

**European *C. elegans*
Neurobiology Meeting**

Book of Abstracts

**9-11 October 2010
Fodele Beach, Crete**

- 1 MAIN ENTRANCE
- 2 MAIN BUILDING ENTRANCE
- 3 RECEPTION
- 4 MAIN BAR
- 5 MAIN RESTAURANT
- 6 INTERNET CORNER
- 7 A LA CARTE RESTAURANT "AMBROSIA"
- 8 SNACK BAR
- 9 BEACH BAR
- 10 DISCO
- 11 MINI CLUB

- 12 MEDICAL CENTER
- 13 FITNESS ROOM (GYM)
- 14 SAUNA
- 15 MAIN SWIMMING POOL
- 16 HEATED SWIMMING POOL
- 17 PLAZZA
- 18 STAR LIGHT BAR
- 19 SWIMMING POOL
- 20 MINI GOLF
- 21 TENNIS COURTS
- 22 BASKET BALL COURT
- 23 MINI MARKET
- 24 HAIRDRESSING SALON
- 25 RENT A CAR
- 26 BEACH
- 27 SEA SPORTS
- 28 AMPHITHEATRE
- 29 LUGGAGE ROOM
- 30 PARKING
- 31 ARCHERY
- 32 BEACH SOCCER
- 33 BEACH VOLLEY
- 34 GAMES ROOM
- 35 CONFERENCE ROOM
- 36 DIVING CENTER
- 37 ATM
- 38 WATERPARK
- 39 SNACK BAR "WINDMILL"
- 40 BOCCIA
- 41 A LA CARTE RESTAURANT



**FODELE BEACH
HOLIDAYS RESORT**
 P.O. BOX 1354
 GR - 71500 HERAKLION
 CRETE - GREECE
 Tel . +302810521251/5
 +302810521351/4
 Fax. +302810521249
 www.fodelebeach.gr
 e-mail: fodele@fodelebeach.gr


FODELE BEACH
 ★★★★★
HOTEL PLAN

Table of Contents

Programme of the Meeting	vii
 Oral Presentations	
Lateral Facilitation between Primary Mechanosensory Neurons Controls Nose Touch Perception in <i>C. elegans</i> , M. Chatzigeorgiou and W. Schafer	11
Optogenetics-based functional analysis of a PVD-mediated nociceptive neuronal network in <i>C. elegans</i> , S. J. Husson, <i>et al.</i>	12
Different Food Types Affect Lifespan through the Sensory Nervous System and the Neuromedin U Receptor <i>nmur-1</i> , W. Maier, <i>et al.</i>	13
Soluble Guanylate Cyclases Specify Oxygen Sensory Neurons in <i>C. elegans</i> , M. Zimmer, <i>et al.</i>	14
DEG-3/DES-2 nAChR in PVD Function and Morphology, E. Cohen, <i>et al.</i>	15
Identifying the Effectors that Enable RHO-1 to Stimulate Neuronal Activity, M. Elmi, <i>et al.</i>	16
<i>C. elegans</i> identifies a novel insecticide target, A. Sluder, <i>et al.</i>	17
Analysis of Cysteine String Protein Function in <i>Caenorhabditis elegans</i> , J. R. Johnson, <i>et al.</i>	18
Mitochondrial Distributions in Neurons are Regulated by Microtubules, G. Sure, <i>et al.</i>	19
Small Heat Shock Proteins Protect Against Necrotic Cell Death, N. Kourtis and N. Tavernarakis	20
The <i>C. elegans</i> Kinesin Motor UNC-104 is Degraded upon Loss of Specific Binding to Cargo, J. Kumar, <i>et al.</i>	21
The Contactin RIG-6 Mediates Axon Outgrowth and Navigation in <i>C. elegans</i> , M. Katidou, <i>et al.</i>	22
Mechanism of Dendritic Arborization, M. Oren-Suissa, <i>et al.</i>	23
Regulation of the Molecular Motor UNC-104(KIF1A) in the Nervous System of <i>C. elegans</i> , C.-P. Shen, <i>et al.</i>	24
Frataxin Suppression Reduces fat Accumulation and Induces Autophagy in a p53-dependent Manner, Independently of a Caloric Restriction-like Response, A. Schiavi, <i>et al.</i>	25
Transgenic Nematodes as a Model for Parkinson's Disease, R. K. Bodhicharla, <i>et al.</i>	26
Identification of Ascr1 as a Gender Specific Mate-finding Cue in the Free-living Nematode <i>Panagrellus redivivus</i> , Andrea Choe, <i>et al.</i>	27
The Neuronal Circuit for the Thermal Avoidance Response in <i>Caenorhabditis elegans</i> , S. Liu, <i>et al.</i>	28
A Computational Model of Associative Learning and Chemotaxis in the Nematode Worm <i>C. elegans</i> , P. A. Appleby and N. Cohen	29
Microfluidic Devices for Neuronal Transport Imaging in <i>C. elegans</i> , S. Mondal, <i>et al.</i>	30
<i>In vivo</i> Optical Projection Tomography (OPT) Imaging in <i>Caenorhabditis elegans</i> , M. Rieckher, <i>et al.</i>	31

