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A Novel Exosome-Mediated Apical Secretion Pathway Involving the V0-sector of the V-ATPase in *C. elegans* Epidermal Cells

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Polarized intracellular trafficking in epithelia is critical in development, immunity and physiology to deliver morphogens, defensins, or ion-pumps to the appropriate membrane domain. However, the mechanisms that control apical trafficking remain poorly defined. *C. elegans* epithelia apically deliver transmembrane (TM) proteins (CHE-14/Disp, VHA-5), and secrete cuticle components, Hedgehog-related peptides (HRPs: WRT-2, WRT-8), and antimicrobial peptides. Hence, at least three kind of apical trafficking events take place in these cells: constitutive delivery of TM proteins at the apical membrane, tightly timed secretion related to molting processes, and regulated apical secretion of antimicrobial peptides induced upon infection. *C. elegans* epithelial cells are thus a privileged model to study apical secretion.

We first show that the membrane-bound V0 sector of the vacuolar H+-ATPase (V-ATPase) is involved in apical secretion, independently from its contribution to the V-ATPase osmoregulatory activity driven by the association of both V0 and V1 sectors. Performing site-directed mutagenesis, we obtained mutants of the “a” subunit of the V0 sector (VHA-5) which are secretion-defective but osmoregulation-proficient, and others that are secretion- proficient but osmoregulation-deficient mutants. Using GFP fusions, we observed that VHA-5, CHE-14/Disp, WRT-2/8 accumulate in discrete organelles containing Hrs/VPS-27 (a marker for multivesicular bodies - MVBs) only in secretion-defective mutants. Immuno-electron microscopy analysis confirmed that these accumulations are MVBs where we localized both VHA-5 and WRT-2::GFP.

Our results unravel a novel apical secretion route mediated by MVBs that can release HRPs-containing exosomes thanks to a V0-specific function of the V-ATPase. Based on the similarity with secretion processes in immune cells, we infected worms with fungi, and observed that secretion-defective VHA-5 mutants appear hypersensitive to infection whereas secretion-proficient VHA-5 mutants do not. It raises the possibility that the pathway we described here is necessary for efficient innate immunity.

We are currently using an RNAi based screening strategy on candidate gene families to search for enhancers or suppressors of VHA-5-secretion mutants. We are looking for cuticle defects, impaired resistance to fungal infection, and/or the accumulation of normally secreted GFP tagged proteins. We already found three candidates that are under further investigation.

BACK

T-002

The Wnt Pathway Controls Engulfment Of Apoptotic Corpses, Spindle Orientation And Migration Through CED-10/Rac

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The recognition and engulfment of apoptotic cell corpses is achieved by at least two parallel pathways in *C. elegans*. These are defined by the proteins CED-1/SREC, CED-6/GULP, CED-7/ABCA1 and CED-2/CrkII, CED-5/Dock180, CED-12/ELMO. The pathways merge at CED-10, which codes for a Rac small GTPase. CED-1 is a scavenger receptor for the recognition of cell corpses in the first pathway. However, the receptor which regulates the second pathway has long remained elusive. Here we show that the receptor MOM-5, a Frizzled homolog of the Wnt pathway in *C. elegans*, functions upstream of the second pathway not only in engulfment but also in spindle orientation and gonadal migration. Therefore, in different developmental processes, CED-10/Rac links polar signals mediated by the Wnt pathway to the modulation of the cytoskeleton.

BACK

T-003

Towards A Pathway for Centriole Duplication

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C. elegans embryos are well-suited for deciphering the process of centrosome duplication in metazoan organisms. We and others have identified five molecules as being crucial for centriole formation in the early *C. elegans* embryo: the kinase ZYG-1 and the coiled-coil proteins SPD-2, SAS-4, SAS-5 and SAS-6. All five proteins are enriched at centrioles and found diffusely in the cytoplasm. Moreover, SPD-2 accumulates at the PeriCentriolar Material (PCM).

We set out to investigate the relationships between these five proteins to better understand how they contribute towards building centrioles. First, we addressed which protein is recruited first to newly formed centrioles. We devised conditions where oocytes express in their cytoplasm one of the five proteins fused to GFP and are fertilized by sperm containing unlabelled centrioles. In this manner, we can assess the timing of centriolar recruitment of each fusion protein. We found that SPD-2 and ZYG-1 are recruited to centrioles shortly after fertilization, during meiosis I, whereas SAS-5 and SAS-6 start to be enriched at centrioles at the end of meiosis II. By contrast, gradual SAS-4 incorporation occurs during the subsequent S phase. We next tested whether this differential timing of recruitment reflects related epistatic relationships. Accordingly, we found that in the absence of SPD-2, the centriolar localization of the four other proteins is abolished, while SPD-2 localization remains unchanged in the absence of either ZYG-1, SAS-4, SAS-5 or SAS-6. Our results also indicate that even though ZYG-1 controls centriolar localization of SAS-5 and SAS-6, there is an unsuspected feedback loop from SAS-5 and SAS-6 that modulates ZYG-1 distribution.

Together with previous observations, these findings lead us to propose the following working model for a pathway of centriole duplication in *C. elegans*. Initially, SPD-2 is loaded to centrioles at the very onset of the duplication cycle. This leads to centriolar recruitement of ZYG-1, which in turns allows SAS-5 to deposit SAS-6 onto the mother centriole or a closely associated structure. The presence of SAS-6 in turn triggers SAS-4 recruitment and formation of a new centriole.

BACK

Identifying Monomethyl Branched Fatty Acids as a Critical Product Of *let-767*, A Short Chain Dehydrogenase

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Recently, metabolic studies in *C.elegans* are catching considerable attention. However, the small molecule products of many enzymes are not known. Generally in a genetic system such as *C.elegans*, these molecules can be identified based on two approaches: i) by analyzing metabolites in mutants; ii) by complementing mutants with downstream products. We have exploited these two approaches in order to study the function of *let-767*, a short chain dehydrogenase/ reductase involved either in sterols or fatty acids metabolism. By complementing *let-767 (RNAi)* developmental arrest with hydrophobic extract from wild type worms, we found that the critical missing products in *let-767 (RNAi)* were the monomethyl branched chain fatty acids (mmBCFA). Consistently, the amount of mmBCFA in triacylglycerols from *let-767 (RNAi)* worms was highly reduced. In addition, we studied the cellular requirements of LET-767 and showed that its activity was important for the apical structure of the intestinal cells. Thus, identification of the product of LET-767 allowed to connect its cellular functions with a class of lipids, products of this enzyme. Our combination of approaches can be used in order to study other metabolic enzymes.

BACK

T-005

Barrier-to-Autointegration Factor Participates Directly in Nuclear Envelope Formation *in vivo* and this Function is Regulated by VRK-1 Protein Kinase

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The nuclear envelope (NE) of eukaryotic cells provides an essential barrier that separates the genome from the cytoplasm. In metazoan organisms the seemingly stable structure of the interphase NE is dynamically re-arranged during the cell division. In mitotic prophase the NE breaks down, while in anaphase and telophase it reassembles around the segregated chromatids. Nevertheless, the precise molecular mechanisms and regulations of the NE reformation are still not known. In order to identify novel candidates potentially involved in NE assembly we analyzed a group of maternal-effect embryonic lethal mutations in *Caenorhabditis elegans* that show nuclear appearance defects. This analysis discovered a point-mutation in *baf-1* gene that encodes the *C. elegans* homologue of human Barrier-to-autointegration factor (BAF). BAF is a small and essential protein that is highly conserved among the metazoan species. BAF can bind simultaneously to DNA and a group of integral nuclear membrane proteins and by doing this it was proposed to have a structural role in NE. By structural and functional studies we found that both *baf-1* mutation and BAF down-regulation by RNA-interference (RNAi) results in strong NE assembly defects. Genetic studies showed that the *baf-1* mutation is temperature sensitive. At permissive temperature (15°C) the *baf-1* homozygous mutant embryos are alive and have a functional NE that is indistinguishable from the wild-type NE. At restrictive temperature (20°C and higher) the embryos die and their NE structure resembles that of the BAF RNAi NE. Biochemical studies showed that the mutant BAF protein fail to be phosphorylated at restrictive temperature, while at permissive temperature it is properly phosphorylated. Furthermore we identified the VRK-1 protein kinase as one of the kinases that phosphorylates BAF. Our studies therefore suggested that the role of BAF in NE formation is regulated by VRK-1 kinase. The temperature sensitivity of the *baf-1* mutation is one of the most appropriate tools to investigate the role of BAF during NE assembly *in vivo*. Shifting up the temperature from permissive to restrictive during time-lapse recordings of the *baf-1* homozygous mutant embryos expressing different GFP-tagged NE marker molecules may answer when and how BAF is involved in this complex mitotic process. In order to do these experiments we developed a novel fast response heating-cooling device suitable for confocal microscopy. Using this device our novel discoveries in this field would be discussed.

BACK

T-006

Centrosomes Dictate Timing of Mitotic Entry in *C. elegans* Embryos

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Mitosis is a tightly regulated process that allows equal repartition of the genetic material to daughter cells. Activation of the Cyclin B1/Cdk1 complex is key for initiating entry into mitosis. Intriguingly, in human cells, the active form of Cyclin B1/Cdk1 is detected first at centrosomes, the major microtubule organizing centre (MTOC) of animal cells. However, it is not known in any systems whether centrosomes are required for promoting mitotic entry. We set out to investigate the mechanisms underlying timing of mitotic entry, in the early *C. elegans* embryo. To this end, we developed a novel assay to monitor timing of mitotic entry with high spatial and temporal resolution. Using this assay, we showed that neither anterior-posterior (A-P) polarity, nor microtubules, dictate timing of mitotic entry. By contrast, we established that centrosome integrity is essential. Moreover, we found that the centrosomal Aurora-A kinase AIR-1 is essential for proper mitotic timing. Finally, by using appropriate mutant embryos, we could demonstrate that centrosomes are not only necessary but also sufficient to promote entry into mitosis. Our findings lead us to propose that centrosomes serve as integrative centres for mitotic regulators and thus dictate the timing of mitotic entry.

BACK

DAB-1 Gives Us New Insights into Trafficking Pathways

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The mammalian Disabled proteins are cargo-specific adaptor proteins that facilitate the endocytosis of lipoprotein receptors. We, and others, have obtained data implicating the sole *C. elegans* Disabled, DAB-1, in the trafficking of the lipoprotein receptors LRP-1/-2 (Kamikura & Cooper, 2003; Holmes & Pettitt, IWM, 2005), suggesting that this is a conserved function of Disabled proteins. We have shown that DAB-1 is expressed in oocytes, early embryos, and in all epithelia of late stage embryos and postembryonic stages. It forms membrane-associated puncta that may correspond to clathrin-coated pits or vesicles. DAB-1 puncta are largely confined to the apical membranes of cuticle-secreting epithelia, such as the hypodermis and pharynx: in contrast they are enriched on the basolateral membranes of intestinal cells. Strong punctate expression is also seen on the surfaces of coelomocytes, consistent with the coelomocyte uptake (Cup) phenotype observed in *dab-1* null mutants. DAB-1::GFP expression during endocytosis in epithelial tissues and coelomocytes is particularly dynamic, and we are currently investigating whether DAB-1 puncta dynamics in the hypodermis are subject to developmental regulation, such as during moulting. We are also examining the role of clathrin and the AP-2 complex on puncta turnover. We have previously identified a synergistic genetic interaction between AP-1-mediated trafficking and DAB-1 mediated endocytosis. Similarly, loss-of-function mutations affecting components of the AP-3 complex are synthetic lethal in combination with dab-1 mutations. This suggests that DAB-1 is a component of a pathway that acts in parallel to AP-1 and AP-3 to traffic molecules from the plasma membrane. Thus, in the absence of AP-1 or AP-3 dependent pathways, some cargoes normally sorted by these adaptors can still reach their normal destinations via DAB-1-dependent endocytosis from the plasma membrane.

We want to identify receptors participating in DAB-1-dependent endocytosis. Coelomocytes endocytose a broad range of substrates suggesting that the receptor(s) involved can bind to a wide range of ligands. Lipoprotein receptors, such as megalin, are known to recognise a broad range of ligands making these good candidates for endocytic receptors acting in coelomocytes. We have identified 16 transmembrane proteins containing the DAB-1-binding motif, [F/Y]xNPxY, in their intracellular domain. Five of these are members of the lipoprotein receptor family: LRP-1, LRP-2, RME-2, T13C2.6 and APL-1. It is likely that not all identified receptors bind only to DAB-1, since NUM-1 and CED-6 have also been shown to bind to NPxY motifs. We are carrying out systematic RNAi knockdown of each of these genes to determine whether this will phenocopy the *dab-1* null phenotype. Combined RNAi of *lrp-1* and *lrp-2* results in a high level of embryonic/larval lethality with a greatly increased frequency of moulting defects, indicating that these molecules have overlapping functions, however coelomocyte endocytosis is not noticeably defective.

BACK

T-008

A Novel Role for Nanos in PAR Protein-Dependent Cell Polarity

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Asymmetric cell division is a fundamental process for the generation of cell diversity during the development of metazoans. We are interested in understanding how cells become polarized and divide asymmetrically.

In the early *C. elegans* embryo PAR proteins are responsible for the establishment and maintenance of cell polarity. PAR-3, PAR-6 and PKC-3 are members of a protein complex localized at the anterior cortex of the embryo (referred to as the PAR-3/6/3 complex); the PAR-2 and PAR-1 proteins are present at the posterior cortex of the embryo.

Mutations that disrupt PAR-2 function result in embryos in which the PAR-3/6/3 complex is mislocalized, leading to polarity defects and embryonic lethality. It has been previously shown that the lethality of *par-2* mutants can be suppressed by reducing PAR-6 levels or by depleting CDC-42. This indicates that the lethality due to the absence of PAR-2 can be suppressed by conditions that affect the levels, the localization and/or the activity of the PAR-3/6/3 complex.

To identify new genes that either regulate or are regulated by the PAR-3/6/3 complex, we have performed a genome-wide RNAi screen looking for suppressors of *par-2* lethality. One of the identified suppressors is NOS-3, the homologue of *Drosophila* Nanos. We find that *nos-3* suppresses most of the phenotypes associated with loss of *par-2* function, including early cell division defects, maternal effect sterility and mislocalization of the anterior PAR-3/6/3 complex. Strikingly, we find that asymmetric localization of PAR-1 at the posterior cortex is not restored. However, PAR-1 activity is essential in *nos-3; par-2* double mutants, suggesting that the function of PAR-1 is independent of its cortical localization. Interestingly, *nos-3* can also suppress the phenotypes associated with a null allele of *par-2*, indicating that NOS-3 impinges on the PAR pathway independently of the PAR-2 protein. Consistent with this, we find that NOS-3 regulates PAR-6 levels in the embryo. We are currently investigating the molecular mechanisms by which NOS-3 controls PAR-6 levels.

BACK

The Mitochondrial Prohibitin Complex Modulates Ageing In *C. elegans*

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The prohibitin complex in eukaryotes consists of two prohibitin subunits (PHB1 and PHB2) that assemble into a macromolecular structure of approximately 1 MD in the inner mitochondrial membrane. The evolutionary conservation and the ubiquitous expression of PHB genes in mammalian tissues suggest an important function for the prohibitin complex. However, the physiological role of the complex has remained elusive. A number of diverse cellular functions have been attributed to these proteins, including a role in cell cycle regulation, receptor-mediated signalling, regulation of transcription, apoptosis and a role in the assembly of mitochondrial respiratory chain complexes.

We are characterizing the function of prohibitin during *C. elegans* development and ageing. The PHB complex is essential for *C. elegans* embryonic development. The postembryonic depletion of PHB proteins results in gonad differentiation defects and sterility, reduced physiological rhythms and oxygen consumption rates, and increased sensitivity to oxidative stress. Muscle mitochondria appear fragmented and disorganized in *phb(RNAi)* animals, suggesting that the prohibitin complex has an important role in establishing or maintaining the integrity of mitochondrial membranes. We are currently investigating the effect of *phb-1* and *phb-2* knock down on the lifespan of wild type animals as well as in different mutant genetic backgrounds. Prohibitin depletion has no effect on the lifespan of N2 animals at 20 degrees, although sometimes a slight reduction in lifespan can be observed. However, *phb-1/2(RNAi)* animals show increased lifespan at 25 degrees as well as increased intrinsic thermotolerance (itt). This indicates that in PHB-deficient animals, changes in temperature have a dramatic effect on mitochondrial function and in ageing. RNAi knockdown of PHB proteins dramatically increases *daf-2(e1370)* lifespan in a *daf-16* dependent manner. However, the increased itt observed in *phb-1/2(RNAi)* animals is partially *daf-16* independent. Further, *phb-1/2(RNAi)* considerably extends the lifespan of the long lived mitochondrial mutant *isp-1(qm150)* as well as the caloric restricted mutant *eat-2(ad465)*. Our results suggest an important role for prohibitins in mitochondrial metabolism that also impinges on the ageing process.

BACK

The LET-418/Mi-2-interacting Protein BRA-1 Links TGF-β Signaling and Dauer Formation with Chromatin Remodeling

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Chromatin structure plays a crucial regulatory role in the control of gene expression in response to developmental signals. Recent advances highlight two important chromatin-remodeling mechanisms involved in the transcriptional process. One of them is an enzymatic system that governs histone modifications, such as acetylation and methylation; the other includes ATP-dependent nucleosome destabilizing activity mediated by members of the evolutionarily conserved SWI/SNF family of proteins. The mammalian chromatin-remodeling Mi-2/NuRD (*nu*cleosome *r*emodeling and histone *d*eactylation) complex contains both, ATP-dependent nucleosome disruption and histone deacetylase activites. The *C. elegans* gene *let-418* encodes an ortholog of the human Mi-2 whose product is the central component of the NuRD complex. Mutations in *let-418* display a pleiotropic phenotype suggesting that it is involved in many developmental processes.

To learn more about the function of LET-418, we searched for interacting proteins. Among the candidates, we found a DAF-1-binding protein, BRA-1 that presents homology with BRAM1 (*B*MP *r*eceptor *a*ssociated *m*olecule), an alternatively spliced form of the putative transcriptional co-repressor and tumor suppressor protein BS69. Co-immunoprecipitation experiments confirmed that BRA-1 forms a complex with LET-418. This interaction is supported by the *in vivo* interaction of BRA-1 with HDA-1 that is a *C. elegans* ortholog of HDAC, another component of the mammalian NuRD complex. It was recently shown that BRA-1 associated with DAF-1/type I TGF-β receptor by pull-down assays suggesting an involvement of *bra-1* in regulating the DAF-7/TGF-β Dauer (Daf) pathway (Morita *et al.,* 2001). The physical *in vivo* interaction between BRA-1 and LET-418/HDA-1/DAF-1 as well as the overlapping expression patterns of *daf-1*, *bra-1* and *let-418* pointed towards a putative function of *let-418* in the Dauer pathway. This was confirmed by the finding that mutations in *let-418* and *bra-1* genes significantly enhanced the Dauer phenotype of dauer-consitutive (*daf-c*) mutant genes. This is in contrast to the current model proposing that *bra-1* negatively regulates DAF-7/TGF-β ligand signaling. Surprisingly, we also found genetic interactions of *let-418* and *bra-1* with mutant alleles of the insulin pathway. The link between insulin-like/ TGF-β signaling and chromatin-remodeling proteins reveals part of a network integrating different aspects in the decision between growth and developmental arrest.

BACK

T-011

Ribosomal Proteins are New Longevity Determinants in *C. elegans*

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Multiple longevity pathways and genes have been identified in *C. elegans*. One example is the conserved DAF-2/insulin/IGF-1 pathway, which modulates lifespan through the downstream transcription factor DAF-16. Other processes, including dietary restriction, affect lifespan in *daf-16* independent ways. The molecular mechanism by which dietary restriction works is largely unknown; however, experiments from other model organisms suggest the involvement of the nutrient sensor TOR (Kapahi *et al*., 2004, Kaeberlein *et al*., 2005).

Through an RNAi screen, we found that RNAi clones for ribosomal proteins (RPs) significantly extend lifespan in a *daf-16* independent fashion. Knockdown of RPs belonging to both the small and the large ribosomal subunits increased lifespan, but only when RPs were inhibited during adulthood. Collectively, these observations reveal a longevity function for ribosomal proteins in adult *C. elegans.* Interestingly, deletion mutants of several ribosomal proteins in yeast have recently been reported to increase lifespan (Kaeberlein *et al*., 2005), indicating that the function of RP in lifespan modulation is conserved.

One major regulator of ribosomal biogenesis and translation is the kinase TOR, which, like RPs, influences *C. elegans* lifespan in a *daf-16* independent fashion (Vellai et al., 2003). We have therefore begun to investigate a potential mechanistic overlap among RPs, TOR, and some of its putative effectors (including S6 kinase and several translation initiation factors).

