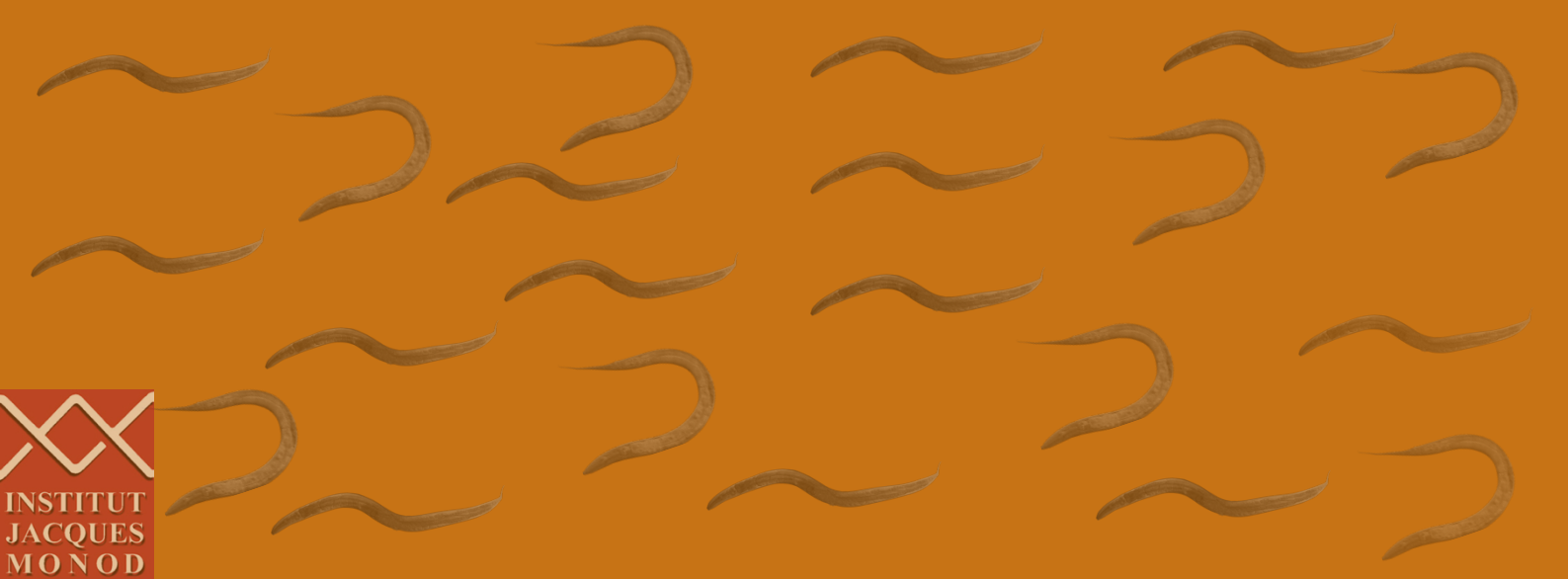
ABSTRACT BOOK

Colloque VerMidi XVIII

9 Janvier 2015 PARIS

INSTITUT JACQUES MONOD



INSTITUT JACQUES MONOD

VerMidi XVIII Program

09:30 - 10:00 **WELCOME BREAKFAST**

10:00 - 10:40 Keynote Lecture: **Pierre GÖNCZY**: Title to be announced

10:40 - 12:00 **SESSION 1 -** Chair: **Nicolas TAVERNIER**

10:40 - 11:00 Emanuel Culetto (I2BC, Paris , France)
The ESCRT II proteins are required for normal muscle function

11-00 - 11:20 Thanh VUONG (IGBMC, Strasbourg, France)
Nano-ablation studies reveal different regulation of mechanical stress anisotropy in the head and in the body during *C. elegans* embryo elongation

10:20 - 11:40 José-Edouardo Gomes (IBGC, Bordeaux, France)
C. elegans as model organism to study purine metabolism disorders

11:40 - 12:00 Marie PIERRON (CGphiMC, Lyon, France)
A novel effector of integrin adhesion complexes is involved in cholinergic synaptogenesis in *Caenorhabditis elegans*

12:00 - 15:00 **LUNCH / POSTER SESSION**

15:00 - 16:20 **SESSION 2 -** Chair: **Gilliane MATON**

15:00 - 15:20 Abderazak DJEDDI (UPMC, Paris, France)
Efficient sperm-inherited organelle clearance relies on LC3-dependent targeting of the autophagosomes to the peri-centrosomal area for their acidification and dispersion among *C. elegans* blastomeres

15:20 - 15:40 Ruddi RODRIGUEZ-GARCIA (IGDR, Rennes, France)
Dynein intermediate light chain tracks microtubule plus end in an EBP-2 dependent manner in *C. elegans* one cell embryo

15:40 - 16:00 François ROBIN (University of Chicago, USA)
Dynamic coupling of actin assembly and Rho activation underlies pulsed contractions in *C. elegans*

16:00 - 16:20 Anne Pacquelet (IGDR, Rennes, France)
PAR-4/LKB1 and anillin prevent myosin from uncoupling mitotic spindle and cytokinetic furrow positions during cell division

16:20 - 16:40 **COFFEE BREAK**

16:40 - 18:00 **SESSION 3 -** Chair: **Benjamin LACROIX**

16:40 - 17:00 Arnaud Hubstenberger (UPMC, Paris, France)
Ribonucleoprotein transitions between soluble, liquid and solid phases during early development

17:20 - 17:40 Aymeric BAILLY (CRBM, Montpellier, France)
A conserved role for deNEDDylating enzyme NEDP1 in apoptosome oligomerisation through NEDD8 chains restriction in response to DNA damage

17:40 - 18:00 Patrick PHILLIPS (University of Oregon, Eugene, USA)
Transgenerational hormesis: testing the adaptive plasticity hypothesis using experimental evolution to heat stress in *C. remanei*

HAPPY HOUR

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ORAL PRESENTATION ABSTRACTS

The ESCRT II proteins are required for normal muscle function

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Abstract Not Available

Nano-ablation studies reveal different regulation of mechanical stress anisotropy in the head and in the body during C. elegans embryo elongation

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Actomyosin forces are the main driver of morphogenesis throughout the animal kingdom. However, their spatial and temporal regulation remain to be explored. During C. elegans embryogenesis, the epidermal actin network forms parallel bundles surrounding the embryos. They are thought to squeeze circumferentially to elongate the embryo in the anterior-posterior direction. We used a laser nano-ablation technique to dissect quantitatively actomyosin-dependent mechanical stress in the epidermis to understand how it drives the embryonic elongation.

The technique consists of cutting a thin line in a labeled actin cortex with an infrared femto-second laser. The opening shape of the cut gives a direct assessment of the mechanical stress and the stiffness. First, we found that before muscle contractions, elongation may not be driven by anisotropy of stress (higher stress in circumferential direction than in anterior-posterior direction) in all lateral epidermal cells, but more likely by the ones in the middle and tail regions. At the same time, there is little stress in dorso-ventral cells in the head. Second, we observed an increase of stress magnitude and anisotropy in the head after the onset of muscle contractions. This phenomenon persists in muscle defective embryos, indicating that it is independent of muscle contractions. Moreover, this increase of stress anisotropy does not depend on the spectrin network. The finding of a significant higher stress in wild type embryos compared to a let-502/rho-kinase mutant supports the validity of the method. We will present a preliminary model explaining the mechanics of the early elongation of C. elegans embryo.

