Scientific community is challenged to find automated and long-term methods to study food intake. Although some long-term methods based on food clearance assays have been reported, they fail in the automated and continuous aspects. Here we describe a method for the analysis and continuous monitoring of worm feeding in 96-well plates. This method is based on a bioluminescence assay that we previously described for measurement of developmental timing in C. elegans. We measure bioluminescence in reporter strains that constitutively and ubiquitously express luciferase and the substrate of the bioluminescent reaction, luciferin, is added in the food. With the luciferin being limiting for the reaction, the bioluminescence signal from individual animals reports differential worm food intake. To validate our system we have monitored bioluminescence in eat-2 mutants. EAT-2 functions post-synaptically in pharyngeal muscle, regulating the rate of pharyngeal pumping. We observe that the bioluminescence signal is reduced in eat-2 mutants. We are working to further validate this method treating worms with serotonin, a neurotransmitter that accelerates pharyngeal pumping. We will present evidence of the suitability of our method to monitor food intake and dissect underlying regulatory mechanisms.
Posttranslational protein modifications (PTPMs) of human transthyretin – a novel approach in transgenic C. elegans CL2008

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Human transthyretin (hTTR) is a visceral protein which facilitates the transport of thyroid hormones in blood and cerebrospinal fluid. It is frequently affected by oxidative posttranslational protein modifications (PTPMs) at a free cysteine residue, and those PTPMs have been shown to alter tetramer stability of hTTR. As the protein is known for its amyloidogenic character, and hTTR fibril formation is implicated in several diseases, we investigated PTPMs of hTTR in vivo. While studies of PTPMs in humans are challenging and time-consuming, we tested a novel approach utilizing the transgenic C. elegans strain CL2008, which is overexpressing hTTR. The expression of hTTR in CL2008 worms was confirmed by PCR and western blot experiments. By real-time RT-PCR, we showed that hTTR expression in L1 and L4 stage nematodes is indistinguishable, enabling studies in both larval stages. We investigated PTPMs of hTTR via MALDI-TOF-MS in worm homogenates after hTTR isolation by immunoprecipitation. While in unstimulated CL2008 worms the native, unmodified hTTR was identified as the major form, stimulation with the oxidant menadione (MND) induced various posttranslationally modified hTTR forms. Namely, we detected a dose-dependent increase in glutathionylated and cysteinylated hTTR after MND incubation, as well as the formation of an hTTR-MND adduct. In addition, MND exerted toxic effects in CL2008 worms, as dose-response survival curves revealed an LD₅₀ of 250 μM following 1 h incubation. However, the incubation with the non-toxic drug D-penicillamine also resulted in pronounced changes within the PTPM pattern, showing that PTPM formation occurs independently from toxic effects. We detected a decrease in cysteinylated hTTR, an increase in sulfonated hTTR and formation of an hTTR-penicillamine adduct after incubation with the thiol-containing drug. We further studied the role of the antioxidant N-acetylcysteine (NAC) as a potential antagonist of the MND-induced effects, which might be attributable to oxidative stress. In fact, co-incubation of NAC and MND revealed a possible protective effect of NAC, characterized by a reduction in MND-induced lethality and PTPMs of hTTR. Altogether, we established a novel model to study kind and degree of drug-mediated oxidative PTPMs of hTTR in vivo using the transgenic C. elegans strain CL2008. We verified the suitability of C. elegans as a model system to investigate drug-modulated PTPMs, enabling high-throughput analyses and screening of compounds. As oxidative PTPMs of hTTR have been identified as potent triggers in the formation of hTTR-related amyloid fibrils, our findings hold future promise for deciphering the mechanisms involved in disease progression secondary to hTTR misfolding.
Modulation of Caveolae by the Insulin/IGF-1 signaling Pathway Regulates Lifespan and Proteostasis in the Nematode C. elegans

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The Insulin/IGF-1 signaling pathway (IIS) is a key regulator of aging, lifespan and stress resistance in worms, flies, mice and presumably humans. Among other genes, IIS reduction modulates the expression levels of lipid modifying enzymes, suggesting that the activity of this pathway affects cellular organization. Moreover, since dynamic lipid assemblies are known to play key roles in the organization of signaling complexes, we examined the possibility that IIS reduction affects membrane organization and protein distribution, thereby influencing longevity and stress resistance. In order to test this hypothesis, we isolated detergent resistant membranes (DRMs) from worm populations that were treated with RNAi toward daf-2 or left untreated and analyzed their protein content by mass spectrometry. Our results show that the protein Caveolin-1 is scarce in DRMs of daf-2 RNAi-treated worms compared to their untreated counterparts. Caveolin-1 is crucial for the formation of caveolae, a subtype of DRMs that among other functions, serve as organizing centers of signaling complexes. The knockdown of cav-1, which is expressed primarily in neurons of the adult worm, extended the nematode’s lifespan mildly while having no effect on the worm’s resistance to heat, radiation or pathogenic bacteria. Interestingly, knocking down cav-1 provided partial protection from proteotoxicity, although not as strongly as daf-2 knockdown. This study points at lipid rafts in general and caveolae in particular as critical centers for lifespan and proteostasis regulation and further strengthens the theme that lifespan and stress resistance are separable.
Unraveling the pathological mechanisms causing splicing-related Retinitis Pigmentosa

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Mutations in splicing factors genes (PRPF3, PRPF4, PRPF6, PRPF8, PRPF31 and SNRNP-200) have been linked to the autosomal dominant form of a rare genetic disease called Retinitis Pigmentosa (adRP). RP is characterized by a progressive visual degeneration produced by the loss of photoreceptors by apoptosis. We are modeling this disease in Caenorhabditis elegans. As a result of our research, we were able to establish two important similarities between splicing-related adRP (s-adRP) and the RNAi phenotypes in worms: (i) there is a cell-type-specific apoptosis and (ii) such apoptosis seems to be associated with transcriptionally active tissues (Rubio-Peña et al, 2015). An intriguing question remains open: how does partial loss-of-function of ubiquitously expressed splicing factors produce severe cell-specific effect? Our transcriptomic data showed a low intron retention in RNAi treated worms that cannot explain the apoptotic outcome. We are currently exploring an alternative mechanism to identify the cause of the tissue specific apoptosis. Along with the apoptotic gene egl-1, the DNA damage response atl-1/ATR gene was found upregulated upon inactivation of s-adRP genes by RNAi. atl-1/ATR responds primarily to DNA lesions caused by replication fork stalling or UV damage. These findings lead us to think that mutations in s-adRP genes may produce an active but inefficient splicing that, in highly transcriptionally active tissues, unchains a number of unfortunate events that include a decrease in the efficiency of transcription, R-Loop formation, replication stress and genomic instability that finally leads to apoptosis. We are investigating the implication of atl-1 signaling and R-loops in the onset of C. elegans phenotypes and therefore in the development of the disease. In such investigations we are taking advantage of tools like CRISPR and analyses of DNA-RNA and DNA-Protein interactions.
C. elegans Integrator Complex characterization

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The Integrator Complex, which is composed by at least 14 subunits in humans, interacts with the CTD domain of RNA polymerase II at the promoter of snRNA genes and is responsible for 3'snRNA processing. This cleavage of the snRNAs into their mature form is thought to occur co-transcriptionally. The Integrator Complex is conserved throughout evolution in metazoans. Predictable protein domains such as ARM (armadillo like repeats), COIL (coiled coil), PHD (plant homeodomain finger), TPR (tetricopeptide repeats), β-lactamase/β-CASP and VWA (von Willebrand type A like domain) can be distinguished within Integrator subunits. Here, we define C. elegans Integrator Complex members by affinity purification and function in 3’ end snRNA processing. Moreover, we suggest that C. elegans Integrator Complex has other roles apart from 3’ snRNA processing. The Integrator Complex might be acting beyond specific conditions producing a transcriptional response.
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The histone demethylase JMJD-1.2 controls axon guidance by regulating Hedgehog-like signaling

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Components of the KDM7 family of histone demethylases play roles in neuronal development and PHF8, a member of this class, is frequently found mutated or deleted in cases of X-linked mental retardation. However, how PHF8 regulates neurodevelopmental processes and contributes to the disease is unknown. Here we show that the catalytic activity of the PHF8 homolog in C. elegans, JMJD-1.2, is required during embryogenesis for correct axon guidance. Loss of jmjd-1.2 results in upregulated expression of two Hedgehog-related proteins, whose ectopic expression was validated to be responsible for the axonal phenotype. Interestingly, reduced expression of C. elegans homologs of genes positively regulating Hedgehog signaling ameliorates the phenotype associated with loss of jmjd-1.2. Thus, our study highlights a novel function of jmjd-1.2 in axon guidance that may be relevant for the onset of X-linked mental retardation and provides compelling evidences of a conserved function of the Hedgehog pathway in C. elegans axon migration.
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Development and Function of the Mystery Cells of the Male: Decoding a Glia-to-Neuron Transition Required for Sex-specific Associative Learning

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Vertebrate neurogenesis occurs through asymmetric divisions of neuroepithelial cells, radial glia and even astrocytes, resulting in self-renewal and the production of a neuron. We have recently discovered the first example of a glia-to-neuron transition in an invertebrate, suggesting that glia act as neural progenitors across metazoan taxa (Sammut et al. 2015). The mystery cells of the male (MCMs) are born from the asymmetric division of the amphid socket (AMso) glial cells, which are fully-differentiated glial cells present in both sexes, that divide in the males, just prior to sexual maturity. The MCM interneurons regulate sexual conditioning (Sammut et al. 2015), a form of associative learning that pairs mate experience with gustatory cues and overrides the aversive association of this gustatory cue with starvation (Sakai et al. 2013). To begin to decode the molecular mechanisms that regulate this glia-to-neuron transition we have performed a GFP-based forward genetic screen for mutants in which the MCMs fail to be specified. We have isolated 10 mutants so far and using a battery of cell-division, glial and neuronal markers we are classifying the stage at which MCM development is affected: Class I: no AMso cell specification; Class II: no AMso division; Class III: AMso division but no MCM neuronal specification; Class IV: no MCM subtype specification. We are initially focusing our attention on Class II and Class III mutants as these are likely to represent genes required to initiate and drive the glia-to-neuron transition. We have previously shown that cell-autonomous manipulations of the sex-determination pathway results in a Class II phenotype and are keen to identify genes that act downstream or in parallel to this pathway. We are using CloudMap to map and clone our mutants (Minevich et al., 2012) and already have good candidates for several of our Class II mutants. This work will uncover the gene regulatory networks that allow the genesis of new neurons from glia. Additionally, we have found that sexual conditioning can occur in response to several volatile olfactory cues such as benzaldehyde and diacetyl. At least one of our mutants is defective in sexual conditioning to both olfactory and gustatory cues, implicating the MCMs in the combination of a wider range of associative cues with mate-experience.
miRNAs are short non-coding RNAs that regulate the gene expression post-transcriptionally. In an effort to study miRNA gene functions, knockouts of miRNA biogenesis genes have been generated, the analysis of the knockouts showed that miRNAs are essential for embryonic and larval development. However, at the same time these findings hampered the efforts to unravel the post-developmental roles of miRNAs. In 2012, a temperature sensitive allele of an essential miRNA biogenesis gene, pash-1 (pash-1ts) has been isolated in C. elegans. Using this allele, miRNA production was ceased post-developmentally by a temperature up-shift, which led to rapid aging and motility defects in the mutant animals. Functional decline in proteostasis network (PN) is one of the contributing factors to aging. PN is composed of various components that ensures proper folding or degradation of proteins to maintain the proteome integrity. In our studies, we first questioned whether the functional decline in the PN is the main cause of accelerated aging in pash-1ts mutant animals. To test this hypothesis, we treated pash-1ts mutant animals with a chemical known to enhance proteostasis. Increase in the proteostasis capacity extended the life span of pash-1ts animals by 40%, but it also rescued the motility defects in these animals by 50-60%. In parallel to these findings, we showed that the loss of miRNA production increases toxicity of the metastable proteins in the muscle cells. In addition, the number of the protein aggregations is higher in the pash-1ts animals compared to wild type animals. Collectively, these data indicate that the absence of miRNAs enhance the misfolding of the protein and suggest that aging phenotype of pash-1ts animals, if not all, is mostly due to a functional decline in proteostasis. The regulation of stress genes involved in the cellular protein quality control is under the control of three main transcription factors, daf-16, hsf-1 and skn-1. RNAi knockdown of these transcription factors in the wild type animals shortens the lifespan, showing that they are important regulators of aging. However, except daf-16, RNAi knockdown of hsf-1 and skn-1 in pash-1ts animals do not show any effect on the lifespan of these mutant animals. Based on these findings, we have postulated that stress response pathways, particularly regulated by hsf-1 and skn-1, are already mis-regulated in pash-1ts animals. We have concluded that miRNA synthesis during the adulthood is essential to maintain the proteome integrity to prevent protein aggregation and suggest that critical cellular components that protect the stability of the proteome become deficient.
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Is there an autonomous ground state for cleavage directions in Caenorhabditis elegans?