We expected that this group of molecules collectively involved in translation would behave similarly to influence longevity. We were therefore surprised to find significant differences in how these molecules affect lifespan. Most importantly, our genetic analysis suggests that TOR affects lifespan by a dietary restriction-like mechanism in *C. elegans*, whereas RPs may function by an as yet unidentified mechanism. TOR's role in dietary restriction might therefore involve TOR-regulated processes other than translation *per se*. We are currently investigating this hypothesis in more detail.

BACK

The Genetics of Natural Variation in the Phenotypic Plasticity of Dauer Larvae Development

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Natural isolates (including N2 and DR1350) of the free-living nematode *Caenorhabditis elegans* vary in their phenotypic plasticity of dauer larvae development. For example, some lines appear to be highly sensitive to dauer inducing conditions while others are less so. We have sought to investigate the causes and consequences of this plasticity.

To determine the genetic basis of this plasticity of dauer development we have undertaken a quantitative trait loci (QTL) mapping based analysis of recombinant inbred lines (RILs) produced from crosses between N2 x DR1350. This has identified several regions containing candidate QTLs that affect the plasticity of dauer development. Nearly isogenic lines (NILs) have been constructed for a candidate QTL on chromosome II, with analysis of these NILs confirming that the region contains genes that affect the plasticity of dauer larvae development. This nearly isogenic region contains a maximum of 441 genes, none of which have previously been identified as being part of the genetic pathway regulating dauer development. Overall, these data have developed a clearer picture of the genetics underlying natural variation in a complex trait and demonstrate that the analysis of natural variation can reveal genes not identified by other genetic approaches.

BACK

T-013

Dauer Larvae Development and Fitness: Does it All Depend on your Environment?

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Natural isolates (including N2 and DR1350) of the free-living nematode *Caenorhabditis elegans* vary in their phenotypic plasticity of dauer larvae development. For example, some lines appear to be highly sensitive to dauer inducing conditions while others are less so. We have sought to investigate the causes and consequences of this plasticity.

To determine the fitness consequences of this plasticity of dauer development we have investigated how lifespan, total fecundity, reproductive schedule and population growth vary in N2 and DR1350 and in N2 x DR1350 recombinant inbred lines (RILs). We have found that the plasticity of dauer larvae development is positively correlated with the population growth rate (as measured by population size after 8 days of growth). Differences in population growth appear not to be dependent on lifetime fecundity or reproductive schedule (number of eggs laid *per* day) *per se*, but rather due to how the reproductive schedule changes in response to reduced food availability. Overall, these results suggest that there may be different reproduction and dauer formation strategies in response to environmental change.

BACK

T-014

Identification of a Novel Protein Kinase that Acts in the DAF-18/PTEN Pathway and Regulates Lifespan

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In *Caenorhabditis elegans*, the insulin/IGF-1 DAF-2 receptor controls entry into dauer and longevity. DAF-2 activates the PI3 kinase homologue AGE-1 which results in phosphorylation and cytoplasmic sequestration of the DAF-16 transcription factor. Activation of the DAF-2 pathway is downregulated by DAF-18 which is encoded by the orthologue of the *PTEN* human tumor suppressor gene. We have previously shown that DAF-18 antagonizes the activity of the PI3 kinase/AGE-1 and that this function requires the DAF-18 lipid phosphatase activity. To better characterize the pathways involving DAF-18, we have initiated a RNAi screen aimed at identifying novel genes that regulate lifespan and that genetically interact with *daf-18*. We focused our initial screening on the *C. elegans* kinome that comprises 438 genes coding for protein kinases. Using this strategy, we have identified a new interactor of *daf-18* that controls lifespan. We found that this kinase acts upstream of the *daf-16*/FOXO transcription factor but in parallel of the canonical *daf-2*/*age-1* pathway. We further established that worms with an inactivation of this gene were resistant to oxidative stress. More information on the nature and the mode of action of this protein kinase will be presented at the meeting.

BACK

T-015

Long Lived *C.elegans* "Mit" Mutants as a Model for Human Mitochondrial Diseases: Friedreich Ataxia

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Friedrecih Ataxia (FA) is the most frequent inherited ataxia and is due to low level of Frataxin, a nuclear encoded mitochondrial protein. In *C.elegnas,* knocking down frataxin by RNAi, similarly to other mitochondrial proteins, increases life span (Ventura et al., 2005). By titrating the concentration of RNAi against different mitochondrial ETC (electron transport complex) proteins, we recently found that there is a concise window of ETC inactivation that prolongs *C.elegans* life span. Lowering ETC components below this window will inevitably lead to decreased life span or arrested animals. Our findings are consistent with the arrested phenotype we observed in the frataxin KO (and with recent findings showing that frataxin RNAi on a RNAi hypersensitive background strain, rrf-3, actually decreases life span) and suggest that mitochondrial functionality has to be optimized in order for *C.elegans* to live longer and be viable. We are currently investigating possible stress response mechanisms, such as antioxidant genes (SOD3 and GST4), heat shock factors (HSP16.2 and hsp6), DNA repair (aconitase and p53) and metabolic pathways (Akt, AMP and MAP kinases), which might be induced to compensate for mitochondrial dysfunction and be responsible for the life span extension in our *C.elegans* model. Our long-lived animals offer an excellent model to look for candidate pathways to be targeted with preventive therapies in the human mitochondrial disease before signs and symptoms become established.

BACK

T-016

Exogenous Stimulation of Muscles and Neurons Via Photoactivation of Channelrhodopsin-2 Triggers Rapid Behavioural Responses

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Except for sensory neurons responding to specific stimuli, there are no satisfying methods available to precisely stimulate certain neurons in *C. elegans*, neither *in vivo*, nor in dissected animals. We developed a method that allows to stimulate distinct neurons (or other excitable cells, i.e. muscle cells) in *C. elegans*, and thus to trigger specific behaviours, simply by illuminating the animals with blue light.

To this end, we heterologously expressed the protein Channelrhodopsin-2 (ChR2), a light-gated cation channel from the green alga *Chlamydomonas reinhardtii*, using cell-(type)specific promoters. Illumination of animals expressing ChR2 in muscles led to immediate muscle contractions and thus to a readily observable shrinking of the whole body, when animals were cultured in the presence of the chromophore all-*trans* retinal (required for ChR2 function). Electrophysiological whole-cell recordings from body-wall muscle cells showed rapid inward currents persisting as long as the illumination.

Animals expressing ChR2 in mechanosensory neurons showed withdrawal behaviours after illumination, that were reminiscent of the response to mechanical stimulation (gentle touch, i.e. or more precisely, tapping of the culture dish). These behaviours could also be triggered in mutants lacking functional MEC-4/MEC-10 mechanosensory ion channels, indicating that the touch receptor neurons were still functionally integrated in their circuit.

Besides sensory neurons, preferential expression and photoactivation of ChR2 in the (backward) command interneurons AVA and AVD (using the *nmr*-1 promoter), also led to rapid reversal behaviour. We are further expressing ChR2 in cholinergic motorneurons and expect to thus trigger acetylcholine release and, consequently, muscle contractions.

In sum, the heterologous expression of ChR2 in *C. elegans* enables the specific stimulation of excitable cells by light, which should allow to trigger any neuron (provided a specific promoter is available) and to study the (behavioural) output of this cell, potentially also by imaging of neuronal activity in downstream cells using the genetically encoded optical Ca^{2+}-sensor Cameleon.

Nagel, Brauner, Liewald, Adeishvili, Bamberg, Gottschalk (2005) *Curr Biol* **15**: 2279

BACK

Regulated Intraflagellar Transport of Gα Subunits in *C. elegans* Sensory Cilia

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C. elegans uses a specific subset of sensory neurons to perceive chemical cues from its environment. Crucial for this perception are the sensory cilia. The cilia of *C. elegans* are divided into two segments: the middle and the distal segment. For proper perception it is essential that signaling molecules are correctly localized. The two segments have different intraflagellar transport (IFT) mechanisms, which would allow regulated localization of signaling components in either compartment. In the middle segment cargo is transported by two members of the kinesin family, kinesin II and OSM-3, working together. In the distal segment however, particles are transported by OSM-3 alone. How transport to either segment specifically is regulated is largely unknown.

To address this question, we focused on the Gα subunits GPA-4 and GPA-15, which may act as cargo for the IFT machinery. An indication that these Gα subunits are transported by IFT is that the presence of dominant active GPA-3 (*gpa-3QL*) interferes with entry of these proteins into the distal segments of the cilia. This effect of *gpa-3QL* requires kinesin II. Similarly, the localization of GPA-4::GFP could be altered by exposing larvae to dauer pheromone. These findings hint to the existence of a novel mechanism that alters the localization of signaling molecules dependent on environmental circumstances.

In order to confirm that these Gα proteins are transported by the IFT machinery, we used confocal microscopy to visualize the movement of GPA-15::GFP in the cilia of sensory neurons, and, by making kymographs, measure their speeds. The speeds that we measured for GPA-15::GFP in wild type animals are consistent with the speed of OSM-3 as described in previous reports (Snow et al., 2004; Ou et al., 2005). Remarkably, in *gpa-3QL* animals, the speed of GPA-15 particles is markedly reduced. This may either be due to a direct effect of *gpa-3QL* on the motility of the individual motors, or due to a shift of GPA-15::GFP transport mediated by OSM-3 to kinesin II mediated transport. We are currently investigating these two possibilities by measuring GPA-15::GFP transport rates in *osm-3* and kinesin II (*kap-1*) mutant animals. In addition, we will determine transport rates of OSM-3::GFP and KAP-1::GFP fusion proteins in *gpa-3QL* animals. We will discuss the latest results.

BACK

T-018

Lunapark: A Link between Synaptogenesis and the Ubiquitin Proteasome System?

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Recently, new mechanisms involving protein degradation through the ubiquitin-proteasome system (UPS) have been shown to be involved in synaptic regulation. Regulation of neuronal function involving UPS encompasses a large number of molecular processes: synapse development, synapse size, vesicle cycling, neurotransmitter release, receptor trafficking, spine size, synaptic plasticity and downstream signalling.
A new mouse gene coding for a protein named Lnp (**L**imb and **n**eural **p**attern) was identified at the vicinity of the Evx2 and the Hoxd cluster. In vertebrates *lnp* is expressed, during embryogenesis, in limbs, eyes, heart, genitalia, and in the nervous system. Moreover its expression in neuronal structures, such as the neural tube, coincides with that of Evx transcription factors that play an important role in the interneuron identity in the spinal cord. In contrast to *evx2* and *hoxd* genes, the function of *lnp* is completely unknown.

To elucidate a possible role of *lnp* in the nervous system we have developed a model system using C*.elegans*.
We have analysed *lnp* loss-of-function mutants and found that they showed an increase in aldicarb resistance, resulting probably from either a presynaptic or/and postsynaptic defect. In a yeast two hybrid screen we have identified a putative interaction partner, the ortholog of the *Drosophila* seven in absentia (SINA) protein. *Sina* encodes a RING/E3 ubiquitin ligase. Studies in mammals and *drosophila* suggest that SIAH regulates neuronal development and function by mediating the ubiquitin-dependent degradation of a number of neuronal target proteins.

In C. *elegans* we have shown that *lnp* expression pattern is related to that of *siah* (**si**na **h**omologue*)*. Broad and probably ubiquitous expression is first visible during gastrulation. Postembryonically, LNP and SIAH reporters are expressed in numerous cells bodies along the ventral cord, around the pharynx and tail and most of them are neuronal cells. Moreover, immunohistochemistry in NGF-differentiated PC12 cells revealed significant colocalisation of these two proteins with synaptophaysin (synaptic vesicle protein) in a punctuate staining pattern in cell bodies and neuritic processes suggesting that LNP and SIAH are associated with vesicular structures like endosomes and synaptic-like microvesicles. We have performed *in vitro* ubiquitination experiments and we have showed that LNP enhances SIAH E3 ubiquitin ligase activity.

Together this suggests that LNP together with SIAH might be part of multisubunit RING finger complexes involved in synaptogenesis.

BACK

Regulation of RHO-1 by G12 Alpha Controlling Neurotransmitter Release

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Gq alpha increases and Go alpha decreases levels of acetylcholine (ACh) release by respectively increasing or decreasing levels of diacylglycerol (DAG) at release sites. The accumulation of DAG at release sites recruits the DAG binding neuromodulator UNC-13, where it is essential for all neurotransmitter release. Data from our lab and others suggests that accumulation of DAG and UNC-13 at release sites is necessary for Gq alpha mediated increase in ACh release. A critical inhibitor of release is a diacylglycerol kinase (DGK-1), which lowers DAG levels by phosphorylation to phosphatidic acid. We have found that DGK-1 is bound by and it's activity inhibited by the single *C.elegans* homolog of the small GTPase RhoA, RHO-1. Thus increases in RHO-1 activity inhibit DGK-1 and cause an increase in ACh release.

Inhibition of endogenous RHO-1 using the *C. botulinum* C3 transferase causes a decrease in locomotion and ACh release suggesting that RHO-1 signals to control locomotion under normal laboratory conditions. What are the upstream regulators of RHO-1? Evidence from mammalian studies suggests that RhoA activity is mainly regulated via RhoGEFs that convert the inactive RhoA-GDP to the active RhoA-GTP. We have therefore been testing for the role of RhoGEFs in control of ACh release. Our studies indicate that the trimeric G-protein G12 alpha (GPA-12) activates RHO-1 via the single RGS RhoGEF (RHGF-1) in *C. elegans*. Over-expressors of constitutively active G12 alpha, GPA-12 (Q205L) have increased ACh release and both mutation of *rhgf-1* and inhibition of RHO-1 by C3 transferase suppresses this effect of GPA-12 (Q205L). We have defined the site of action of RHGF-1 to the cholinergic motor neurons for it's effect on ACh release. Co-incident with it's effect on ACh release GPA-12 (Q205L) causes an increase in UNC-13S puncta located at release sites that is also suppressed by the *rhgf-1* mutation. Both recruitment of UNC-13S to release sites and increases in ACh release by GPA-12 (Q205L) expression are blocked by a mutant of UNC-13 which is unable to bind DAG. Interestingly our previous results show that RHO-1 increases ACh release by both UNC-13 dependent and independent pathways whereas GPA-12 acts only via the UNC-13 dependent pathway suggesting that GPA-12 activates only a subset of neuronal RHO-1 signalling pathways.

These results show there is a third G protein coupled pathway controlling DAG mediated ACh release in the motor neurons. G12 alpha activates RHGF-1 converting RHO-1 to its active GTP bound form enabling it to inhibit DGK-1 thus increasing DAG levels. The increase in DAG causes UNC-13 recruitment to the membrane, increasing synaptic vesicle fusion and neurotransmitter release.

BACK

Novel Role for G Protein and MAP Kinase Signaling in Salt-Chemotaxis

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We discriminate three responses of *C. elegans* to NaCl: (1) chemo-attraction to concentrations of 0.1 to 200 mM; (2) avoidance of concentrations higher than 200 mM, probably due to osmotic avoidance; (3) avoidance of otherwise attractive NaCl concentrations after prolonged pre-exposure, which is called gustatory plasticity. We are using behavioral assays and molecular biological techniques to unravel the mechanisms behind these processes.

Previously it was found that the ASE cells are the main salt sensors in *C. elegans*, but also a role for ADF, ASG, ASI, and ASK has been found (Bargmann *et al.*, 1990). Chemotaxis to NaCl is mediated by calcium and cGMP signaling. Previously a role was found for the cGMP gated channel TAX-2/TAX-4 and TAX-6 calcineurin in salt detection (Coburn & Bargmann, 1996; Komatsu *et al.,* 1996; Kuhara *et al.,* 2002; Hukema *et al.*, 2006). We found that these molecules seem to function in the same pathway, since *tax-4 tax-6* double mutants show the same response to NaCl as the single mutants: no chemotaxis to 0.1-10 mM and still a small response to 10-100 mM. The chemotaxis defect of *tax-4* mutants could partially be rescue with the ODR-4 promoter. Expressing *tax-4* under both the ODR-4 promoter as well as GCY-32 promoter gave the same rescue for chemotaxis, but also resulted in stronger avoidance after pre-exposure. This suggests that *tax-4* functions in amphid sensory neurons in salt chemotaxis and in the body cavity neurons AQR, PQR, URX in gustatory plasticity. Although a role in other cells cannot be ruled out.

Additionally, we found a new role for the G alpha protein ODR-3, the TRP channel OSM-9, and the guanylate cyclase GCY-35 in chemotaxis. Single mutants for these genes only had a defect in gustatory plasticity, but not in chemotaxis. However, in the double mutants *tax-4 gcy-35*, *tax-6 odr-3, tax-4 odr-3,* and *tax-6 osm-9* chemotaxis was completely abolished.

Fuerthermore, we found a role for MAP kinases in chemotaxis to NaCl. Mutants for the MAPKKK *nsy-1* and the MAPKK *sek-1* both show a defect in chemotaxis to NaCl, which is comparable to the defect of *tax-2, tax-4,* and *tax-6* mutants. We are currently analysing double mutants and performing cell specific rescue experiments to further elucidate the role of these MAP kinase proteins in chemotaxis to NaCl.

BACK

Impaired Processing of FLP and NLP Precursors in *C. elegans* Lacking Active Prohormone Convertase 2 (EGL-3) and Carboxypeptidase E (EGL-21): Mutant Analysis by Mass Spectrometry

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Biologically active (neuro)peptides are synthesized as larger proprotein precursors which are further processed by the concerted action of a cascade of enzymes. Among these, proprotein convertase 2 (PC2), which cleave the precursors after dibasic residues, and carboxypeptidase E (CPE), which remove these basic amino acids from the carboxyterminals, play a pivotal role. We have examined the role of the *C. elegans* orthologues PC2/EGL-3 and CPE/EGL-21 in the processing of FMRFamide-like peptide (FLP) precursors and neuropeptide-like protein (NLP) precursors. Recently, we performed the peptidomic analysis of *C. elegans* by two dimensional nanoscale liquid chromatography - Quadrupole Time-Of-Flight tandem mass spectrometry (2D-nanoLC – Q-TOF MS/MS). This setup yielded the identification of sixty endogenously present (neuro)peptides. We now expand this list of identified peptides by using an off-line approach in which HPLC fractions are analyzed by a Matrix Assisted Laser Desorption Ionisation – Time Of Flight Mass Spectrometer (MALDI-TOF MS). This way we compared the peptide profile of different *C. elegans* strains, among which PC2/EGL-3 and CPE/EGL-21 mutants. This differential peptidomics approach now unambiguously proves the role of these enzymes in the processing of FLP and NLP precursors.

BACK

T-022

G Proteins and Environmental Cues Regulate the Localization of Ciliary Signalling Molecules

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Sensory perception requires the proper localization of signalling molecules in sensory cilia. Intraflagellar transport (IFT), a system essential for the assembly and maintenance of cilia, plays a critical role in trafficking cargo. Although many IFT proteins have been identified, not much is known about the regulation and specificity of IFT. We were intrigued by the finding that gain-of-function mutation of the Gα protein GPA-3 in *C. elegans* (*gpa-3QL*) causes a dye filling defect (Zwaal et al., 1997, Genetics). Since many dye filling defects are caused by mutations in genes important for IFT, we hypothesized that *gpa-3QL* might affect IFT.

Defects in the IFT machinery often strongly affect the microtubular axoneme of the cilia. However, we found no defects in the microtubular axoneme of *gpa-3QL* animals. Next, we looked at more specific IFT defects in the cilia of *gpa-3QL* animals. To this end we determined the localisation of several sensory sensory Gα subunits using GFP fusion constructs, since they are possible cargo molecules. Surprisingly, *gpa-3QL* interfered with entry into the distal segments of the cilia of three sensory Gα proteins, GPA-4, GPA-9 and GPA-15, but did not affect other Gα proteins. In wild type animals GPA-4 and GPA-15 can be transported in the cilia middle segments by both kinesin II and OSM-3 kinesin motor complexes, and in the distal segments by OSM-3. In *gpa-3QL* animals the two Gα proteins are predominantly transported by kinesin II; OSM-3 mediated transport can only be seen in the absence of kinesin II. However, we found no evidence that *gpa-3QL* affects the two kinesin complexes, the retrograde motor complex or several other IFT proteins. In contrast, *gpa-3QL* strongly affects the localization of OSM-5, the *C. elegans* homologue of the mouse Polaris/Tg737 IFT protein.

We propose a model in which G protein mediated signals regulate the localization of specific signalling molecules in the cilia by modulating, via OSM-5/Polaris, the assembly of motor protein - cargo complexes. Finally, we show that a similar effect on the localization of GPA-4 and GPA-15 in the cilia can be accomplished by exposing *C. elegans* larvae to dauer pheromone. We hypothesize that our findings reveal a novel mechanism that allows structural plasticity in response to environmental cues.