C. elegans as model organism to study purine metabolism disorders

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Living organisms sense constantly their own physiological status and suronding environment, and regulate their metabolism accordingly. In particular, regulation of biosynthesis pathways can depend on availability of nutriments. Our work focus on Purine biosynthesis, a process highly conserved from bacteria to humans. It is composed of the de novo and recycling pathways, and deficencies on specific enzymes of pathway cause severe human diseases. Although purine metabolism has been extensively characterized in unicellular organisms bacteria and yeast, few studies have been reported using animal model organisms. We therefore developped a C. elegans model to study pathologies associated with purine metabolism. We performed a phenotypic analysis of C. elegans mutants (or RNAi) deficient for crucial enzymes ADSL (involved both in the purine recycling and de novo synthesis), PPAT (de novo synthesis only), ATIC (de novo) and ADSS (recycling only). We observed defects on post embryonic development, germline proliferation, locomotion behaviour, fertility and lifespan. For the most part these phenotypes are separable; our results indicate that accumulation of intermediate metabolite SAICAR causes shortened life span, AICAR accumulation causes reduced fertility and abnormal locomotion, and deficiencies on the purine recyling pathway cause abnormal post-embryonic development, strong defects on locomotion and hinder germline proliferation. Our work allowed to establish a workable basis to analyze purine metabolism in the animal model organism C. elegans.

A novel effector of integrin adhesion complexes is involved in cholinergic synaptogenesis in Caenorhabditis elegans

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At Caenorhabditis elegans neuromuscular junctions (NMJs), acetylcholine receptors (AChR) cluster in front of acetylcholine release sites. AChR localization is essential for excitatory neurotransmission. To identify novel mechanisms involved in the formation and maintenance of cholinergic synapses, we performed a genetic screen based on the direct visualization of fluorescently tagged AChR. Mutations causing abnormal AChR localization were identified using whole genome sequencing.

We identified a novel gene, rsu-1, specifically required for the localization of AChR clusters at NMJs formed between SAB neurons and head muscles. In rsu-1 null mutants, multiple AChR clusters form at non-synaptic sites of the muscle cell membrane. These ectopic clusters emerge from the aggregation of normally diffuse extrasynaptic receptors and the mislocalization of synaptic clusters. RSU-1 is a small cytoplasmic protein composed of LRR protein-protein interaction domains and functions cell autonomously. RSU-1-GFP localizes at dense bodies, M-lines and muscle cell intercellular junctions in a pattern identical to that observed for PAT-3/β-integrin. This localization is dependent on a physical interaction with one of the major component of integrin adhesion complexes. Consistently, RSU-1 was shown to influence integrin-dependent cell adhesion in other model organisms. However, immunofluorescence experiments and translational reporters revealed that integrin adhesion complexes are properly formed in this mutant context and do not colocalize with AChR clusters.

These results indicate that active mechanisms are required to prevent abnormal clustering of AChR outside of the synapse. We are currently testing how RSU-1 indirectly might control AChR localization by regulating the interaction of muscle cells with the extracellular matrix.

Efficient sperm-inherited organelle clearance relies on LC3-dependent targeting of the autophagosomes to the peri-centrosomal area for their acidification and dispersion among C. elegans blastomeres

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Abstract Not Available

Dynein intermediate light chain tracks microtubule plus end in an EBP-2 dependent manner in C. elegans one cell embryo.

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Abstract Not Available

Dynamic coupling of actin assembly and Rho activation underlies pulsed contractions in C. elegans

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Actomyosin-based pulsed contractions are a widespread and highly dynamic form of self-organized contractility that underlie numerous morphogenetic events during early development. However the mechanisms that govern pulsed contractions remain poorly understood.

We combined single molecule imaging and single particle tracking analysis to quantify actomyosin assembly/disassembly in relation to the onset and terminations of pulsed contractions. We found that sharp increases in assembly/recruitment rates and stability of both F-actin and Myosin II precede the onset of contraction by ~ 6 second. These data rule out models for the initiation of pulsed contractions in which local contraction concentrates factors that promote further contraction, and instead suggest mechanisms in which phasic modulation of assembly/disassembly drive initiation and termination of pulsed contractions. To address this further, we measured the relative timing of appearance/disappearance for different components of pulsed contractions. Strikingly, F-actin, Myosin and the multivalent scaffold Annillin-1 accumulate and disappear with nearly identical timing during each pulsed contraction. However the active form of their common upstream regulator Rho-1 appears before all three and well before the onset of observable contraction, suggesting that pulsed accumulation of active Rho is a key timer for pulsed contractions. Finally, consistent with these observations, neither Myosin nor Anillin are required for pulsed accumulations of Rho-1.

In conclusion, our data show that pulsed contractions do not arise through contractile instabilities, tension-based stabilization or dynamical clustering of cortical actomyosin. Instead, we show that pulsed contractions are preceded and driven by local, autocatalytic pulses of activated RhoA, independently of Myosin and Anillin.

PAR-4/LKB1 and anillin prevent myosin from uncoupling mitotic spindle and cytokinetic furrow positions during cell division

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Abstract Not Available

Ribonucleoprotein transitions between soluble, liquid and solid phases during early development

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During development, the gene expression network must be synchronized, adapted to a changing environment, and marked by cell lineage and past history. As part of this process, RNAs are covered by proteins to form ribonucleoproteins (RNPs) that will regulate the RNA fate of: storage, decay, expression, repression, or localization. RNPs can further co-assemble into diverse granules, some of which are as large as the nucleus. These supramolecular polymers are compartmented into subdomains, and devoid of surrounding membrane. We explore the emerging properties of these macroscopic co-assemblies that can reach 10 um in size.

Using C. elegans germline as a model, we show that the fluidity of repressed mRNPs in the cytosol is tightly controlled during development and that mRNPs can undergo phase transitions between diffuse states, liquid droplets, and solid aggregates. We explore the mechanisms by which phase transitions are controlled using genetic screen, live imaging, biophysical and biochemical complementary approaches. We propose a model in which conformational switches of “prion like” peptides control the supramolecular polymerization that drive RNP liquid condensation or solidification. In contrast, RNA helicases can actively destabilize RNP structure to liquefy RNP aggregates.

Furthermore we show that droplet size and viscosity regulate RNP exchanges with the cytosol, while liquid-liquid demixing sorts RNA processing pathways. Because phase transitions segregate or mix RNPs, change their local concentrations and potential binding partners, and modify subcellular localization, we predict that phase transitions should impact whether RNA information is retained or degraded, expressed or repressed during development.

A conserved role for deNEDDylating enzyme NEDP1 in apoptosome oligomerisation through NEDD8 chains restriction in response to DNA damage

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NEDD8 is a Ubiquitin-like molecule that covalently modifies substrate proteins. The best characterised target for NEDD8 is the cullin family of proteins, component of the Cullin-Ring E3 ligases. However, recent studies have indicated a more diverse signalling function for NEDDylation.

Through a combinatorial approach that exploits C. elegans genetics, quantitative proteomics and human cells in culture, we discovered an evolutionarily conserved signalling module that regulates apoptosis in response to DNA damage through NEDDylation.

We found that deletion of the NEDD8-specific protease NEDP1 causes a nearly complete resistance to DNA damage induced apoptosis in C. elegans and human cells. By combining worms genetics and quantitative proteomics (SILAC) we found that the key role of NEDP1 is to prevent the formation of poly-NEDD8 chains through lysines K11/K48, which is required for the induction of apoptosis upon DNA damage. Importantly, NEDP1 does not affect the activation of the checkpoint pathway or DNA repair. Instead, NEDP1 controls the oligomerisation of the apoptosome core component APAF1/CED-4 in the cytoplasm upon DNA damage.

In conclusion, we unveiled a conserved function of the NEDD8-specific protease NEDP1 in the DNA damage-induced apoptosis through restriction of NEDD8 chain formation and control of apoptosome formation upon DNA damage.