Poster Presentations

P01	Mutagenic Screen for Rescue of Neurotransmission and Locomotion Defects in <i>unc-18</i> (<i>e81</i>) Null Alleles, V. Martin and J. W. Barclay	35
P02	GTL-1 together with TRPA-1 Mediates PVD's Response to Cold Temperatures, E. Cohen, <i>et al.</i>	36
P03	Emodepside is a Resistance Breaking Anthelmintic Drug with Selective Toxicity to the Parasite's SLO-1 Calcium-activated Potassium Channel, A. Crisford, <i>et al.</i>	37
P04	The Role of the <i>Caenorhabditis elegans</i> Globin Family in the Response to Hypoxia, S. De Henau, <i>et al.</i>	38
P05	A Worm Model of Tauopathy, C. Fatouros, <i>et al.</i>	39
P06	Investigating the Role of UNC-31 in Glutamatergic Signaling in the Pharynx, C. Franks, <i>et al.</i>	40
P07	Characterizing the Role of Prolyl Hydroxylation during Neuronal Development, N. Torpe and R. Pocock	41
P08	Investigating the Effects of Compounds with Anthelmintic Potential: Amidantel, Bay d 9216 and Tribendimidine, M. Joyner, <i>et al.</i>	42
P09	Cell Fate Determination of Oxygen and Carbon Dioxide Sensing Neurons in <i>C. elegans</i> K. Langebeck-Jensen, <i>et al.</i>	43
P10	Investigating the Functional Roles of the FLP-8 Neuropeptide in Dauer-induced Stress Responses, J. G. Petersen, and R. Pocock	44
P11	The Golgi Protein SQL-1/GMAP210 Modulates Intraflagellar Transport in <i>C. elegans</i> Suzanne Rademakers, <i>et al.</i>	45
P12	Molecular Mechanisms of Salt Taste, Oluwatoroti O Umuerrri, <i>et al.</i>	46
P13	<i>Caenorhabditis elegans</i> as a Model of Homocystinuria: Characterization of Nematode Cystathionine Beta-synthase, Roman Vozdek, <i>et al.</i>	47
P14	A High-throughput whole Genome Sequencing Pipeline to Identify Genes Involved in <i>C. elegans</i> Aggregation Behaviour, Katherine P. Weber, <i>et al.</i>	48
List of Participants		49

European *C. elegans* Neurobiology Meeting

9-11 October 2010

Fodele Beach Hotel, Crete, Greece

Programme of the Meeting

Saturday, 9 October

16:00-18:00 Registration

Platform session 1: **Sensory Mechanisms**

Chair: **Sandhya Koushika**

18:00 Welcome note

18:10 **Marios Chatzigeorgiou** [MRC Laboratory of Molecular Biology, UK]

“Lateral facilitation between primary mechanosensory neurons controls nose touch perception in *C. elegans*”

18:35 **Steven Husson** [Goethe University Frankfurt, Germany]

“Optogenetics-based functional analysis of a PVD-mediated nociceptive neuronal network in *C. elegans*”

19:00 **Wolfgang Maier** [Friedrich Miescher Institute for Biomedical Research, Switzerland]

“Different food types affect lifespan through the sensory nervous system and the neuromedin U receptor *nmur-1*”

19:25 **Manuel Zimmer** [Research Institute of Molecular Pathology (IMP), Austria]

“Soluble guanylate cyclases specify oxygen sensory neurons in *C. elegans*”

20:00 Dinner

Sunday, 10 October

Platform session 2: **Neuronal Cell Biology, Development & Disease models I**

Chair: **Nataschia Ventura**

09:00 **Emiliano Cohen** [Hebrew University, Israel]

“DEG-3/DES-2 nAChR in PVD function and morphology”

09:25 **Muna Elmi** [University College London, UK]

“Identifying the effectors that enable RHO-1 to stimulate neuronal activity”

09:50 **Anthony Flemming** [Syngenta, UK]

“*C. elegans* identifies a novel insecticide target”

10:15 Coffee break

10:45 **James Johnson** [University of Liverpool, UK]

“Analysis of cysteine string protein function in *Caenorhabditis elegans*”