BACK

T-023

MEL-28, a Novel Nuclear Envelope and Kinetochore Protein Essential for Zygotic Nuclear Envelope Assembly

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The nuclear envelope (NE) of eukaryotic cells mediates nucleo-cytoplasmic transport and contributes to control of gene expression. In most eukaryotes, the NE breaks down and is then reassembled during mitosis. Assembly of nuclear pore complexes (NPCs) and the association and fusion of nuclear membranes around decondensing chromosomes are tightly coordinated processes. Here we report the identification and characterization of MEL-28, a large conserved protein essential for the assembly of a functional NE in *C. elegans* embryos. RNAi depletion or genetic mutation of *mel-28* severely impairs nuclear morphology and leads to abnormal distribution of both integral NE proteins and NPCs. Light microscopy and transmission electron microscopy analysis demonstrate that MEL-28 localizes at NPCs during interphase, at kinetochores in early to mid mitosis then is widely distributed on chromatin late in mitosis. We show that MEL-28 is an early assembling, stable NE component required for all aspects of NE assembly. Based on its dynamic localization, we suggest that MEL-28 may link chromatin to the assembling NE.

BACK

T-024

The Establishment of Cortical Polarity in One-Cell Embryos: A Genomics Approach

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The establishment of the first axis of polarity in the C. elegans embryo is indicated by the formation of two cortical domains corresponding to the anterior and posterior of the embryo. These two domains are marked by different complements of PAR proteins: PAR-3 and PAR-6 localize to the anterior cortex; PAR-1 and PAR-2 localize to the posterior cortex. It remains unknown how the PAR proteins recognize the anterior and posterior cortices. However, a coincident establishment of contractile polarity, mediated by the asymmetric distribution of an acto-myosin contractile network, indicates that cortical activity provides upstream cues for PAR protein segregation. We are interested in understanding how cortical polarity is established in one-cell embryos. We have assessed the effect of approximately 150 genes on embryonic polarity using RNAi-mediated depletion and time-lapse imaging of GFP-PAR-2. The subset of genes analyzed exhibited contractility and/or polarity phenotypes in genome-wide RNAi-based phenotypic screens. We have analyzed our data for 36 individual phenotypes ranging from ruffling and pseudocleavage to centrosome rotation and spindle displacement, allowing us to group the genes into 18 phenotypic categories. These phenotypic categories reveal several important aspects of polarity establishment, including thresholds of contractility during polarization. For instance, reducing cortical contractility prevents ruffling but not PAR-2 polarity, while eliminating cortical contractility prevents both ruffling and PAR-2 polarity. We present a generalized model (and the genes required) for polarity establishment in the one-cell embryo: 1) centrosome activation, 2) acto-myosin contraction, 3) centrosome migration to the cortex, 4) initiation of posterior domain formation, 5) posteriorization, 6) spreading of the posterior cortical domain, 7) pseudocleavage, and 8) regulation of posterior domain size.

BACK

T-025

Protein O-fucosylation is Important for Normal Distal Tip Cell Migration

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Protein glycosylation is crucial for the development and growth, as well as the normal functioning and survival of an organism. However, in most instances we do not know the exact function and the mechanism of action of glycan modifications at the molecular level. We want to elucidate the function of glycosylations that occur on thrombospondin type 1 repeats (TSRs). TSRs are cysteine- and arginine-rich modules of about 60 amino residues in length that are part of many proteins with important physiological functions, like e.g. inhibition of angiogenesis, cell- and axonal guidance and regulation of complement in higher organisms.

TSRs undergo two different types of glycosylation: i) C-mannosylation of tryptophan residues and (ii) the addition of O-linked fucose to serine and threonine residues, followed by extension with a single glucose to form Glc-beta1,3-Fuc-O-Ser/Thr. We have begun to study the latter process in *C. elegans,* in order to reveal its possible function. The *C. elegans* genome encodes 29 TSR-containing proteins, some of which have been found to be important for normal development, like UNC-5, GON-1, ADT-1 and NAS-36.

We have demonstrated that *C. elegans pad-2* encodes the fucosyltransferase (POFUT-2) that modifies TSRs. Extracts from HEK293T cells overexpressing PAD-2 show a 10-fold increase in transferase activity towards a TSR. Furthermore, product analysis by mass spectrometry of isolated peptides showed the fucose to be linked to the correct Thr residue. In contrast, no increase in TSR fucosylation was observed by overexpressing *C. elegans* POFUT1, the enzyme that fucosylates EGF-like repeats (encoded by C15C7.7). We are characterizing the *pad-2(tm1756)* mutant that we received from the NBP, Japan. The *tm1756* strain is homozygous viable and lacks all fucosyltransferase activity towards a TSR. The mutant does not show embryonic lethality nor morphogenetic defects (Menzel et al., Genomics 84: 320, 2004), but a precocious dorsal migration of the anterior gonad arm is observed. The latter is reminiscent of the phenotype resulting from the precocious expression of UNC-5 in DTCs (Su et al., Dev. 127: 585, 2000). Genetic experiments show that *unc-6*, *unc-5* and *unc-40* genes are epistatic to *pad-2*, suggesting that *pad-2* is acting as an inhibitor of the netrin pathway in DTCs. We are now testing whether the TSRs of *C. elegans* UNC-5 are fucosylated *in vivo* and whether this modification is necessary for the *pad-2*-mediated inhibition of the DTC ventro-dorsal migration. Preliminary evidence indicates that *C. elegans* also contains the glucosyltransferase activity that attaches glucose to the fucose It will be of interest to examine its possible function.

BACK

The Transmembrane Nucleoporin NDC-1 is required for Nuclear Envelope Assembly *in vivo* and *in vitro*

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The nuclear envelope of eukaryotic cells consists of structurally and functionally distinct lipid bi-layers, the outer and the inner nuclear membrane. While the outer nuclear membrane is continuous with the endoplasmic reticulum, the inner nuclear membrane has a unique set of transmembrane proteins, some of which interact with chromatin and/or the nuclear lamina. Inner nuclear membrane and outer nuclear membrane are joined at the sites of nuclear pore complexes, macro-molecular structures that perforate the nuclear envelope and are composed of a set of proteins called nucleoporins. Nuclear pore complexes function as gatekeepers for controlled exchange of large molecules (>30kDa) between nucleus and cytoplasm. At the onset of mitosis in metazoa the nuclear envelope disassembles: While the membranes are, at least in mammals, reabsorbed into the endoplasmic reticulum, nuclear pore complexes disassemble into mostly soluble nucleoporins At anaphase, membranous structures harbouring inner nuclear membrane proteins are recruited back to chromatin, and subsequently form a closed membrane in a series of fusion events. At the same time nuclear pore complexes are assembled into the reforming nuclear envelope.
Sequence comparison revealed that factors involved in nuclear envelope formation are highly conserved through the animal kingdom. Much of our knowledge of post-mitotic nuclear envelope assembly comes from *in vitro* experiments using *Xenopus laevis* egg extracts. Upon incubation of cytosol and a membrane fraction prepared from these eggs with sperm chromatin as a template, nuclei form *in vitro*. These nuclei contain a closed nuclear envelope and nuclear pore complexes that are able to import proteins. On the other hand, *C. elegans* is regarded as an excellent system to study metazoan early development in living animals. Thus the combination of both systems, frog egg extracts and worm embryo live imaging, provides an outstanding experimental setup for studying nuclear envelope assembly in detail *in vitro* and *in vivo*. For example, it has been shown in both systems that the small GTPase Ran is critically involved in mitotic processes such as spindle formation and nuclear envelope assembly.
Here we report the initial characterization of a novel metazoan transmembrane nucleoporin, NDC-1 (B0240.4). Ndc1p is known in yeast as a component of the spindle pole body and the nuclear pore complex. As such, NDC-1 is a nuclear envelope resident protein and co-localizes with nuclear pore complex marker proteins in worm embryos, frog cells and *in vitro* assembled nuclei. Antibody depletion from frog egg extracts following reconstitution of nuclei results in severe defects of nuclear envelope and nuclear pore complex morphology as well as nuclear import deficiencies. Similarly, NDC-1 depletion by RNAi and time-lapse imaging of fluorescent marker proteins in *C. elegans* embryos has revealed a number of defects connected to nuclear envelope abnormalities, e.g. membrane segregation defects, abnormally small pro-nuclear size or formation of multi-nucleated cells. We conclude that NDC-1 is a novel transmembrane nucleoporin required for both nuclear pore complex function and post-mitotic nuclear envelope assembly *in vivo* and *in vitro*.

BACK

MAA-1, A Novel Acyl-CoA Binding Protein Involved in Endosomal Vesicle Transport in *C. elegans*

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The formation of transport vesicles during membrane trafficking requires an intricate interplay between many proteins including those coating the vesicles, adaptor proteins that recruit components of the coat, and small GTPases involved in initiation of vesicle formation. In addition to the protein factors, membrane bilayer lipids are increasingly being recognised as molecules participating actively during vesicle formation. For example, formation of transport carriers is promoted by the hydrolysis of acyl-CoA lipid esters *in vitro*. The mechanisms by which these lipid esters are directed to the appropriate membranes during vesicle biogenesis is not yet understood. Here we present the first study of MAA-1, a novel member of the acyl-CoA-binding protein family. We show that in *C. elegans*, MAA-1 is membrane associated and localizes to intracellular membrane organelles in several polarised tissues in *C. elegans*. MAA-1 binds fatty acyl-CoA *in vitro* and the ability to form high affinity ligand-protein complexes is necessary for function. Lack of *maa-1* reduces the rate of endosomal recycling and enhances phenotypes caused by *rme-1*, an EH domain protein involved in endosomal recycling. Our results are consistent with a role for acyl-CoA and MAA-1 during endosomal recycling *in vivo*.

BACK

T-028

Genes Required for Glycosphingolipid Synthesis are Dispensable in Most *C. elegans* Cells

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One of the central challenges in biology is to understand the precise function and regulation of the huge variety of complex lipids found in animal cells. Many lines of evidence suggest that glycosphingolipids (GSLs), complex glycosylated lipids derived from ceramide, participate in several key cellular events leading to neuronal development, cell growth and proliferation, apoptosis and cell migration. Although the precise function of GSLs is unclear, it has been proposed that GSLs form microdomains, or “rafts”, that would provide the structural environment for interactions between ligands, plasma membrane receptors, and downstream molecules. This would improve or allow signalling. However, it has been difficult to prove that these microdomains are functionally relevant or whether they exist.

To clarify whether GSLs exert essential functions in *C. elegans* cells, we inactivated ceramide glucosyl transferase (CGT), an enzyme needed for GSL biosynthesis. Analysis of the complete genome sequence revealed that *C. elegans* has three CGTs: *cgt-1* (T06C12.10), *cgt-2* (F20B4.6), and *cgt-3* (F59G1.1). Inactivation of each single *cgt* gene does not cause any visible phenotype. However, double mutant animals in which two CGTs, *cgt-1* and *cgt-3,* are both knocked out, stop growing at the L1 larval stage, undergo progressive paralysis, and eventually die.

We carried out knockdown experiments at different time points and found that *cgt* activity is required embryonically or within the L1 larval stage. We used constructs expressing fully functional CGT-1::GFP and CGT-3::GFP proteins to determine the localization of CGT-1 and CGT-3. We found that the pharyngeal intestinal valve and a cell/small group of cells in the tail (probably the intestinal-rectal valve) are the only tissues that express both *cgts*, suggesting that these epithelial tissues might be the focus of the lethal *cgt* phenotype. To test whether this is the case, we used a variety of promoters to drive expression of *cgt-1* and *cgt-3* in specific tissues. We could rescue the growth arrest phenotype associated with *cgt* inactivation by only expressing *cgt-1* or *cgt-3* in the pharyngeal intestinal valve and in the intestinal-rectal valve. Because expression in these tissues could also rescue the lethality associated with inactivation of all 3 *cgts*, we conclude that *cgts*, and thus also GSLs, are dispensable in most *C. elegans* cells.

BACK

VPS-32 is an Endosomal Protein Essential to Maintain the Integrity of Epithelial Cells during *C. elegans* Development

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Class E VPS (Vacuolar Protein Sorting) proteins are involved in the formation of MultiVesicular Body in yeast. They are components of the Endosomal Sorting Complexes Required for Transport (ESCRTI, II and III). We have previously shown that the inactivation of the *C. elegans* class E *vps* genes resulted in various phenotypes ranging from lethality to an absence of obvious phenotype (1). In particular VPS-27 is involved in endosomal and autophagic pathways and its inactivation affects cholesterol traffic and larval molting (1). We present here the characterization of VPS-32, a component of ESCRT III. Phylogenetic and functional analyses of the two *vps-32* homologues present in the *C. elegans* genome indicated that only one encodes a functional protein, meanwhile the other is a pseudogene. Using antibodies and VPS-32::GFP fusion proteins, we observed that VPS-32 is expressed in vesicular structures and enriched in epithelial cells during the morphogenesis of the embryo.

vps-32(RNAi) and *vps-32* null mutant animals arrest their development at mid-embryogenesis and early L1 stage respectively, with a disorganisation and degradation of the epidermis. Moreover, *vps-32* RNAi feeding of larvae result in L4 arrest with the same epidermal defect associated with abnormal molting. Optical and electron microscopy analyses of *vps-32* animals, revealed the presence of enlarged VPS-27-positive endosomes and an excess of autophagy. Theses results indicate that, similarly to several VPSE, VPS-32 protein is required for endosomal and autophagic pathways. However despite these similarities, inactivation of *vpsE* genes results in different developmental phenotypes. A model to explain the heterogeneity between *vpsE* phenotypes will be presented.

(1) Roudier N., Lefebvre C. and Legouis, R. (2005). *Traffic* 6, 695-705.

BACK

T-030

Dynamic Expression of Homeobox Genes during *C. elegans* Embryogenesis

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Homeobox genes encode transcription factors of critical importance for development. Systematic studies of dynamic, spatio-temporal gene expression patterns of developmental control genes have been rare so far in C. elegans, We have started to investigate the expression patterns of particular homeobox genes during development using 2-channel 4D microscopy. The PCR-based cloning strategy adapted from Oliver Hobert allows the production of transcriptional fusions in which the upstream regulatory regions of each gene is placed in frame with the translational initiation ATG of the reporter gene GFP. The homeobox::GFP reporter constructs are being used to investigate the expression pattern in larval stages, and more importantly, also to examine the embryonic expression pattern using live GFP time-lapse recordings. We have been recording 4D stacks interspersed with GFP for the following genes ceh-5, ceh-33, ceh-34, ceh-41, ceh-44, and ceh-45, ceh-30, NPax, ceh-6, ceh-32, ceh-26. Additional homeobox genes are in line to be processed. Analysis of our 4D recordings has revealed in many instances early dynamic expression that fades again in later development, whose functional significance is currently unclear. Possibly these events in early development set up patterns, regions and cell fates that are not reflected by the expression seen later in larval stages.

Still, many of the genes start their expression pattern later in embryogenesis. Nevertheless, we find very intriguing expression patterns which show dynamic changes of cell position, incipient expression in a very wide range of cells with no apparent connection, or slowly increasing expression during late embryogenesis. Our recordings reveal a large array of different dynamic strategies that the C. elegans embryo employs to execute its developmental program.

To aid in our analysis, we are developing several software tools. Virtual Wormbase (celegans.sh.se) is the major tool on which we are working to ultimately convert GFP expression data into digitized data that can be submitted to Wormbase. We also have developed additional tools to convert our recorded stacks in different formats and produce quicktime movies of the 5 to 10 Gig large datasets that one recording produces. More recently, we also started to increase the image data density (number of slices) to allow better lineaging.

BACK

Suppressor Genetics Identify Activation Mutations in Effectors of AGE-1/PI3 Kinase Signaling in *C. elegans*

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A conserved insulin-like signaling pathway controls larval development, stress resistance and adult lifespan in *Caenorhabditis elegans*. Signaling downstream of the DAF-2/insulin receptor requires AGE-1, the catalytic p110 subunit of PI3 kinase, which in turn activates serine/threonine kinases, AKT-1, AKT-2 and PDK-1. Activated AKT-1 and AKT-2 phosphorylate and antagonize DAF-16, a FOXO transcription factor. Mutations in components of the DAF-2/insulin-like signaling pathway can lead to a several fold extension in lifespan and constitutive dauer arrest. Here we describe a genetic screen for suppressors of the constitutive dauer larval arrest phenotype of *age-1(mg109)* animals. From approximately 20,000 haploid genomes, we identified 40 suppressor mutations and molecularly characterized 5 of these. We identified two mutations in *daf-16*. In addition, we identified two mutations that were activating alleles of *akt-1* and *pdk-1*. Finally, we describe a fifth mutation, *mg227*, located on chromosome X, that does not correspond to any known dauer genes. Lifespan analysis of the suppressor mutants showed that *mg227* enhanced the *age-1(mg109)* long lifespan phenotype, whereas, *akt-1(mg247)* and *pdk-1(mg261)* did not affect lifespan of *age-1(mg109)*. Both of the *daf-16* alleles suppressed the *age-1(mg109)* long lifespan phenotype. *mg227, akt-1(mg247)* and *pdk-1(mg261)* did not alter the enhanced stress resistance phenotype of *age-1(mg109)*. Consistent with previous reports, the two *daf-16* alleles suppressed the enhanced stress resistance phenotype of *age-1(mg109)*. Furthermore, genetic epistasis analysis using RNAi revealed that bypass of dauer arrest in *akt-1(mg247);age-1(mg109)* animals was dependent on the presence of *pdk-1*. Similarly, dauer bypass in *age-1(mg109);pdk-1(mg261)* animals was dependent on *akt-1*. Interestingly, dauer bypass in *age-1(mg109); mg227* animals required only *akt-1*, and *pdk-1* activity was dispensable in this background. The interdependence of *akt-1* and *pdk-1*, even in activated forms, supports the existence of AGE-1-independent pathways for these phospholipid-dependent kinases.

BACK

T-032

A Conserved Function of the Retromer Complex in Long-Range EGL-20/Wnt Signaling

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Several aspects of *C. elegans* development are regulated by Wnt signaling. One such process is the migration of the Q neuroblasts, which is controlled by EGL-20/Wnt signaling. To identify regulators of the EGL-20 pathway, we performed a genome-wide RNAi screen for genes that affect Q daughter cell (Q.d) migration. Knock-down of *vps-35* results in anterior localization of the QL.d, a phenotype that suggests a defect in EGL-20 signaling. VPS-35 is a member of the retromer, a multi-protein complex involved in retrograde transport between the late endosome and the Golgi. Interestingly, the *vps-35* null phenotype is very similar to that of *egl-20*. Thus, in *vps-35* mutants *mab-5* is not expressed in QL, the final positions of the QR.d and the HSN neurons is shifted towards the posterior, the polarization of the V5 division is defective and postdeirid formation is not inhibited after V6 ablation. As some of these phenotypes are BAR-1/beta-catenin independent, this suggests that the retromer complex acts early in the EGL-20 signaling cascade. This was confirmed by epistatic analysis. Tissue specific rescue and partial mosaic analysis showed that *vps-35* is required and sufficient in the cells that express EGL-20 (a group of cells located in the tail). This suggests that VPS-35 may be required for the production of a functional Wnt. Using RNAi in human cell lines and morpholino mediated knock down in *Xenopus tropicalis*, we could confirm and complement the results obtained in the worm, showing that this particular function of the retromer complex is conserved. Loss-of *vps-35* strongly affects EGL-20 but not, or only mildly, LIN-44 and MOM-2. As EGL-20 is the only Wnt that is known to act over a relatively long distance, we investigated the effect of the *vps-35* mutation on secreted EGL-20 localization. Expression of a tagged EGL-20 protein followed by immunostaining showed that EGL-20 forms a punctate anteroposterior concentration gradient that ranges from the EGL-20 producing cells in the tail to the mid-body. Strikingly, this gradient was absent in *vps-35* null mutants. As we did not observe any accumulation of EGL-20 in the producing cells, EGL-20 secretion is probably not affected. These results demonstrate that the ability of Wnt to regulate long-range patterning events is dependent on a critical and conserved function of the retromer complex within Wnt producing cells. Putative mechanisms of retromer function in long-range Wnt signaling will be discussed.

BACK

T-033

A Systematic Analysis of Nonsense Mediated mRNA Decay in *C.elegans*

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Nonsense-mediated mRNA decay (NMD) pathway selectively degrades mRNAs harboring premature termination codons (PTCs). Seven genes (*smg1-7*) essential for NMD were originally identified in the nematode *Caenorhabditis elegans*, and orthologs of these genes have been found in many other species. A crucial aspect of NMD is the mechanism of PTC definition. In human cells, it has been established that pre-mRNA splicing plays a critical role in defining PTCs, and this is mediated by the components of the exon junction complex (EJC) assembled upstream of exon-exon boundaries. In *Drosophila* cells, definition of a PTC occurs independently of exon boundaries, and accordingly, the components of the EJC are dispensable for NMD (Gatfield et al., 2003, EMBO J., 22:3960). Although the requirement for *smg* genes for NMD in *C.elegans* is well documented, little is known about the mechanism of PTC definition in this organism.