Transgenerational hormesis: testing the adaptive plasticity hypothesis using experimental evolution to heat stress in C. remanei

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Organisms are frequently better at surviving an extreme environmental stress after being subjected to a similar stress briefly and/or at sublethal doses. This increase in resistance via prior exposure is known as hormesis. Parents encountering stressful environments could potentially influence the phenotype of their offspring in a form of transgenerational hormesis or phenotypic plasticity. These parental effects have the potential to be adaptive if offspring are thereby better able deal with future stressors. Here, we test for the existence of anticipatory parental effects in the heat stress response in the highly polymorphic nematode Caenorhabditis remanei. Rather providing an anticipatory response, parents subject to a prior heat stress actually produce offspring that are actually less able to survive a severe heat shock. Selection on heat shock resistance within the larvae via experimental evolution leads to a loss of sensitivity (robustness) to environmental variation during both the parental and larval periods. Whole genome transcriptional analysis of both ancestor and selected lines shows that there is weak correspondence between genetic pathways induced via temperature shifts during parental and larval periods. Overall, parental effects can evolve very rapidly via selection acting directly on offspring.

POSTER ABSTRACTS

Poster Board # **Title**

P1: At the frontiers of locomotion performances

P2: Reproductive consequences of early-life stress exposure: Within- and transgenerational effects in C. elegans

P3: The adaptation of cryo-sectioning procedures to *C. elegans* research

P4: Dimensional scaling of microtubule assembly rate

P5: Integrated signaling networks in innate immunity

P6: Balancer-free hands-off selection of adsl heterozygous mutant using CRISPR-Cas9 system

P7: The National Functional Genomics Platform

P8: Innate Host Defense Requires TFEB-Mediated Transcription of Cytoprotective and Antimicrobial Genes

P9: *C. elegans'* native gut microbiome

P10: MegaTIC: a dual selection-based strategy for genome engineering in *C. elegans*

P11: Characterization of genome instability in a H3K4 methylation defective mutant.

P12: Regulation of the GPR protein during asymmetric cell division.

P13: Drug screening for mitochondrial diseases related to POLG mutations using Saccharomyces cerevisiae and Caenorhabditis elegans

P14: AICAR effects on the Ubiquitin Proteasome System and on the Methyl cycle

P15: Functional caracterisation of the SET1/MLL complex in the C. elegans germline

P16: Toward the identification of genetic pathways involved in muscle aging in Caenorahbditis elegans

P17: Mitochondria Dynamics and Muscle Degeneration in C. elegans

P18: The axon guidance receptor Ryk impairs neuronal resistance capacity by repressing FOXO activity during the early phases of huntingtin pathogenicity

P19: Mitochondrial ROS mediated muscle degeneration in hlh-1(cc561);dys-1(cx18) Caenorhabditis elegans mutant

P20: Monitoring the first steps of the host response to fungal infection and wounding in c. elegans epidermis

P21: Quantitative Analysis of the Polarization mechanism of a field of Neuronal Precursors in C.elegans
P22: Identifying nipi-3-dependent elements required for the stability and translation of cebp-1 mRNA

P23: Dissecting a worm killer

P24: Analysis of chromatin factors involved in the asymmetric divisions of neuronal precursors

P25: Systems modeling and network-based approaches for basic and translational research in Huntington's disease

P26: Analysis of activity-dependent synaptogenesis at the SAB neuromuscular junction.

P27: Impaired mitochondrial stress response in C. elegans dystrophin-dependent muscle degeneration.

P28: Role of acto-myosin based force production in cell invasion in Caenorhabditis elegans

P29: Genetic and developmental mechanisms underlying sperm size variation in Caenorhabditis nematodes.

P30: Microtubule dynamics and mechanical forces in the one-cell C. elegans embryo

P31: crld-1 is an evolutionarily conserved gene required for the biosynthesis of AChRs

P32: Recombinant inbred lines for quantitative locus mapping

P33: Atypical autophagy of polarized sperm-inherited mitochondria

P34: Caenorhabditis elegans expressing the Saccharomyces cereviasiae NADH alternative dehydrogenase Ndi1p as a tool to identify new genes involved in complex I related diseases

P35: Astral microtubules displaying two different dynamical behaviours may perform different functions during the division of C. elegans one-cell embryo.

P36: Cdk1 phosphorylates SPAT-1/Bora to trigger PLK-1 activation and drive mitotic entry in early C. elegans embryos.

P37: The NEDD8 inhibitor MLN4924 increases the size of the nucleolus and activates p53 through the ribosomal-Mdm2 pathway

P38: Chromosome Segregation in the C. elegans Oocyte: Functional Analysis of CLASP

P1: At the frontiers of locomotion performances

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The development, rise and decay of major physiological functions and performances throughout life is a common feature shared most living organisms. Several studies described the relation between such trajectories and age through sport performances. Dan. H. Moore developed such a description with a bi-exponential equation describing the development of track and field events (Dan H. Moore, Nature 1975). This function was recently adjusted to an extended range of sports revealing a deterministic asymmetrical envelope.

We investigated this relation for different species and performance traits. We developed experimental settings with C. elegans to describe free-activity and maximal speed. The dynamics of growth and decay processes revealed a similar asymmetric envelope with a growth phase much faster and shorter than degeneration. This investigation underlines the similarity of envelopes and paves the way for an extending exploration through the description of maximal performance traits in different species and at different scales.

P2: Reproductive consequences of early-life stress exposure: Within- and transgenerational effects in C. elegans

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Life history events can be recorded not only as memories, but also as characteristic patterns of chromatin modification in our genome and are frequently associated with changes in gene expression. How environmentally-triggered, non-genetic effects are molecularly transmitted over multiple generations and whether regulation of such epigenetic inheritance shows genetic variation in nature represent key questions in current biological research. Yet, empirical evidence for epigenetic inheritance is still very scarce as few adequate study systems exist in which to genetically dissect this phenomenon. Here we ask how a single stress experienced early in life can affect the development of an individual and subsequent generations. We address this question by characterizing natural genetic variation in epigenetic inheritance systems in the nematode Caenorhabditis elegans. In addition to the lab reference strain, (N2), many natural, genetically distinct isolates of C. elegans have been collected throughout the world, allowing us to test whether regulation of epigenetic inheritance shows genetic variation in nature, and thus may be subject to natural selection.

P3: The adaptation of cryo-sectioning procedures to *C. elegans* research

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Classical TEM approaches have proven to be indispensable for *C. elegans* cell and developmental biology research. Though the resin embedded samples are frequently used for immuno EM labeling, cryo-preparation by Tokuyasu is generally more efficient for the higher precision labeling. Despite the fact that cryo-sectioning and *C. elegans* have now coexisted for over 40 years, this method has not been transferred, mainly due to technical difficulties related to sample fixation and cryo-sectioning orientation steps. For Tokuyasu preparation samples are mildly fixed in paraformaldehyde, infiltrated with saturated sucrose solution as cryo-protectant and hardened by freezing in liquid nitrogen, so to be sectioned in a low temperature (-120°C). The minimal use of chemicals and absence of resins that might block the immunogenic sites enables higher labeling efficiency.
We have developed a Tokuyasu procedure for *C.elegans* using embedding that facilitates samples orientation for cryo-sectioning. Because of the limitations of *C. elegans* preparation for chemical fixation, we developed the fast-hybrid rehydration technique following high pressure freezing-freeze substitution cryoimmobilization. Using this procedure we were able process both larvae of earlier stages and embryos, otherwise very difficult to prepare by standard chemical fixation. We tested the efficiency of immuno labeling with several generic and *C. elegans* specific antibodies. Immuno-labeling of semi-thin sections with the fluorescent antibodies and ultra-thin sections with gold conjugates gave very satisfying results. We succeeded in preserving GFP signal in intact samples, on cryo-sections and obtained efficient labeling with immuno-gold. Improved immuno-gold labeling together with efficient immuno-fluorescence retention on cryo-sections provides a great potential for further studies using direct correlative light and electron microscopy.