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How do the cells know their highly specific cleavage directions in the early embryonic stages? Do these arise by the modification of a ground state, or are they specified de novo in each cleavage round? To address this question we used manipulated or mutant embryos and recorded all cell cleavages by 4D-microscopy. The derived coordinates were then analysed by bioinformatics. So far it is known that a cell polarity signal for the AB descendants derives from the P1-blastomere and its germ line descendants. To eliminate the polarity signal to a minimum level the P1-blastomere was removed using a fine needle. This results in diverse cell cleavage orientations up to the 8-AB cell stage. The only remaining principle is that blastomeres divide orthogonally to the orientation of the penultimate and the prior cell cleavages. Hyman and White have described this very pattern for normal embryos in 1987. This raises the question, whether this orthogonal pattern is an autonomous ground state for the cleavage direction in the absence of any other polarity signal, or whether another polarity signal exists, which regulates the so-called orthogonal cleavage pattern. Candidates are the par- genes establishing the initial embryonic polarity, where so far the main focus of interest is only in the first and second cleavage of the embryo. Our analyses, now including the fourth cleavage round, show, that in par-3 or par-6 mutants the 4-AB descendants divide nearly parallel to the cleavage direction of the AB blastomere. Strangely enough this suggests that these genes are responsible for the left-right component of the orthogonal cleavage pattern.
Iron starvation-induced mitophagy mediates lifespan extension upon mitochondrial stress in C. elegans.

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Frataxin is a nuclear-encoded mitochondrial protein that regulates the biogenesis of iron-sulfur-cluster containing proteins and, as a consequence, the functionality of the mitochondrial respiratory chain (MRC). Complete absence of frataxin is lethal in different species including the nematode Caenorhabditis elegans (C. elegans). Similar to other proteins which regulate mitochondrial respiration, severe frataxin deficiency leads to pathology in humans - Friedreich’s ataxia, a life-threatening neurodegenerative disorder - and to developmental arrest in C. elegans. Interestingly, partial frataxin depletion extends C. elegans lifespan, and a similar anti-aging effect is prompted by reduced expression of other mitochondrial regulatory proteins from yeast to mammals. The beneficial adaptive responses to mild mitochondrial stress are still largely unknown and, if characterized, may suggest novel potential targets for the treatment of human mitochondria-associated, age-related disorders. Here we provide evidence that in C. elegans a hypoxia-like iron starvation response is causally involved in the lifespan-extending effect elicited by frataxin suppression and identify mitochondrial autophagy (mitophagy) as part of this beneficial response. Furthermore, we identify that mitophagy is induced in an evolutionarily conserved manner upon frataxin silencing and show for the first time that, similar to mammals, it is activated in response to mitochondrial stress in a Parkin/pdr-1 Pink/pink-1 and Bnip3/dct-1 dependent manner in C. elegans. Our findings provide further insight into molecular mechanisms of mitochondrial stress control of longevity and point to mitophagy as a novel potential therapeutic target for FRDA and possibly other mitochondrial associated diseases.
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Differences in the genetic control of early egg development and reproduction between C. elegans and its parthenogenetic relative D. coronatus

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Diploscapter coronatus is the closest known relative of C. elegans with parthenogenetic reproduction. Recently, we sequenced its genome (Hiraki et al., in prep.). Looking at oogenesis and embryogenesis in D. coronatus we observed a number of distinctive features. These include just 2 chromosomes in the diploid set, absence of meiotic pairing, differences in the mitosis/meiosis decision and altered establishment of a-p asymmetry. In a genomic comparison between D. coronatus and C. elegans (plus other nematodes), we found that certain genes required for the processes mentioned above in C. elegans are absent in D. coronatus. This is particularly obvious for genes involved in the first embryonic division exemplifying that alternative molecular mechanisms may accomplish the same essential developmental steps. In a second approach, we sequenced the mRNA content of early D. coronatus embryos and compared it with similar stages in C. elegans (Hashimshony et al., 2012) and Ascaris suum (Wang et al., 2014). We identified 350 orthologous clusters with nearly 1,500 genes expressed in the early D. coronatus embryo but not in the other two considered nematodes. In non-nematode organisms many of such transcripts have been associated with chromosome structure and behavior or DNA transcription, replication and repair. Significantly overrepresented gene ontology (GO) terms in the D. coronatus data set support the need for gene products involved in chromosome behavior during this early time window. Only in a small number of cases orthologs were neither detected in other nematodes nor in more distantly related reference systems like Drosophila, zebrafish or mouse. This suggests taxon-specific roles which need to be revealed by future functional studies.
Dynein dynamics during mitosis in the C. elegans one-cell embryo

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Asymmetric cell divisions are essential for the generation of cellular diversity during development. Mitosis of the C. elegans one-cell embryo is studied as a classical example of an intrinsic asymmetric division, in which anterior-posterior polarity is set up prior to mitosis by the cortical PAR protein complexes. This polarity axis subsequently dictates the asymmetric positioning of the spindle during mitosis, which results in the formation of a larger anterior and a smaller posterior blastomere. Positioning of the mitotic spindle is governed by pulling forces that act on dynamic astral microtubules at the cell cortex, with higher forces being generated in the posterior compared to the anterior. An evolutionarily conserved force generator complex consisting of Gα, GPR-1/2PINS/LGN and LIN-5Mud/NuMA proteins is required for the generation of these pulling forces, acting downstream of PAR polarity. In the currently prevailing model, the force generator complex recruits cytoplasmic dynein, a minus-end directed molecular microtubule motor protein, to the cell cortex. Dynein subsequently associates with microtubules in an end-on conformation, which generates pulling forces by both microtubule depolymerisation and minus-end directed movement. Extensive genetic and biochemical experiments support this model, but the exact dynamics of cortical pulling force generation remain unknown. In order to study these events in more detail, we fused mcherry and egfp to the endogenous dynein heavy chain dhc-1 gene using CRISPR/Cas9-mediated genome editing. Spinning disk and TIRF imaging of early embryos expressing fluorescent protein-tagged DHC-1 revealed the presence of dynein diffusely in the cytoplasm and specifically at kinetochores, the nuclear envelope, cell cortex and mitotic spindle. Simultaneous dual-color imaging showed that dynein strongly colocalises with dynamic microtubule plus ends that radiate outwards from the centrosomes to the cortex in mitosis. This mitotic localisation was also observed for the dynactin subunits DNC-1p150Glued and DNC-2p50/dynamitin. Plus-end-tracking of dynein can be completely abolished by both RNAi-mediated knockdown and CRISPR/Cas9-mediated knock-out of ebp-2EB1. Interestingly, this does not perturb cortical dynein localisation nor anaphase pulling forces, as shown by spindle severing experiments. In addition, we generated a knock-out strain for ebp-1/3 and ebp-2, which is viable as a homozygous mutant, surprisingly showing that C. elegans does not rely on the presence of EBs and dynein plus-end-tracking for its embryonic development and survival.
Muscle degeneration is a common feature of muscle aging and muscular dystrophies such as Duchenne Muscular Dystrophy (DMD). Our lab has established a Caenorhabditis elegans mutant with progressive dystrophin-dependent muscle degeneration: the dys-1(cx18);hlh-1(cc561) double mutant worm called the C. elegans DMD model. This model exhibits dramatic changes in mitochondrial morphology compared to wild-type worms suggesting a deregulation in the fusion/fission mitochondrial balance. My goal is to shed light on the role of mitochondria dynamics in the molecular mechanisms leading to muscle degeneration. We first focused on DRP-1, a protein required for mitochondrial fission. We introduced in the C. elegans DMD model a null mutation drp-1(tm1108). The DMD;drp-1(tm1108) mutant showed reduced mitochondrial fragmentation compared to DMD mutants. Furthermore, DMD;drp-1(tm1108) mutants exhibited reduced muscle degeneration and increased mobility. DRP-1 is well known to be implicated in cell death processes. In C. elegans, the pro-apoptotic function of DRP-1 is dependent on its cleavage by the CED-3 caspase. We generated transgenic DMD;drp-1(tm1108) lines expressing DRP-1 with mutated CED-3 cleavage site. Interestingly, our data suggest that DRP-1 cleavage by CED-3 is required for DRP-1 to decrease muscle degeneration. Altogether, the pro-fission and pro-apoptotic protein DRP-1 seems to be implicated in pathological muscle degeneration. We then aimed at identifying other genetic suppressors of dystrophin dependent muscle degeneration playing a role in mitochondria dynamics. Large screens on the C. elegans DMD model had allowed for the identification of 28 genes, which decrease muscle degeneration after RNAi knock-down and which are known to function in mitochondria biology. We tested these 28 RNAi candidates for an effect on mitochondria morphology of DMD worms. We found that knock-down by RNAi of wah-1 (AIF homolog) and let-363 (mTOR homolog) can reduce mitochondrial fragmentation of DMD mutants. Our findings provide the first evidence that WAH-1 affects mitochondria morphology in muscle cells. Moreover, reducing wah-1 or let-363 mRNA levels in the absence of DRP-1 can further decrease muscle degeneration of the C. elegans DMD model suggesting that WAH-1 and LET-363 act at least in part in distinct molecular pathways than DRP1. As mitochondria processes are highly conserved among species, our study of mitochondria dynamics is likely to be relevant for Human muscle degeneration.
LITE-1, a photosensor of *Caenorhabditis elegans*, as a novel optogenetic tool

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The gustatory receptor family member LITE-1 is an unusual GPCR with 8 transmembrane helices found in *C. elegans*, with homologues also in *Drosophila*, which enables UV and blue light detection. LITE-1 is necessary for a proper avoidance response to short wavelength light. In addition, in the presence of food the pharynx stops pumping in response to light, this is mediated both by LITE-1 and GUR-3, a related sensor (Bhatla and Horvitz, 2015). The mechanism of light sensation by LITE-1 is still under debate. It is speculated that the light induced generation of reactive oxygen species is the mechanism how LITE-1 detects light (Liu et al., 2010, Bhatla and Horvitz 2015). It is not known whether it carries a chromophore, or whether it forms an intrinsic chromophore from its own amino acids. The signal transduction of LITE-1 is only partially established to date. A model is suggested whereby LITE-1 transduces light signals in the ASJ neuron through G-protein signaling, which leads to cGMP generation followed by opening of cGMP-sensitive CNG channels (Liu et al., 2010). We ectopically expressed LITE-1 in pharyngeal (PMC) and body wall muscle cells (BWM). In electropharyngeograms (EPGs) the pharynx, ectopically expressing LITE-1 in PMC, stops pumping when stimulated with blue light, whereas wild type shows an increased pump rate upon light stimulation. In contrast, a short light pulse increases the pump activity of LITE-1 expressing but not of wild type pharynxes. Interestingly, ectopic expression of LITE-1 in BWM leads to robust overall body contraction upon blue light stimulation (Edwards et al., 2008), which lasts for several minutes. Based on the facts that TAX-2/-4 channels are absent in muscles and that light-evoked cGMP production in BWM via the light activated guanylyl cyclase rhodopsin CyclOp (Gao et al., 2015) does not evoke contractions, we suggest a signal transduction different from that in neurons. By RNAi, we tested the contribution of different Gα proteins expressed in muscles on LITE-1 mediated effects, as well as other potential mediators of the pathway. Currently we are investigating further steps of the signal transduction as well as a putative mechanism of light sensation. Elucidating the pathway of ectopic LITE-1 signaling may allow establishing it as a novel optogenetic tool.
Sublateral motor neurons control sleep-specific flipping behavior