Here, we have conducted a systematic analysis of the cis-acting sequences and trans-acting factors that are required for PTC recognition in the nematode *C.elegans*. We have used transgenic *C. elegans* strains expressing GFP/LacZ reporter, either in a wild-type version, or harboring premature termination codon (PTC). We show that introduction of a PTC into the reporter induces a robust NMD response, as determined by the lack of GFP expression and decreased level of the corresponding mRNA. Use of cDNA reporters established that introns are not required to define a PTC, and we show that accordingly EJC components are not essential for NMD in this organism. We will discuss the use of these reporters in a genome-wide RNAi-based screen to identify novel genes that contribute to the process of nonsense-mediated decay.

BACK

Identification and Characterization of *mtm-1,* A Novel Member and Negative Regulator of the Engulfment Signaling Pathway in *C. elegans*

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The proper removal of apoptotic cells is critical during embryonic development and in tissue homeostasis to prevent inflammation and autoimmunity. However, the molecular details of the "engulfment machinery" are not fully understood. The powerful genetics of the small nematode *C. elegans* has been used to identify genes involved in the programmed clearance of apoptotic cells during development and in the adult germ line. Nine genes were isolated which act in two partially redundant pathways. One group is composed five genes: the small GTPase MIG-2/RhoG and its nucleotide exchange factor UNC-73/TRIO, as well as the adaptor protein CED-2/CrkII and the bipartite RacGEF complex CED-5/Dock180 and CED-12/ELMO. In the second pathway, CED-1/CD91/LRP/SREC/EATER functions as a transmembrane receptor which might recognize the apoptotic cell. The CED-7/ABCA1 transporter is likely important for membrane dynamics, and plays an additional critical role in dying cells. CED-6 and its human homologue GULP encode a signaling adaptor molecule whose function is evolutionary conserved and which physically interact with CED-1/CD91/LRP/SREC/EATER. Both pathways converge at the level of CED-10/Rac. However, the signaling pathway between CED-6 and CED-10, as well as events downstream of CED-10 have yet to be identified.

To identify negative regulators of engulfment, we used the vital dye Acridine Orange (AO) to identify mutations that suppress the engulfment defect of *ced-6(n1813)* mutants. We identified 13 potential suppressors that restore partial engulfment of germ cell corpses and also show a decreased number of persistent cell corpses during embryonic development. We found that the strongest suppressor, *op309,* is a hypomorphic allele of *mtm-1*, a phosphatidyl-inositol-3-phosphatase. *mtm-1(309)* is a recessive mutation and acts zygotically. Furthermore kinetic 4D lineage reveals that the engulfment efficiency in *mtm-1; ced-6* animals is restored compared to *ced-6*. Epistatic analysis suggest that *mtm-1* acts upstream of the ternary complex *ced-2, ced-5* and *ced-12,* in parallel to *mig-2*. Interestingly, we found that *mig-2(gf)* phenocopies *mtm-1(lf)* and also partially suppresses persistent cell corpses in *ced-1*, *ced-6, ced-7* and *ced-10* embryos. Additionally actin polymerisation around germ cell corpses is partially restored in *mtm-1; ced-10* animals. We will show evidence that in *mtm-1(op309)* animals engulfment signaling is increased. This "promotion" of engulfment might be a prerequisite for therapeutics designed to induce the clearance of "unwanted" cells.

BACK

wTF2.0: A Fundamental First Step for Mapping the *Caenorhabditis elegans* Transcription Factor Localizome

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The Localization of Expression Mapping Project (LEMP) aims to use cloned *C.elegans* promoter fragments to generate a genome-wide set of expression patterns – termed the Localizome. The Promoterome currently contains promoter fragments of up to 2kb from about 6,500 of the approximately 20,000 identified genes in the *C.elegans* genome. Using the Multisite Gateway system these promoter fragments are re-cloned upstream of GFP within an *unc-119* rescue vector and the resulting constructs used to transform *unc-119* mutant nematodes by a modified ballistic DNA transfer technique.

The current focus of the LEMP is on *C.elegans* transcription factor genes with the view that these expression pattern data will be essential for mapping transcription regulatory networks. While *C.elegans* gene models have undergone continuous refinement, the extant lists of predicted *C.elegans* transcription factors (collectively referred to as wTF1.0) were in need of updating. We have identified a compendium of 934 transcription factor genes (referred to as wTF2.0) by interrogating Wormbase version 140 for proteins with appropriate Gene Ontology (GO) terms and then manually removed false positives (369) and added false negatives (373). The results of various bioinformatic analyses using wTF2.0 as well as how the list can be used to investigate regulatory networks will be discussed. We expect wTF2.0 to be a dynamic resource due to regular updating of the *C.elegans* genome annotation as well as changes in the functional annotations of protein domains. wTF2.0 will be accessible to the scientific community via an online interface where input is possible. wTF2.0 provides a starting point to decipher the transcription regulatory networks that control metazoan development and function.

BACK

Evolutionary Resistance of Some Transcription Factor Gene Families to Gene Duplication Events

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The Promoterome is a library of DNA fragments cloned using the Gateway recombination system for genome scale analyses and containing the promoter regions of more than 6,500 *C. elegans* genes[1]. Promoterome promoter fragments from genes encoding regulatory transcription factors have been assayed for their ability to drive reporter gene expression in *C. elegans* with expression observed for 94% (193 of 206). This success rate was much higher than the 49% success rate obtained in a previous study[2] in which 364 *C. elegans* genes, effectively selected at random, had been assayed by the reporter approach. In the previous study, reporter fusions had been generated by a shot-gun cloning approach and with different vectors, but these distinctions did not appear to be the explanation for the differing success rates as assay of another set of promoters from the Promoterome library gave the same low success rate. Rather, the higher success rate seemed due to the selection of regulatory transcription factor genes as the target of our study.

Analysis of the earlier results[2] had suggested the main reason for the low success rate might be large numbers of pseudogenes being identified as real genes in the annotation of the *C. elegans* genome. The presence of such a high number of pseudogenes would require a high rate of gene duplication during *C. elegans'* recent evolutionary history. Might *C. elegans* transcription factor genes be resistant to such gene duplication events such that the annotated transcription factor gene families would include far fewer pseudogenes than other *C. elegans* gene families?

To explore the evolutionary history of *C. elegans* transcription factor genes a comparison was made with *Caenorhabditis briggsae*. While only 60% of all annotated *C. elegans* genes have *C. briggsae* orthologues[3], in many regulatory transcription factor gene families the proportion of *C. elegans* genes with *C. briggsae* orthologues is much higher. Genes within these families appear to have undergone few evolutionarily successful gene duplication events since the divergence of *C. elegans* and *C. briggsae*, suggesting dosage for these transcription factors may be critical on an evolutionary scale. While complex transcription factor regulatory networks may provide robustness to developmental control, they may provide resistance to evolutionary change by gene duplication.

1. Dupuy *et al*. ('04) Gen. Res. 14: 2169. 2. Mounsey *et al*. ('02) Gen. Res. 12: 770. 3. Stein *et al.* ('03) PLoS Biol. 1: 166

BACK

T-094

Subnuclear Positioning Changes upon Activation of Transcription in *C. elegans*

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Active and inactive genes are not randomly positioned in the eukaryotic nucleus, yet the impact of their localization is still not completely understood. Moreover, it is not yet established in the context of a whole organism with differentiated cells whether nuclear positioning of genes precedes or results from functional chromatin characteristics such as timing of replication or transcriptional potential. We use the worm to study nuclear dynamics in the context of a multicellular organism with specialized differentiated cells.
We created strains expressing GFP-lacI under transcriptional control of an ubiquitous promoter by injection and integration. Surprisingly, embryos and larvae expressing GFP-lacI show two bright nuclear spots per nucleus. By genetic and FISH analyses, we show that the array itself is recognized by the GFP-lacI protein. This is likely to be due to the presence of a single lacO site in the sequence of the plasmids used to create these arrays. Since most of the arrays created for the last 20 years involve plasmids containing lacO sites, this is a powerful technique to identify arrays localization when genes coded by the arrays are either active or inactive. Indeed, quantitative microscopic analysis shows that the positioning of genes varies in the nuclei of differentiated cells depending on the activity of the integrated promoter.
Using this technique, we will now study the localization of a pharyngeal specific gene array *in vivo* during differentiation of the pharynx. Future studies will relate the DNA replication timing with expression patterns and subnuclear positioning.

BACK

Self-Assembly of Centralspindlin, a Conserved Complex of Mitotic Kinesin and RhoGAP, Critical for Cytokinesis

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Antiparallel microtubule structures play critical roles in cytokinesis. The central spindle contributes to specification and promotion of cleavage furrow ingression. The midbody is indispensable for completion of cytokinesis. Centralspindlin, a protein complex of ZEN-4/Pavarotti/MKLP1 mitotic kinesin and CYK-4/RacGAP50C/MgcRacGAP RhoGAP, is an evolutionarily conserved critical factor for the assembly of these structures. It accumulates on the central spindle during anaphase and concentrates to the center of the midbody during telophase. In addition to the bundling of microtubules, this specific localization of centralspindlin is critical for cytokinesis since it contributes to the local activation of Rho through direct interaction with Pebble/ECT2 RhoGEF at furrow specification and to the recruitment of the exocyst-SNARE complexes through centriolin at abscission. However, the mechanism by which centralspindlin accumulates exclusively to the central spindle and later to the center of the midbody, and not to other microtubule structures, is not known. We have found that centralspindlin undergoes self-assembly into higher order structures under physiological conditions. A region was identified in the putative coiled coil of ZEN-4, the mitotic kinesin subunit in *C. elegans*, that is critical for the self-assembly but dispensable for dimerization and the complex formation with CYK-4. A centralspindlin complex with ZEN-4 subunit lacking this region showed much weaker microtubule bundling activity than a complex with self-assembly-competent ZEN-4. A self-assembly-deficient zen-4 was less effective at rescuing zen-4 null animals than a wild type zen-4. These results indicate that self-assembly of centralspindlin is functionally important in vivo. We are currently analyzing the effect of self-assembly on the subcellular localization of centralspindlin.

BACK

A Conserved Role of *C. elegans* CDC-48 in ER-Associated Protein Degradation

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Protein degradation mediated by the ubiquitin/proteasome system is essential for the elimination of misfolded proteins from the endoplasmic reticulum (ER) to adapt to ER stress. It has been reported that the AAA ATPase p97/VCP/CDC48 is required in this pathway for protein dislocation across the ER membrane and subsequent ubiquitin dependent degradation by the 26S proteasome in the cytosol. Throughout ER-associated protein degradation, p97 cooperates with a binary Ufd1/Npl4-complex. In *Caenorhabditis elegans* two homologs of p97, designated CDC-48.1 and CDC-48.2, exist. Our results indicate that both p97 homologs interact with UFD-1/NPL-4 in a similar CDC-48$^{UFD-1/NPL-4}$ complex. RNAi mediated depletion of the corresponding genes induces ER stress resulting in hypersensitivity to conditions which induce increased levels of unfolded proteins in the ER lumen. Together, these data suggest an evolutionarily conserved retro-translocation machinery at the endoplasmic reticulum.

BACK

C. elegans NUM-1 Modulates Endocytosis by Targeting the TAT-1 Phospholipid Flippase

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We have previously reported that *C. elegans* NUM-1 modulates both endocytosis and the activity of LIN-12, a receptor that mediates a number of cell fate specification events in the worm. In order to understand better how NUM-1 functions, we carried out a yeast 2-hybrid screen for interacting proteins. One clone we isolated encodes TAT-1, a transmembrane P-type ATPase. Work in *S. cerevisiae* has shown that these proteins function as so-called inward phospholipid flippases to concentrate specific phospholipids to the cytosolic leaflet of biological membranes. They are known to be associated with endosomes and to be required for correct vesicle trafficking. We have found that TAT-1 is expressed in tissues in which NUM-1 functions. Mapping of the regions required for binding revealed that the PTB domain of NUM-1 associates with an NXXF motif at the C-terminus of TAT-1. RNAi experiments show that *tat-1* affects membrane trafficking in *C. elegans*. Our results suggest that NUM-1 functions in the worm by modulating TAT-1 activity.

BACK

T-040

Mechanical Oscillations during Asymmetric Spindle Positioning Require a Threshold Number of Active Cortical Force Generators

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Background. Asymmetric division of the *C. elegans* zygote is due to the posterior-directed movement of the mitotic spindle during metaphase and anaphase. During this movement along the anterior-posterior axis, the spindle oscillates transversely. A theoretical analysis indicates that oscillations might occur as a result of the concerted action of many cortical force generators that pull on astral microtubules in a tug-of-war situation. This model predicts a threshold of motor activity below which no oscillations occur.

Results: We have tested the existence of a threshold by using RNA interference to gradually reduce the levels of GPR-1 and GPR-2 that are involved in the G-protein-mediated regulation of the force generators. We found an abrupt cessation of oscillations as expected if the activity drops below a threshold. Furthermore, we could account for the complex choreography of the mitotic spindle - the precise temporal coordination of the build-up and die-down of the transverse oscillations with the posterior displacement - by a gradual increase in the processivity of the force generators during metaphase and anaphase.

Conclusions: The agreement between our results and modeling suggests that the same motor machinery underlies two different spindle motions in the embryo: the equal and opposite motors on each side of the AP axis drive oscillations whereas the imbalanced motors in the two halves of the embryo drive posterior displacement.

BACK

T-041

Mechanisms and Functions of Homotypic Cell Fusion

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Cell fusion is found in germlines, muscles, bones, placentas and stem cells. In *C. elegans*, the sperm and eggs and one third of all somatic cells fuse during development.

To dissect the pathway of cell fusion, we use genetic and kinetic analyses using live confocal and multiphoton microscopy to find early and late intermediates of cell membrane fusion. We explore cell fusion in developing embryos using a semi-automated system to simultaneously monitor the rates of multiple cell fusion processes in epithelia. We found that fusion in the epidermis comprise mechanistically distinct stages of initiation and completion of membrane fusion. The stages of cell fusion are differentially blocked or retarded in *eff-1* and *idf-1* mutants. We generate kinetic cell fusion maps for embryos grown at different temperatures. Surprisingly, different sides of the same cell differ in their fusogenicity: while the left and right membrane domains are fusion-incompetent, the anterior and posterior membrane domains fuse with autonomous kinetics in wild-type embryos.

We demonstrate that EFF-1 transmembrane type I proteins are homotypic fusogens using a new in vitro heterologous system and mosaic analysis in *C. elegans*. Thus, EFF-1-mediated cell fusion is different than heterotypic viral and intracellular membrane fusion. We will discuss how the homotypic nature of cell-cell fusion may be another efficient mechanism to control the size and shape of syncytia.

BACK

Autophagy Contributes to Necrotic Cell Death in *C. elegans*

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Autophagy is the main process for bulk protein and organelle degradation and recycling under conditions of extracellular or intracellular stress (accumulation of damaged organelles/cytoplasmic components, nutrient deprivation, hypoxia, high temperature, overcrowding). It is evolutionarily conserved in all eukaryotes and its deregulation has been associated with mammalian diseases, such as cancer, muscular disorders and neurodegeneration. However, whether autophagy protects or contributes to neurodegeneration remains unclear.

Necrotic cell death has been implicated in acute neurodegenerative episodes such as ischemic stroke. In *C. elegans*, diverse genetic and environmental stimuli (*mec-4(d), deg-3(d),* hyperactive Gas, hypoxia) trigger necrotic degeneration. We are investigating the possible role of autophagy in the execution of necrotic cell death inflicted by such insults. To this end, we have conducted similarity searches and identified more than 12 orthologs of yeast autophagy-related genes (*atg*) in *C. elegans*. Genetic lesions or RNAi with genes involved in autophagy induction, formation of autophagosomes and retrieval of ATG proteins, resulted in attenuation of necrotic neurodegeneration triggered by various stimuli. Necrotic death was also decreased after treatment of animals with the specific autophagy-inhibitor 3-methyladenine (3-MA). Monitoring of autophagosome formation in neurons undergoing necrosis, using fluorescent autophagy markers, revealed an increased number of autophagic vacuoles at initial stages of degeneration. We have previously shown that necrosis requires the function of intact lysosomes. Neuronal survival was further enhanced in genetic backgrounds where both autophagy and the lysosomal catabolic mechanisms were impaired. Our findings directly implicate autophagy in the process of necrosis.

BACK

T-043

Expression of Mammalian GPCRs in *C. elegans* Generates Novel Behavioural Responses to Human Ligands

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G protein coupled receptors (GPCRs) play a crucial role in many biological processes and represent a major class of drug targets. However purification of GPCRs for biochemical study is difficult and most methods of screening receptor-ligand interactions require cultured cells and endotoxin free compounds. In contrast, *Caenorhabditis elegans* is a soil dwelling nematode that feeds on bacteria and uses GPCRs expressed in chemosensory neurons to detect bacteria and environmental compounds. Here we report that expression of the mammalian somatostatin receptor (Sstr2) and chemokine receptor 5 (CCR5) in gustatory neurons allow *C. elegans* to specifically detect and respond to human somatostatin and MIP-1α respectively in a simple avoidance assay. The endogenous signalling components involved in this remarkable promiscuity of interaction, spanning 800 million years of evolution, are investigated. This system has practical utility in ligand screening. Using structure:function studies, we identified key amino acid residues involved in the interaction of somatostatin with its receptor. This *in vivo* system, which imparts novel avoidance behaviour on *C. elegans*, can therefore be used in screening impure GPCR ligands, including the identification of bacterial clones expressing agonists within recombinant libraries.

BACK

Neuropeptide Y-like Receptors NPR-2 and NPR-1 Act Antagonistically to Control Movement

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In order to survive and reproduce, animals have to respond appropriately to changes in their environment by adapting behaviours such as feeding, locomotion, foraging for food and egg-laying. Studies on *C. elegans* have shown that neuropeptides can signal these changes and so influence the behavioural state of the worm. For example, neuropeptides encoded by the *flp-1* gene have a role in switching off egg-laying in the absence of food. We are interested in the role of Neuropeptide Y-like receptors (NPRs) in the regulation of worm behaviour. Here we present work on the role of NPR-2 in the control of movement.

We have investigated the role of *npr-2* using deletion allele *ok419*, a putative null. The *npr-2* mutant moves slowly (~50 % of wild type rate) and is uncoordinated, both on plates and in liquid thrashing assays. Furthermore, *npr-2* animals are resistant to the acetylcholine esterase inhibitor aldicarb. We have carried out reporter expression studies for *npr-2* and these show that the gene is expressed in neurones in the head and nerve cord, probably in a subset of cholinergic motorneurones. This strongly suggests that NPR-2 facilitates movement, probably through facilitation of ACh release at the neuromuscular junction.

We further asked whether a closely related NPY-like receptor, NPR-1, also contributes to the coordination of movement as it is expressed in the GABAergic motorneurones (1). The NPR-1 receptor has been implicated in social feeding behaviour (2). We carried out a genetic analysis of the *npr-2* mutation in combination with different alleles of *npr-1.* This analysis showed that the locomotion defects are only apparent in an *npr-1* gain of function (F215V) background found in N2 animals. Thus, NPR-2 and NPR-1 play an antagonisitc role in the regulation of movement. We propose a model where NPR-1 215V inhibits and NPR-2 facilitates movement, either directly or by inhibiting NPR-1 215V. We are currently investigating whether NPR-2 and NPR-1 also have a role in regulating feeding. We would further like to know what the environmental cue is that activates the NPR-2 signalling pathway.

We thank the BBSRC for financial support and the *C. elegans* Knockout Consortium, CGC and Mario de Bono for strains.

(1) Coates and De Bono (2002), Nature 419, 925-929
(2) De Bono and Bargmann (1998), Cell 94, p 679-689

BACK

T-045

Evolutionary Conservation of Synaptic Tetraspan Vesicle Membrane Proteins but Lack of Defects in Mutant *C. elegans* Strains

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Tetraspan vesicle membrane proteins (TVPs) comprise a major portion of synaptic vesicle proteins, yet their contribution to the synaptic vesicle cycle is poorly understood. TVPs are grouped in three mammalian gene families, the physins, gyrins and secretory carrier associated membrane proteins (SCAMPs). Remarkably, each family is represented by only a single polypeptide in *C. elegans* and all nematode TVPs co-localize to the same vesicular compartment when expressed in mammalian cells. To examine their function, *C. elegans* null-mutants were isolated for each gene. Triple mutants were generated by combination of all three mutant alleles. Careful analyses of these worms revealed no morphological or behavioral phenotypic defects. Nervous system architecture and synaptic contacts appear normal in the triple mutants. Mutant worms moved normally and released normal levels of neurotransmitter as assayed pharmacologically and electrophysiologically. Worms also responded normally to chemical or mechanosensory stimuli. We therefore conclude that TVPs are not needed for the basic neuronal machinery. Current transrciptome analyses identify potential regulatory components whose expression is altered in the mutant strains.