P4: Dimensional scaling of microtubule assembly rate

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One fascinating property of cells is their capacity to assemble large structure with given and proper size on the micrometer range from molecular units of only few nanometers. It has been shown that several organelles such as nucleus, mitochondria and mitotic spindle are able to adapt their size to cell volume, but the mechanisms controlling organelle scaling remain elusive. The mitotic spindle is a conserved structure among eukaryotes that partition the genetic material during cell division. Since the mitotic spindle is mostly composed of dynamic polymer formed by an assembly of tubulin dimers, it has been suggested that the remodeling capacities of microtubules could be responsible for the control of spindle size. The aim of our work is to understand if and how microtubule dynamics achieve the dimensional challenge of spindle assembly when cell vary in size.

By measuring microtubule dynamics during early embryonic development of C. elegans in which spindle dimensions scale with blastomere size, we demonstrated that microtubule assembly rate scale with cell size. To verify that this property was not embryo-dependent we used the C. elegans vulval epithelium as somatic model for cell size scaling. We found that microtubule assembly rate scale with cell size in both embryonic and somatic tissue but that the scaling factors are distinct. Our results suggest that microtubule assembly rate regulation is not only dependent on cell dimensions but is in addition regulated by cell fate or cell lineage determinants. Possible mechanisms by which microtubules could sense cell volume will be discussed.

P5: Integrated signaling networks in innate immunity

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Upon fungal infection C. elegans up-regulates the expression of many antimicrobial peptide genes. We are building an integrated gene regulatory network to represent this gene induction, integrating in-house and public data from various techniques, including genome-wide RNAi screens, RNA-seq, miRNA -seq and Chip-seq. To identify key genes of the network, we carried out several genetic screens and recently, a genome wide RNAi screen (Zugasti et al. Nature Immunol. 2014). From the latter we found 360 RNAi clones that block the immune response. Our new tool, CloneMapper, (Thakur et al. G3, 2014; http://bioinformatics.lif.univ-mrs.fr/RNAiMap/) allowed the target genes to be identified. To explore the dynamics of gene expression upon infection, we ran RNASeq analyses at different times post-infection, identifying different clusters of Differential Expressed Genes (DEG). To identify their biological roles we are performing functional enrichment analysis using an in-house collection of ~4000 functional classes. To identify the potential regulators of these cluster of DEGs, we are analyzing 240 modENCODE chipseq datasets for 92 TFs, mainly using RSAT. At the same time I am also looking for the enrichment of DEGs among the lists of potential targets of each TF (as identified by ChIPseq). My final objective is to integrate information from these different datasets with protein-protein interaction data to build a robust regulatory network that explains the regulation of these defense genes upon infection.

P6: Balancer-free hands-off selection of adsl heterozygous mutant using CRISPR-Cas9 system

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Balancer chromosome are rearranged chromosome, making cross-over events unlikely and used to maintain, througt an heterozygous state, mutation inducing sterile or lethal phenotype when homozygous. adsl gene encodes an enzyme involved in purine biosynthesis. KO worms for this gene are sterile, making it impossible to study without the use of Balancer chromosome. However, using this method, only 1/16 of indivuals are homozygous and non-marked, making it difficult to analyse for metabolic studies.

We used CRISPR-Cas9 genome editing system to target adsl gene, replacing it with a Neo selection marker, selecting heterozygote, and then add a fluoresent CDS to the remaining copy, throught a 2A peptide linker. We obtained a strain that allowed us to keep the heterozygous state and select specifically homozygous individuals (non-fluorescent worms). Those individuals will be use to perform metabolic analyses.

We believe new genome editing tools can be used to create better, more reliable genetic system to study mutation inducing sterile or lethal phenotypes.

P7: The National Functional Genomics Platform

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A couple of years ago, the worm platforms in Villeurbanne and Marseille federated to form a national facility that is part of the IBiSA infrastructure network (www.ibisa.net). Together these platforms offer a wide range of services to European worm groups.

The Villeurbanne facility maintains a large Mos1 transposon insertion library generated by the NEMAGENETAG consortium. These strains are referenced in Wormbase (ttTi alleles), and can also be searched using a dedicated tool (www.ciml.univ-mrs.fr/applications/MosLocator). They can be used as the starting point for targeted genome engineering (via MosTIC and MosDel). The facility additionally proposes a service of custom genome modification using CRISPR. Depending on demand, these services can include repair template construction. We also welcome students/researchers who want to learn these techniques.

Both the Villeurbanne and Marseille platforms are equipped with COPAS worm sorters. These can be used for high-throughput genetic, RNAi or small molecule screens. The Marseille platform has just acquired a new sorter, equipped with 2 solid-state lasers, offering much improved sensitivity and compatible with a broader range of fluorophores, including mCherry. With the dedicated TECAN robot, large-scale screens can be handled with relative ease. Currently, a single-pass genome-wide RNAi screen can be completed in 5 weeks. A number of custom instruments have been made expanding the screening possibilities, including F3 clonal screens, and automated determination of lifespan (hundreds of conditions in parallel).

Details of the platforms and the services they offer can be found at:

http://ums3421.univ-lyon1.fr/

http://www.ciml.univ-mrs.fr/technology/c-elegans-functional-genomics

P8: Innate Host Defense Requires TFEB-Mediated Transcription of Cytoprotective and Antimicrobial Genes

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Animal host defense against infection requires the expression of defense genes at the right place and the right time. Understanding such tight control of host defense requires the elucidation of the transcription factors involved. By using an unbiased approach in the model Caenorhabditis elegans, we discovered that HLH-30 (known as TFEB in mammals) is a key transcription factor for host defense. HLH-30 was activated shortly after Staphylococcus aureus infection, and drove the expression of close to 80% of the host response, including antimicrobial and autophagy genes that were essential for host tolerance of infection. TFEB was also rapidly activated in murine macrophages upon S. aureus infection and was required for proper transcriptional induction of several proinflammatory cytokines and chemokines. Thus, our data suggest that TFEB is a previously unappreciated, evolutionarily ancient transcription factor in the host response to infection.

P9: *C. elegans'* native gut microbiome

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Most animals host numerous strains of bacteria. Changes in the numbers and types of these bacteria can exert a strong influence over host health and disease. Bacterial-gut-community members have in particular been demonstrated to play a role in a wide range of disorders. Despite growing recognition of the importance of the gut-bacterial-community, the role of many commensal (non-host-harming) bacteria remains largely undescribed. We examine *Caenorhabditis elegans* for naturally gut-associated microbes based on samples collected in the field. It is important to study naturally associated microbes because closely related strains of bacteria can vary greatly in pathogenicity, making it very difficult to choosing meaningful laboratory strains of bacteria for experiments. We used *C. elegans* sampled from their natural environment because those maintained in the lab lose their associated gut-bacteria due to frequent bleaching.

Here we leveraged historical frozen collections from our laboratory as well as freshly sampled nematodes never cultured on *E. coli* to assess microbial diversity associated with a variety of visible gut phenotypes. One striking visible association concerns bacteria that adhere to the apical side of intestinal cells. We isolated genomic DNA from surface-sterilized worms, and then amplified bacterial rDNA by PCR with targeted primers designed against the conserved regions surrounding the V4 variable region of the 16S rRNA gene to characterize gut-associated bacteria.

Follow-up includes using Fluorescent *In Situ* Hybridization (FISH) to quantify relative abundance and within-organism location of the various bacterial strains detected in a subset of the original sample. This study seeks to begin documenting the role of associated gut microbiota in *Caenorhabditis*, establishing them as a genetic model system to study gut-associated bacterial communities.