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Animals can be in different states such as sleep and wake and display behaviors that are specific for these states. However, little is known about how behaviors become state-specific. Here we investigated a turning behavior during sleep in C. elegans. Worms usually lie on their side and propel by sinusoidal body movements generated by dorso-ventral muscle contractions. This two-dimensional locomotion is caused by activity in the dorsal and ventral cord and is fairly well understood. Worms also display three-dimensional movements but the neural circuits are much less understood. During larval sleep, worms often turn from one side to the other, and this behavior is almost never found during wake. Neither the neuronal substrates of this flipping behavior are known nor do we understand why this behavior is sleep-specific. We performed a genetic screen for mutants that do not flip during sleep and identified the conserved NK-2 class homeobox protein CEH-24 to be crucially required for flipping. CEH-24 is expressed in sublateral motor neurons, which innervate the four body muscle quadrants. It is required for the formation of sublateral processes and for the expression of acetylcholine, which in turn is required for flip induction. Most important are the SIA neurons, which activate during flipping but also during wake. Optogenetic activation of the SIAs during sleep causes flipping but activation during wake does not. Whether or not SIA activation leads to flipping or not appears to depend on the global activity levels of neurons or muscles. Thus, we show that flipping during sleep is triggered by sublateral neuron activation. Flipping is state-specific because it requires reduced global activity of excitable cells as a permissive signal.
A chaperone complex consisting of Hsp70, a J-protein and Hsp110 is able to prevent and reverse the aggregation of Htt amyloid fibrils

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In order to be biologically active, proteins need to fold into a unique three-dimensional structure. If this folding process fails, proteins undergo the risk to aggregate. Protein aggregation, however, can be cytotoxic and amyloid aggregates represent the hallmark of several neurodegenerative diseases. To maintain cell viability, cells are equipped with a sophisticated chaperone network that supports de novo and refolding, prevents unfolding, misfolding and aggregate and can promote disaggregation. One prominent example for a proteotoxic disease is Huntington’s disease where mutated Huntingtin forms amyloid fibrils in neuronal cells that results in cell death. The first exon of Huntingtin encodes for a consecutive poly glutamine stretch. Generally, this stretch has fewer than 36 repeated glutamines, which results in production of the soluble cytoplasmic protein Huntingtin. However, a sequence of 36 or more glutamines causes instability of the protein and results in the formation of robust and SDS resistant fibrils. We used proteins encoded by the first exon of Huntingtin with 48 consecutive glutamine residues in in vitro assays and could demonstrate that a chaperone triage consisting of Hsp70, J-protein and Hsp110 can efficiently prevent its aggregation. As C. elegans encodes for four cytosolic Hsp70 yet numerous cytosolic J-proteins we tested different combinations and could reveal that the combination of Hsp110 with the constitutively expressed Hsp70, HSP-1, and the B-type J-protein DNJ-13 was most effective in prevention of aggregation. Furthermore, we could demonstrate that a 4-fold excess of chaperones over substrate is required for absolute suppression of Htt fibril formation and the concentration of the J-protein seems to be the limiting factor. Prevention of aggregation is dependent on an active ATPase cycle of the chaperones. Our experiments are supported by EM images that also show that this combination can effectively prevent the aggregation and demonstrate the physical interaction between the fibrils and and the chaperones by immunogold labeling. The same triage of chaperones appears to not only prevent the aggregation of Huntingtin but additionally can disassemble existing Huntingtin fibrils. Our experiments were complemented by in vivo experiments in C. elegans strains that express Huntington exon 1 in their muscle cells. Upon knockdown of the chaperones, aggregation was significantly enhanced. Taken together we could identify a chaperone complex that can effectively prevent and reverse the formation of cytotoxic amyloid fibrils.
Poster abstracts in alphabetical order (first author)

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Using CRISPR/Cas9 to model splicing-related diseases in *C. elegans*

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By taking advantage of CRISPR/Cas9, we aim to produce pathological human mutations in *C. elegans* splicing factors. We are currently focusing our work on two of such proteins: PRP4K and T08A11.2/SF3B1. The PRP4 kinase (*prpf-4/PRPF4B*) is a splicing regulatory kinase that participates in different steps during the removal of introns from pre-mRNA. Studies in fission yeast and human cells indicate that this protein is also required for proper chromosomal segregation, and it might be involved in signaling pathways and chromatin remodeling processes. In addition, altered PRP4K expression levels may influence the response to certain chemotherapeutic agents in different cancer types. However, the functions of this protein in multicellular organisms are not yet fully understood. In *C. elegans*, *prpf-4(RNAi)* causes pleiotropic phenotypes, including sterility and embryonic lethality. We are using the CRISPR/Cas9 system to generate mutants lacking the kinase activity, null mutants and reporter strains, which will be very helpful to describe PRP4K functions in the worm. In addition, we are interested in studying the splicing factor T08A11.2/SF3B1, which is part of the U2 snRNP. This protein facilitates branch point identification during the early steps of the splicing reaction. Importantly, SF3B1 is mutated in 10-15% of chronic lymphocytic leukemias (CLL) and in 20-30% of myelodisplastic syndromes (MDS), and could be used as a prognosis marker. Most of the identified mutations are located in the C-terminal region of the protein, being K700E (*C. elegans* K718E) the most common substitution. Our goal is to introduce this and other prevalent point mutations in the *C. elegans* SF3B1 ortholog by CRISPR/Cas9 in order to study their functional impact. Moreover, we will be able to use these mutants to test the effects of drugs on such mutants in a multicellular context. We will also explore possible genetic interactions between SF3B1 mutations and mutations in other genes that co-occur in different cancer types.
Pro-longevity mitochondrial disturbance promotes BRC-1/BRD-1 dependent and independent resistance to genotoxic stress.

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Background: The efficiency of resistance towards infections and stress (e.g. DNA damage agents) deteriorate with aging and interventions that promote healthy aging are typically associated with increased resistance to different types of stress. Emblematic examples of this paradigm have been extensively characterized in the nematode Caenorhabditis elegans (C. elegans) where lifespan extending mutants have been at first often identified in screening for enhanced resistance to stress. Paradoxically, alteration of core cellular functions such as translation, mitochondrial metabolism, the IGF/insulin pathway, and DNA integrity induce the expression of cellular stress responses which in turn are often causally involved in animal longevity and resistance to stress. Objectives: Here we investigated whether long-lived C. elegans (Mit) mutants resulting from partial reduction of mitochondrial functionality have altered systemic or germline resistance to genotoxic stress and whether these effects are simply associated or causally involved in their extended longevity. Materials and Methods: To this extent, animal’s resistance to genotoxic stress was characterized by quantifying germ cell apoptosis and somatic sensitivity to radiation, and a library of dsRNA against DNA maintenance genes was screened in search of possible molecular players. Results: We showed for the first time that pro-longevity mitochondrial stress confers resistance to germ cells physiological and DNA-damage induced apoptosis. Notably, this protective effect is not due to impaired apoptotic machinery but rather to more efficient genomic maintenance apparatus and we found that BRCA/BRD tumor suppressor genes are required for this germline protection. On the other hand BRCA/BRD genes were not essential for the extension of lifespan and somatic resistance of animals with reduced mitochondrial activity. Conclusion: With this study, we showed that, at least in animals with reduced mitochondrial activity, the mechanisms activated to safeguard progeny against genomic instability are different from the one required for somatic maintenance and lifespan extension thus suggesting that genes which contribute to the conservation of the species likely did not evolve with the ones to extend lifespan, a very likely process from an evolutionarily point of view. Keywords: Mitochondria, frataxin, aging, apoptosis, cell proliferation, DNA-damage, BRCA/BRD.
Insulin signaling links metabolic state to systemic arousal in *C. elegans*

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Neural circuits integrate external and internal signals to inform behavioural decisions. Food search behaviours need to balance energy expenditure during neural processing and motor activity with energy conservation. Ambient oxygen (O₂) is a sensory cue used by worms to instruct food search behaviours. We use O₂ stimulation to characterise the behaviour of worms as they are subjected to starvation of increasing durations. Unlike well-fed worms, 1h fasted worms exhibit enhanced responses to an O₂ concentration downshift. This is suppressed upon 16h starvation; instead, animals are prone to enter a reversible state of sleep-like quiescence, which is further enhanced upon O₂ downshift. The timescale of starvation-controlled behaviours requires insulin signaling as insulin receptor mutants (daf-2) display the behaviour of 16h starved WT worms already upon 1h fasting. This early starvation behaviour requires DAF-2 in the nervous system. Expression of daf-2 only in sensory neurons suppresses quiescence, suggesting that wakefulness in *C. elegans* depends on food availability and insulin signaling acting at the sensory level. To understand the underlying network mechanisms we perform brain-wide single cell resolution Ca²⁺ imaging. While brain-wide network dynamics underlie wakefulness, starvation promotes periods of inactivity among almost all neurons; however, upon arousal the network re-engages in dynamic activity. In summary, we report that starvation systemically affects the brain and promotes quiescence, which likely serves the purpose of energy conservation in a stimuli-free environment. Our results suggest that food availability mediated by elevated insulin signaling serves as arousal cue, thereby preventing quiescence. With lasting starvation, insulin signaling ceases, during which state remaining sensory inputs like high O₂ maintain the aroused state. When O₂ concentrations drop, quiescence occurs. This supports the idea of quiescence as default brain state in the absence of arousing cues.
A sensitive mass-spectrometry-based method identifies metabolic changes of life history traits in C. elegans

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Abnormal nutrient sensing and nutrient metabolism are a hallmark of the aging process and the development of age-related disease. Although studies in the worm C. elegans have identified various metabolic genes that influence worm development and lifespan, few tools are available to measure the metabolic consequences of such perturbations in life history. We therefore developed and validated a highly sensitive mass spectrometry (MS)-based method for the identification of major metabolite classes in worms. Using (UPLC-)tandem-MS we detected 44 fatty acids (FAs) and 19 amino acids (AAs) in a sample that is equivalent to approximately 500 worms. To confirm the validity of the method, we analyzed the changes in FAs and AAs in worms deficient in mdt-15 and bcat-1 respectively, which encode enzymes important to lipid and branched-chain AA catabolism. As a consequence, mdt-15 RNAi worms accumulate C18:0 FA at the expense of polyunsaturated FAs, while bcat-1 RNAi worms accumulate BCAAs. We then applied this method to analyze the changes in metabolite profiles throughout development and aging. The abundance of FAs was low during the larval stages and followed by an increase in most of FA species, which reached the peak at day 7 of adulthood. Most AAs reached the peak during the later larval stages and decreased in adult phase, except for aspartate and glycine, which remained low in development and early adulthood, and significantly increased in later age. Furthermore, by exposing worms to E. coli strain HT115 or strain OP50 as food, we demonstrated that worm FA and AA composition reflects the diet they eat. Altogether, this MS-based method is a powerful tool to perform worm metabolomics for studies focusing on aging and metabolic challenges.
Role of innate immune factors in DNA damage responses of *Caenorhabditis elegans*

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DNA damage can be sensed in the *Caenorhabditis elegans* germline by RAD-5 and components of the 9-1-1 complex (HUS-1, MRT-2 and HPR-9). After damage recognition, *C. elegans* germ cells activate check point signaling that lead to either transient cell cycle arrest and DNA repair mechanisms or apoptosis. The p53 homolog CEP-1 mediates only the apoptotic response upon DNA damage, however it is dispensable for cell cycle arrest and repair. Studies on the *C. elegans* germline were thus valuable for a better understanding of apoptosis regulation and further investigations are still required for the identification of novel factors that might be involved in this process. In addition, recent evidence indicates that DNA lesions in the germline induce systemic stress resistance through the activation of innate immune factors. While the mechanisms through which genome instability promotes DNA damage checkpoint signaling are established, the role of innate immune pathways and their activation upon DNA damage remains unclear. Here, we apply the germline of *C. elegans* to investigate the function of major immune factors and their potential targets in regulating the responses to DNA damage. Via RNA sequencing transcriptome analysis and the wormpath algorithm, we were able to generate a network of DNA damage-responsive genes and hope to identify pathways that might link the components of innate immunity with DNA damage check point signalling.
Linking Inhibition of Induced Transdifferentiation with Proteostasis

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Regulations of pluripotency and cellular reprogramming have been extensively studied on the level of transcriptional regulation. However, transcription factors (TFs) required for induction of specific cell differentiation programs during development are often inefficient in imposing those on other differentiated cells. It has recently been shown that inhibitory mechanisms can be involved in preventing these TFs from driving their differentiation programs in ectopic cells (Tursun et al. 2011, Patel et al. 2012). In order to identify such factors that contribute to safeguarding cells against ectopic fates, we performed RNAi screens. We used transgenic animals expressing CHE-1, the ASE neuron fate-inducing TF, under the heat shock promoter and GFP under the ASE neuron-specific promoter \(gcy-5\) (\(hsp-16.2p::che-1\), \(gcy-5p::gfp\)). After ubiquitously inducing \(che-1\) mis-expression, \(gcy-5::gfp\) is visible in a few additional head neurons, but no ectopic induction in other tissues is detectable.