11:10 **Sandhya P. Koushika** [National Centre for Biological Sciences, India]

“Mitochondrial distributions in neurons are regulated by microtubules”

11:35 **Nikos Kourtis** [Institute of Molecular Biology and Biotechnology, FORTH, Greece]

“Small heat shock proteins protect against necrotic cell death”

13:00 Lunch

Platform session 3: **Neuronal Cell Biology, Development and Disease Models II**

Chair: **Tibor Vellai**

- 14:00 **Sandhya P. Koushika** [National Centre for Biological Sciences, India]
“The *C. elegans* kinesin motor UNC-104 is degraded upon loss of specific binding to cargo”
- 14:25 **Markella Katidou** [Institute of Molecular Biology and Biotechnology, FORTH, Greece]
“The contactin RIG-6 mediates axon outgrowth and navigation in *C. elegans*”
- 14:50 **Benjamin Podbilewicz** [Technion-Israel Institute of Technology, Israel]
“Mechanism of dendritic arborization”
- 15:15 *Coffee break*
- 15:45 **Oliver Wagner** [National Tsing-Hua University, Taiwan]
“Regulation of the molecular motor UNC-104(KIF1A) in the nervous system of *C. elegans*”
- 16:10 **Natascia Ventura** [University of Rome, Tor Vergata, Italy]
“Fratxin suppression reduces fat accumulation and induces autophagy, in a p53 dependent manner, independently of a caloric restriction-like response”
- 16:35 **Rakesh Bodhicharla** [University of Nottingham, UK]
“Transgenic nematodes as a model for Parkinson’s disease”
- 17:00 **Poster session** with *Coffee break*
- 20:00 *Dinner*

Monday 11 October

Platform session 4: **Behaviour & Methods/Technology**

Chair: **Manuel Zimmer**

- 09:00 **Andrea Choe** [CALTECH University, USA]
“Identification of Ascr1 as a gender specific mate-finding cue in the free-living nematode *Panagrellus redivivus*”
- 09:25 **Shu Liu** [Albert Ludwig Univ. Freiburg, Inst. Fuer Biology III/ZBSA, Germany]
“The neuronal circuit for the thermal avoidance response in *Caenorhabditis elegans*”
- 09:50 **Neta Cohen** [University of Leeds, UK]
“A computational model of associative learning and chemotaxis in the nematode worm *C. elegans*”
- 10:15 *Coffee break*
- 10:45 **Sudip Mondal** [National Centre for Biological Sciences, India]
“Microfluidic devices for neuronal transport imaging in *C. elegans*”
- 11:10 **Matthias Rieckher** [Institute of Molecular Biology and Biotechnology, FORTH, Greece]
“*In vivo* Optical Projection Tomography (OPT) imaging in *Caenorhabditis elegans*”
- 11:35 Closing remarks
- 13:00 *Lunch*

Abstracts
Oral Presentations

Lateral Facilitation between Primary Mechanosensory Neurons Controls Nose Touch Perception in *C. elegans*

Marios Chatzigeorgiou and William Schafer

Cell Biology Division, MRC Laboratory of Molecular Biology, Hills Road, Cambridge, UK

The nematode *C. elegans* senses head and nose touch using multiple classes of mechanoreceptor neurons that are electrically-coupled through a network of gap junctions. Using *in vivo* neuroimaging, we have found that one sensory neuron class, the multidendritic FLP nociceptors, respond to harsh touch throughout their receptive field but respond to gentle touch only at the tip of the nose. Whereas the FLP harsh touch response depends solely on cell-autonomous mechanosensory channels, gentle nose touch responses require the activities of additional mechanoreceptor classes, OLQ and CEP, which are electrically-coupled to FLP through a gap junction network. Conversely, FLP activity is required to indirectly facilitate nose touch and harsh head touch responses in the OLQs, demonstrating that information flow across the network is bidirectional. Thus, nose touch perception involves a hub-and-spoke network that allows individual sensory neurons to integrate information from multiple interconnected mechanoreceptors.