BACK

T-046

The Role of Polyunsaturated Fatty Acids in Neurotransmitter Release

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Long chain polyunsaturated fatty acids (PUFA), fatty acids with several double bonds, are highly enriched at nerve terminals and are required for efficient neurotransmission. Indeed, alteration of PUFA levels has been associated with several diseases leading to cognitive impairment, a phenomenon associated with defective neurotransmission. However, the precise function of PUFAs at the nerve terminal is unclear.
To clarify this, we isolated *C. elegans* strains depleted of PUFA by inactivating the gene *fat-3*, which encodes an enzyme essential for PUFA biosynthesis. *fat-3* mutants animals don't show developmental defects of the nervous system. However, they release abnormally low levels of neurotransmitter at cholinergic and serotonergic neuromuscular junctions. This is associated with a dramatic decrease in the number of synaptic vesicles at presynaptic sites, suggesting that PUFA-depleted animals have a number of synaptic vesicles insufficient to support normal neurotransmitter release.

The depletion of synaptic vesicles at pre-synaptic sites in *fat-3* mutants could be caused by one or a combination of different defects. First, the biogenesis of synaptic vesicle precursors in the neuronal cell body could be impaired. Second, the transport of synaptic vesicle precursors from the cell body to sites of release could be hampered. Third, retrieval of synaptic vesicle components from the presynaptic plasma membrane could be defective. This would result in less synaptic vesicle components being available for vesicles reformation.
To determine whether *fat-3* mutants are defective in synaptic vesicle biogenesis, transport or recycling, we generated *fat-3* worms expressing synaptic vesicle components tagged with GFP. Since synaptic vesicle components are tightly associated with synaptic vesicle membranes, their distribution faithfully reflects the distribution of synaptic vesicles and their precursors. Ultrastructural and confocal analysis revealed that synaptic vesicles are produced at normal levels and correctly transported to nerve terminals. This suggests that *fat-3* mutants are not defective in the biogenesis of synaptic vesicles or in their transport from the soma. However, the synaptic vesicle component synaptobrevin/VAMP is mislocalized in *fat-3* mutants implying defects in the retrieval of synaptic vesicle components from the plasma membrane. Impaired retrieval would lead to inefficient synaptic vesicle recycling.

Our data suggest that the defects in synaptic vesicle recycling are responsible for the abnormally low number of synaptic vesicles observed in *fat-3* mutants. We propose that these defects are caused by the altered biophysical properties associated with PUFA depletion from synaptic vesicle membranes.

BACK

T-047

Rho is a Pre-Synaptic Activator of Neurotransmitter Release at Pre-Existing Synapses in *C.elegans*

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Rho GTPases have important roles in neuronal development but their function in adult neurons is less well understood. We demonstrate that pre-synaptic changes in Rho activity at *C.elegans* neuromuscular junctions can radically change animal behaviour via modulation of two separate pathways. In one, pre-synaptic Rho increases acetylcholine (ACh) release by stimulating the accumulation of diacylglycerol (DAG) and the DAG-binding protein UNC-13 at sites of neurotransmitter release; this pathway requires binding of Rho to the DAG kinase DGK-1. A second DGK-1-independent mechanism is revealed by the ability of a Rho inhibitor (C3 transferase) to decrease levels of release even in the absence of DGK-1; this pathway is independent of UNC-13 accumulation at release sites. We do not detect any Rho induced changes in neuronal morphology or synapse number, thus Rho facilitates synaptic transmission by a novel mechanism. Surprisingly, many commonly available human RhoA constructs contain an uncharacterised mutation that severely reduces binding of RhoA to DAG kinase. Thus a role for RhoA in controlling DAG levels has not been previously appreciated.

BACK

T-048

Early Embryonic Programming of Postmitotic Laterality of Chemosensory Neurons in *C. elegans*

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Neuronal laterality is a common feature of many nervous systems. In *C. elegans*, the bilaterally symmetric gustatory neuron pair ASEL/ASER displays directional asymmetric expression of several cell fate markers and sense and discriminate distinct inputs, thereby diversifying behavioural response. We have previously shown that the ASE cell fate decision is a bistable system. The output of this bistable system is regulated postmitotically by a cell-autonomous double-negative feedback loop involving the microRNAs *lsy-6* and *mir-273* and their respective target transcription factors *cog-1* and *die-1*. However, it is currently unclear what biases the outcome of this bistable system. We are investigating whether ASE laterality is established during embryogenesis and can envisage two possible mechanisms. The ASE cells descend from asymmetric lineages during embryogenesis suggesting the asymmetry in the specific pattern of cell divisions may determine ASE laterality in a lineage dependent or lineage intrinsic manner. Alternatively, a specific cell non-autonomous "asymmetry signal" may exist.

To distinguish between these mechanisms we have used a combination of genetics and laser ablations to manipulate the early embryonic lineages of various ASE reporter strains. We show that ASE laterality is dependent on early embryonic asymmetry but find no evidence for any specific "asymmetry signal". ASE cells produced from ASEL lineages generated ectopically on the right side of the embryo still acquire a left fate and vice versa. Ablations that leave only the ABalp (ASEL precursor) or the ABpra (ASER precursor) blastomere alive at the AB^8-cell stage show that laterality is intrinsic to these blastomeres. These experiments strongly suggest that adult ASE laterality is specified in a lineage dependent manner by the mechanisms that establish the fates of the AB^8-cell blastomeres. ASE laterality is not dependent on POP-1 levels at the AB^8-cell stage but is specified even earlier at the AB^2-cell stage as a function of the ABa/ABp lineage decision. By transforming the ABa/ABp blastomere fates at the 4-cell stage we show that ASE laterality is dependent on the previously described Notch signal that establishes the ABa/ABp fate difference by down-regulating *tbx-37* and *tbx-38* in the ABp blastomere only. This Notch signal acts both temporally and genetically upstream of the bi-stable loop to bias this loop nine rounds of cell division later in the post-mitotic ASE cells. Our data demonstrate that the adult laterality of the ASE neurons is specified embryonically at the 4-cell stage.

BACK

C. elegans Intersectin: A Synaptic Adaptor Protein Coupling Endocytic Membrane Traffic to Exocytosis

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Intersectin family proteins are scaffold proteins believed to play a role in endocytosis. Intersectins have a modular organization with two N-terminal EH domains (Eps15 homology), a central coiled-coil region and five SH3 domains in the C-terminal region. A long, brain-specific isoform contains at the C-terminal an additional DH domain (Dbl homology), a PH (Pleckstrin homology) and a C2 domain. Intersectin proteins were identified by several groups based on their ability to bind proteins involved in a variety of processes, such as endocytosis, exocytosis, signal transduction pathways and actin remodeling. Recently, *in vivo* studies in *Drosophila melanogaster* indicated a function for Dap160/Intersectin in synaptic development and in the stabilization of an endocytic macromolecular complex, since the levels of several endocytic molecules such as dynamin, synaptojanin and endophilin are severely reduced in dap160 mutant NMJs (Marie et al. 2004; Koh et al., 2004).

We will present the analysis of Y116A8C.36 gene, encoding for ITSN-1 protein, the unique orthologue of the intersectin family in *C. elegans.* We will discuss our data showing that 1) ITSN-1, differently then in *Drosophila melanogaster,* is not an essential gene in *C.elegans*. 2) ITSN-1 protein is mainly expressed in neuronal cells and it is localized in synaptic vesicle-rich regions. 3) ITSN-1 plays a role in synaptic transmission, controlling acetylcholine release. 4) *itsn-1* genetically interacts with *dyn-1* and *ehs-1* genes, both implicated in compensatory endocytosis at the synapses. 5) ITSN-1 protein interacts *in vivo* with EHS-1 and Dynamin proteins.

Our *in vivo* studies challenge the participation of ITSN-1 protein in exocytic processes and in the control of synaptic vesicle cycling suggesting a role for ITSN-1 in temporal and spatial coordination of exocytosis and endocytosis.

BACK

T-050

C. elegans ASIC-1 is required for Associative Learning and Memory in *C. elegans*

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Acid-sensing ion channels (ASICs) are members of the DEG/ENaC superfamily of ion channels. DEG/ENaC ion channels have been identified in a variety of organisms ranging from nematodes to humans and are involved in diverse cellular processes such as ion homeostasis and sensory transduction. ASIC ion channels have been implicated in synaptic plasticity, learning and memory in mammals.

ASIC-1, a novel DEG/ENaC protein and close homologue of mammalian ASIC, is highly expressed in *C. elegans* dopaminergic neurons and is localized at synapses. Dopamine biosynthesis is normal in animals lacking ASIC-1. We find that ASIC-1 is required for conditioning to several chemical attractants, while it is dispensable for chemotaxis. Consistently, ASIC-1 is also required for associating the presence of food with the rearing temperature. Such observations indicate that ASIC-1 functions at dopaminergic neuron synapses to contribute to the capacity of the animal for associative learning. Therefore, ASIC roles in associative learning and memory might be conserved from nematodes to mammals, thus, making *C. elegans* an attractive model in which to dissect the relevant mechanisms.

BACK

T-051

Giving Worms the Hump: Genome-Wide Screen for Novel Molecules that Regulate the Cadherin-Catenin Complex in *C. elegans*

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Epidermal morphogenesis is responsible for the change in shape of the *C. elegans* embryo from an ovoid into a worm, and involves two cadherin-dependent events: ventral enclosure and elongation. We have previously shown that animals homozygous for a hypomorphic alpha-catenin mutation (*fe4*) provide a sensitive genetic background to identify molecules that contribute to cadherin-catenin complex function (Pettitt *et al.,* 2003). These primary results have provided the rationale for a genome-wide RNAi screen for *C. elegans* genes whose loss of function enhances the *fe4* phenotype (Cox and Hardin, 2005, personal communication). We have recently completed a screen of Chromosome III, from which we identified 17 genes whose knock-down by RNAi gives a wild-type phenotype in N2, but which enhances the penetrance and severity of the *fe4* mutation. Most of the genes have homologues in other animal phyla: these include molecules predicted to be involved in cell adhesion and actin organisation, and two genes encoding conserved proteins of unknown function. We have focused our attention on five molecules due to their possible involvement in cell adhesion and actin organisation, and their level conservation with molecules from other phyla. These molecules are: FLI-1 (homologue of *Drosophila* Flightless I), TAG-213 (homologue of one of the Tho complex proteins), Y71H2AM.17 (contains a HMG box domain and is related to SWI/SNF proteins), F26F4.1 and ZK637.2 (both are of unknown function but are highly conserved across species). We are in the process of determining the cellular and molecular basis of the genetic interactions between these genes and *hmp-1*. For instance, regulators of gene expression (i.e *tag-213* and Y71H2AM.17) may directly regulate the expression of cadherin-catenin complex components, or may result in partial defects in epidermal differentiation.

FLI-1 localises to the cytoplasm of hypodermal cells in embryos undergoing morphogenesis. As has been found previously (Lu and Lundquist, IWM 2005), it is also expressed throughout postembryonic development in pharyngeal and muscle cells, and in neurons. We believe FLI-1 contributes to the regulation of actin dynamics at cadherin junctions, such that when *fli-1* function is reduced in *hmp-1(fe4)* worms (which already display minor defects in actin filament organisation), elongation is severely compromised. We are currently examining genetic interactions between *fli-1* and genes encoding other proteins thought to modulate actin filaments at cadherin junctions.

BACK

T-052

Phosphorylcholine-Modified Proteins of *Caenorhabditis elegans* are Specific to Developmental Stages

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The free-living nematode *Caenorhabditis elegans* has been found to be an excellent model system for developmental studies [1] investigating parasitic nematodes [2] and drug screening [3]. Structural analyses of glycoconjugates derived from this organism revealed the presence of nematode specific glycosphingolipids of the arthro-series, carrying, in part, phosphorylcholine (PC) substituents [2]. PC, a small haptenic molecule, is found in a wide variety of prokaryotic organisms, i. e. bacteria, and in eukaryotic parasites such as nematodes. There is evidence that PC-substituted proteins glycolipids are assumed to be responsible for a variety of immunological effects including invasion mechanisms and long-term persistence of parasites within the host [4]. In contrast to PC-modified glycosphingolipids [5], only a limited number of PC-carrying (glyco)proteins were identified so far [6-9].
We have analysed the expression of PC-modified proteins of *C. elegans* during developmental stages using two dimensional SDS-Page separation, 2D-Western-blot and MALDI-TOF mass spectrometry.
The pattern of PC-modified proteins was found to be stage specific. The PC-modification on proteins was most abundant in the egg and dauer larvae-stages followed by the adult-stage and L4. Only small amounts of the PC-substitution were found in L3 and L2. In L1 we couldn´t detect any PC-Modification. The prediction of the cellular localisation of the identified proteins revealed a predominant cytosolic and mitochondrial occurrence of the PC- modification. Most of the identified proteins are involved in metabolism or in protein synthesis.

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BACK

T-053

hedgehog-Related Genes Have Essential Functions in the Extracellular Matrix

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The *C. elegans* genome encodes about 60 *hedgehog*-related genes in 4 families: quahog, warthog, groundhog, and ground-like. Here we report functional studies for *wrt-6*, *wrt-5* and *qua-1*, and show how they play essential roles in the extracellular environment. *wrt-6* is primarily expressed in the sensilla socket cells and mutants display Dyf, Osm and Daf-d phenotypes. The cilia structure is normal, as shown by EM and confocal microscopy. However, the sheath cell channel is expanded and the dendrites of the sensory neurons do not form tight bundles. Using the socket cell marker Punc-53::GFP we observe morphological defects in the channel. The Dyf phenotype can be rescued in L4 or young adults by a hsp::WRT-6 construct, suggesting that there are no major developmental defects in cilia or cell fate specification. WRT-6 point mutations have identified several functionally important residues in the WRT domain. The WRT domain alone is not able to rescue the Dyf phenotype, the HOG domain is required. Replacing the WRT domain of WRT-6 with that of WRT-5 is able to rescue the Dyf phenotype, suggesting that the WRT domain structures are similar and can tolerate numerous substitutions. The translational GFP fusions have revealed that WRT-6 is associated with the cuticle in the channel of amphids and phasmids and in the vulval labia. WRT-5 lacks the Hint/Hog domain and is expressed in seam cells, the pharynx, pharyngeal-intestinal valve cells, neurons, neuronal support cells, the excretory cell, and the reproductive system. WRT-5 protein is secreted into the extracellular space during embryogenesis. Furthermore, during larval development WRT-5 protein is secreted into the pharyngeal lumen and the pharyngeal expression changes in a cyclical manner in phase with the molting cycle. *wrt-5* mutations cause a cold-sensitive embryonic lethality. Animals that hatch exhibit variable abnormal morphology, e.g., bagging worms, blistering, molting defects, or Roller phenotypes. Using AJM-1::GFP we observed severe cell boundary abnormalities in the arrested embryos. AJM-1:GFP protein is also misplaced in pharyngeal muscle cells in the absence of WRT-5. WRT-5 may be an essential cofactor for cell fusion at low temperatures. *qua-1* is expressed in hypodermal cells, rectal cells, sensilla support cells. *qua-1*::GFP undergoes cyclical changes during development in phase with the molting cycle. *qua-1* mutants display a 100% penetrant Mlt phenotype. EM reveals double cuticles due to defective ecdysis and abnormal alae structures, but no obvious defects in the hypodermis. QUA domain-only::GFP and full-length QUA-1::GFP fusion constructs are secreted and associated with the overlying cuticle. QUA-1::GFP rescues the mutant phenotype, but QUA domain-only::GFP causes dominant Mlt phenotypes in N2.

BACK

T-054

The Planar Cell Polarity Protein VANG-1 interferes with Intercalation Events During Epithelial Morphogenesis In *C. elegans*

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C. elegans VANG-1 is the only homolog of the *Drosophila* planar cell polarity (PCP) protein Strabismus/Van Gogh. We originally identified VANG-1 as a putative binding partner of DLG-1 (Discs large), a MAGUK protein that acts as a molecular scaffold for the establishment of the apical junction in all epithelia of the *C. elegans* embryo. The phenotype observed in *vang-1* mutant embryos show intercalation defects during epithelial morphogenesis of the intestine and the hypodermis. Similar phenotypes arise in *lin-17* (*frizzled*) mutants or after RNAi against *dsh-2* (*dishevelled*), indicating core components of the PCP pathway in other systems to be involved in cell intercalation in the *C. elegans* embryo. In addition, VANG-1 and DSH-2 do colocalize and the asymmetric distribution of VANG-1 in epithelia depends on DSH-2. Taken together these results suggests that planar cell polarity might exist in *C. elegans*.

BACK

T-055

The ATPase ASNA-1 Positively Regulates Insulin Secretion in *C. elegans* and Mammals and Promotes Growth Non-autonomously

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C. elegans embryos that hatch in the absence of food arrest growth during the first larval stage (L1). While much has been learned about the later diapause, called dauer that worms enter on encountering adverse conditions little is known presently about the mechanisms governing L1 arrest. We have identified and characterized a gene, *asna-1*, which when mutated, causes a complete but reversible arrest at the L1 stage even in the presence of food. We find that *asna-1* encodes an ATPase that functions non-autonomously to regulate larval growth. *asna-1* is expressed in a restricted set of sensory neurons and in the intestine; cells that produce insulin. *asna-1* mutants have reduced insulin signaling while overexpression of *asna-1* mimics the effects of overexpressing insulin. The human homologue *ASNA1*, which rescues the *C. elegans* mutant phenotype, is expressed at high levels in human pancreatic eta cells but not in other pancreatic endocrine or exocrine cell types. Increase or decrease of *ASNA1* function in mouse insulinoma cells causes an increase or decrease in insulin secretion respectively. Similarly, reducing *asna-1* gene function in *C. elegans* causes a decrease in levels of secreted insulin. We propose that ASNA1 is a novel evolutionarily conserved modulator of insulin signaling.

BACK

Developmental Regulation of UNC-45 Mediated Myosin Assembly

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In muscle, selective protein degradation by the ubiquitin-proteasome system is required to mediate the destruction of the sarcomeric structure, to regulate the maintenance and remodelling of the sarcomere and to ensure the development of muscle, the major component of the myofibrillar apparatus. The myosin chaperone UNC-45 plays a crucial role in the assembly of myosin into thick filaments and our recent work revealed that UNC-45 protein levels are subject to stringent regulation by two specific E3 ubiquitin ligases, CHN-1 and UFD-2. These two E3 enzymes form a novel E4 complex responsible for the multiubiquitylation of UNC-45, earmarking it for terminal protein degradation by the 26S proteasome.

We have further investigated the roles of CHN-1 and UFD-2 during the process of UNC-45 dependent myosin assembly in *C. elegans*. Our current investigations revealed that movement defects of *unc-45* thermosensitive (*ts*) mutants are suppressed in animals lacking either CHN-1 or UFD-2 and perturbation of UNC-45 protein level regulation by CHN-1 and UFD-2 results in severe movement defects specifically at the transition from L4 to young adult larval stage. This suggested that UNC-45 might be regulated *in vivo* by muscle specific co-expression of both E3 ubiquitin ligases in a developmentally regulated manner. We could support this notion by showing that UNC-45 protein levels increase during larval development and is highest at the L4 larval stage during the exponential growth phase of muscle thick filaments and degraded thereafter. Northern blot analysis identified an up-regulation of both *chn-1* and *ufd-2* transcripts specifically at the young adult larval stage, after body wall muscle development has occurred. In addition, our data suggest that *unc-45*, *chn-1* and *ufd-2* are not involved in myosin disassembly, suggesting their role to be specific for the developmental process but not for the degradation or reorganization of the sarcomere. Importantly, both genetic and biochemical interactions of UNC-45, UFD-2 and CHN-1 are found from worm to man, indicating a conserved function of these components in muscle development across evolution.

BACK

Role of Runx and CBFbeta Homologues in the Control of Cell Proliferation in *C. elegans*

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The seam cells of *C. elegans* are a good model system for understanding how cell fate and proliferation are regulated and coordinated during development. These hypodermal blast cells divide in a reiterated self renewing stem cell-like pattern at each larval stage producing an anterior cell that differentiates and fuses with hyp7 and a posterior seam cell that divides again in the same way. Some of the seam cells also undergo extra proliferative symmetrical divisions in L2 larvae and in L3 males. We wish to understand how this pattern of cell division is programmed during development.

We identified the Runx homologue, *rnt-1*(pka *mab-2*) as a rate-limiting regulator of cell proliferation that specifically functions in seam cells. We also showed that the cyclin dependent kinase inhibitor orthologue, CKI-1 is negatively regulated by *rnt-1* revealing a possible mechanism by which this Runx factor regulates cell division. We have recently discovered that the timing of *rnt-1* overexpression is critically important for its hyperplastic effects. In addition it seems that RNT-1 may be inactivated by passage through S phase: a pulse of *rnt-1* overexpression in G2/M induces an extra round of division whereas overexpression prior to normal S phase entry fails to induce extra division. We have also found that the asymmetry of division may be overcome by *rnt-1* overexpression – the anterior cell normally differentiates and fuses with the hyp7 syncytium but in response to high levels of RNT-1 it is capable of remaining as a seam cell and dividing again. We propose that this failure to differentiate is caused by inappropriate cell cycle re-entry programmed by *rnt-1*.