P10: MegaTIC: a dual selection-based strategy for genome engineering in *C. elegans*

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A growing number of techniques have emerged to customize C. elegans genes by homologous recombination, including MosTIC and more recently CRISPR. In most cases isolation of engineered animals relies on PCR detection of the engineered locus, which can be more or less tedious depending on the frequency of the recombination events. To facilitate the detection of engineered animals, we developed a new technique, called MegaTIC (for Meganuclease excision-induced Transgene-Instructed gene Conversion) that relies on the insertion of a dual selection cassette containing both positive and negative selection markers. This technique comprises 2 steps. First, the MegaTIC cassette is inserted in the target locus by MosTIC or CRISPR. Positive selection of insertion events is based on resistance to hygromycin. Second, the MegaTIC cassette is excised by the meganuclease I-SceI to introduce customized gene modifications. Non-engineered animals ubiquitously express miniSOG from the MegaTIC cassette and can be efficiently counter-selected by exposure to blue-light that causes toxic production of singlet oxygen. PCR is used to confirm locus engineering. MegaTIC strategy was used to tag unc-49, which encodes subunits of a GABA receptor, acr-16 which encodes a subunit of the levamisole-insensitive nicotinic receptor and crld-1 which encodes an ER protein. Recombinant worms were found in 7%, 22% and 29% of blue-light illuminated plates deriving from independent P0s injected to modify unc-49, acr-16 and crld-1, respectively.

Our results show that this dual selection strategy is efficient and could be used to parallelize gene engineering by minimizing screening time.

P11: Characterization of genome instability in a H3K4 methylation defective mutant.

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Post-translational histone modifications play a crucial role in controlling DNA metabolic processes including transcription, replication and repair. In a set-2/SET1 mutant where methylation of lysine 4 of histone 3 (H3K4) is largely impaired, we observed genome instability and hypersensitivity to DNA damage agents. We will present data showing that neither DNA damage signaling nor apoptosis are affected in the absence of H3K4 methylation whereas kinetics of DNA damage repair seems to be modified.

P12: Regulation of the GPR protein during asymmetric cell division.

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The position of the mitotic spindle dictates the cell division plan. For cells having a precise orientation within a tissue, spindle positioning must be tightly controlled to prevent loss of cell polarity and cell detachment. An evolutionary conserved molecular complex is responsible for spindle orientation. One member of that complex, the GoLoco-containing protein GPR, is found in a restricted cell cortical area, towards which the spindle reorients. One of its functions is to recruit the molecular motor dynein to the cortex. Despite its essential function, GPR is still poorly characterized. We propose to unravel the mechanisms of GPR temporal and spatial regulation in the one-cell stage C. elegans embryo. In this cell, spindle movements can be easily quantified and constitute a clear readout of the GPR-containing complex activation. We found that embryos from different nematode species display different spindle movements, which are accompanied by sequence divergence of GPR. We use these variants to correlate phenotypes, GPR localization and sequence divergence to identify GPR regulatory elements. We also perform protein replacement between species, as well as analysis of protein chimeras. Finally we plan to use optogenetics in order to control GPR localisation temporally and analyze the consequences on pronuclei and spindle movements during the first embryonic division.

P13: Drug screening for mitochondrial diseases related to POLG mutations using Saccharomyces cerevisiae and Caenorhabditis elegans

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Mitochondrial function requires a set of respiratory chain subunits, some of them are encoded by the mitochondrial genome. Thus, mtDNA instability may results to OXPHOS failure, one of the causes of various metabolic diseases that are currently incurable. The Polymerase gamma (Pol γ) protein replicates the mtDNA and is important for mtDNA inheritance and maintenance. Most known mutations in the POLG gene are pathogenic and it is nowadays one of the major locus of human mitochondrial diseases. In this study, we have blind-screened 1200 chemical molecules using two yeast mutants in the MIP1 gene (POLG ortholog in yeast). We showed that two positive hits, MRS2 and MRS3, rescued various respiratory phenotypes on yeast mip1 mutant in the polymerase domain, e.g., increase in oxygen consumption, decrease of petite colonies production (colonies with mtDNA rearrangement or loss) and increase of Mip1 protein steady-state level. These positive hits were further tested and validated on a Caenorhabditis elegans animal model of the POLG mutation (brood-size augmentation, prolonged longevity and mtDNA content increase). Furthermore, our preliminary result also showed an increase of the mtDNA content on POLG patient's fibroblast upon treatment with MRS3. These results demonstrated that a “yeast-to-nematode” drug-screening scheme is one of the promising approaches to identify drug candidates for mitochondrial diseases associated with POLG mutations.

P14: AICAR effects on the Ubiquitin Proteasome System and on the Methyl cycle

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AICAR (5-Phosphoribosyl-4-carboxamide-5-aminoimidazole), also known as ZMP, is an intermediate metabolite on the de novo purine biosynthesis pathway. In addition to it's intermediate metabolite role AICAR is also a regulatory small molecule. It was shown namely to regulate specific transcription factors in yeast to upregulate purine synthesis, it is also a well known activator of the AMPK a by mimicking AMP. In our lab we are interested in understanding the biological properties of AICAR. Specifically we aim at identifying the molecular targets of AICAR and its mechanism of action. Previous work in our laboratory using yeast and mammalian cells identified S-Adenosyl-Methionine (SAM) Synthetase and the Ubiquitin pathway E1 enzyme UBA1 as potential targets of AICAR. We tested whether these two AICAR targets are conserved in C. elegans. AICAR seems to activate SAM synthetase - the enzyme catalyzing the production of SAM, the sole known donor of the Mehyl group in methylation reactions. We show that AICAR partially rescues the fertility defects of a deletion mutant of sams-1 (one of the five genes encoding SAM synthetases in the C. elegans genome), consistent with an increase of SAM synthetase activity induced by AICAR. UBA1, on the other hand seems to be inhibited by AICAR. Consistent with this hypothesis, AICAR increases the embryonic lethality of heterozygotes carrying one deletion allele of uba-1 (the sole gene encoding the ubiquitin E1 in C. elegans). We are currently testing this hypothesis through biochemical approaches.

P15: Functional caracterisation of the SET1/MLL complex in the C. elegans germline

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Methylation of histone H3 Lys4 (H3K4me) is associated with active transcription in all species, and is catalyzed by highly conserved multiprotein complexes known as Compass or SET1/MLL. Work from sveeral labs using both yeast and mammalian cells has led to a detailed description of the SET1/MLL complexes. Biochemical analysis has shown that interaction between a common subset of conserved proteins play an important role in regulating histone methyltransferase activity, with other components potentially contributing to tissue-specificity or developmental regulation. This specificity has emphasized the need to study the function of individual subunits of the complex in a developmental context. We have previously shown that SET-2, the sole SET1 homologue in C. elegans, and ASH-2, another member of the SET1/MLL complex play different roles in H3K4me during development, particularly in the germline.

In order to better understand the regulation of H3K4 particularly in the germline, we have decided to develop a proteomic approach to identify SET-2 molecular partners both in the germline and in the soma. Our project is based on the expression of two set-2 tagged versions, constructed by the CRISPR/Cas-9 technology : one under the control of its own promoter and the other one under the control of a germline specific promoter. These two versions will the immunopurified and the complex composition will be analyzed by mass spectrometry. Interesting partners will then be caracterized using both genetic and biochemical approaches. This study will give a better understanding of the molecular mechanisms responsible for H3K4me regulation in different tissues .

P16: Toward the identification of genetic pathways involved in muscle aging in Caenorahbditis elegans

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Aging is accompanied by a progressive loss of muscle mass and function, termed sarcopenia. In human, this disease is responsible for a decrease in mobility leading to a reduction in the quality of life and to dependency that increases the need for care. Epidemiological studies further suggest that skeletal muscle aging is also a risk factor for the development of several age-related diseases such as diabetes, cancer, Alzheimer's disease, and Parkinson's disease.