Optogenetics-Based Functional Analysis of a PVD-Mediated Nociceptive Neuronal Network in *C. elegans*

Steven J. Husson¹, Wagner Steuer Costa¹, Jeffrey N. Stirman², Joseph D. Watson³, W. Clay Spencer³, Millet Treinin⁴, David M. Miller³, Hang Lu², Alexander Gottschalk^{1,5}

¹Institute of Biochemistry, Goethe-University Frankfurt, Germany, ²Interdisciplinary Bioengineering Program, School of Chemical & Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA, USA, ³Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN, USA, ⁴Department of Medical Neurobiology, Hadassah Medical School, Jerusalem, Israel, ⁵Frankfurt Molecular Life Sciences Institute, Goethe-University, Germany

The two sensory PVD neurons envelop the nematode with highly branched dendritic arbors and were reported to be responsible for the sensation of harsh touch, whereas six other touch receptors respond to gentle touch. Expression of the blue light-activated depolarizing channelrhodopsin-2 in the PVD neurons and subsequent photoactivation allow us to mimic the harsh touch response, while leaving the other mechanoreceptors silent. Acute escape responses were monitored for different light stimulation protocols using spatially restricted illumination and automated video-tracking. To identify genes involved in this nociceptive neuronal network, we used RNAi to knockdown expression of genes that are specifically upregulated in a microarray data set derived from PVD neurons. RNAi of genes that mediate outgrowth of the PVD branches, such as *mec-3* and *unc-86*, impaired PVD-mediated escape responses and were therefore identified as key determinants of PVD function. This was also the case for voltage-gated calcium channel subunits when performing cell-specific knock down, emphasizing that signalling downstream of PVD mainly occurs via synaptic contacts with the command interneurons and not through gap junctions. This hypothesis was further verified by PVD-specific expression of Tetanus toxin light chain, which inhibits synaptic transmission by cleaving synaptobrevin, as this also abolished the optically stimulated escape responses.

Different Food Types Affect Lifespan through the Sensory Nervous System and the Neuromedin U Receptor *nmur-1*

Wolfgang Maier, Bakhtiyor Adilov, Martin Regenass, Joy Alcedo

Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

The lifespan of an organism, just like other traits, is not only a function of its genes, but rather of the interactions between its genes and its environment. Food is an important environmental factor that regulates lifespan. In organisms ranging from yeast to mammals, restriction of food levels can extend lifespan. In addition, lifespan of *C. elegans* can be modulated by the ablation of specific gustatory and olfactory neurons, which have been proposed to affect lifespan independently of food level restriction.

We have found that the lifespan of wild-type worms is food-type dependent and varies on different strains of *E. coli* in the absence of food-level restriction. We also discovered food type-dependent lifespan effects for sensory genes, like *osm-3*, a well-characterized cilium-structure gene, and the neuronally expressed gene *nmur-1*, a neuropeptide receptor gene with homology to mammalian neuromedin U receptors. Our findings demonstrate that, at least in worms, lifespan is not only determined by the amount of food but also the type of food experienced by the animal. We also show that these food type-dependent effects on lifespan require the sensory nervous system and neuropeptide signaling. In addition, we provide evidence that the lipopolysaccharide structure of *E. coli* is one of the bacterial factors that define food types.

Soluble Guanylate Cyclases Specify Oxygen Sensory Neurons in *C. elegans*

Manuel Zimmer*, Jesse M. Gray, Navin Pokala, Andy J. Chang, David S. Karow, Michael A. Marletta, Martin L. Hudson, David B. Morton, Nikos Chronis, and Cornelia I. Bargmann

Laboratory of Neural Circuits and Behavior, The Rockefeller University, New York, USA.

*Current affiliation: Research Institute of Molecular Pathology, Vienna, Austria

We are studying the nematode *C. elegans* as a model system to understand the function of neural circuits. Specifically, we are interested in how neurons interpret sensory information in order to generate the correct behavioral output. *C. elegans* detects a variety of sensory cues in its environment, including oxygen.

We have used a simple behavioral assay to measure the animals' locomotion speed in response to changing oxygen levels. Using this assay we discovered that a single sensory neuron BAG mediates transient slowing in response to a decrease from hyperoxic to preferred oxygen levels. In contrast, the sensory neuron URX is required for a similar slowing response when oxygen levels increase from preferred to hyperoxia.

In order to directly measure neuronal activity we have expressed the genetically encoded fluorescent Ca^{2+} indicator G-CaMP in these neurons. To deliver oxygen to the animal we designed a sealed microfluidic device that allows the immobilization of animals under an epifluorescence microscope. Using this system, we found that BAG generates Ca^{2+} signals when oxygen concentrations decrease. Conversely, URX generates Ca^{2+} signals when oxygen concentrations increase.

The genome of *C. elegans* contains multiple genes encoding soluble guanylate cyclases (sGCs). sGCs can bind directly to molecular oxygen and have been suggested as the primary oxygen sensory molecules in *C. elegans*. By analyzing mutants, we have found that the expression of different sets of sGCs in BAG and URX is critical for determining whether these neurons sense increases or decreases in environmental oxygen levels, thus providing a molecular code for sensory specificity.

Our studies provide insight into how changes in oxygen levels are differentially interpreted by a circuit of specialized sensory neurons in order to generate correct behavioral responses.