We have discovered that the CBFbeta homologue, *bro-1*, is also required for seam cells to divide correctly. This implies that these two proteins interact in the same way as in other systems, namely that CBF beta binds to the Runt domain and increases Runx DNA binding affinity. Intriguingly, however, the proliferative effect of overexpression of *bro-1* appears not to be abrogated by *rnt-1* loss of function, indicating that *bro-1* may be capable of exerting its effects in a *rnt-1*-independent manner. This result is unexpected as CBF beta proteins have not been previously shown to function separately from Runx factors. We are currently confirming this result and attempting to understand the additional mechanism by which *bro-1* acts.

BACK

Defects in *C. elegans* Nucleotide Excision Repair Lead to Hypermutability, Growth Arrest and Degradation of RNA Pol II

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In eukaryotes, UV-induced photolesions are repaired through the multistep nucleotide excision repair (NER) pathway. In humans, the absence of NER activity results in the disorder xeroderma pigmentosum (XP). XP patients suffer from extreme photosensitivity, a high incidence of skin cancer and neurological defects. Most studies of NER have employed the single-celled yeast or somatic cell lines. To understand how defects in NER affect the development and survival of a multicellular organism, we have characterised a UV hypersensitive and NER defective mutant, *rad-3* [1].

We have established that the *rad-3* gene encodes the human XPA homolog (K07G5.2), a DNA-binding protein that is essential in the early steps of NER. We also obtained a deletion mutant from the KO consortium, *xpa-1(ok698)*. Both alleles of *xpa-1* are likely to be null because they encode truncated proteins, which are missing essential domains. In the absence of UV exposure, *xpa-1* worms develop normally; we have shown that *xpa-1*(*ok698*) mutants have a low rate of spontaneous mutation and are not hypersensitive to reactive oxygen species (ROS). By contrast, when *xpa-1* animals are exposed to UV irradiation, they undergo an immediate growth arrest and decline in survival, and become hypermutable. Surprisingly, transcriptionally quiescent dauer stage larvae survive after receiving UV doses that are lethal to worms at other stages of development. We have successfully rescued the UV sensitivity of both *xpa-1* mutants using a XPA-1::GFP construct; XPA-1::GFP is expressed in nuclei throughout development, and in most tissues.

We further show that the UV-induced growth arrest and subsequent death of *xpa-1* worms is correlated with transcriptional inhibition. This inhibition is probably due to the stalling of transcription complexes on unrepaired UV-lesions, a phenomenon that leads to the proteolytic degradation of the large subunit of RNA Polymerase II [2]. In support of this hypothesis we show that the steady-state levels of Pol II decline in *xpa-1* worms after UV exposure. Finally, we demonstrate that the degradation of Pol II is dependent on the activity of an Rsp-5 like E3 ubiquitin ligase, encoded by the *wwp-1* gene. The UV sensitivity of the *wwp-1 xpa-1* double mutant is greater than either single mutant alone. These results, together with studies in yeast, establish that the ubiquitylation and degradation of Pol II aids the survival of organisms after they are exposed to UV irradiation. [1] Hartman *et al. Genetics* 122, 379-85. (1989), [2] Ratner *et al. J Bio Chem* 273, 5184-89. (1998).

BACK

Using *C. elegans* Chromosome Substitution Strains to Map Innate Immunity Loci

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The genome of the Hawaiian natural isolate (CB4856) of *Caenorhabditis elegans* contains thousands of SNPs that differ from Bristol N2. Wicks *et al*. proposed a method for mapping mutations generated in the N2 background by genotyping snip-SNPs in crosses between mutants and the Hawaiian strain [1]. However, some polymorphisms lead to functional differences between the two strains that affect a variety of phenotypes such as social feeding, germline RNAi, and immunity [2-4]. We found that the Hawaiian strain has increased susceptibility to *Pseudomonas aeruginosa*. In order to identify the loci responsible for the Hawaiian sensitivity, recombinant inbred lines between Hawaiian and N2 were created. Analysis of these lines shows that changes at multiple loci cause the observed Hawaiian pathogen sensitivity phenotype. Separately, we have EMS mutagenized N2 worms to obtain mutants with enhanced susceptibility to pathogen (Esp); however, we have been unable to map these mutants using Hawaiian due to Hawaiian's sensitivity to pathogen.

In order to map our Esp mutants and to identify the Hawaiian immunity loci, we have created N2/Hawaiian chromosome substitution strains (CSSs). Each of these six strains (five autosomes and the X chromosome) contains a single homozygous Hawaiian chromosome substituted into an N2 background. CSSs have been used in mice to map quantitative trait loci, and we reasoned that a similar strategy could identify loci that contribute to the Hawaiian phenotype [5]. Importantly, those CSSs that are like N2 for sensitivity to pathogen can be used to map Esp mutations that occur on the same chromosome.

We will report on using the CSSs to identify Hawaiian specific innate immunity loci and the mapping of Esp mutants from our mutagenesis screen. We believe that these CSSs will be a valuable resource for other researchers who have been unable to use the Hawaiian strain for mapping their own mutants due to the differences in phenotypes between Hawaiian and N2.

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BACK

T-060

The Acyltransferase BUS-1 Acts in the Rectal Epithelium to Mediate Sensitivity of *C. elegans* to the Bacterial Pathogen *Microbacterium nematophilum*

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In mammals, the skin and the epithelia lining mucosal tissues act as a primary nonspecific barriers serving to sense potentially harmful pathogens. In genetic screens we have identified *bus-1*, a novel acyltransferase 3 family member, whose product is required for the nematode sensitivity to the bacterial pathogen *Microbacterium nematophilum*. Mutations in *bus-1* prevent bacterial attachment and subsequent post-anal cell swelling in *C. elegans*. *bus-1* encodes a transmembrane protein, with a domain present in many uncharacterized *C. elegans* proteins.

Consistent with its function in hindgut establishment/maintenance, the *Cel-bus-1* is expressed during late embryogenesis and post-embryonically, in the rectal epithelium.
The specific expression pattern observed with reporter constructs makes *bus-1* a valuable hindgut marker for morphological and developmental studies, and in particular, in situations where the shape or the dimension of the rectal epithelial cells is to be inspected.

We show that incorrect specification of rectal epithelium in *egl-38* mutants has both positive and negative effects on *bus-1* expression. In contrast, expression is not affected by *egl-5*. *bus-1* is also repressed by two transcription factors *mab-23* and *mab-9*. Furthermore, we have observed that mutations in *mab-9* render the worms hypersensitive to *M. nematophilum.* We constructed double mutants of *bus-1* and *mab-9* and observed that the bacterial adhesion to the rectum is restored in these animals, showing that *bus-1* is not essential for infection.

Bus-1 mutants exhibit no obvious defects, other than infection resistance. Nevertheless, *bus-1* is highly conserved. We found a *Cel-bus-1* ortholog that functionally rescues *Cel-bus-1* mutants, and shows the same rectal expression pattern, plus expression in vulval cells. We propose that BUS-1 is involved in post-translational modifications of a surface-exposed molecule, which acts as player in *Cel-M. nematophilum* recognition events. Our findings have implications for our understanding of host factors required for bacterial adhesion to epithelial mucosa.

BACK

T-061

Novel Genes Specifically Affecting DNA Damage Induced Apoptosis Define Pathways Acting Upstream and Downstream of *cep-1*/p53

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Failure to properly respond to DNA damage is implicated in tumorigenesis and a number of genetic instability disorders. As part of the DNA damage response, checkpoint pathways are needed to detect DNA damage and relay a signal that elicits cell cycle arrest as well as DNA repair and/or programmed cell death. While it is known that the transcriptional induction of *egl-1* by the *C. elegans* p53 homologue *cep-1* is required for the subsequent activation of the core apoptotic machinery, the signaling network connecting damage sensing and the cell death machinery remains ill-defined. To further our understanding of the pathways leading to DNA damage induced apoptosis we pursue both candidate gene and forward genetic approaches.

By scanning a series of mutations in candidate genes we found the *C. elegans* sirtuin *sir-2.1*, a NAD+ dependent protein deacetylase and homologue of yeast Sir2p to be required for DNA damage induced apoptosis, while other DNA damage responses and developmental apoptosis were at wild type levels. Furthermore, elevated levels of germ cell apoptosis in *ced-9(n1653ts)* and *gla-1(op234)* mutants were not suppressed by mutations in *sir-2.1*, indicating that germ cells are in principle able to undergo apoptosis. To address where *sir-2.1* might act in the DNA damage pathway, we assayed the irradiation dependent transcriptional induction of the *cep-1* target *egl-1*, which we found to be at wild type levels. These results indicate a role for *sir-2.1* in activating the core apoptotic machinery downstream or in parallel to *egl-1* induction, yet upstream of CED-3 activation. To help us further define the function of *sir-2.1* in DNA damage induced apoptosis we have generated antibodies against *sir-2.1* and are currently generating antibodies against the *C. elegans* core cell death components.

Our finding that pathways leading to DNA damage induced apoptosis are more complex than previously anticipated were further corroborated by finding 5 mutants not allelic to either *cep-1* or *sir-2.1* but also defective exclusively in DNA damage induced apoptosis. Out of these only one is unable to induce *egl-1* in response to irradiation. Interestingly, two of the mutants, *yp23* and *yp51*, interact genetically with *cep-1* as *cep-1*/+; *yp23*/+ and *cep-1*/+; *yp51*/+ double heterozygotes show a dramatic reduction in DNA damage induced apoptosis. We are currently mapping those mutants.

BACK

PMK-1 MAP Kinase Pathway in Response and Defense to Pore-Forming Toxins

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Pathogenic bacteria can produce a wide range of gene products that contribute to their virulence. The most common virulence factors produced by pathogenic bacteria are pore-forming toxins (PFTs). *Bacillus thuringiensis* is a soil pathogen that produces a family of PFTs called Cry toxins. Cry toxins work by binding to receptors on the surface of the host's intestine and inserting into the bilayer to form a pore (~2nm) in the host cell membrane. Our lab has shown that the nematicidal Cry toxin Cry5B is toxic to *C. elegans* in a dose-dependent manner. In an effort to learn how animal cells deal with and protect themselves against PFTs, we are using Cry5B and *C. elegans* to identify and characterize host genes and pathways that protect the animal against PFTs.

From our studies, we have discovered that the p38 MAP kinase cascade (NSY-1/SEK-1/PMK-1) helps protect *C. elegans* from Cry5B toxin. We also found that the protective function of this pathway is conserved in a mammalian cell line exposed to a PFT from a bacterium that causes human disease. We further investigated the role of this pathway in Cry5B defense using a combination of microarray analysis and reverse genetics to find the transcriptional targets of the pathway that are important to toxin defense. This analysis led to the identification of the *ttm* gene class (toxin-regulated target of MAP kinase).

We have recently found that Cry5B exposure induces phosphorylation of the *C. elegans* PMK-1 MAP kinase, indicating that the host responds to toxin by activating this protective pathway. Furthermore, we have determined that the NSY-1 MAP3K is not absolutely required for activation of PMK-1 in Cry5B treated animals, indicating an alternative upstream activator for this pathway that has yet to be discovered. In an attempt to identify some of the genes responsible for toxin-responsive activation of PMK-1, we have begun a genome-wide RNAi screen for animals that are hypersensitive to Cry5B and unable to activate PMK-1 in response to Cry5B exposure. This screen has thus far identified over 70 new genes involved in toxin defense, including one that is important for activation of PMK-1 by Cry5B.

BACK

SNPping the *nipi*'s

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Infection of worms by bacterial or fungal pathogens causes the induction of specific response genes (1, 2; see abstract by Wong et al.). Infection with the fungus *Drechmeria coniospora* results in a strong up-regulation of genes encoding antimicrobial peptides: the CNC/caenacins and certain NLPs expressed in the hypodermis. We are undertaking a dissection of the signalling pathways involved in the activation of the gene *nlp-29*.

We have constructed a transgenic strain IG274 containing two integrated reporter constructs. One, p*nlp-29::GFP*, is strongly induced upon fungal infection, the other p*col-12::dsRed* gives constitutive red fluorescence in the hypodermis and acts as an internal control signal. Reporter gene expression can be easily quantified using the Union Biometrica COPAS sorter. We found that p*nlp-29::GFP* is not induced by a number of bacterial pathogens nor by stresses such as starvation, heat-shock or heavy metal exposure. On the other hand, physical injury and high osmolarity also provoke a rapid induction of p*nlp-29::GFP*.

We have conducted an EMS screen to identify mutants that do not express p*nlp-29::GFP* following infection with *D. coniospora* and recovered 6 alleles that fall into 5 complementation groups. We are concentrating on the characterisation of the three that give most penetrant phenotype. These *nipi* (No Induction of Peptide after *Drechmeria* Infection) genes map to regions that do not contain genes known to be involved in innate immunity. Interestingly, none show an alteration in their response to osmotic stress. And while *nipi-1* and *nipi-2* no longer respond to wounding, injury-induced up-regulation is still seen in *nipi-3*. This indicates that these responses are genetically separable.

Through SNP mapping, we have narrowed down the localisation of two alleles to distinct regions of the X chromosome, within an interval of some 20 genes. Progress on the identification of these genes will be reported.

1. Mallo et al. Curr Biol 12, 1209 (2002). **2**. Couillault al. Nat Immunol 5, 488 (2004).

BACK

__T-064__

Identification of Evolutionarily Conserved DNA Damage Response and Radiation Protection Pathways by Genome-Wide RNAi

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Cancer treatment often involves exposure to ionizing radiation or radiomimetic chemotherapeutics. The current challenge in radiation oncology is to identify the most important targets that increase cytotoxicity towards tumor cells but spare normal tissue.

In this study, we present the first comprehensive screen for genes that protect cells against ionizing radiation in an intact animal, the nematode *C. elegans*. A total of 45 *C. elegans* genes was identified in a genome wide RNA interference screen for increased sensitivity to ionizing radiation. These include orthologs of well-known human cancer predisposition genes (e.g. ATM) as well as novel genes, including human disease genes not previously linked to defective DNA damage responses.
Knockdown phenotypes include defects in DNA damage induced cell cycle arrest and apoptosis, and hypersensitivy to chemotherapeutics.

Almost all genes are conserved across animal phylogeny and their relevance for humans was directly demonstrated by showing that their knockdown in human cells results in radiation sensitivity, indicating that this set of genes is important for future cancer profiling and drug development.

BACK

The *lin-35*/Rb and PTGS/RNAi Pathways Cooperate to Regulate the Onset of Endocycles in *C. Elegans*

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The intestinal lineage of *C. elegans* provides an excellent model to examine how developmental cues control the cell cycle due to the invariant, developmentally-regulated cell cycle transitions characteristic of these cells. We have isolated 5 mutants that show irregularities in the control of intestinal nuclear division timing during post-embryonic development. We classified these mutants based on whether they possessed more intestinal nuclei (*rr33* and *rr45*) or less intestinal nuclei (*rr42, rr43* and *rr44*) than wild-type. Since all these mutants hatch with the wild-type complement of intestinal cells they all affect the cell cycle transition from mitosis to karyokinesis and finally the onset of the endocycles that normally occurs at the first larval stage. By performing lineage analysis on *rr33* mutants we found that the intestinal nuclear divisions occur normally at the L1 stage, but they do not make the appropriate transition to endocycle that usually occurs at the L1 moult. Consequently they undergo an extra round of nuclear division early in the L2 stage and become tetranucleate. We mapped this *rr33* mutant close to *dpy-5* on LGI and we performed systemic RNAi with all the predicted genes in this interval to determine if any of these candidates could phenocopy *rr33*. Only C32F10.2 (RNAi) phenocopied the *rr33* phenotype and this gene corresponds to *lin-35*: the *C. elegans* orthologue of the Retinoblastoma(Rb) gene. *lin-35/*Rb is part of the synMuv B complex, which is required for the proper regulation of vulva cell fates by inhibiting the ras signaling pathway. We found that all synMuv B components tested so far enhance the intestinal phenotype of *lin-35(rr33)*, suggesting that the same complex that is required for vulva specification is also required for the appropriate regulation of intestinal nuclear divisions.

The reiteration of a L1-specific division during the L2 stage (heterochronic phenotype) prompted us to look at some of the components of the miRNA as well as PTGS/RNAi pathway. We found that the *lin-35(rr33); al! g-1(gk214)* double mutant is synthe! tically lethal, whereas neither of the single mutants show this phenotype suggesting that these two genes might function in a similar essential pathway or process in addition to their roles in regulating nuclear division. We also found that components of the endoRNAi pathway (*rrf-3 and eri-3*) suppress the intestinal phenotype of *lin-35(rr33)* suggesting that DCR-1 protein play a role in this transition and is limiting. When the endoRNAi pathway is abrogated DCR-1 becomes more available for the microRNA or exoRNAi pathway to restore the wild-type complement of intestinal division. We therefore conclude that LIN-35 and probably other components of the synMUV B are required for the timely cell cycle transition of the intestinal cells at the L1 stage and that *lin-35* cooperates with components of the PTGS and/or microRNA pathway to appropriately regulate this cell cycle transition.

BACK

T-066

Distinct and Redundant Functions for *C. elegans* HP1 Proteins in Post-Embryonic Development

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The highly conserved HP1 family proteins play an important role in the dynamic organization of nuclear architecture and in the epigenetic control of gene expression. Although most species contain more than one HP1 family member which differ in their chromosomal distribution, it is not known to what extent their function is redundant or specific in a developmental context.

C. elegans has two HP1 homologues, HPL-1 and HPL-2. While *hpl-2* is required for the maintenance of a functional germline and for vulval development by acting in the Rb related synMuvB pathway, no obvious function has so far been attributed to HPL-1. We report the characterization of an *hpl-1* null allele. We show that while the absence of *hpl-1* alone results in no obvious phenotype, *hpl-1;hpl-2* double mutants show synthetic, temperature sensitive phenotypes including larval lethality and severe defects in the development of the somatic gonad. Furthermore, we find that *hpl-1* has an unexpected role in vulval development by acting redundantly with *hpl-2*, but not other genes previously implicated in vulval development. Localization studies show that like HPL-2, HPL-1 is a ubiquitously expressed nuclear protein. However, HPL-1 and HPL-2 show clear differences in their localization. This is most clearly evident in embryos, where HPL-1 and HPL-2 are found concentrated in non-overlapping nuclear foci. We find that the LIN-13 protein, which forms a complex with HPL-2, is absolutely required for recruitment of HPL-2, but not HPL-1, in these foci. Our results show that HPL-1 and HPL-2 play both unique and redundant functions in post-embryonic development. Altogether, these studies contribute to an understanding of the function of HP1 proteins in gene regulation throughout development, and in a more general way on how a general chromatin interacting protein may play specific roles in given developmental pathways.

BACK

T-067

The Glycosyltransferase BUS-8 is Essential for Epidermal Morphogenesis

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In *C. elegans* the epidermis is a simple epithelium. It is derived from a sheet of cells on the dorsal side of the embryo that spread to enclose the ventral side prior to elongation. The epidermis is then covered by the cuticle, an extracellular structure.

We independently isolated alleles of *bus-8* in screens for mutants resistant to infection by *Microbacterium nematophilum* and for nicotine hypersensitivity. Further alleles were obtained from a non-complementation screen, and a deletion (putative null) allele was provided by Shohei Mitani.

bus-8(null) animals arrest during embryogenesis. We have shown that this is due to a defect in ventral enclosure, leading to extrusion of the body contents through the anterior of the ventral side during early elongation. This is reminiscent of mutations in the cadherin *hmr-1*. Weaker alleles of *bus-8* show a variety of larval defects, including hypersensitivity to a variety of drugs. We have examined the structure of the cuticle and epidermis by electron microscopy. The epidermis is strikingly disordered. We also see abnormalities in the surface coat and cuticle. We propose that, due to their abnormalities in epidermal morphogenesis, *bus-8* mutants are more permeable to small molecules.

We have cloned *bus-8*. It encodes a putative glycosyltransferase. This suggests that glycosylation may have a role in mediating cadherin-based adhesion. We will discuss our progress at relating the molecular identity of *bus-8* to its roles during development.

BACK

The Transcription Factor EGL-43 is Necessary for Anchor Cell Invasion during Vulval Development

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For proper egg-laying by the hermaphrodite, the uterus has to become firmly connected to the vulva during late larval development. The anchor cell (AC), a specialized cell in the somatic gonad, coordinates vulval and uterus development and initiates the formation of the connection between these two tissues.

During the L3 larval stage, the AC induces six neighboring ventral uterine (VU) cells to adopt the Pi cell fate. Around the same time of development, the basal laminas separating the uterus from the vulva begin to dissolve and the AC invades the vulval tissue by extending a process between the primary cells (Sherwood et al., 2003). In this way, the AC connects the vulval and uterine tissues.