Our screen identified three different ubiquitin-proteasome system (UPS) genes, \(eel-1\) (encodes a predicted Hect E3 ubiquitin ligase HUWE1), \(hecd-1\) (HECTD1 ubiquitin ligase homolog), and \(math-33\) (homolog of Ubiquitin-specific protease 7). When the transcription factor CHE-1 is broadly expressed, the depletion of each of them allowed ectopic induction of the ASE-specific neuronal marker \(gcy-5::gfp\) in the germline, suggesting a role of posttranslational modifications in inhibiting induced transdifferentiation.
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Study of dynamics and regulation of purinosome, the multienzyme complex of de novo purine synthesis, in Caenorhabditis elegans

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Purines are essential building blocks of nucleic acids and cofactors and they are universal carriers of chemical energy in all organisms. Sufficiency of purines is in equilibrium among de novo purine synthesis (DNPS), salvage pathway and excretion. DNPS is provided by cascade of ten enzymatic steps. Four of these enzymes are multifunctional and thus they organize into dynamic multienzyme complex called purinosome. Purinosome formation was initially observed in transiently transfected cell lines with fluorescently labeled proteins of DNPS in purine-depleted medium. We previously confirmed the purinosome formation by immunochemistry using fixed HeLa, HepG2, HEK293 and Saos-2 cell lines, human fibroblasts and keratinocytes. But there is still little known about its proper composition, dynamics and types of cell regulation. Caenorhabditis elegans is promising in vivo model for study of this enzymatic complex. We prepared a model carrying translational reporters of two enzymes of the DNPS pathway – phosphoribosylpyrophosphate transferase tagged with green fluorescent protein and triGART tagged with red fluorescent protein mCherry. The transgenic worms are observed by confocal microscopy. We study worms in different larval stages in certain time period. Our preliminary results show increased colocalization of fluorescent signals of these two reporters in body wall muscle and pharyngeal muscle of the worms. There are visible differences in starved worms (no food or medium without additive chemicals) or in dauer larvae compared to well-fed animals. We supposed that the nutrient deprivation should let to increased forming of purinosome as in other studies. We are going to observe changes in purinosome formation in different conditions – feeding by purine metabolites, purine analogues etc. At the same time we can measure final products of purine metabolism by HPLC and LC-MS/MS to see differences in metabolome profile of those differently fed worms. This work was supported by Ministry of Health of the Czech Republic, grant nr. 15-28979A. All rights reserved.
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Effects of 2-O-β-D-glucopyranosyl-carboxyatractyligenin from raw coffee in C. elegans

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Various plant extracts are used as supplements in the human diet to support weight reduction and healthy living. Raw coffee (Coffea arabica) based dietary supplements are advertised due to its content of antioxidants like chlorogenic acid and various flavonoids. However, they also contain high concentrations of 2-O-β-D-glucosyl-carboxyatractyligenin (CATC-Gluc), a derivative of the phytotoxin carboxyatractyloside, which was found to be a potent inhibitor of adenine nucleotide translocase (ANT) in isolated mitochondria from mouse tissue [1, 2]. CATC-Gluc is bioavailable in humans supplemented with raw coffee extract, and we wondered about its systemic impact. The pure compound 2-O-β-D-glucosyl-carboxyatractyligenin was isolated from raw coffee and purified by chromatographic techniques as reported recently [1, 2]. To analyse the effects of CATC-Gluc in vivo, C. elegans were treated with the pure compound in NGM agar at concentrations comparable to the CATC-Gluc content of raw coffee based dietary supplements. E. coli OP50 do not metabolize the mother compound, leading to a high bioavailability of CATC-Gluc in the nematodes found by LC-MS/MS analysis, and quantified using gibberellic acid as the internal standard. CATC-Gluc induced a decrease in adult lifespan and in reproduction rate. These changes seem to be mostly driven by partial inhibition of ANT leading to a manifested shift in the cellular ADP/ATP ratio. The present study gain first insights into potential negative physiological side-effects of raw coffee containing dietary supplements on survival and reproduction of eukaryotic organisms. [1] Lang R, Fromme T, Beusch A, Wahl A, Klingenspor M, Hofmann T. 2-O-β-D-Glucopyranosyl-carboxyatractyligenin from Coffea L. inhibits adenine nucleotide translocase in isolated mitochondria but is quantitatively degraded during coffee roasting. Phytochemistry. 2013. 93:124-35. [2] Lang R, Fromme T, Beusch A, Lang T, Klingenspor M, Hofmann T. Raw coffee based dietary supplements contain carboxyatractyligenin derivatives inhibiting mitochondrial adenine-nucleotide-translocase. Food Chem Toxicol. 2014.70:198-204.
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Natural Variation in Acute Stress Resistance, DR and Lifespan in a Multi-Parent Recombinant Inbred Line Panel in C. elegans

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Stress and lifespan are closely related, with many mutations that alter lifespan also affecting stress-response and nutrient sensing. Experiments on stress can therefore provide insight into the mechanisms of ageing and C. elegans is an important model species for such studies. Most studies in C. elegans have however been limited to using just one canonical strain (N2) and many studies of natural variation have relied on differences between N2 and a small number of other genotypes. Given the N2 was maintained in the continuous culture for about 13 years it is likely that it has adapted to laboratory conditions. This potentially constrains the detection and functional analysis of allelic variants that could play major roles in determining the control of complex traits. Knowing that genetic variation can significantly influence stress response a closer look at the freshly derived wild isolates is needed to obtain more realistic picture. Here, we present an analysis of multiple acute stress responses (cold shock, heat shock and oxidative stress), of dietary restriction and of lifespan for a new panel of 200, sequenced and genotyped recombinant inbred lines (RILs) without N2. These RILs are derived from four wild isolates which are representative of genotypically distinct groups of C. elegans isolates from France. The RILs are therefore good representatives of natural genetic variants and do not contain the lab adaptation alleles found in N2. Our analyses identify extensive variation between the RILs, identify quantitative trait loci (QTLs) for all traits, and identify co-localising QTLs for several sets of traits. In conjunction with the gene expression data available for these RILs, we are now analysing candidate genes for these QTLs.
iglr-2 is a paqr-2 Genocopy Important for Membrane Homeostasis

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We previously showed that PAQR-2 regulates membrane homeostasis, specifically inducing fatty acid desaturation when increased membrane fluidity is required. To identify novel components of the paqr-2 pathway that are important for glucose tolerance, we sought mutants that have phenotypes identical to that of paqr-2, i.e. paqr-2 "genocopies". These novel mutations may affect genes that act together with or downstream of PAQR-2. A forward genetics screen of over 80 000 mutagenized haploid genomes yielded 5 mutants with phenotypes identical to that of paqr-2: three were alleles of the gene iglr-2 and two were novel alleles of paqr-2 itself. The iglr-2 mutant alleles are remarkably similar to paqr-2 in all assays tested: they exhibit the same cold sensitivity, tail tip defect, excess of saturated fatty acid, decreased fat-7 expression, sugar intolerance, as well as reduced brood size and slow growth rate. This striking similarity in phenotypes suggests that iglr-2 and paqr-2 act together as a complex or act in a simple direct sequence, one being downstream of the other. One prediction from these models is that the double mutant should exhibit the same phenotypes as the single mutants. This is indeed the case. Genetic evidence therefore suggests that iglr-2 and paqr-2 act in a mutually dependent way during cold adaptation, tail tip morphogenesis, regulation of fatty acid composition, and sugar tolerance. IGLR-2 is predicted to consist of an intracellular C-terminal domain, a single transmembrane domain, and an extracellular part with an immunoglobulin (Ig)-like domain and several leucine rich repeats (LRRs). We used Bimolecular Fluorescence Complementation analysis (BiFC) to test whether PAQR-2 and IGLR-2 actually interacts with each other. BiFC is a powerful method to visualize protein interactions in vivo that relies on fusing two separate portions of the Venus yellow fluorescent protein to each putative protein partner: physical interaction between the partners brings the complementary fragments of the fluorescent protein in close proximity, allowing its assembly and fluorescence. BiFC shows that PAQR-2 and IGLR-2 interact on cell membranes. We conclude that PAQR-2 and IGLR-2 can form a complex on plasma membranes, and that this likely explains the genetic evidence for mutual dependence.
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Somatic stress responses and changes in odor preference in response to benzaldehyde

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Every living organism has to effectively distinguish beneficial and harmful conditions to survive. To understand the mechanism of this decision we have to examine a whole organism from the level of cells to behavior. The nematode *Caenorhabditis elegans* changes its preference to odors after negative conditioning with starvation or learn to avoid the smell of pathogenic bacteria after infection. A yet unexplained phenomenon in *C. elegans* is an aversive response to high concentrations of otherwise attractive odors. Aversion indicates possible harmful conditions, which also evoke stress responses at the cellular level. In *C. elegans* a major signaling protein of cellular stress is JNK-1, which is a direct activator of the DAF-16, FoxO3 orthologue transcription factor, required for cellular homeostasis, immunity and longevity. In this work we explore possible connections between cellular stress responses and the aversive response to high concentration of the odorant, benzaldehyde. We examined the effects of benzaldehyde to N2 wild-type animals with both light- and fluorescent microscopy. The nuclear translocation of DAF-16 occurs upon various environmental stresses, such as heat shock, oxidative or osmotic stress, therefore a DAF-16::GFP expressing strain (TJ356) was applied as an indicator of cellular stress conditions. To investigate the mechanism of aversion, we assayed the kinetics of olfactory preference change to benzaldehyde in wildtype, *daf-16* and *jnk-1* mutant strains. We found that concentrated benzaldehyde treatment for one hour has robust effects on both L1 and adult worms. We observed decreased motor activity, and increased aging-related autofluorescence in worms. The same treatment caused massive translocation of DAF-16::GFP to the nucleus already after 15 minutes. DAF-16 translocation was time and concentration dependent. We confirmed previous findings that wild-type worms show an initial attraction to benzaldehyde, but their response turns into aversion after one hour in the presence of the odorant. This change in odor preference was absent in the *jnk-1* mutant strain after one hour. The *daf-16* mutant performed similarly to wild-type, which implies that JNK-1 acts on a different signalling pathway to promote aversion. Our work on other stress-responsive pathways, including the chaperone Hsp90 is under way. Based on our results, we conclude that benzaldehyde acts as a toxic agent and causes decreased movement in *C. elegans* while it increases aging-related autofluorescence and DAF-16 nuclear translocation. A possible interpretation of our results is that the change in benzaldehyde preference is caused by toxic effects and it is signalled to the nervous system through JNK-1, to change behavioral strategy. Our findings can contribute to understand the neuro-endocrine mechanisms behind the communication between somatic cells and neurons upon stress.
“Muscle to Epidermis” mechanotransduction pathways involved in \textit{C.elegans} embryonic elongation