Several mechanisms, including mitochondria default, apoptosis or alteration of muscle protein turnover, have been associated to sarcopenia. However the physiological importance of these events in the etiology of sarcopenia remains to be investigated.

We are using C. elegans to identify genetic pathways involved in muscle aging. For this purpose we first concentrated on the characterization of biomarkers of muscle aging in C. elegans. Good candidates should fulfill two criteria: changing with chronological age and being associated to functional decline, i.e. loss of mobility. We have defined a time course of events that take place during muscle aging starting from a decrease in the expression of some, but not all muscle genes expression, followed by a change in mitochondria morphology and an impairment of muscular proteostasis. We are now using those biomarkers as a read out to perform a genetic screen in order to identify genes that modulate sarcopenia.

P17: Mitochondria Dynamics and Muscle Degeneration in C. elegans

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Muscle degeneration is a common feature of muscle **aging** and muscular dystrophies such as **Duchenne Muscular Dystrophy (DMD)**. Currently, subcellular mechanisms of muscle degeneration remain poorly understood. Our lab has established a Caenorhabditis elegans mutant with progressive dystrophin-dependent muscle degeneration: the DMD worm model dys-1(cx18);hlh-1(cc561) double mutant**.** The DMD mutant worm model exhibits increased mitochondrial fragmentation compared to wild-type worms suggesting a deregulation in the fusion/fission mitochondrial balance. **My goal is to understand the role of mitochondria dynamics in the molecular mechanisms leading to muscle degeneration.**

To study the link between mitochondria dynamics and muscle degeneration, we first focus on **DRP-1,** required for mitochondrial outer membrane fission. Muscle tissues of drp-1(tm1108) mutant worms, that lack DRP-1, show an absence of degeneration over aging and reduced mitochondrial fragmentation compared to those of wild-type animals. Moreover, dys-1(cx18);hlh-1(cc561);drp-1(tm1108) mutant worms exhibit a better mobility and less muscle degeneration than dys-1(cx18);hlh-1(cc561) mutant worms. All together, **DRP-1** seems to be implicated in both **dystrophin and aging-dependent muscle degeneration**.

We also perform experiments with **WAH-1,** the worm homolog of the mammalian Apoptosis-Inducing Factor (AIF). Interestingly, treatment with wah-1 RNAi decreases mitochondria circularity in DMD mutant and wild-type worms and reduces dystrophin-dependent muscle degeneration. Our findings provide the first evidence that WAH-1 affects **mitochondria dynamics** in muscle cells.

All together, our data converge toward a key role of DRP-1 and WAH-1 in molecular mechanisms that lead to dystrophin and aging dependent muscle degeneration.

P18: The axon guidance receptor Ryk impairs neuronal resistance capacity by repressing FOXO activity during the early phases of huntingtin pathogenicity

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The ability of neurons to cope with the chronic stress induced by mutant protein expression in Huntington's disease (HD) may determine the course of their decline and eventual demise. Although the pathophysiological importance of these stress responses has been previously shown, little is known about the pathways that regulate neuronal homeostasis during the early phases of mutant huntingtin pathogenicity and how this might impact on the overall capacity of neurons to maintain function in HD.

We found that the Wnt receptor Ryk, a protein important for neurogenesis, is increased in several models of HD, which may occur prior to or during the early phases of the disease process as suggested by C. elegans and mouse models of HD. Interestingly, increased levels of Ryk repress activity of the FOXO proteins, a family of transcription factors that play an important role in cell survival/longevity, neuronal homeostasis and protection. Ryk directly represses FOXO protective activity through its intracellular domain, a g-secretase cleavage product. This highlights the regulation of HD neuron survival by a Ryk pathway that is distinct from canonical Wnt/Ryk signaling. From our findings, we postulate that neurons are unable to develop an efficient FOXO-mediated survival response during the early phases of the pathogenic process in HD (Tourette et al., PLoSBiol 2014). This suggests that restoring FOXO-mediated stress response has significant therapeutic potential and that FOXO-interaction networks might contain important indicators of drug protection. Additionally, our findings support the possibility that FOXO-interaction networks might contain genes that are involved in disease modification.

P19: Mitochondrial ROS mediated muscle degeneration in hlh-1(cc561);dys-1(cx18) Caenorhabditis elegans mutant

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Mitochondria dysfunctions are involved in muscle disorders, like muscular dystrophies. The development of treatments against theses pathologies is challenging, in part due to various primary genetic defects affecting different cellular functions. Our aim is to identify common mitochondrial suppressors of muscular dystrophies.

We used Caenorhabditis elegans mutant animals that present a mutation in the C. elegans ortholog of myogenic transcription factor MyoD, hlh-1 associated with another mutation in particular genes involved in muscle physiology: ortholog of human dystrophin (**dys-1)** affected in Duchenne and Becker dystrophies, sarcoglycan-alpha (**sgca-1),** calpaïne-3 (**clp-7**), lamine-A/C (**lmn-1**), four and a half LIM - FHL-1 - (**lim-9**) or glycosyltransferase (**lge-1**) affected in Limb-Girdle, Emery-Dreifuss or congenital muscular dystrophy. All the double mutant animals exhibited progressive muscular degeneration and paralysis and thus represent nematode models for human muscular dystrophies.

First, we focused on mitochondrial ROS production. Preliminary results suggested that treatments with NAC or vitamin C, two powerful antioxidants, or with the mitohormesis inducer paraquat, decreased muscle degeneration by 20% in hlh-1(cc561);dys-1(cx18) double mutant animals compared to untreated hlh-1(cc561);dys-1(cx18) worms.

Second, we will examine mitochondrial-dependent programmed cell death (PCD) and determine the relevance of cell death related proteins for muscle degeneration in hlh-1(cc561) models.

Our study will establish whether manipulating mitochondrial ROS production and PCD can reduce or reverse progression of muscle degeneration in muscular dystrophies.

P20: Monitoring the first steps of the host response to fungal infection and wounding in c. elegans epidermis

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Sterile wounding or infection of C. elegans by the fungus D. coniospora leads to a rapid increase in the transcription of antimicrobial genes in the epidermis. This is an interesting system for study because it can be activated at will. We are investigating it at different levels, combining cell biology with genetics and physiological analyses of the whole organism. In order to understand the signalling network that controls these responses, several genetic screens have been performed. Many of the players have been identified (1) and it was shown that part of the innate immune response, the one convergent on the p38 MAPK pathway, is triggered both by D. coniospora infection and injury.

Recently in the lab, we identified the GPCR DCAR-1and its ligand HPLA, as being involved in triggering the immune response (3). Because we previously demonstrated that genetic inactivation of vesicle trafficking abrogates immune signalling (2), it's relevant to monitor the subcellular localization and dynamics of DCAR-1 and otherknown signalling proteins, as well any cytoskeleton reorganization that might accompany infection or injury.

Using spinning disc microscopy, we have observed that the apical localization of a fluorescently tagged DCAR-1 receptor, which is localized to the apical membrane of the main epidermal syncytium hyp7, is distributed in a heterogeneous manner, between relatively large and stable assemblages or in very mobile small vesicles. The exact nature and functional importance of these different forms are currently being characterized.

P21: Quantitative Analysis of the Polarization mechanism of a field of Neuronal Precursors in C.elegans

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Cell and tissue polarities are essential for the functions of animals and their misregulations are often associated with pathologies. Recent studies in both vertebrates and invertebrates suggest that gradients of Wnt ligands can polarize fields of cells, however how the positional information conveyed by the gradient of Wnt ligands is translated into cell polarization is poorly understood.