In an RNAi-based screen for genes regulating vulval morphogenesis, we have found that knock-down of *egl-43* causes a penetrant protruding vulva (Pvl) phenotype because the connection between the uterus and vulva is not formed correctly. *egl-43* encodes a zinc finger transcription factor homologous to the human EVI1 proto-oncogene. Closer examination showed that in *egl-43* RNAi animals the basal lamina under the AC is not removed, which prevents the AC from invading the vulval tissue. In addition, *egl-43* RNAi animals show defects in the specification of the Pi cell fate. Similar defects in AC invasion have been reported for *fos-1* mutants (Sherwood et al., 2005), and like *fos-1*, a transcriptional *egl-43p::gfp* reporter is expressed at high levels in the AC and at lower levels in the adjacent VU cells. Moreover, *egl-43p::gfp* expression in the AC is 5-fold reduced in a *fos-1(0)* background, indicating that FOS-1 promotes AC invasion at least in part by up-regulating *egl-43* expression in the AC.

BACK

Interaction Between Nicotinic and Insulin Pathways during Post-Embryonic Development

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C. elegans develops through four larval stages (L1 to L4) separated by molts. The identity of larval stages is mostly determined by stage-specific expression of heterochronic genes, which constitute an intrinsic genetic timer. However, extrinsic cues such as food availability or population density also modulate developmental rate by mechanisms that remain largely unknown. To investigate a potential role of the nervous system in the temporal regulation of *C. elegans* development, we pharmacologically manipulated nicotinic neurotransmission, which represents a prominent signaling component in the *C. elegans* nervous system.

We previously demonstrated that exposure to the nicotinic agonist DMPP during post-embryonic development is lethal at the L2/L3 molt. Specifically, DMPP delays cell divisions and differentiation during the L2 stage but does not affect the timing of the molt cycle, hence causing exposure of a defective L3 cuticle to the environment after the L2/L3 molt. Nicotinic acetylcholine receptors (nAChRs) containing the UNC-63 subunit are required, most likely in neurons, to trigger DMPP-induced developmental delay. Using a forward genetic screen, we identified six genes whose mutations confer resistance to DMPP, including the nuclear hormone receptor *daf-12*. DAF-12/NHR is a key regulator of the dauer versus reproductive L3 choice. DAF-12 is also required to implement DMPP-induced developmental delay.

DAF-12/NHR integrates signals from the DAF-2/InsR (Insulin Receptor) and the DAF-7/TGFβ pathways to take the dauer decision. *daf-2* and *daf-7* mutants form dauer larvae regardless of environmental conditions. However, we showed that *daf-2* mutants are DMPP resistant whereas *daf-7* are not. Therefore DMPP sensitivity is independent of dauer formation but specifically requires DAF-2/InsR signaling. The transcription factor DAF-16/FOXO is the major output of DAF-2/InsR signaling in *C. elegans*. We demonstrated that DAF-16 activation is sufficient but not necessary to confer DMPP resistance. This suggests that additional pathways function in parallel to DAF-16/FOXO to control L2 developmental timing. Indeed, the DMPP resistant mutants isolated in our screen suppress constitutive dauer formation of specific *daf-2*/InsR alleles. Genetic interaction experiments suggest that these genes might be part of a DAF-16 independent DAF-2/InsR pathway. Using mutants causing degeneration of various neurons and genetic ablation, we further demonstrated that 8 neurons including two ciliated neurons in the head are required to implement DMPP toxicity. Interestingly, insulin signaling has been shown to function in these neurons to regulate dauer entry. Altogether, our results indicate that DAF-2/InsR signaling interacts with nicotinic signaling to control dauer and non-dauer development.

BACK

An Ancestral Module Patterns a Derived Nematode Vulva Equivalence Group

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Nematode vulva formation provides a paradigm to study the evolution of pattern formation and cell fate specification. The *Caenorhabditis elegans* vulva is generated from three of six equipotent cells that form the so-called vulva equivalence group. During evolution, the size of the vulva equivalence group has changed: *Panagrellus redivivus* has eight, *C. elegans* six and *Pristionchus pacificus* only three cells that are competent to form vulval tissue. In *P. pacificus,* the reduction of the vulva equivalence group is achieved by programmed cell death of individual precursor cells.

We have identified the genes controlling this cell death event and the molecular mechanism of the reduction of the vulva equivalence group. Mutations in *Ppa-hairy*, a gene which is unknown from *C. elegans*, result in the survival of two precursor cells, which expands the vulva equivalence group. Mutations in *Ppa-groucho* cause a similar phenotype. *Ppa*-HAIRY and *Ppa*-GROUCHO form a molecular module that represses the Hox gene *Ppa-lin-39* and thereby, reduces the size of the vulva equivalence group. The *C. elegans* genome does not encode a similar *hairy*-like gene and no typical HAIRY/GROUCHO module exists. Instead, the size of the *C. elegans* vulva equivalence group is regulated by the EGF and WNT signaling pathways.

Therefore the **derived vulva equivalence group** of *P. pacificus* is **patterned by an ancestral hairy/groucho module**.
I will also present a highly speculative model of what kind of adaptive values might be associated with the *Pristionchus* specific reduction of the size of the vulva equivalence group.

BACK

T-071

Interplay Between the Nuclear Receptor NHR-25 and β-Catenin Signaling during Cell Fate Decision in the Gonad of *C. elegans*

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Molecular mechanisms that specify cell fates during asymmetric cell divisions are critical for differentiation. In the somatic gonad precursors (SGPs) of *Caenorhabditis elegans*, a β-catenin pathway plays a crucial role in the proximal-distal fate decision. The Wnt nuclear effector TCF/POP-1 and β-catenins WRM-1 and SYS-1 are the key molecules for this decision. Impaired function of all of these genes leads to a Sys (Symmetrical sisters) phenotype, when all SGPs adopt the same, proximal fate. Thus, no distal tip cells (DTCs) that would lead germline differentiation and elongation of the gonadal arms are formed in hermaphrodites. Here, we show that a loss of the nuclear receptor NHR-25 (a homolog of SF-1 and Ftz-F1) causes an extra DTC phenotype, where up to four DTCs form at the expense of proximally fated the anchor cell. This "all-distal" Sys phenotype is thus opposite to known loss-of-function phenotypes of genes in the β-catenin pathway. Our data show that a balance between the β-catenin/POP-1 activity and the action of NHR-25 is required for the proper establishment of both the distal and proximal cell fates. *pop-1(q645)* mutants that never form DTCs and consequently lack gonadal arms develop DTCs, extend the gonadal arms, and become fertile when *nhr-25* is silenced in them. By using cell transfection techniques, co-immunoprecipitation and yeast two-hybrid assays, we show that this interaction is coordinated by mutual modulation of NHR-25 and POP-1 activities through direct contacts of NHR-25 with the distinct β-catenins WRM-1 and SYS-1. The crosstalk between nuclear receptor and β-catenin signaling thus establishes the proper axis of the entire organ.

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BACK

COQ Genes: Ubiquinone Biosynthesis, Gonad Development and Fertility Establishment

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Coenzyme Q (Ubiquinone, Q) is a lipidic compound involved in electron transport in mitochondrial respiratory chain. Q has been found to be related with physiological processes like resistance against oxidative stress and aging and its deficiency derives in neurodegenerative diseases in humans.

Although Q biosynthesis pathway has been characterized in yeast, its regulation is still unclear. Ten genes (*coq1* through *coq10*) have been described to participate in Q biosynthesis in *S. cerevisiae*. Based on RNAi experiments we identified six genes involved in Q biosynthesis in *C. elegans*, homologues to yeast *coq* genes. Silenced *C. elegans* individuals showed reduced production of reactive oxygen species and extended life-span.

To study COQ proteins expression and regulation we generated COQ::GFP transgenic animals for *coq-3, coq-5, coq-6* and *coq-8* genes. COQ-3, COQ-5 and COQ-6 proteins participate catalytic steps of Q biosynthesis. COQ-8 protein has unassigned function although it similarities with regulatory kinase proteins and is a candidate for Q biosynthesis regulation.

Genes *coq-3, coq-5* and *coq-6* showed a similar constitutive expression pattern in tissues with high requirements of energy whereas COQ-8 protein showed a differential expression pattern during development and aging. COQ-8 expression correlated with Q content reported during life cycle.

A *coq-8*-deleted mutant strain showed defects in tissues where COQ-8 was expressed, defective gonad development and reduced progeny that does not complete embryonic development. This embryonic arrest correlated with the stage when COQ-8 protein expression was first detected in embryos.

These results suggest that either COQ-8 protein or bioenergetics may be involved in gondadogenesis and fertility establishment.

BACK

T-073

Robustness and Evolution of *Caenorhabditis* Vulva Development

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We study *C. elegans* vulva development as an example of a biological process that is robust to stochastic noise and variations in the external environment. The vulva is formed by three precursor cells aligned in the ventral epidermis. P6.p usually adopts a 1° fate, P5.p and P7.p a 2° fate. In addition, P4.p and P8.p (and P3.p when it does not fuse to the epidermal syncytium) are competent to form vulval tissue but normally adopt a non-vulval 3° fate. The spatial fate pattern (‘332123’) is organized around the gonadal anchor cell that emits a LIN-3/EGF signal. Cell fate patterning involves a well-characterized network of intercellular signaling pathways, including EGF/Ras, Notch and Wnt signaling. The cell fate pattern is quasi-invariant within and among wild isolates of different *Caenorhabditis* species.

We measured the actual precision of this vulval fate patterning process and characterized deviant patterns in six laboratory environments (standard laboratory conditions at 15°C, 20°C and 25°C; liquid; dauer; starvation in L2). Overall, we find that errors that result in a defective vulva occur at low frequency (<0.1%) in all environments and that the rates and patterns of variants depend on the environment and genetic background for different wild isolates of *C. elegans* and *C. briggsae*. For example, under the L2 starvation conditions, N2 individuals are prone to miscenter their vulva on P5.p (instead of P6.p), a variation rescued by P4.p competence, whereas the *C. elegans* isolate JU258 shows a dramatic increase in P4.p and P8.p fusion, but rarely miscenters the vulva, and *C. briggsae* AF16 is prone to miscenter its vulva on P7.p. In addition, P3.p fusion decreases upon starvation in AF16 while it increases in N2 and JU258. The interplay between variations in centering and competence is likely to be relevant to the evolution of the system.

Buffering of the system to environmental variations may result in the buffering of some genetic variation, thus allowing the evolution of developmental processes without phenotypic change (silent/cryptic evolution). To reveal cryptic variation within *C. elegans*, we introgressed vulva mutations into six divergent genotypes (CB4856, JU258, PS2025, AB1, PB303, PB306) and found that the phenotypic effect of a mutation varies greatly with the genotype. To reveal cryptic variation among *Caenorhabditis* species, we ablated the anchor cell at successive timepoints during vulval induction, which uncovers a temporal series of P(5-7).p cell fate patterns, starting from ‘333’ and ending with the correct ‘212’ pattern. P(5-7).p adopt an intermediate ‘222’ fate pattern in *C. briggsae* and in 'basal' *Caenorhabditis* species, as in many other nematode genera. Direct transition to the correct ‘212’ fate pattern is found in a group of species including *C. elegans*, and a ‘232’ intermediate pattern is found in *C. remanei*. We thus reveal candidate changes in the relative activities of different vulva signaling pathways, in the absence of change in the final phenotype.

BACK

T-074

Computational Modeling and Analysis of *Caenorhabditis elegans* Vulval Development

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Caenorhabditis elegans vulval development provides an important paradigm for studying the process of pattern formation during animal development. Although many gene interactions involved in this process have been identified, how they orchestrate to generate a robust pattern is not yet completely determined. Using the language of *Reactive Modules* we have developed a discrete, dynamic, and state-based computational model according to the current understanding of the mechanisms underlying VPC pattern formation. Our model represents the crosstalk between the epidermal growth factor receptor (EGFR) and LIN-12/Notch signaling pathways, which patterns the fates of the six vulval precursor cells (VPC). The model is capable of reproducing up-to-date experimental data of VPC fate specification, as well as to predict the outcomes of new experiments and to point out mechanistic gaps that have not yet been investigated. Analysis of our model using the *model-checking* technique has provided new biological insights that may aid our understanding of VPC fate specification. Specifically, our model predicts that EGFR signaling negatively regulates *lateral signal target* (*lst*) genes, which highlights a new putative negative feedback loop that is currently under experimental validation. In addition, our model analysis shows that most mutations affecting vulval development lead to stable fate patterns in spite of variations in synchronicity between VPCs. The analysis further suggests that unstable fate patterns are caused by variations in the timing of the lateral signal relative to the down-regulation of *lin-12* activity. These novel insights propose new avenues to explore experimentally, and may further pave the way for better understanding of the mechanisms underlying precise pattern formation during animal development.

BACK

T-075

MOG-3 Links Sex Determination to GLP-1/Notch Signaling

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The hermaphroditic nematode *Caenorhabditis elegans* sequentially produces both sperm and oocytes from a single pool of germ cell precursors. After the brief period of spermatogenesis during the fourth larval stage, the adult produces oocytes for the rest of its life. The events that regulate the fate of germ cells are at least in part controlled post-transcriptionally: While the entry into meiosis and its progression require the silencing of the *glp-1* mRNA, the switch from spermatogenesis to oogenesis is dependent on post-transcriptional repression of the *fem-3* mRNA.

To understand the molecular mechanisms that govern *fem-3* repression, several *trans*-acting factors have been identified in genetic and biochemical screens. The *mog* genes have been shown to repress *fem-3* mRNA through it's 3' untranslated region. Four cloned *mog* genes code for nuclear proteins that are homologous to RNA processing factors. In addition these MOG proteins directly bind to the nuclear zinc finger protein MEP-1 that is also required for *fem-3* repression.

We have cloned the *mog-3* by SNP mapping and candidate gene approach. *mog-3* encodes a conserved nuclear protein that shares weak homology to a spliceosomal complex protein in fission yeast. A *mog-3::gfp* transgene is expressed in the nuclei of many somatic cells. MOG-3 binds to MEP-1 in a similar fashion as the other MOG proteins. Similar to other *mog* genes *mog-3* also play a role in the mitosis to meiosis switch.

To investigate the molecular function of MOG-3, a yeast two-hybrid system was used to screen the interacting factors. One of the interacting protein (CIR) is a co-repressor of LAG-1. CIR also interacts with MEP-1. Since MEP-1 is a part of the NuRD complex, we speculate that CIR and MOG-3 with MEP-1 may form a complex to regulate target genes in the GLP-1/Notch pathway.

Keywords & Abbreviations: Germline, Sex determination, Post-transcriptional regulation, *mog* (masculanization of germ line), *fem* (feminization), *mep* (*mog* interacting and ectopic P granules), *gld* (germ line defective), SNP (Single Nucleotide Polymorphism) and NuRD (nucleosome remodeling and deacetylation), *glp* (germline proliferative) and Notch signaling.

BACK

Changes in Gonad Arm Extension and the Evolution of Gonad Morphology in Nematodes

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P. pacificus is a comparative system that compliments *C. elegans* and is the second model nematode that allows detailed forward genetic and molecular analysis of developmental mechanisms. Like *C. elegans*, *P. pacificus* is a hermaphroditic species with a two-armed gonad. The two U-shaped arms are rotationally symmetrical and open at the center of the animal into a common uterus. We have shown that the gross morphology, the somatic tissues, and germ line of *P. pacificus* differs from that of *C. elegans* in many ways[1]. Among the most obvious of these changes is the path of gonad arm extension. Two principal differences are apparent. First, in comparison to *C. elegans*, the gonad arms make a novel extension back to the ventral side of the animal when they meet over the vulva. Second, the *P. pacificus* gonad arms turn dorsally earlier as a percentage of body length in comparison to *C. elegans*.

In wild-type *P. pacificus* animals, a gonad arms extends ventrally ~70% of the time. The novel ventral migration in *P. pacificus* is regulated by putatively novel signaling systems in comparison to *C. elegans*. Laser ablation of the entire vulva (i.e. all the vulva precursor cells, VPCs, early and the VPC descendants late in development) results in all gonad arms failing to extend ventrally. Ablation of subsets of the VPCs and descendants results in intermediate percentages of gonad arms failing to extend ventrally. This suggests a quantitative signal from the vulva to the distal tip cells that guide extension. Surprisingly a second signaling center maybe involved in the ventral extension as well. When one arm of the gonad is ablated the remaining arm fails to extend ventrally ~80% of the time. Thus there is evidence for crosstalk between the gonad arms. We hope to elucidate the molecular basis of these signaling mechanisms in the future.

The *dim-1* recessive loss-of-function mutation, for **di**stal tip cell **m**igration defective, affects the dorsal gonad arm migration. In Dim-1 animals, the gonad arms fail to turn dorsally; instead they continue to migrate along the ventral body wall. They also fail to migrate back towards the center of the animal; instead one arm terminates at the base of the pharynx and the other at the anus. The *dim-1* locus maps to a very small region of *Ppa*-chromosome II. We are attempting to isolate the locus to a single BAC and to analyze potential candidate genes. Cloning of *dim-1* will provide insight into regulatory changes resulting in an altered timing/position of the dorsal gonad arm extension in *P. pacificus* relative to *C. elegans*.

1. Rudel, D., Riebesell, M., & Sommer, R.J. 2005. Dev. Biol. 277, 200-221.

BACK

T-077

The Novel Cytoplasmic Poly(A) Polymerase GLD-4 Regulates Germ Cell Fate Decisions in *C. elegans*

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The adult *C. elegans* germ line is an excellent model to study the fine balance existing between proliferation and differentiation, forces necessary for tissue generation and homeostasis. A constant mitotic germ cell pool is maintained through proliferative signals emanating from the distal tip cell, which functions as a somatic stem cell niche. Further proximally, germ cells enter meiosis and subsequently differentiate into gametes in a sex-specific manner. We are interested in the molecular controls of these germ cell fate decisions. Our work focuses on post-transcriptional RNA mechanisms. It is the action of conserved translational repressors and activators that build a molecular switch governing the entry from mitosis into meiosis. One of the important players is GLD-3, a Bicaudal-C type RNA-binding protein, which acts in a complex together with GLD-2, a cytoplasmic poly(A) polymerase, to promote entry into meiosis. Germ cells in *gld-2* or *gld-3* loss-of-function mutants over proliferate and fail to enter meiosis appropriately. Further analysis of *gld-3* led us to discover *gls-1*, which encodes a protein that interacts with GLD-3 and appears to have opposite functions from the *gld-3* gene. Germ cells in *gls-1* mutants exit mitosis and enter meiosis precociously.

In order to understand the molecular role of this antagonism we employed a yeast 2-hybrid screen using GLS-1 as bait and recovered multiple cDNAs encoding a novel conserved protein, termed GLD-4. Database searches revealed a strong similarity of GLD-4 to members of the cytoplasmic poly(A) polymerase protein family. GLD-4 contains a nucleotidyltransferase domain and a PAP associated domain similar to GLD-2. Also like GLD-2, GLD-4 lacks an obvious RNA-binding domain. We have raised a polyclonal antibody against GLD-4 and find it highly enriched in the germ line where it localizes to the cytoplasm. In particular, we find GLD-4 to be an integral part of P-granules at all stages of embryonic and postembryonic germ cell development, thus suggesting a possible role for GLD-4 in germ cell development.

We carried out RNAi experiments to characterize *in vivo* roles of *gld-4* and discovered its requirement for germ cell proliferation, a function that seems to be shared with *gls-1*. We identified a germ cell proliferation defective mutation in *gls-1(ef4)* which is unable to bind to GLD-4 in Y2-Hybrid experiments but is still expressed. Taken together, we propose that GLD-4 is a putative cytoplasmic poly(A)polymerase that activates mRNAs encoding germ cell fate determinants required for the mitosis/meiosis decision in conjunction with GLS-1 to promote mitosis.

BACK

T-078

Using *Caenorhabditis elegans* to Study Cellular Responses to Endogenous DNA Damage

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DNA repair pathways and intracellular signalling cascades contribute to DNA damage response (DDR) mechanisms that determine cellular responses to DNA damage. *C.elegans* has been used successfully to study DDR in response to DNA damage induced by physical or chemical agents. Whether DDR is activated in response to the most abundant forms of DNA damage, arising spontaneously through the reaction of water and reactive oxygen species with DNA, is poorly understood. Compared with treatment with physical or chemical DNA damaging agents, the study of DDR in response to endogenous lesions must overcome technical problems with respect to inducing sufficiently high levels of DNA lesions to allow studies of downstream responses. We have exploited RNA interference (RNAi) technology in *C.elegans* to modulate dUTP pools in order to achieve incorporation of uracil above background levels. Here, we present data describing the phenotypical consequences of increased incorporation of uracil into DNA. The role of DNA repair and DNA damage checkpoints in activating DNA damage response pathways in response to uracil will be presented.