DEG-3/DES-2 nAChR in PVD Function and Morphology

Emiliano Cohen¹, Marios Chatzigeorgiou², Bill Schafer², and Millet Treinin¹

¹Department of Medical Neurobiology, Institute for Medical Research Israel Canada, Hebrew University-Hadassah Medical School, Jerusalem, Israel; ²Cell Biology Division, MRC Laboratory of Molecular Biology, Cambridge, UK

DEG-3 is a nicotinic acetylcholine receptor subunit that can mutate to cause neuronal degeneration. DEG-3 and DES-2 are subunits of the DEG-3/DES-2 nAChR that is expressed in several sensory neurons including PVD. PVD neurons are multi-dendritic poly-modal nociceptors that mediate responses to harsh touch and to cold temperatures and that also regulate the animal's movement and posture. DEG-2 staining and a functional DES-2::GFP fusion show that the DEG-3/DES-2 receptor decorates the entire sensory arbor of PVD. To understand the role of DEG-3 and DES-2 in the sensory dendrites of PVD neurons we examined phenotypes of mutations affecting these genes. Analysis of these mutants reveals similar phenotypes to these of animals lacking PVD suggesting an important role for the DEG-3/DES-2 receptor in PVD function. Moreover, calcium-imaging shows reduced responses of mutants to harsh touch. Thus the DEG-3/DES-2 receptor appears to affect the sensitivity of PVD to sensory stimulus. In addition analysis of PVD morphology shows a late onset defect in morphology of PVD arbors, suggesting a role for this receptor in maintaining PVD morphology. Overall our work demonstrates a central role for a nicotinic acetylcholine receptor in the function and structural integrity of a poly-modal nociceptor.

Identifying the Effectors that Enable RHO-1 to Stimulate Neuronal Activity

Muna Elmi, Andrew Porter, Rachel McMullan, Stephen Nurrish

MRC Laboratory of Molecular Cell Biology, University College London, UK

RHO-1, the *C.elegans* homologue of mammalian RhoA, regulates a wide variety of signal transduction pathways. In adult animals, RHO-1 is involved in neuronal activity stimulating neurotransmitter release at synapses. We have previously shown that in cholinergic motoneurons expression of constitutively activated RHO-1 (caRHO-1), induces the accumulation of diacylglycerol by inhibition of diacylglycerol kinase 1 (DGK-1), resulting in increased ACh release and a faster movement with a loopy phenotype. Although DGK-1 inhibition is important for RHO-1 signaling expression of a caRHO-1 mutant unable to bind DGK-1 can still stimulate ACh release and loopy locomotion. This suggests other downstream pathway(s) for RHO-1 signaling.

In order to identify other targets of RHO-1 we have conducted a genetic screen for suppressors of caRHO-1 that no longer have the loopy phenotype. Animals with caRHO-1 were EMS mutagenized and non-loopy mutant animals were selected. As a secondary screen we confirmed that our mutants could suppress loopy locomotion from caRHO-1 expressed from the heat-shock promoter. Heat-shock expressed caRHO-1 also has effects outside of the nervous system including a dar (deformed anal region) phenotype. We selected those mutants able to suppress the loopy locomotion but not the dar phenotype caused by caRHO-1.

From this suppressor screen we have identified 12 strong suppressors. One mutant, *nz94*, has a mutation in the *unc-80* gene causing a premature stop. UNC-80 along with UNC-79 are important regulators of the neuronal ion channel NCA-1/2. Mutations in any of these genes suppress the caRHO-1-induced loopy locomotion, while expression of UNC-80 in the motor neurons is sufficient to rescue the phenotype in *nz94* mutants. A model by which RHO-1 could alter activity of the NCA-1/2 ions channels will be presented.

Another suppressor mutant identified in the screen, *nz110*, was subjected to whole genome sequencing to identify possible downstream targets of RHO-1. One of the hits from the list of mutated genes is *unc-31*, which functions in the synaptic release of dense core vesicles. In our mutant suppressor, there is a missense mutation in the MHD domain, which is believed to be required for the interaction with syntaxin. UNC-31 cDNA-rescue experiments of *nz110* are currently underway in our lab.

Our results lead us to propose that both ion channels and neuropeptide release are required downstream of RHO-1 for it to alter locomotion behaviour.

***C. elegans* Identifies a Novel Insecticide Target**

A. Sluder², R. Clover², S. Shah¹, M. She¹, L. Hirst¹, P. Cutler¹, T. Flury³, C. Stanger¹, **A. Flemming¹**, F. Earley¹, E. Hillesheim⁵ and L.-P. Molleyres⁴.