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Actomyosin contraction drives \textit{C. elegans} embryonic elongation solely upto 1.7 fold, till muscles become active and drive the elongation further. Muscle contraction induced tension leads to GIT-1 recruitment and remodelling of Hemidesmosomes, ultimately contributing to \textit{C. elegans} elongation. Loss of muscle activity in the embryo results in elongation arrest at 2 fold or paralysed at two fold (PAT). Surprisingly GIT-1 null mutant does not display 2 fold arrest or PAT phenotype and is completely viable. This led us to predict the existence of parallel pathways potentially relaying the mechanical effect of muscles. To identify them and the players involved, a genome wide RNAi screen was performed on a git-1 mutant background looking for enhancers of Body morphology defects (BMD), PAT or elongation arrest phenotypes. Subunits of the dynein/dynactin complex, phosphatase 2A (PP2A), spectrins (alpha and beta), capping proteins, cyclins were some of the most promising candidates based on their interaction with git-1 and egl-19, a pore forming complex alpha subunit of voltage gated Ca$^{2+}$ channel. Most of the dynein/dynactin mutants are lethal, so we studied the interaction of git-1 with a weak thermosensitive allele (egl-50) of the dynactin complex subunit ARP-1. To further understand the interaction and what components are being affected, we combined fluorescent markers of adherens junctions, hemidesmosomes and the extracellular matrix (ECM) with egl-50;git-1. Further crosses of git-1 with sur-6 (subunit of PP2A) and dnc-1 (subunit of Dynein) are undergoing to elaborate the interactions. This will lead us to a better understanding of muscle tension induced embryonic elongation.
Natural variation in dauer formation and salt tolerance of Pristionchus pacificus

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Phenotypic plasticity can help organisms to adapt to different environments by forming more than one phenotype from a single genotype. Dauer larvae are a fascinating example of this phenomenon. They are formed under stressful conditions as an alternate third larval stage of free-living nematodes like Pristionchus pacificus (Ppa). They are highly long-lived and extremely stress resistant. Previously, natural variation studies showed that dauers of one Ppa strain, RS5134B, survive significantly longer than those of the standard laboratory strain PS312. RS5134B also forms more dauers in response to its own pheromone and that of PS312 than the latter. Strains like RS5134B are therefore described as “Escapers”, since they escape the harsh environment by entering the dauer phase, whereas isolates like PS312 are “Cheaters”, since they cheat the pheromone communication system. How do Escapers survive longer than Cheaters? Would they also differ in stress tolerance? What would be the nature and dynamics of such a trait? To answer these questions at a molecular level, I conducted an RNASeq analysis of differentially expressed genes. Based on PFAM domain studies and one-to-one orthologs, I selected two genes for further studies: hgo-1, significantly upregulated in Escaper dauers, and ctbp-1, highly expressed in Cheater dauers. The candidate genes were validated by qPCR. hgo-1 encodes for a putative ortholog of human homogentisate oxidase, an enzyme involved in the catabolism of Phe and Tyr. Enzymes in this pathway in general, and HGO-1 in particular, have been suggested to play a role in stress tolerance in plants, worms and hibernating stages of mammals. ctbp-1 codes for a C-terminal binding protein, which has been shown to negatively influence life span in C. elegans (Cel). hgo-1 was suggested to have a role in salt tolerance in Cel adults. I developed a liquid culture assay for studying this stress in Ppa dauers. Escapers recover significantly better than Cheaters, and dauers of Cel, when exposed to acute shocks (1 hour) of extremely high concentrations (1M-3M) of sodium chloride (NaCl), surviving up to 4 hours at 3M NaCl. However, chronic exposure of 24 hours at lower levels of NaCl (20mM-900mM), and heat and oxidative shocks, failed to elicit this dichotomy. Adults of the two strains do not differ in survival in response to either chronic or acute salt stress. Abundant natural variation exists among dauers of more than 20 strains of Ppa for tolerance to salt shock. Pre-adaptation of dauers of either type at 400 mM NaCl for 1 hour enhanced their recovery after longer exposure (2 hours) of 3M NaCl. hgo-1 expression, as quantified by qPCR, is positively correlated with duration and level of exposure of salt shock. It is enhanced further upon pre-adaptation of dauers, peaking at 90 minutes of exposure of 3M NaCl. Thus, Ppa Escaper dauers survive better than Ppa Cheater dauers and Cel dauers under extreme saline conditions for short periods of time, and there is no difference in response to long exposures of mild salinity. This differential recovery of Ppa dauers to salt shock is stress- and stage-specific. hgo-1 is an interesting candidate to study the underlying mechanism of stress tolerance, while work on ctbp-1 aims to unravel the negative regulators of longevity of Ppa dauers using the well-established Ppa molecular toolkit.
Impact of individual GLD-1 binding sites in target 5' and 3' UTRs

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Untranslated regions (UTRs) of messenger RNAs (mRNAs) provide the landing platforms for RNA binding proteins and small RNAs, the major effectors in post-transcriptional gene regulation. While 3' UTRs are well-studied, much less is known about regulatory interactions within 5' UTRs. Furthermore, virtually all studies which dissect function of regulatory sites in 3' UTRs do not take into account possible interactions with 5' UTRs. GLD-1 is a conserved RNA binding protein (RBP) and a translational repressor with essential roles in C. elegans germ cell development. We could show in an earlier study (Jungkamp et al., 2011) that GLD-1 binds highly conserved sites in 5' UTRs in addition to in part previously described 3' UTR sites. Using targeted insertion via the MosSCI system, we systematically test individual sites in 5' and 3' UTRs. Our data indicate that sites in 5' UTRs can mediate specific and strong translational repression. Furthermore, considerable derepression is only observed when mutating all binding sites in both UTRs of a transcript, suggesting that it is important to investigate 5' and 3' UTRs in combination.
Cell cycle-independent role of the pre-replication complex during anchor cell invasion

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Anchor-cell (AC) invasion is serving as an excellent model of study fundamental aspects of cell invasion, a process occurring during normal development as well as during tumor metastasis. By screening the *C. elegans* orthologs of genes up-regulated in invasive human melanoma, we have identified *mcm-7* as a regulator of AC invasion. *mcm-7* encodes a component of the pre-replication complex, which is required for the licensing of replication origins during the G1 phase of the cell cycle. However, the invading AC never replicates its DNA and remains arrested in G1. Moreover, AC-specific RNAi of *mcm-7* causes a similar invasion defect as systemic RNAi, suggesting a cell cycle-independent and cell-autonomous function of MCM-7. RNAi of other components of the pre-replication complex such as *cdc-6, cdt-1* or *orc-2* causes a similar phenotype. We thus propose that components of the pre-replication complex have adopted a cell-cycle independent function to regulate cell invasion.
Thermo-sensitive (TS) mutants have always provided an incredible resource in studying the roles of genes in specific developmental contexts as well as in genetic screens. On the same line, methods which permit conditional gene expression have been regarded as a precious resource for understanding gene functions. Unfortunately, TS mutations do not exist for the great majority of genes and conditional gene expression methods have been restricted, so far, to somatic cells. Even when possible, conditional gene expression has never provided a way to tightly control transcriptional levels and over-expression methods have frequently been the only choice to use. We propose a new method to conditionally control gene expression of virtually any kind of gene in a TS manner. The method is based on the use of a TS allele of the smg-1 gene, which codes for one of the components of the nonsense-mediated mRNA decay (NMD), and the add-in of a so-called “NMD element” at the end of the coding region of the gene of interest. It is important to note that getting rid of the NMD pathway in Caenorhabditis elegans does not cause major defects in the animal development, making our system a powerful and amenable way of controlling gene expression and to create TS null or gain-of-function alleles.
Optogenetic analysis of previously unstudied AS neurons in the locomotor circuit

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Caenorhabditis elegans moves by generating waves of dorso-ventral bends along its body. Head and ventral nerve cord (VNC) circuits control the animal’s undulations. The core components of the motor circuit revealed include five pairs of premotor interneurons, which integrate inputs from sensory and upper layer interneurons and five types of motoneurons found in ensembles or subcircuits, repeating 9-11 times from the “neck” to the tail of the animal (White et al., 1986; Haspel, O’Donovan, 2011). For 11 cholinergic AS neurons in VNC surprisingly no physiological data of their role in locomotion is available to date. In this study we analyzed the locomotion circuit with emphasis on the previously “understudied” AS motor neurons by optogenetic tools. Expression of optogenetic tools in AS neurons was achieved either by broad expression and reduction in subsets of cells, or using the Q system (Wei et al., 2012). Depolarization of AS neurons by channelrhodopsin-2 (ChR2), using selective illumination (Faumont et al., 2011) showed much less contraction of the body, when compared to activation of all cholinergic motorneurons, had less effect on speed, and caused dorsally biased turning movement. In contrast, worms expressing ChR2 in all cholinergic motorneurons ceased the motion due to spastic paralysis / hypercontraction of all muscles. Surprisingly, acute hyperpolarization of AS neurons by the Cl-conducting anion channel rhodopsin ACR-1 (Sineshchekov et al., 2015) led to partial body contraction, while animals with ACR-1 expressed and stimulated in all cholinergic motorneurons elongated; macroscopically, in both cases, animals reduced their speed and stopped. Expression of the histamine-gate Cl-channel HisCl1 (Pokala et al., 2014) in AS and all cholinergic interneurons also caused ceased locomotion of the worms. The data supports the hypothesis that AS neurons significantly contribute to locomotion process. Currently, we address the role of AS neurons by Ca2+ imaging in free-moving animals, both in the AS neurons (using GCaMP6), and in the post-synaptic muscle (using calcium indicators combined with rhodopsins in the AS neurons). The particular role of AS neurons and the synaptic partners will be discussed.

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In Situ and In Vivo miRNA Target Validation Studies: Many Hands Make Light Work and Great Contributions

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The ambiguous nature of miRNA:target base-pairing has made it hard to define strict, “programmable” rules that would allow computer algorithms to predict miRNA targets with high confidence. The algorithms of competing software packages differ, and therefore produce lists of putative miRNA targets that aren’t always comparable. These facts have led to an “industry standard:” computationally predicted miRNA targets require experimental validation in order to establish definitively whether or not a gene is specifically targeted by a given miRNA. Wonderfully, miRNA:target validation experiments are relatively simple, inexpensive (at least in preliminary phases), and are wonderful “feet-wetting” projects for undergraduate students who want to do research in preparation for subsequent training. Such studies are particularly attractive for researchers at small universities for two reasons. 1) One big obstacle for researchers at small universities is the availability of funding support. Limited funding leads to limited research, and limited numbers of students that are offered the privilege of getting critical pre-graduate, hands-on research experience. With thousands of predicted miRNA:target interactions, validation studies provide a seemingly limitless amount of work to offer undergraduate researchers at a very limited monitary cost to the laboratory/university. 2) While the work of miRNA:target validation is critical to the miRNA field, few if any laboratories are dedicated solely to the purpose of performing validation studies. Laboratories that do participate in these studies fill a significant niche in the field and provide meaningful contribution to the miRNA community. The work described here summarizes some of the findings of undergraduate researchers at Mount St. Mary’s University in their efforts to experimentally validate predicted miRNA targets.
Axonal calcium imaging in freely moving animals