BACK

T-079

Systematic Mapping of Genetic Interactions in *C. elegans* Suggests a New Paradigm for Human Genetic Disease

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Most heritable traits, including disease susceptibility, are affected by the interactions between multiple genes. However, we still understand very little about how genes interact since only a minute fraction of possible genetic interactions have been explored experimentally. To begin to address this, we are using RNA interference to identify genetic interactions in *C. elegans*, focussing on genes in signalling pathways that are mutated in human diseases. We tested ~65,000 pairs of genes for possible interactions and identify ~350 genetic interactions. This is the first systematically constructed genetic interaction map for any animal. We successfully rediscover most components of previously known signalling pathways; furthermore, we verify 9 novel modulators of EGF signalling. Crucially, our dataset also provides the first insight into the global structure of animal genetic interaction maps. Most strikingly, we identify a class of highly connected 'hub' genes: inactivation of these genes greatly enhances phenotypes resulting from mutations in many different pathways. These hub genes all encode chromatin regulators, and their activity as genetic hubs appears conserved across metazoans. We propose that these genes function as general buffers of genetic variation and that these hub genes will act as modifier genes in multiple, mechanistically unrelated genetic diseases in humans.

BACK

T-080

C. elegans Models of Protein Misfolding Diseases

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Aggregation of misfolded proteins occurs in various age-related neurodegenerative disorders, including Parkinson's, Alzheimer's, and Huntington's disease. To understand how cells protect themselves against misfolded proteins, we search for genes that enhance or prevent protein aggregation. C. elegans strains expressing polyglutamine stretches fused to YFP with visible, age-dependend protein aggregation are used as a genetic model. Using a genome-wide RNAi screen, we have previously identified 186 genes that, when knocked down, cause premature protein aggregation. These genes include genes involved in protein synthesis, folding, degradation and RNA synthesis and processing. [1]

Conversely, we performed a forward mutagenesis screen to identify genes that, when mutated, suppress age-dependent polyglutamine aggregation. For one suppressor mutant, in which aggregation is suppressed by more than 75%, we have now identified the responsible mutation. This mutation is a missense mutation in a gene encoding a protein of unknown function that is highly conserved between C. elegans and humans. Knock-down by RNAi of the same gene in wild-type worms yielded a similar reduction in aggregation, suggesting a loss-of-function mutation. We are currently further characterizing this mutant and the remaining suppressor mutants. In addition, to establish whether the genes we have identified are specific for polyglutamine aggregation or whether they comprise of a general protein homeostatic buffer, we have developed a worm model for aggregation of alpha synuclein, which occurs in Parkinson's disease. Altogether our results will provide insight into cellular protection against misfolded proteins and yield targets for therapy against protein misfolding diseases.

[1]**Nollen E.A.A.,** Garcia S.M., van Haaften G., Kim S., Chavez A., Morimoto R.I., Plasterk R.H. **(2004)** Genome-wide RNA interference screen identifies previously undescribed regulators of polyglutamine aggregation. **Proc. Natl. Acad. Sci. U.S.A.** 101(17):6403-8.

BACK

T-081

Down-Regulation of CHN-1, a Chaperone-Associated E3-Ligase, Suppresses Muscle Degeneration in DMD Model of *C. elegans*

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CHN-1, the *C. elegans* ortholog of human CHIP (carboxyl terminus of HSC70-interacting protein), is a chaperone-associated ubiquitin ligase, which mediates the attachment of a ubiquitin chain to a client protein and thereby stimulates its degradation by proteasome. Recently, *C. elegans* CHN-1 in a complex with ubiquitin conjugating enzyme UFD-2, was reported to multiubiquitylate myosin chaperone UNC-45 and the absence of CHN-1 partially suppress myofibril disorganization and paralysis phenotype of *unc-45* mutants (ref.). Since ubiquitin-proteasome system is the major protein degradation pathway in muscle cells (1), we started to analyse other *C. elegans* mutants that have been associated with defective muscle structures or functions. Among these are mutations in *dys-1*, encoding the *C. elegans* homologue of human dystrophin. Mutations in dystrophin cause Duchenne muscular dystrophy. Previous experiments by the Segalat lab have shown that combining dystrophin and MyoD mutants in *dys-1(cx18);hlh-1(cc561ts)* animals results in a synthetic phenotype, causing muscle degeneration and immobility at late larval stages. Strikingly, our experiments indicate that inactivation of *chn-1* -/- also is able to suppress the dystrophin phenotype, significantly reducing muscle decay and resulting in improved locomotion and egg-laying. A detailed analysis of the dystrophic phenotype of *dys-1(cx18);hlh-1(cc561ts)* animals revealed a loss of 13% of all body-wall muscles already at day 2 of adulthood, whereas muscle degeneration is reduced to 4% in animals with additional CHN-1 deficiency. As a consequence, the triple mutant animals perform remarkably better in a food race assay, an indicator of improved muscle activity.

Our data suggest that the precise regulation of proteolysis in the muscle may be an effective way to delay muscle degeneration in disease. Transcriptome profiling allowed us to identified genes that are either up- or downregulated as a consequence of the suppressor mutation. We are currently engaged in identifying their roles in this process.

[1]Hoppe T, Cassata G, Barral JM, Springer W, Hutagalung AH, Epstein HF, Baumeister R. Regulation of the myosin-directed chaperone UNC-45 by a novel E3/E4-multiubiquitylation complex in C. elegans.Cell. (2004) 118(3), 337-49.

BACK

Regulation of BRC-1 - Dependent Ubiquitylation at DNA Damage Sites

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The BRCA1 tumour suppressor and its heterodimeric partner BARD1 constitute an E3-ubiquitin (Ub) ligase and function in DNA repair by unknown mechanisms. We have previously described *C. elegans* BRCA1 and BARD1 orthologues (*brc-1* and *brd-1*, respectively) that possess many of the functional domains present in their human counterparts, including RING, ankyrin, and BRCT domains (Boulton *et al*., 2004). Consistent with conserved roles in DNA repair, BRC-1 and BRD-1 interact to form a heterodimer via their respective RING domains.

To explore the mechanistic role of BRC-1 and BRD-1 in DNA repair processes we have characterized a *C. elegans* BRC-1/BRD-1 complex (CeBCD) purified by tandem immunoaffinity before and at different time points after IR-treatment. This approach is a first for *C. elegans* and demonstrates that protein complexes purified in this manner are amenable to biochemical analysis and can be used in combination with genetics and cell biology to accelerate functional discoveries. We present evidence that the CeBCD complex possesses an E3-Ub ligase that is activated on chromatin in response to IR-treatment and further demonstrate that the DNA damage checkpoint promotes association of the CeBCD complex with E2-Ub conjugating enzyme, Ubc5(LET-70), to form an active E3-Ub ligase in response to DNA damage. We also show that ubiquitylation events at DNA damage sites require *brc-1*, *brd-1*, *ubc5(let-70)*, *mre-11* and *atl-1*, thus providing *in vivo* evidence to support our biochemical analysis.

Boulton, S.J., Martin, J.S., Polanowska, J., Hill, D.E., Gartner, A. and Vidal, M. (2004) *Curr Biol*, 14, 33-39.

BACK

T-083

Gene Requirements for UV-Induced Apoptosis

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Activation of checkpoints following DNA damage is important to ensure efficient repair processes. The physiological importance of these response pathways is illustrated by a number of genetic disorders, such as ataxia telangiectasia (A-T) or xeroderma pigmentosum (XP), which result in multiple pathological features including predisposition to cancer.

We studied the effects on the *C. elegans* germ line of ultraviolet light, which causes a pattern of DNA damage distinct from the one observed following ionizing radiation. We identified a signaling pathway that senses UV-induced damage, and conveys this information to the apoptotic machinery. Recognition of UV lesions by the nucleotide excision repair (NER) machinery is required not only for DNA repair, but also to activate downstream signaling pathways that lead to apoptosis. We will also present our results about the interplay between the NER pathway and other DNA damage response pathways that participate in the UV response in *C. elegans*.

BACK

T-084

Sex-Dependent Resistance to the Fungal Pathogen *Cryptococcus neoformans*

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Cryptococcus neoformans is the causative agent of cryptococcosis, a fatal fungal disease of immunocompromised patients. Our group and others have made use of *C. elegans* as an alternative host for *Cryptococcus*, in order to investigate the molecular basis of host-pathogen interactions.

Using this system, we now report that male *C. elegans* show greater resistance to killing by *Cryptococcus* than hermaphrodite animals and that this resistance can be induced in hermaphrodite animals by inappropriate activation of the male sex-determination pathway. Resistance is molecularly determined, rather than resulting from behavioural changes or reproductive differences, and requires the activity of the stress-response transcription factor DAF-16.

Finally, we demonstrate that resistance to *Cryptococcus neoformans* correlates broadly with longevity within the *Caenorhabditis* genus. Our results suggest that many of the molecular determinants of longevity and immunity are the same and that differential regulation of these determinants may underlie much sex-dependent and species-dependent variation.

BACK

T-085

CB4856 /N2 Mapping Populations: A New Powerful Resource for Fine-Mapping of Complex Traits

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Genetic analysis of quantitative traits such as feeding behaviour and neurological disorders makes increasingly use of segregant populations derived from distinct evolutionary lineages of *Caenorhabditis elegans*. The type and size of the mapping population determine to a large extent the power of QTL detection. To facilitate mapping strategies we have developed two permanent mapping populations from the cross between the canonical strain N2 (Bristol) and strain CB4856 (Hawaii): more than 1000 recombinant inbred lines (RI) and 100 introgression lines (IL). RI population (approximately 90 fully genotyped lines) consists of homozygous individuals obtained by selfing of F1 hybrid for 20 generations and comprises of ~50% of each parental genome in different combinations. The IL population (all 100 genotyped) consists of lines that contain a single homozygous chromosome segment of CB4856 strain introgressed into the background of N2 covering in total 95% of the CB4856 genome. These populations overcome typical shortcomings of many previous mapping populations like low resolution power, overshadowing effect of major QTL on minor QTL or interaction between unlinked QTLs and therefore provide a powerful resource for dissection of many behavioural, physiological and biochemical traits. We show properties of ILs as compared to recombinant inbred (RI) population in QTL mapping of the response to extreme temperature stress (60ºC).

BACK

Nemadode Gene-Tagging Tools and Resources

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We are involved in a collaborative effort funded by the EU 6th Framework Programme to optimize and improve the efficiency of transposon-mediated mutagenesis methodologies based on the *Mos1* transposable element. In addition, we are developing alternative approaches both for mutagenesis and transgenesis in *C. elegans* using the Minos and Piggybac transposon systems. Our goal is to exploit these tools to generate and evaluate a comprehensive collection of transposon-tagged mutants with the ultimate aim of achieving comprehensive coverage of the *C. elegans* genome. Our progress towards meeting these goals will be presented. We anticipate that our efforts will complement and extend already existing resources for the study of gene function, which include the CGC collection of mutants, various other sources of deletions and transposon mutations and RNAi libraries.

BACK

T-087

What Can A Bright Worm Tell Us About Its Biology?

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We are interested in how a whole organism responds to environmental stress at a genetic level. To address this question, we have made a brightly luminescent strain of *C. elegans* carrying the American firefly *luc* gene, fused to *gfp* under the control of the *sur-5* promoter. Luciferase catalyses the oxidation of the exogenous luciferin substrate and generates light by an ATP-dependent reaction. We exploit bioluminescence as a sensitive indicator of health following exposure to stress. Our aim is to develop an efficient assay to carry out a genome-wide screen for genes involved in the response of *C. elegans* to unfavourable environmental conditions.

Here we show that 19h exposure to sub-lethal levels of a heavy metal results in reduced light emission in a concentration dependent manner. This response correlates well with slower feeding, as assessed by the optical density of the bacterial suspensions on which the worms were cultured. We also tested the impact of the metal on development and reproduction after approximately 3 days exposure. Metal concentrations that affect development and reproduction in longer exposures correlate well with those that give rise to changes in luminescence in the19 h assays.

Ongoing work is aimed at testing the link between light output and cellular ATP at the whole organism level. The *in vitro* ATP content of metal exposed N2 worms decreases with increasing metal concentrations, as previously observed by luminescence. We are at present investigating how the drop in ATP levels relates to changes in biomass. In addition, we are investigating the stability of expression of luciferase in our strain under standard metal exposure conditions, by measuring GFP fluorescence.

Finally, we will discuss the use of our strain as a physiological and metabolic reporter in combination with genome-wide RNAi to investigate the involvement of particular genes in the stress response. Our approach is also of significance in other research areas such metabolic research, ageing, interactions with microbial pathogens, etc.

BACK

A Mass Spectrometry Method to Detect and Quantitate Low Abundance Proteins in a *C. elegans*

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The detection of low abundance proteins in complex mixtures can be a tedious, expensive and time-consuming ordeal. Current approaches rely on the use of antibodies to detect the protein of interest by Western blotting. An alternative approach is the mass spectrometry equivalent of a Western blot. In this approach, a unique tryptic peptide(s) for a protein of interest is monitored by high resolution selected reaction monitoring (SRM) mass spectrometry, and the peptide precursor ion, fragment ion, and retention time facilitate the detection and quantitation of the protein in a mixture. We applied this technique to the *C. elegans* proteins DAF-16, a FOXO transcription factor and AKT-1, a serine/threonine kinase, both of which are essential components of the *C. elegans* insulin/IGF-1 signaling pathway. To test the detection of DAF-16 and AKT-1 in *C. elegans* lysate, 77 ug of total protein was injected per μLC-MS/MS analysis. We were able to easy detect both endogenous DAF-16 and AKT-1 with a short 15 min chromatography gradient. Additionally, we can measure the absolute protein abundance using standard addition of synthetic peptide standards of DAF-16 and AKT-1. We will present experimental confirmation of both the detection and quantitation of both of these proteins. Also, we will validate our mass spectrometry method by directly comparing traditional Western blots and "mass spec westerns".

BACK

*Mos*TIC: A Novel Tool to Engineer the *C. elegans* Genome by Homologous Recombination

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Techniques based on homologous recombination provide a means to engineer custom mutations at specific loci and are widely used to study gene functions in most eukaryotic model organisms. Unfortunately, in *Caenorhabditis elegans*, homologous recombination between the genome and exogenous DNA fragments is very inefficient and only few examples of genome engineering by homologous recombination have been documented so far. We have developed a novel technique to introduce targeted gene modifications in the *C. elegans* genome using transgene-instructed gene conversion following *Mos1* transposon excision.

Mos1 is a drosophila transposon that can be mobilized by expressing the Mos transposase in the *C. elegans* germline. Transgene-instructed DSB repair requires two steps: (1) generation of a DSB at the locus of interest by excision of a pre-existing *Mos1* insertion and (2) repair of this DSB by gene conversion using an engineered transgene as a template. To establish this technique, we used *Mos1* insertions in the *unc-63* and *unc-5* genes. Following expression of the Mos transposase in the germline, we were able to demonstrate gene conversion events between the chromosome and a transgene containing only 3 kb of homologous sequences. This technique, that we called *Mos*TIC (*Mos1* induced transgene instructed gene conversion), allowed (i) the introduction of point mutations, (ii) the engineering of deletions, and (iii) the knock-in of *gfp* in the *unc-5* gene. All these events were recovered at a frequency ranging from from 5.10^{-5} to 5.10^{-4} per generation. Analysis of the conversion tracts indicated that 500 bp on each side of the *Mos1* insertion site were copied from the transgene into the chromosome in 50 % of the *Mos*TIC events.

*Mos*TIC requires a *Mos1* insertion in the vicinity of the genomic region to manipulate. Such insertions can be recovered in genetic screens using *Mos1*-mediated mutagenesis. In addition, progress of the NEMAGENETAG consortium to produce a comprehensive library of localized *Mos1* insertions will provide a means to manipulate most *C. elegans* genes with the *Mos*TIC technique.

BACK

WormBase: Recent and Future Developments

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WormBase is a database concerned primarily with providing current and comprehensive information on the genome and genetics of *Caenorhabditis elegans*. As more nematode worm genomes are being sequenced WormBase attempts to make this data available to the scientific community in a convenient and familiar fashion. We are constantly developing and evaluating new analyses and tools to help users make the most of this wealth of data. Recent developments have included the incorporation of external data sets such as InParanoid, COGS and TreeFam, Vancouver fosmid mappings and nematode EST clusters from NEMBASE and Nematode Net, amongst others.

As well as adding new data and features, our recent user survey indicated that some of the things we've been doing for a long time are still important. Correct gene structures were a high priority for the majority of respondents. We constantly review these in light of new data and user submissions. As more worm genome sequences become available they will give us more valuable comparative data for determining gene structures and other genomic sequence features. WormBase will play a central role in making the new genome data available in a timely manner. We will also be taking advantage of the COMPARA system developed for EnsEMBL to analyse and display this multispecies data.

To increase the accessibility of this data we have developed WormMart, a BioMart based data-warehouse including an easy-to-use wizard style query tool. The functionality of GBrowse has been enhanced, giving the ability to create genetic and physical maps. Web site speed has been an issue in the past and we have addressed this by restructuring the web site code, hardware and data management strategies. The establishment of a second European mirror in the UK should be of particular help to conference delegates.

BACK

Generic Method for *C. elegans* Gene Tagging by *in vivo* Recombineering in *E. coli*

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Here we present a fast and generic protocol for production of tagged transgenes for bombardment-based transformation in *C. elegans* by engineering of genomic BAC clones. The tag is inserted with minimal disturbance to the endogenous genomic context. The gene is then subcloned into an unc-119 based shuttle vector. All steps are done by in vivo recombineering in *E. coli*. Large DNA sequences can be modified in this way, and the method is applicable for virtually any gene of interest. All regulatory regions from 5' and 3' UTRs, introns etc. are present in the transgenic construct, and are likely to result in a correct expression pattern.

As a proof of principle we used the RPCI-94 *Caenorhabditis briggsae* BAC library. We GFP tagged and subcloned a relatively large (16 kbp) gene. Transformation efficiency by bombardment was similar to that of the commonly used pAZ132 vector. GFP expression patterns were reproducible and similar to that of a published promoter:GFP reporter construct. To show that the *C. briggsae* transgene is functional in *C. elegans* we knocked down the endogenous gene by RNAi. This makes the transgene the only expressed copy of the gene, essentially simulating a knock-in situation. The RNAi induced phenotype was rescued by the transgene in most of the animals.

While the ability to knock down the endogenous gene is a big advantage of the cross-species transgenes, *C. elegans* clones would still be preferable for most studies. Recently a genomic fosmid library for *C. elegans* was announced. Our protocol can be directly applied to these clones.

The method is fast - tagging and subcloning can be accomplished within a week.
The high efficiency of recombination in E. coli makes it possible do all steps in liquid culture, only checking the final construct for correct recombination. We are currently setting up the conditions for 96 well format tagging that would allow us to automatically process a large number of genes in parallel. We aim to establish a library of tagged transgenic constructs covering the whole genome as a community resource.

BACK

T-092

Analysing the Redundancy of Duplicated Genes and the Evolutionary Conservation of Genetic Interactions Using Combinatorial RNAi in *C. elegans*

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Systematic analyses of loss-of-function phenotypes have been carried out for almost all genes in *S. cerevisiae*, *C. elegans,* and *D. melanogaster*, and there are major efforts to make a comprehensive collection of mouse knockouts. While such studies greatly expand our knowledge of single gene function, they do not address redundancy in genetic networks, nor do they attempt to identify genetic interactions. Developing tools for the systematic mapping of genetic interactions is thus a key step for exploring the relationship between genotype and phenotype. We thus sought to establish protocols for targeting multiple genes simultaneously by RNA interference (RNAi) in *C. elegans* to provide a platform for the systematic identification of genetic interactions in this key animal model system.
We set up conditions for RNAi that allow us to target multiple genes in the same animal ('combinatorial RNAi') in a high throughput setting and to detect the great majority of previously known synthetic genetic interactions. We then used this assay to test the redundant functions of genes that have been duplicated in the genome of *C. elegans* since divergence from either *S. cerevisiae* or *D. melanogaster*, and identified 16 pairs of duplicated genes that are at least partially functionally redundant. Intriguingly, 14 of these redundant gene pairs were duplicated before the split of *C. elegans* and *C. briggsae* 80-110 million years ago. Our data provide the first systematic investigation into the redundancy of duplicated genes in any organism and strongly support population genetics models, which suggest that redundancy can be maintained over substantial periods of evolutionary time.
Furthermore, we set out to test whether systematically compiled yeast genetic interaction data can predict genetic interactions in the worm. We will present these data.

BACK

T-093

The Integrative Interaction Map for *Caenorhabditis elegans*

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C. elegans is a popular model system for the systematic analysis. However, relatively little is known for its networks of genetic interactions. We hereby construct an integrative interaction map for C. elegans by integrating diverse functional genomics
Datasets. We will also present some of the novel tools in querying the interaction map for answering powerful biological hypotheses.

BACK