¹Department of Bioscience, Syngenta, Bracknell, Berkshire, United Kingdom; ²Cambria Biosciences, Woburn, MA, United States; ³Biochemistry, Syngenta, Basel, Switzerland; ⁴Chemistry, Syngenta, Stein, Switzerland; ⁵Biology, Syngenta, Stein, Switzerland

Spiroindolines, an exploratory insecticide class, were shown to induce coiling symptomology in the nematode *Caenorhabditis elegans*, which is characteristic of some genetic lesions affecting neuromuscular function. A genetic screen identified mutations giving rise to resistance which mapped to a single gene. Overexpression of wild type or variant forms of the homologous gene in *Drosophila melanogaster* also conferred resistance to Spiroindolines. Expression of this gene in cell culture generated a high affinity binding site for Spiroindolines with characteristics very similar to that seen in insect tissues.

Analysis of Cysteine String Protein Function in *Caenorhabditis elegans*

James R. Johnson, Robert C. Jenn, Jeff W. Barclay, Robert D. Burgoyne, and Alan Morgan

The Physiological Laboratory, Department of Physiology, University of Liverpool, L69 3BX

Cysteine string protein (CSP) is an evolutionarily conserved synaptic vesicle protein. In both *Drosophila* and mouse, CSP deletion mutants are characterised by impaired SNARE complex assembly, severe pre-synaptic neurodegeneration and premature mortality. These phenotypes support the proposal that CSP functions as a pre-synaptic co-chaperone through a DNA-J domain-dependent interaction with the ubiquitous chaperone Hsc70 to maintain the integrity of exocytotic proteins at the synapse. Consistent with this, we find that either RNAi knock-down or deletion of the single CSP homologue in *C. elegans*, *dnj-14*, result in a reduced lifespan of the animal, with knock-out worms living approximately half as long as wild-types. Compared to wild-type worms, *dnj-14*-null animals showed a significant reduction in locomotion (a behavioural read-out of synaptic activity). This was partially overcome by raising the external Ca^{2+} concentration, as seen in both fly and mouse models. Furthermore, this defect in locomotion was found to be highly age-dependent. This corresponded with a severe age-dependent reduction in neuronal health and neuro-muscular synaptic activity, as indicated by a pan-neuronal GFP marker and aldicarb assays respectively, in *dnj-14*-null worms. These data suggest that, like its CSP homologues, DNJ-14 plays a critical role in the maintenance of pre-synaptic exocytotic machinery via Hsc70 and that its loss leads to impaired synaptic activity, severe neurodegeneration, and increased mortality. This may provide us with a new model with which to understand the mechanisms of age-dependent neurodegeneration.

Mitochondrial Distributions in Neurons are Regulated by Microtubules

Guruprasada Sure, Eva Romero, Anjali Awasthi, Sudip Mondal, **Sandhya P. Koushika**

Neurobiology, NCBS-TIFR, Bellary Road, Bangalore-560065

Mitochondria are important for neuronal function partly due to their ability to generate ATP and buffer calcium at synapses. However, the volume of mitochondria at synaptic regions is smaller than the volume along the axon, highlighting the importance of axonal mitochondria. We studied the distribution of mitochondria in touch receptor neurons of *C. elegans* using a transgenic strain that marks the matrix of all mitochondria with GFP. Using this strain we observed a linear correlation of mitochondrial number with the length of the neuronal process and established that distributions of mitochondria along the neuronal process are not random. The distributions are regulated such that two neighbouring mitochondria maintain a certain minimum distance between them. We show that number of mitochondria along the axon is regulated by the activity of the Kinesin-I and dynein motors. The distributions of these fewer mitochondria in Kinesin-I mutants remain close to those observed in wild type neurons. By comparison, in microtubule mutants the number of mitochondria in the axon increases and their distributions become more random. Namely, parts of these distributions in microtubule mutants can be fit to random distributions obtained by simulations. Analysis of electron micrographs of wild type touch receptor neurons reveals the presence of filamentous structures that connect mitochondria to both microtubules as well as to the plasma membrane. These filamentous structures may underlie the regulated distributions observed in neurons. We also observe that mutants that have many inappropriately positioned of mitochondria in axons have quicker desensitization to gentle touch stimulation, suggesting a role for axonal mitochondria in neuronal function.