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To correlate natural behavior with fast neuronal activity fluctuations in a non-invasive manner is one of the great challenges in neurobiology. Sensitive genetically encoded calcium indicators (GECIs), targeted to single neurons of interest, now facilitate robust quantitative monitoring of calcium dynamics. Recent developments combining calcium imaging in the whole brain of Caenorhabditis elegans nematodes, using nuclear localized GECIs, and simultaneous behavioral tracking of freely moving animals is currently unraveling the neuronal ensembles regulating worm locomotion. However, as calcium transients in the axon are expected to be most indicative of temporally relevant activity in the neuron (i.e. directly correlated with transmitter release), a microscopic setup for axonal calcium imaging in freely moving worms has been optimized in our lab. Our system allows fast automated tracking of a fluorescent marker while maintaining a broad field of view with sufficient spatial resolution for both somal and axonal calcium imaging. This approach enables measuring the spatiotemporal extent of axonal calcium dynamics as well as synchronously monitoring accurately timed locomotory behavior. Optogenetic stimulation of single neurons furthermore revealed their -thus far unknown- roles in the control of worm locomotion. Simultaneous quantitative behavioral tracking analysis demonstrated their involvement in for instance the fine-structure of body bending or overall velocity. This 'reverse-optogenetics' approach thus enables us to identify interesting candidate interneurons and link their functionality to the regulation of downstream motorneurons mediating locomotion. Measuring the spontaneous activity of these interneurons in freely moving worms with our automated axon calcium imaging setup is used to validate findings from optogenetic experiments and to study the natural function of these neurons in more detail. Correlation of the spatiotemporal calcium dynamics along an axon with behavioral parameters like speed, body angles, length or forward-backward transitions permits an in depth analysis of higher order neuronal regulation of natural locomotion in C. elegans.
Poster abstracts in alphabetical order (first author)

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DLK-1/p38 MAP kinase signaling controls cilium length by regulating RAB-5 mediated endocytosis

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Cilia are sensory organelles present on almost all vertebrate cells. Cilium length is constant, but varies between cell types, indicating that cilium length is regulated. How this is achieved is unclear, but protein transport in cilia (intraflagellar transport, IFT) plays an important role. Several studies indicate that cilium length and function can be modulated by environmental cues. As a model, we study a C. elegans mutant that carries a dominant active G protein \( \alpha \) subunit (gpa-3QL), resulting in altered IFT and short cilia. In a screen for suppressors of the gpa-3QL short cilium phenotype, we identified uev-3, which encodes an E2 ubiquitin-conjugating enzyme variant that acts in a MAP kinase pathway. Mutation of two other components of this pathway, dual leucine zipper-bearing MAPKKK DLK-1 and p38 MAPK PMK-3, also suppress the gpa-3QL short cilium phenotype. However, this suppression seems not to be caused by changes in IFT. The DLK-1/p38 pathway regulates several processes, including microtubule stability and endocytosis. We found that reducing endocytosis by mutating rabx-5 or rme-6, RAB-5 GEFs, or the clathrin heavy chain, suppresses gpa-3QL. In addition, gpa-3QL animals showed reduced levels of two GFP-tagged proteins involved in endocytosis, RAB-5 and DPY-23, whereas pmk-3 mutant animals showed accumulation of GFP-tagged RAB-5. Together our results reveal a new role for the DLK-1/p38 MAPK pathway in control of cilium length by regulating RAB-5 mediated endocytosis.
The yolkless nematode: brain control of reproduction through the CEH-60 transcription factor

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Vitellogenesis or maternal yolk production is considered a driving force in the reproduction of all egg-laying animal species. C. elegans possesses six vitellogenin genes, coding for three distinct yolk proteins. Their function seems clear: after production in the intestine and deposition in the pseudocoel, yolk proteins accumulate in the oocyte through receptor mediated endocytosis, where they provide essential nutrients to the growing embryo. Recent observations however, question whether this is the case: mutants that lack almost all yolk protein content, still reproduce perfectly fine.

A newly identified regulator of vitellogenesis, CEH-60, is expressed in the head region of the worm and potentially acts as a transcription factor. ceh-60 mutants lack almost all yolk proteins, but produce a normal number of viable offspring. We observed that these animals also show decreased oxidative stress resistance, highlighting the possible antioxidative properties of yolk proteins. Furthermore, we observed that ceh-60 mutants fail to survive starvation during the first larval stage (L1 diapause), suggesting a role for yolk proteins in larval, rather than embryonic, survival. To identify the molecular players and biochemical pathways involved in CEH-60 signaling from the brain to the pseudocoel, we performed a differential proteomics study in wild-type worms vs. ceh-60 mutants. Our study raises several questions about the regulation of vitellogenesis, the nature of yolk proteins, and ultimately, the life-driving force that is reproduction.
A neuropeptide Y/neuropeptide F-like signaling system in C. elegans

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Neuropeptides represent a large and diverse group of signaling molecules that are mainly produced by neurosecretory cells. They may act as fast neurotransmitters, neuromodulators or neurohormones, thereby regulating fundamental physiological processes such as feeding, locomotion, reproduction, social behavior and learning and memory formation. The C. elegans genome contains at least 120 neuropeptide precursor genes encoding more than 250 bioactive peptides. The majority of neuropeptides exert their function by binding to plasma membrane-associated receptors known as G-protein coupled receptors (GPCRs), of which about 150 receptor genes have been predicted in C. elegans. Coupling of the putative receptors and their natural ligand(s) remains a challenging task, reflected by the small number of peptide GPCRs being deorphanized so far. We performed a large-scale deorphanization screen and thereby identified several evolutionary conserved neuropeptide systems in C. elegans, including a novel pathway related to mammalian neuropeptide Y (NPY) and insect neuropeptide F (NPF) signaling. In order to gain clues as to the biological function of this neuropeptide signaling system, the spatial expression of the NPY/F precursor and receptor genes was examined using fluorescent reporter constructs. These expression patterns suggest a role for NPY/F signaling in the regulation of feeding behavior and chemotaxis to water soluble attractants in C. elegans.
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RBBP-5, a component of the core complex required for histone 3 lysine 4 methylation, maintains redox balance and prevents premature ageing

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Depleting WDR-5 or ASH-2 extends lifespan in C. elegans. The longevity phenotype has been shown to be transgenerationally inherited for up to four generations after which normal lifespan resumes. WDR-5, ASH-2 and RBBP-5 form the core complex required for histone 3 lysine 4 methylation (H3K4me). We have assessed the longevity of a strain containing a predicted null allele of rbbp-5 and surprisingly found that it is short-lived. In a bid to understand the mechanism behind lifespan regulation by this complex, we have tested the difference between long-lived wdr-5 (-) and short-lived rbbp-5 (-) worms. Firstly, we found that both wdr-5 (-) and rbbp-5 (-) worms display a slight delay in their, otherwise normal, larval development. Secondly, we used a panel of GFP reporter strains to assess the stress response in a wdr-5 (-) and rbbp-5 (-) background. We checked the endoplasmic reticulum unfolded protein response (UPR), the mitochondrial UPR and the levels of reactive oxygen species (ROS). Strikingly, ROS, assayed via the detoxification enzyme SOD-3, was the only assayed stress response that appeared to be altered in either genotype. Although both wdr-5 (-) and rbbp-5 (-) mutant worms displayed an increase in expression of the sod-3::GFP reporter gene, rbbp-5 (-) showed a significantly higher expression of sod-3 compared to wdr-5 (-) worms. Finally, we used an alternative ROS reporter, grx1-roGFP2, to assess directly the glutathione redox state. We found that rbbp-5 (-), but not wdr-5 (-) worms display an altered glutathione redox state. In conclusion, rbbp-5 (-) phenotypes are consistent with premature ageing. Despite approximately normal development, rbbp-5 (-) worms live shorter than wild type worms, which may result from the deleterious effects of an altered ROS state. This work was supported by the Biotechnology and Biological Sciences Research Council doctoral training programme (BB/J014478/1)
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The embryonic sheath is a load-bearing protective layer translating mechanical forces to C. elegans embryonic elongation

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During morphogenesis, the role of apical epidermal structures like the cytoskeleton and junctions has been well characterized. In contrast, little is known about how the apical extracellular matrix (aECM) and its remodeling contribute to morphogenesis. The embryonic sheath – the aECM wrapping around C. elegans embryo has been proposed to be a force-transmitting component during its elongation (Priess and Hirsch 1986), however this hypothesis has not been demonstrated. We decided to investigate the composition, organization of the embryonic sheath and how this layer could act mechanically to promote embryonic elongation. In an RNAi screen for transmembrane or secreted proteins affecting embryonic morphogenesis, we identified NOAH-1 and NOAH-2, two ZP (Zona Pellucida)-domain containing proteins. The localization of endogenous NOAH-1 and NOAH-2 reporters at the apical epidermal surface and in the extra-embryonic space suggested that they are embryonic sheath components. The two proteins are enriched in force-bearing structures, such as seam cells and hemidesmosomes, and showed a remarkable reorganization during late phase of the embryonic elongation (after 2-fold embryo stage) to become circumferentially oriented like actin bundles. This remodeling was promoted by muscle contractions. Genetic analysis established that NOAH-1 and NOAH-2 contribute to embryonic elongation and integrity after the 2-fold stage in a redundant manner with other putative extracellular matrix proteins SYM-1, LET-4 and FBN-1, since double defective embryos arrested elongation and burst at an earlier stage than single mutants. As epidermal actomyosin contractility and muscle contractions mechanically drive embryonic elongation, we looked at their mechanical interactions with the embryonic sheath. Using laser nano-ablation, we demonstrated that tension on the embryonic sheath was linearly dependent on actomyosin tension, implying that the sheath relayed actomyosin forces. Interestingly, depleting extracellular matrix proteins impeded muscle contractions, because muscle attachment was affected. In summary, our studies have shown that the embryonic sheath is a key component that transmits actomyosin and muscle forces to promote C. elegans embryonic elongation, while also ensuring mechanical integrity of the embryo submitted to tension. Reference: Priess, J. R. and D. I. Hirsh (1986). "Caenorhabditis elegans morphogenesis: the role of the cytoskeleton in elongation of the embryo." Dev Biol 117(1): 156-173.
Acute cAMP neurotransmission modulation via neuropeptidergic signaling and increased vesicle loading

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Chemical synaptic transmission, i.e. regulated release of neurotransmitter, depends on depolarization and can be addressed by optogenetics, e.g. by channelrhodopsin-2 (ChR2). Rhodopsin based approaches lead to strong neuronal de- or hyperpolarization, overriding intrinsic activity in the neuronal circuit. In contrast, optogenetic manipulation of cyclic AMP (cAMP) signaling augments cholinergic synaptic transmission in a mild way, leading to enhanced but coordinated network output; yet the underlying molecular mechanisms are unclear. We used optogenetic stimulation of cAMP signaling by Beggiatoa spp. photoactivated adenylyl cyclase (bPAC) in C. elegans cholinergic motor neurons to modulate synaptic output. Through behavioral, electrophysiological and electron microscopic analysis, we identified synapsin (snn-1) as a mediator of cAMP effects on rate and quantal size of transmitter release, i.e. miniature postsynaptic current (mPSC) events. This contrasts ChR2 based stimulation, where increased mPSC probability, but not quantal size increase is observed. cAMP enhanced synaptic vesicle (SV) fusion probability by increasing the SV priming rate, partly depending on mobilization mediated by synapsin and leading to increased mPSC rate. cAMP further evoked dense core vesicle (DCV) release of neuropeptides, unlike stimulation using channelrhodopsin. cAMP signaling caused DCV redistribution that was affected by synapsin-dependent capture at the synapse. In snn-1 mutant synapses, DCVs were reduced and dispersed abnormally, but were abundant in cell bodies. Furthermore, snn-1 mutants were unable to release neuropeptides upon bPAC photostimulation measured by fluorescent protein uptake by coelomocytes. Reduction of neuropeptidergic signaling by unc-31 mutants (Ca²⁺-dependent activator protein for secretion, CAPS) abolishes mPSC quantal size increase upon bPAC photoactivation. Through electrophysiological and pharmacological assays we could show that the modulation of the vesicular acetylcholine transporter is required for the increase in mPSCs quantal size observed in wild type synapses. cAMP therefore acts in two different time domains to augment synaptic output in cholinergic neurons of C. elegans. We reason that cAMP increases mPSCs rate by SV priming probability while cAMP induced neuropeptidergic signaling is an automodulatory signal relayed by a GoS independent pathway to increase mPSC quantal size.
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Investigating the role of the ARF GTPase arf-3 in regulating seam cell development and secretion