One factor that limits our understanding of tissue polarization is the lack of quantitative data on the behavior and interactions between the molecular players in tissue polarization processes occurring in vivo. Using loss and gain of function approaches we have observed that a neuronal precursor field of the C.elegans embryo may be polarized by a gradient of three Wnt ligands acting via a Wnt receptor of the Frizzled family.

We are currently using in vivo imaging techniques (spinning disc confocal, light sheet microscopy and Fluorescent Correlation Spectroscopy) to analyze this polarization process in vivo in a quantified manner.

P22: Identifying nipi-3-dependent elements required for the stability and translation of cebp-1 mRNA

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Regulation of mRNA stability and translatability provides a mechanism to govern gene expression with temporal and spatial precision, and is essential for situations such as stress or infection. 3'UTRs are widely known to be primary targets for post-transcriptional gene regulation. We have previously reported a role in C. elegans innate immunity for nipi-3, homolog of the human Tribbles 1(1). In response to fungal infection, nipi-3 is required for up-regulation of anti-microbial peptides. nipi-3 was found to act upstream of a p38 MAPK cascade that is important for responses to injury and infection. How these kinases affect gene expression upon infection is, however, still unknown. We previously reported the identification of cebp-1 as a genetic suppressor of nipi-3(2). CEBP-1 is homologous to the mammalian C/EBPδ, which has been studied for roles including stress(3). In C. elegans, cebp-1 functions in the nervous system during developmental and adult stages(4), but roles in other tissues have not been described. We find that nipi-3 negatively regulates cebp-1 mRNA levels and this regulation may occur through cebp-1's 3'UTR. cebp-1's 3'UTR has several regions of conservation among nematodes and mammals, suggesting functional conservation of the cis elements as well as the trans factors. We are currently determining the cis elements of cebp-1's 3'UTR that mediate its control by nipi-3 in infection as well as investigating RNA binding proteins that could be involved in this pathway in innate immunity. These studies will further elucidate conserved mechanisms contributing to the regulation of innate immunity.

P23: Dissecting a worm killer

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We have studied intensively the infection of C. elegans by Drechmeria coniospora (see Labed & Pujol (2012), Journal of Invasive Fungal Infections). We have recently initiated a project to dissect the molecular and cellular basis of D. coniospora's capacity to infect worms and resist host immunity.

Combining DNA and RNA sequencing with optical mapping (in collaboration with the Genoscope), we assembled a draft genome of 33Mb, and predicted some 11,000 protein-coding genes. The majority of the predicted proteins (6,500) contains conserved domains and could be annotated with Pfam and GO database terms.

To undertake functional studies of D. coniospora, we have developed methods to engineer the fungal genome. We have adapted a standard protoplast transformation protocol for use with D. coniospora. As a proof of principal, we produced a transgenic fungus that expresses GFP under the control of the D. coniospora beta-tubulin promoter. To demonstrate the feasibility of knocking-out specific genes, we chose to target the D. coniospora orthologue of the gene so1. In Fusarium oxysporum, so1 mutants are deficient in hyphae fusion, giving a phenotype that can be easily scored. As in F. oxysporum, the D. coniospora so1 mutant is deficient for germling fusion. Progress on functional studies of so1 mutant will be reported.

In the future, we plan to use the genome sequence to identify potential virulence factors and to knockout specific genes and assay the consequences on the different steps of fungal infection.

P24: Analysis of chromatin factors involved in the asymmetric divisions of neuronal precursors

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In both vertebrates and invertebrates, postmitotic neurons are often generated by asymmetric divisions of neuronal progenitors such as neural stem cells. We have previously established that in C. elegans the asymmetric divisions of neuronal precursors are regulated by an asymmetric activation of the wnt pathway. More recently we have identified, during a genetic screen, mutants in the chromatin regulator complex PRC1 that affect the generation of some neurons by asymmetric divisions. We are currently investigating how these chromatin factors affect this asymmetric divisions mechanism.

P25: Systems modeling and network-based approaches for basic and translational research in Huntington's disease

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As a growing and more diverse amount of genetic and molecular profile data are generated in several models of Huntington's disease (HD), the potential of these datasets for enhancing HD research has significantly increased. Network-based analysis has the capacity to identify robust HD-associated rules and signatures from complex datasets. These rules and signatures can in turn be exploited for better understanding HD biology on a global scale as well as for prioritizing models and genes around questions of specific interest, thereby supporting decision making in HD research. To support biomedical intelligence in HD, we have developed Biogemix, a network-based data integration framework for precise rule/pattern extraction across models and species. We used this framework for integrating greater than 16 publically available datasets including transcriptomic and gene perturbation data from human, mouse and nematode.

We found previously suspected as well as novel HD-associated features, the latter providing new insights into the global effects of mutant huntingtin pathogenicity. Additionally, we managed to determine functional distances between experimental models of HD and assess the biological relevance of individual models of HD, functional profiles of biological modifiers and shared/unshared properties between pathological and compensatory/survival genes. Our results validate the value and precision of the Biogemix framework for actionable knowledge in HD. As larger HD datasets become available, our results suggest that applying the Biogemix approach to the analysis of these datasets will provide deep insights into the essential features of mutant huntingtin pathogenicity while providing strong guidance to translational and pharmacological research in HD.

P26: Analysis of activity-dependent synaptogenesis at the SAB neuromuscular junction.

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Activity is used to shape neuronal networks. To identify the rules and mechanisms involved in activity-dependent synaptogenesis, I studied the SAB system of C. elegans. There are three SAB neurons that send anterior processes in the head and form synapses at the surface of head muscle cells. During development the morphology of the SABs is modified after electrical silencing of these muscles, suggesting the existence of an unknown retrograde signal (Zhao, 2000). Using fluorescently-tagged acetylcholine receptors, we observed SAB overgrowth and ectopic synapses in unc-13 and unc-18 mutant worms where neuromuscular transmission was disrupted, in agreement with previously published results.

To study the development of SAB neuromuscular junctions, we are currently trying to implement microfluidic devices to trap a worm and image it under the microscope at several time points throughout its lifetime without affecting its development.

Finally, we are trying to independently control the activity of head muscles and SAB neurons using optogenetics. Channelrhodopsin-2 and Arch or Mac provide a means to depolarize or hyperpolarize the muscle cells in a temporally defined fashion, respectively. We are also considering using the inhibitory Drosophila HisCl1 histamine-gated chloride channel to control the activity of specific neurons or muscles (Pokala, 2014).

References:

Pokala, N., Liu, Q., Gordus, A., and Bargmann, C.I. (2014). Inducible and titratable silencing of Caenorhabditis elegans neurons in vivo with histamine-gated chloride channels. PNAS 111, 2770–2775.

Zhao, H., and Nonet, M.L. (2000). A retrograde signal is involved in activity-dependent remodeling at a C. elegans neuromuscular junction. Development 127, 1253–1266.

P27: Impaired mitochondrial stress response in C. elegans dystrophin-dependent muscle degeneration.

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Muscle degeneration can be induced by a variety of mutations causing genetic diseases, notably muscular dystrophies, or by physiopathological processes such as aging. Our project aims at determining the mitochondrial pathology shared by different types of muscle degeneration to develop therapeutic strategies targeting mitochondria. Our group has established and characterized a C. elegans model for dystrophin-dependent muscle degeneration, the dys-1(cx18); hlh-1(cc561) double mutant worm, mimicking Duchenne Muscular Dystrophy (DMD). Evidence demonstrates drastic mitochondria morphology changes in this model. Furthermore, various genetic or pharmacologic manipulations of mitochondria in dys-1(cx18); hlh-1(cc561) double mutant worms can suppress muscle degeneration. All together, this suggests that loss of dystrophin function induces a mitochondrial stress in muscle cell. Recently, we investigated the stress responses of dys-1(cx18); hlh-1(cc561) double mutant worms and of its counterpart dys-1 (cx18) and hlh-1 (cc561) simple mutants. We showed that muscle degeneration observed in dys-1(cx18); hlh-1(cc561) double mutant worms is accompanied by an inability to trigger mitochondrial stress response, especially mitochondrial Unfolded Protein Response (UPR). Furthermore, reducing mitochondrial UPR is sufficient to induce muscle degeneration in wild-type worms. Next, we will analyze the effects of hsp-60 knockdown on muscle degeneration over aging. In parallel, we will overexpress hsp-60 in particular tissues such as intestine, muscles and neurons of dys-1 (cx18); hlh-1(cc561) double mutant worms using specific promoters and test the effects on muscle degeneration and animal fitness. Our study is likely to shed light on the role of mitochondrial stress response in muscle degeneration.