Small Heat Shock Proteins Protect Against Necrotic Cell Death

Nikos Kourtis and Nektarios Tavernarakis

Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Heraklion, Crete, GREECE

Necrotic cell death contributes to severe pathological conditions in humans such as trauma, stroke and neurodegenerative diseases. The molecular mechanisms underlying necrosis are not fully understood. The heat shock response is a highly conserved gene expression program, which is engaged under conditions of stress and orchestrates the coordinated expression of specific genes that protect cells against various stressors. We are investigating the role of the heat shock response in necrotic cell death. We find that activation of the heat shock response pathway by means of a brief heat shock treatment strongly suppresses necrotic cell death caused by harsh environmental conditions, toxic channels, or hypoxia, in *C. elegans*. This protective effect is not due to delay of necrosis initiation or removal of the necrosis initiating insult. Elimination of heat shock factor 1 (HSF-1), the master transcription regulator which orchestrates the heat shock response in *C. elegans*, abolishes the protective effect of heat shock. By contrast, overexpression of HSF-1 suppresses necrosis. While screening for potential mediators of the protective effect of heat shock, we found that the gene encoding for the small heat shock protein HSP-16.1 is specifically required for the protective effect of heat shock on necrosis. Moreover, overexpression of HSP-16.1 provides protection against necrotic cell death and circumvents the requirement for heat shock response activation. Further characterization of the protective action of the small heat shock proteins revealed that those proteins modulate calcium release from the Golgi apparatus. Elucidation of the protective mechanism of the heat shock response and HSP-16.1 in necrosis may facilitate the development of intervention strategies aiming to counter necrotic cell death.

The *C. elegans* Kinesin Motor UNC-104 is Degraded upon Loss of Specific Binding to Cargo

Jitendra Kumar, Bikash Chowdhary, Raghu Metpally, Sowdhamini Ramanathan, Qun Zheng, Michael L. Nonet, Dieter R. Klopfenstein, **Sandhya P. Koushika**

Neurobiology, NCBS-TIFR, Bellary Road, Bangalore-560065

UNC-104 is a Kinesin-3 motor that transports synaptic vesicles from the cell body towards the synapse by binding to PI(4,5)P₂ through its PH-domain. The fate of the motor upon reaching the synapse is not known. We found that wild type UNC-104 is degraded at synaptic regions through the ubiquitin pathway and is not retrogradely transported back to the cell body. As a possible means to regulate the motor, we tested the effect of cargo binding on UNC-104 levels. The *unc-104(e1265)* allele carries a point mutation (D1497N) in the PI(4,5)P₂ binding pocket of the PH-domain, resulting in greatly reduced preferential binding to PI(4,5)P₂ *in vitro* and presence of very few motors on pre-synaptic vesicles *in vivo*. *unc-104(e1265)* animals have poor locomotion irrespective of *in vivo* PI(4,5)P₂ levels due to reduced anterograde transport. Moreover, they show highly reduced levels of UNC-104 *in vivo*. To confirm that loss of cargo binding specificity reduces motor levels, we isolated two intragenic suppressors with compensatory mutations within the PH domain. These show partial restoration of *in vitro* PI(4,5)P₂ binding specificity and presence of more motors on pre-synaptic vesicles *in vivo*. These animals show improved locomotion dependent on *in vivo* PI(4,5)P₂ levels, increased anterograde transport and partial restoration of UNC-104 protein levels *in vivo*. For further proof we mutated a conserved residue in one suppressor background. The PH domain in this triple mutant lacked *in vitro* PI(4,5)P₂ binding specificity and the animals again showed locomotory defects and reduced motor levels. All allelic variants show increased UNC-104 levels upon blocking the ubiquitin pathway. These data show that inability to bind cargo can target motors for degradation. In view of the observed degradation of wild type UNC-104 in synaptic regions, this further suggests that wild type UNC-104 motors may get degraded at synapses upon release of cargo.

The Contactin RIG-6 Mediates Axon Outgrowth and Navigation in *C. elegans*

Markella Katidou^{1,2}, Nektarios Tavernarakis¹ and Domna Karagogeos^{1,2}

¹Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Crete, Greece; ²University of Crete, School of Medicine, Department of Basic Sciences, Crete, Greece

Immunoglobulin superfamily Cell Adhesion Molecules (IgCAMs) are key regulators of nervous system development. The contactin subgroup of IgCAMs consists of GPI-anchored glycoproteins implicated in axon outgrowth, guidance, fasciculation and neuronal differentiation. The intracellular mechanism by which contactins facilitate neuronal development is not understood. To gain insight into the function of contactins, we characterized RIG-6, the sole contactin of *C. elegans*. Here, we show that RIG-6 affects longitudinal axon outgrowth of mechanosensory neurons in a cell autonomous manner. Furthermore RIG-6 is implicated in axon guidance along the circumferential axis. We find that RIG-6 facilitates accurate nervous system patterning by prohibiting commissural dendrite convergence and branching, as well as axon cross-over at the ventral nerve cord. RIG-6 expression levels are critical for nervous system architecture. Upregulation of this contactin causes several behavioral deficits such as abnormal locomotion, egg laying and defecation. In addition to its neuronal function, RIG-6 is also involved in non-neuronal cell migration and morphogenesis. Our data suggest that RIG-6 and the cytoplasmic protein UNC-53 synergize to direct axon guidance and branching along both the anterior-posterior and dorso-ventral direction. This implies that UNC-53/NAV2 proteins may contribute to relay signaling via contactins.