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We have recently identified the promoter region of arf-3, a small GTPase implicated in intracellular trafficking, as the highly tissue-specific driver of the seam cell marker scm::gfp, an enhancer-trap derived transcriptional reporter commonly used in the study of the development of the stem-like seam cells in C. elegans. This raises obvious questions about possible functions of arf-3 in seam cell development. Knockdown of arf-3 by RNAi leads to variable seam cell numbers suggesting a possible failure in the regulation of asymmetric seam cell divisions during larval development. Here we describe the generation of a knockout strain of arf-3 by Cas9 mediated deletion of the arf-3 coding region. In addition we describe on-going co-localisation studies of arf-3 with other intracellular trafficking components in order to determine the nature and likely function of prominent arf-3 vesicles observed with an arf-3 translational reporter.
Dissection of the genetic architecture in *Caenorhabditis elegans* underlying protein misfolding pathological mechanisms

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Neurodegenerative diseases like Alzheimer's-disease (AD) and Parkinson's-disease (PD) represent an increasing threat to human health. Despite progress in identification of several AD and PD related genes, the effect of alleles underlying protein misfolding in these diseases remains unclear. Given the molecular conservation in essential signalling between the model organism *C. elegans* and humans, transgenic worms that express aggregating proteins have been created for studying the onset and progression of these diseases and the functional alleles that affect them. For example, analysis of transgenic worms expressing the human synaptic protein alpha-synuclein, of which accumulation is causative of PD, have identified links between its proteotoxicity and insulin/insulin-like growth factor (IGF), mitochondrial dysfunction, and aging. The evolutionary forces drive genome generation, and natural variants show impacts on molecular and cellular processes, leading to complex organismal phenotypes in plants, worms, flies, rodents as well as humans. However, previous researches that solely rely on induced mutants in canonical N2 limit the ability to explore how naturally varying alleles alter signalling pathways, including investigation on protein misfolding disease pathological mechanisms. Our research aims to go beyond classical mutant screens on the genetic pathway analysis of complex traits, *i.e.* phenotypic differences among individuals, to also consider the allelic interactions in different genetic backgrounds. We have therefore introgressed the PD transgene (unc-54: α-Syn:YFP) into four different wild isolate genetic backgrounds. Analysis of life history in these new introgressed lines indicates that the transgene effects vary greatly depending on the background. This is a necessary and important step to generally understand the influence of natural variation of the genetic background on the disease progressing mechanism.
Analysis of activity-dependent synaptogenesis at the SAB neuromuscular junction.

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In various systems, the activity of neurons can shape network connectivity and regulate synaptic strength, thus providing the basis for learning and memory. In C. elegans, only few systems have been clearly documented to be regulated by activity for synaptic formation and function. We are currently investigating the effects of activity on the connectivity of SAB motoneurons. Zhao and Nonet (2000) demonstrated that activity can regulate SAB morphology: electrical silencing of the head muscle cells innervated by the SAB neurons caused morphological defects of the SABs, suggesting the existence of an unknown retrograde signal. Using fluorescently-tagged acetylcholine receptors, we observed SAB overgrowth and ectopic synapse formation in unc-13 and unc-18 mutant worms where neuromuscular transmission was disrupted, in agreement with previously published results. Furthermore, the inhibition of acetylcholinesterase enzymatic function, either using ace-2;ace-1 double mutants or by direct pharmacological inhibition with aldicarb, leads to a decrease in the number of acetylcholine receptors (AChR) at the neuromuscular junction, suggesting an activity-dependent regulation of the AChR number. To achieve a fine-tuned control of muscle and neuron activity, we are developing lines expressing channelrhodopsin-2 (ChR2), archaerhodopsin-3 (Arch) and HisCl1 in specific subsets of cells. ChR2 and Arch are optically controlled and can respectively activate or hyperpolarize muscle or neuronal cells, while HisCl1 is a histamine-gated chloride channel leading to electrical hyperpolarization of a cell. Finally, to allow longitudinal study of the SAB NMJs development, we are currently trying to implement microfluidic devices to trap a worm and image it under the microscope at several time points throughout its lifetime without affecting its development. References: Zhao, H., and Nonet, M.L. (2000). A retrograde signal is involved in activity-dependent remodeling at a C. elegans neuromuscular junction. Development 127, 1253–1266.
A ring trial to assess the reproducibility of metabolomic studies in *Caenorhabditis elegans*

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Metabolomics, the systematic analysis of metabolites within an organism, cell, or tissue, is gaining more and more attention in the *Caenorhabditis elegans* research community. In the last 10 years the number of studies published using metabolomics has increased and they now cover a wide variety of questions. Although the number of concept papers and technical developments is rising, no assessment of the biological variation of metabolite content or quantitative reproducibility between labs has been published so far. Here, we present our results from a first ring trial using metabolomic and lipidomic analysis to compare wildtype N2 and *daf-2(e1370)* worms from several labs. In order to eliminate the variation between worm populations fresh worm stocks were ordered from the *Caenorhabditis* Genetics Center (CGC University of Minnesota, Minneaspolis, USA) and sent to all participating partners. These worms were handled by each laboratory according to their standard protocol. Both strains were grown at 20°C until adulthood, recovered from plates, flash frozen in liquid nitrogen and sent for metabolomics analysis. Samples from all labs were extracted on the same day using MeOH:H\(_2\)O (1:1, v:v) for metabolites, followed by Methyl-tert-butylether (MTBE) for lipid extraction. The different fractions were analyzed by UPLC-UHR-ToF-MS non-targeted metabolomics and lipidomics using reversed phased chromatography. Quality control (QC, pooled extracts) samples from each lab and a total QC were used to evaluate technical and biological variability. Our first results indicate that samples from different labs slightly differ in the amount of detected features and also yield different significantly altered features when comparing wildtype N2 and *daf-2(e1370)* worms. A core set of similar changes does, however, exist. Defining the robust features in metabolic analyses will allow results from different labs to be compared with more confidence.
O-GlcNAc-modification of SKN-1 regulates oxidative stress defense and ageing in C. elegans

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The transcriptional factor SKN-1 induces the transcription of the conserved phase-2 detoxification genes that play key roles in oxidative stress resistance and ageing in C. elegans. Phosphorylation of SKN-1 mediated by several signaling pathways is important for its functional regulation. It has been reported that the glycogen synthase kinase-3 (GSK-3) prevents SKN-1 from accumulating in nuclei and functioning constitutively in the intestine through phosphorylation at Ser483 and Ser487 of the protein. However, whether other post-translational modifications of SKN-1 affect its function remains unclear to date. A dynamic cycle of O-linked N-acetylglucosamine (O-GlcNAc) addition and removal to the hydroxyl groups of serine or threonine residues is catalyzed by O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA), respectively. O-GlcNAcylation is known to modulate protein functions, including protein-protein interaction, protein stability and subcellular localization. Recent evidence shows that C. elegans ogt-1 and oga-1 mutants alter the phenotypes of lifespan and UV stress susceptibility. However, factors modified by O-GlcNAcylation to regulate ageing and stress resistance are poorly characterized. We found in this study that the O-GlcNAc transferase OGT-1 mediated the O-GlcNAc-modification of SKN-1 at Thr470 and Ser493 by in vitro O-GlcNAcylation assay and mass spectrometry analysis. Depletion of O-GlcNAcase oga-1 increased O-GlcNAcylation level of SKN-1 and caused the accumulation of SKN-1 in intestinal nuclei, which resulted in promoted longevity and the resistance to oxidative stress. Meanwhile, both lifespan and oxidative stress defense were reduced along with the decrease of O-GlcNAcylation level of SKN-1 in ogt-1 mutants. In mammalian cells, the SKN-1 homolog Nrf2, was also O-GlcNAc-modified by OGT, and the O-GlcNAcylation level of Nrf2 was up-regulated when the cells were under oxidative stress. Moreover, the results showed that O-GlcNAc-modification of SKN-1 blocked GSK-3 phosphorylation of SKN-1 at Ser483 upon oxidative stress. This study reveals a mechanism by which O-GlcNAc-modification regulates oxidative stress defense and ageing. The observed impact of O-GlcNAc cycling on transcriptional factor SKN-1 in C. elegans has important implications for studying the functions of O-GlcNAc-modification in human age-related diseases, such as diabetes and neurodegenerative disorders.
SGK-1 regulates intracellular vesicular trafficking in the intestine of C. elegans

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In mammals, the serum- and glucocorticoid-inducible kinases (SGKs) has been shown to regulate a wide variety of ion channels that participate in the regulation of transport, hormone release, neuroexcitability, inflammation, cell proliferation, and apoptosis. We previously showed that SGK-1, the only Caenorhabditis elegans homolog of human SGKs, acts in parallel to the AKT kinases to mediate DAF-2 signaling. In addition, SGK-1 acts downstream of the mTORC2 kinase Rictor/RICT-1 and is also downstream of the cold-sensitive TRPA-1 calcium channel and calcium-sensitive PKC-2 in a signaling pathway that responses temperature change. Thus, SGK-1 integrates several pathways in the regulation of metabolism, development, longevity, and stress response in C. elegans. We now provide evidence that SGK-1 may also be a regulator of endomembrane trafficking in polarized epithelial cells. Misexpression of sgk-1 results in a vacuolization phenotype that we have used to systematically screen for modifier genes by RNAi. We have identified several candidates supporting a role of SGK-1 in the regulation of intracellular vesicular trafficking. We will provide our current knowledge of sgk-1 function in this process.
A bacterial metabolite that alters fat metabolism and delays nematode growth and development

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Violacein is a biologically active purple metabolite produced by various genera of soil and marine bacteria. Despite the effects of violacein in a variety of organisms that range from bacteria to mammals, and the study of violacein as a possible cancer therapeutic, the molecular mechanism of its effect is not well understood. In order to understand the molecular mechanism of violacein’s effect in the physiology of a whole organism, we used several closely related nematode species, including the well-established genetic model organism Caenorhabditis elegans. We made a violacein-producing E. coli strain of OP50 and found that violacein significantly delayed the growth and development of C. elegans, as well as the related nematodes C. briggsae and two species of Pelodera. Pristionchus pacificus was resistant to the effects of violacein, and C. remanei was partially resistant. In C. elegans, violacein exposure resulted in smaller body length and width, distended intestine, and dose-dependent delay in development, with higher concentrations completely arresting development. Larvae exposed from the L4 stage showed egg retention leading to internal hatching, and near complete loss of fat storage, phenotypes reminiscent of worms in a state of malnutrition. Sterile adults exposed to violacein showed decreased lifespan. We found an upregulation of GFP-tagged marker genes of starvation such as lgg-1 and nlp-29 and a downregulation of the toxicity marker gene gst-4. Intriguingly, we observed that internally-hatched larvae emerging from the wormbag were more resistant to the effects of violacein. Supplementing violacein-exposed C. elegans with either whole worm extracts, worm lipid extracts, or purified oleic acid, but not stearic acid, can similarly improve the growth and development of violacein-fed worms. Furthermore, violacein exposure downregulates expression of the fat-7 gene, which encodes a delta-9 fatty acid desaturase that converts stearic acid into oleic acid. We are currently investigating how violacein regulates fat-7 expression and alters fat production, and the possible role of fats in detoxification pathways. Interestingly, we find violacein localized to intestinal vesicles in violacein-resistant nematodes P. pacificus and C. remanei, and a less-toxic version of violacein called deoxyviolacein localized to birefringent gut granules in the C. elegans intestine.
Ultrastructural analyses of synapses photostimulated with Channelrhodopsin (ChR2)