P28: Role of acto-myosin based force production in cell invasion in Caenorhabditis elegans

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Basement membrane (BM) is a dense 2-dimensional sheet of specialized extracellular matrix that separates epithelia from underlying tissue. The penetration of cells through BM barriers, called "invasion", is an important process during normal tissue development and in cancer metastasis. Much has been understood concerning the genetics and signaling of how holes are formed in BM during invasion. However less is clear about the physical forces involved: how the molecular motor myosin participates in BM removal and how actin polymerization contributes to the invasive process. To address this question, we study an invasion event during vulva development in Caenorhabditis elegans, anchor cell (AC) invasion. As a first approach, we use tissue-specific RNAi directed against different cytoskeleton proteins and evaluate the effect of these treatments on egg-laying ability. Our results suggest that the actin assembly factors WAVE and WASP, actin filament capping protein and myosin are important for egg-laying. Now we are characterizing how knock-down of these proteins affects AC invasion by imaging hole opening in fluorescent BM. Next we will fluorescently label relevant proteins to examine their dynamics and localization during BM opening. To have spatial and temporal control over knock-down during AC invasion, we will use optogenetic inhibition, like CALI (Chromophore Assisted Laser Inactivation) against GFP and mini-SOG. We will also perform FRET force sensor experiments with vinculin, a component of cell-BM adhesions, to monitor force development throughout the invasion. These experiments will result in a better understanding of the role of physical forces in cell invasion events.

P29: Genetic and developmental mechanisms underlying sperm size variation in Caenorhabditis nematodes.

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Sperm competition is a major evolutionary force driving diversity in sperm morphology. Such sperm competition exists in C. elegans where larger male sperm consistently outcompetes hermaphrodite sperm, and larger male sperm outcompetes smaller male sperm. Variation in male sperm size among different wild isolates of C. elegans may thus have evolved in response to different degrees of sperm competition. To characterize natural genetic variation in C. elegans male sperm size, we have quantified this phenotype in a collection of 100 genetically-divergent C. elegans wild isolates. This analysis revealed considerable variation in male sperm size, however, a Genome-Wide Association Mapping Study did not allow detection of potential genomic regions underlying this variation. Surprisingly, however, this survey revealed significant differences in male sperm size of different strains derived from the N2 reference strain, pinpointing potential candidate genes involved in sperm size regulation. We are currently exploring how specific candidate genes affect spermatogenesis and sperm maturation, to ultimately define molecular events controlling sperm size variation. In addition, we will present data on the co-evolution of male and hermaphrodite sperm in C. elegans, C. briggsae and C. sp. 11; we further discuss interspecific evolution of sperm size in gonochoristic Caenorhabditis species, which have been shown to exhibit a significantly larger mean sperm size than males of androdioecious species. We have extended these comparative analyses by integrating newly discovered species in the context of a recently established molecular phylogeny.

P30: Microtubule dynamics and mechanical forces in the one-cell C. elegans embryo

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During mitotis, chromosomes are connected to a microtubule-based spindle. The displacement of the spindle poles and/or the activity of kinetochore microtubules generate mechanical forces that segregate the chromatids. Using laser destruction of the centrosomes during C. elegans mitosis, we showed that neither of these mechanisms is necessary to achieve proper chromatid segregation. We demonstrated that an outward force generated by the spindle midzone is sufficient for anaphase in mitotic cells. We next identified candidate molecules involved in this outward force. We found that SPD-1/MAP-65 and BMK-1/kinesin-5 act as a brake opposing the force generated by the spindle midzone. Conversely, we revealed that CYK-4/RhoGAP and two microtubule-growth and nucleation agents, Ran and CLASP, are necessary for the establishment of the outward force. Besides their established role during spindle formation, we uncovered a new function for Ran and CLASP during anaphase.

We will also present a new tool to optically control microtubule dynamics with high spatiotemporal precision and full reversibility. Our Photostatins PST-1 molecule promote microtubule depolymerisation when irradiated with 390-430 nm light, but quickly revert to an inactive form in the dark or by irradiation with 500-530 nm light. Using C. elegans embryos, we show that PST-1 controls mitosis with single-cell resolution and with a temporal response on the scale of seconds. This unique tool opens the way to the study of MTs dynamics in the C. elegans spindle and more generally hold promise as a new class of precision chemotherapeutics

P31: crld-1 is an evolutionarily conserved gene required for the biosynthesis of AChRs

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Reduction of acetylcholine receptor (AChR) number at the neuromuscular junction is responsible for myasthenic syndromes. Symptomatic treatments rely mostly on acetylcholinesterase inhibitors. Alternative therapeutic strategies could be based on modulation of AChR metabolism. We use the nematode C. elegans as a model system to identify new genes that regulate AChR biosynthesis and recycling.

By genetic screen for mutants with a partially decreased sensitivity to the cholinergic agonist levamisole, we identified mutations in the crld-1 gene. crld-1 is the ortholog of the Creld1 and Creld2 human genes (1). Creld1 mutations are associated with developmental cardiac defects (2,3) and Creld2 is implicated in ER stress and UPR through a putative protein disulphide isomerase (PDI) activity (4,5).

We analyzed the crld-1 function that was uncharacterized in C. elegans. By the MosTIC engineering technique we tagged the N-terminal of crld-1 and found that it is ubiquitously expressed with an evident ER pattern. By immunochemistry we observed a decreased number of L-AchRs in crld-1 null mutants and by western blot analysis we confirmed this result and found that CRLD-1 is important for the stability of unassembled L-AChR subunits.

Moreover by sequence alignment comparisons we identified motifs typical of PDI protein in the sequence of C. elegans crld-1; by CRISPR technology we mutated one of these PDI motifs to test if the PDI function is conserved in nematode.

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P32: Recombinant inbred lines for quantitative locus mapping

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I will describe the construction of a hybrid population of C. elegans that resulted from an intercross of 14 wild isolates and maintenance during 140 generations at high populations sizes and significant outcrossing in the laboratory, from which we derived about 300 inbred lines. These inbred lines have been Ilumina genome sequenced and characterized for several phenotypic traits. I will show the possibility for high resolution mapping of quantitative trait loci, by association mapping, that is achieved with this set of inbred lines.

P33: Atypical autophagy of polarized sperm-inherited mitochondria

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Abstract Not Available

P34: Caenorhabditis elegans expressing the Saccharomyces cereviasiae NADH alternative dehydrogenase Ndi1p as a tool to identify new genes involved in complex I related diseases

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Abstract Not Available

P35: Astral microtubules displaying two different dynamical behaviours may perform different functions during the division of C. elegans one-cell embryo.

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Abstract Not Available

P36: Cdk1 phosphorylates SPAT-1/Bora to trigger PLK-1 activation and drive mitotic entry in early C. elegans embryos.

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Abstract Not Available

P37: The NEDD8 inhibitor MLN4924 increases the size of the nucleolus and activates p53 through the ribosomal-Mdm2 pathway

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Abstract Not Available

P38: Chromosome Segregation in the C. elegans Oocyte: Functional Analysis of CLASP

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