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Synaptic vesicles undergo cycles of exocytosis and endocytosis to signal to target cells via neurotransmitters and neuropeptides, and to sustain their activity upon prolonged excitation. In order to study vesicle fusion and endocytic retrieval events, electron microscopy (EM) generates high resolution snapshots of the process of neuronal transmission, providing ultrastructural information such as vesicle distribution, abundance, status and identity. However, specimen preparation of EM micrographs limits the scope of ultrastructural studies. Fast and non-invasive stimulation methods are needed for optimal time-dependent analysis of synaptic recycling. The development of optogenetic tools enabled to combine high pressure freezing/freeze substitution EM (HPF/FS) and light stimulation of neurons to study active neurotransmission at high temporal precision and to produce high-resolution structural information of Caenorhabditis elegans synapses. Optogenetic hyperstimulation of cholinergic motorneurons expressing ChR2(H134R) resulted in reduced number of docked and total synaptic vesicles. This is due to an increase of fusion events, consistent with increased frequency of miniature postsynaptic currents (mPSCs) (Kittelmann et al., 2013). Interestingly, large intracellular vesicular structures are formed in response to prolonged photostimulation. This type of vesicles is unlike endosomes which appear irregular in shape, have a denser core and sometimes are observed to traverse synapses as a tubular structure. Large vesicles induced by hyperstimulation constitute a regular shape, large diameter, clear core and sometimes show almost perfect spherical structures. At intermediate times, we observe vigorous endocytosis that apparently leads to co-endocytosis of parts of the neighboring cell. To better understand the nature and the function of the large vesicles, we have crossed mutants of various proteins with roles in the pre-synapse with ChR2 transgenic animals, and tested them in HPF-EM analyses, following photostimulation. Furthermore, focused ion beam scanning electron microscope (FIB-SEM) was used for better reconstruction of entire cholinergic synapses, and to enable calculations of the equilibrium of membranes in vesicular, endocytic or plasma membrane fractions. Refs: Kittelmann et al. PNAS 2013 Aug 6;110(32):E3007-16
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Investigations into the hypoxia-responsive chromatin regulatory factors

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Chromatin regulatory factors are involved in hypoxia-dependent phenotypes. The transition between different chromatin states are mediated via three main mechanisms. Nucleosome remodelling by ATP-dependent chromatin regulatory factors, post-translational modifications of histones and regulation of histone variants. Some of these chromatin regulatory factors have been reported with roles in hypoxia response one of which is the ISWI homolog *isw-1*. Knock-down of *isw-1* renders animals resistant to hypoxic conditions. In the light of these findings, we are investigating the link between hypoxia and chromatin. Depletion of several chromatin regulatory factors regulated differentiation of certain group of cells in a hypoxia-dependent manner. We are currently investigating the genetic basis of these phenotypes. This work is supported by a grant (114Z163) from The Scientific and Technological Research Council of Turkey (Tubitak).
The GATA transcription factor ELT-2 mediates strain-specific immune responses

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Invertebrates were believed to rely exclusively on common responses of the innate immune system to fight the great diversity of pathogenic threats they encounter. However, recent evidence suggests that invertebrate taxa are able to mount pathogen- and even strain-specific immune responses, but the underlying molecular mechanisms are unknown. To test the hypothesis that the C. elegans immune response is pathogen strain-specific, we analyzed the transcriptomes of worms infected with two pathogenic (BT18247 and BT18679) and one non-pathogenic (BT407) Bacillus thuringiensis (BT) strains. We found that while the expression of the majority of genes was generally regulated by infection with both pathogenic BT strains, 9.2% of infection responsive genes were differentially regulated between BT18247 and BT18679. To further investigate the molecular mechanisms underlying this strain-specific response, we searched for transcription factors regulating the strain-specific expression changes. We found that the AP-1 component gene jun-1 regulates the general response to pathogenic BT. In contrast; the GATA transcription factor gene elt-2 mediates strain-specific responses to BT18247 and BT18679: elt-2 RNAi knockdown worms were highly resistant to BT18247 infection, but highly susceptible to BT18679. Through epistasis analysis, we further found that the p38-MAPK pathway acts either in parallel to or directly interacts with elt-2 in response to the pathogen BT18679, but is not required for the resistance to BT18247. The DAF-2/ILR pathway however is involved in the response to BT18247, likely acting downstream of elt-2. Because the pathogenic strains BT18247 and BT18679 differ in the number and type of crystal pore-forming toxins (Cry) they produce, we are currently investigating the C. elegans response to specific Cry toxins. We found that the role of elt-2 in the defense against BT18679 is specific in conferring resistance to the Cry toxin Cry21Aa2. The response of C. elegans to BT infection is thus strain-specific. Our results further suggest that ELT-2 mediates the specificity of the response, likely in interaction with different signaling cascades. We are currently conducting a candidate mutant screen to identify genes that suppress the elt-2 (RNAi) high resistance phenotype on BT18247. In this way we expect to gain a more detailed insight into the mechanisms by which elt-2 mediates the particular interaction with BT18247.
Pharyngeal morphogenesis of the *C. elegans*

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The *C. elegans* pharynx is a powerful system to study organogenesis. Studies of pharyngeal morphogenesis by Portereiko and Mango (2001) defined three temporally separated steps termed reorientation, epithelialization, and contraction. In the first step, anterior pharyngeal epithelial cells are converted from a radial configuration into two parallel rows through changes in cell shape, location, and apicobasal polarity. In the second step, termed epithelialization, reoriented pharyngeal cells attach to the neighboring arcade cells, which are anterior to the pharynx, to form a contiguous epithelial tube connecting the nascent buccal cavity (mouth) to the intestine. In the third step, termed contraction, localized forces pull the arcade and pharyngeal epithelial cells closer together, and the pharynx shifts en masse lightly toward the anterior. This initiates the conversion of the primordial pharynx from a ball of cells into an elongated bilobed tube connecting the mouth to the intestine. We are characterizing a *C. elegans* thermosensitive mutant *t2160* which has morphogenesis defects. The mutant worms develop with unattached pharynx- pun phenotype. *t2160* pharyngeal morphogenesis were visualized using a pha-4-driven plasma membrane–GFP reporter that is expressed exclusively within cells of the pharyngeal primordium, intestine, and arcade cells (Portereiko and Mango 2001). We note that equivalent levels of expression of the Ppha-4::membrane–GFP reporter in wild type and *t2160* mutants indicates that a reduction or loss of *t2160* activity does not affect the specification of the pharyngeal primordium. For further characterize pharyngeal defects in *t2160* mutants, we use a fluorescence reporter for two well-characterized junctional complexes in *C. elegans*, a classical cadherin–catenin complex (HMR-1/cadherin, JAC-1/p120-catenin, HMP-1/α-catenin and HMP-2/β-catenin) and the more basal AJM-1 and DLG-1/discs large complex. Cloning of the mutant has been performed by one-single step whole genome sequencing. In this strategy, *t2160* mutant has been crossed with Hawaiian *C. elegans* strain CB4856. F2 progeny of this cross were analyzed for their distribution of SNP markers. Genomic regions close to the *t2160* mutation show a decreased incidence of Hawaiian vs. Bristol SNPs. Portereiko M. F., Mango S. E., 2001 Early morphogenesis of the *Caenorhabditis elegans* pharynx. Dev. Biol. 233: 482–494
Cytochrome P450 (CYP)-dependent eicosanoids are epoxygenated and/or hydroxylated metabolites of long-chain polyunsaturated fatty acids (LC-PUFAs). They are important lipid mediators participating in numerous physiological and pathological processes in mammalian systems. The CYP-eicosanoid cascade is initiated by the release of LC-PUFA from cell membrane phospholipids by a phospholipase A2, the CYP-dependent eicosanoids biosynthesis and the interaction of eicosanoids with so far unknown cell-surface receptors that are part of the G-protein coupled receptor (GPCR) family. Recent studies revealed that the nematode Caenorhabditis elegans also produces a broad spectrum of CYP eicosanoids, in particular, epoxy- and hydroxy-metabolites derived predominantly from eicosapentaenoic acid (EPA). One revealed physiological function in the nematode is involved in the regulation of pharyngeal pumping. 17,18-epoxyeicosatetraenic acid (17,18-EEQ), as the most abundant CYP eicosanoid in C. elegans, mimics the stimulating effect of serotonin and rescues LC-PUFA deficient strains from their pumping impairments. Here we tested the hypothesis that 17,18-EEQ serves as second messenger in the underlying signaling pathway and exerts its effect by binding to a specific membrane G-protein-coupled receptor in the pharynx of nematode. We performed a biased screen of selected gene knockout strains displaying behavioral impairments in the pharyngeal pumping by feeding these worms with red fluorescent beads. The results showed that the knockout strains nmur-2(ok3502), npr-24(ok3192), exp-2(sa26), eat-2(ad465) and egl-30(ad806), respectively, were unable to respond to 17,18-EEQ treatment. Moreover, manual counting of the pharyngeal pumping frequency in the presence or absence of supplemental 17,18-EEQ was performed in addition. Two of the candidate genes are coding for GPCRs, which indicates that these proteins might be involved in the 17,18-EEQ signaling of C. elegans.
Late Abstracts

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Maintenance of ER homeostasis through the exogenous RNAi pathway in *Caenorhabditis elegans*

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To maintain ER homeostasis eukaryotic cells harbor a quality control system that eliminates misfolded or unassembled proteins from the endoplasmic reticulum (ER) through a process called ER-associated protein degradation (ERAD). While many components of the ERAD machinery are well characterized in yeast and mammalian cell culture, little is known about ERAD in multicellular organisms. To analyze the function and organization of the ERAD machinery during development and aging in a multicellular organism different genetic screening approaches and massspectrometrie analyses are conducted in the well established model organism *Caenorhabditis elegans*. Both strategies allowed the identification of yet uncharacterized components of the ERAD pathway in *C. elegans*. Surprisingly, many of those components belong to the exogenous RNAi pathway. Thus, this study demonstrate a connection between the exogenous RNAi pathway and ER homeostasis.
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Exploring the substrate specificity of the *C. elegans* atypical calpain CLP-1

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The global rise in the proportion of aged individuals has led to an increased awareness of age-related degenerative diseases. *C. elegans* has become a popular model for studying the molecular mechanisms of ageing and age-related diseases.

Calpains are regulatory Ca$^{2+}$ activated cysteine proteases present in nearly all eukaryotes\(^1\). Misregulation of calpain activity resulting from dysregulation of Ca$^{2+}$ homeostasis has been implicated to be a causative agent in age-related pathologies such as Alzheimer’s disease and sarcopenia. However, it has been difficult to identify the substrates targeted by age-dependent calpain activation, as cleavage specificity is determined by substrate tertiary structure and less so by primary sequence\(^2\).

Calpains consist of a core catalytic domain and other discrete domains. Our present understanding of calpain activity is based primarily on studies of ‘typical’ calpains that contain an EF hand domain; less is understood about the 6 human genes encoding ‘atypical’ calpains lacking this domain. The *C. elegans* genome lacks typical calpain genes, yet encodes 10 atypical calpain genes (*clp-1* to *clp-7, F44F1.3, T11A5.6, W05G11.4*), which we have shown are differentially expressed in hypodermis, muscle and other tissues\(^3\). It was found that overexpression of the protein CLP-1 in body wall muscle caused muscle degeneration and paralysis in 1.5% of adults. The level of paralysis increased to 23.5% when CLP-1 was overexpressed in an *egl-19*(gf) mutant, which has elevated intracellular Ca$^{2+}$ levels. Muscle degeneration and paralysis was not observed when catalytically inactive CLP-1 was overexpressed. It was also found that the paralysis phenotype was suppressed when aspartyl protease activity was suppressed by RNAi, suggesting that overactivation of CLP-1 might upregulate the necrotic pathway.

We speculate that CLP-1 may be proteolysing key structural proteins in the sarcomere. To identify potential CLP-1 substrates, we are taking a proteomic approach by screening for CLP-1 binding partners using co-immunoprecipitation followed by mass spectrometry. To facilitate this study, we have expressed an epitope-tagged catalytically inactive CLP-1 in body wall muscle. We hypothesised that potential CLP-1 substrates would retain the ability to be bound by inactive CLP-1 yet fail to be cleaved. Potential CLP-1 substrates identified from this screen will be validated by quantitative Western blotting.

Refs.