Mechanism of Dendritic Arborization

Meital Oren-Suissa¹, David H. Hall², Millet Treinin³, Gidi Shemer^{1,4} and Benjamin Podbilewicz¹

¹Department of Biology, Technion-Israel Institute of Technology, Haifa 32000, Israel; ²Center for *C. elegans* Anatomy, Dominick P. Purpura Department of Neuroscience Albert Einstein College of Medicine, Bronx, NY 10461, USA; ³Department of Physiology, Hebrew University-Hadassah Medical School, Jerusalem 91120, Israel; ⁴Current address: Department of Biology, University of North Carolina at Chapel Hill 27599, USA

To explore how dendrites form trees (arbors) we use dynamic live imaging of *C. elegans* neurons. Recent studies showed that two PVDs and two FLPs neurons have complex dendritic morphology that was initially missed by TEM serial sections. We show that the homotypic cell-cell fusion transmembrane fusogen protein, EFF-1, restricts and maintains a stereotyped pattern of repetitive branching of two PVDs essential for reception of strong mechanical stimuli. *eff-1* mutants produce neurons with disorganized and hyperbranched structural units reminiscent of candelabra we term menorahs. When expressed in the PVDs, EFF-1 is sufficient to reduce the number of branches and to rescue disorganized menorahs. We found that EFF-1 controls the balance between the number, structure and function of menorahs mainly by stimulating the retraction of defective outgrowth, but also by fusing abnormal branches and forming loops that restrict further growth. We show that EFF-1 mediates auto-cell fusion, bends dendrites, and maintains complex branched neuronal structures.

In addition to *eff-1*-dependant loop formation, we found that multiphoton laser nanosurgery of dendrites induces regeneration by fusion between severed neurites. In cases where broken neurites fail to fuse, compensatory sprouting and menorah-menorah fusion can overcome degeneration. Thus, fusion emerges as a quality control mechanism that acts to limit growth and repair severed dendrites.

We show that loss-of-function mutations in *fmi-1* (seven-pass cadherin), *sax-7* (L1 cell adhesion molecule) and *nhr-25* (nuclear hormone receptor) result in impaired menorah formation. *fmi-1* and *nhr-25* act to stimulate arborization, whereas *sax-7* is required for primary outgrowth, and proper localization of mature dendrites.

Thus, the fusogen EFF-1, two neuronal adhesion proteins and a nuclear hormone receptor are essential for dendritic arborization and detection of strong mechanical stimuli. We have uncovered genetic pathways that drive the generation and maintenance of complex stereotyped arborized nociceptors in *C. elegans*.

Regulation of the Molecular Motor UNC-104(KIF1A) in the Nervous System of *C. elegans*

Che-Piao Shen, Gong-Her Wu, Chih-Wei Chen, Ying-Cheng Yan, Yu-Shin Huang, Chien-Yu Chang and **Oliver I. Wagner**

National Tsing Hua University, Institute of Molecular and Cellular Biology & Department of Life Science, Hsinchu, Taiwan (R.O.C.)

Neuronal axons are densely packed with tubulovesicular structures, cytoskeletal scaffolds and molecular motors bound to synaptic precursors. Considering the extensive length and small diameter of axons coupled with the amount of material that must be transported, it is not surprising that disruption in trafficking lead to defects causing numerous neurodegenerative diseases. Many of these neurodegenerative diseases are related to defects in microtubule-based motors and their cargos. *C. elegans* kinesin-3 motor UNC-104(KIF1A) is essential for transporting synaptic precursors to synapses while only little is known how motor activity is regulated. We have shown recently that the active zone protein SYD-2(liprin- α) acts as both, a cargo and a UNC-104 motor regulator (Wagner et al., 2009, PNAS). As SYD-2 exhibits a LIN-2(CASK) and a UNC-10(RIM) binding site we now investigate the effect of these two active zone proteins on UNC-104's transport characteristics. We present data how *lin-2* and *unc-10* mutations affect UNC-104's axonal motility (and its cargo synaptobrevin-1) as well as axonal clustering. We further provide yeast-two hybrid data on specific interactions between LIN-2 and UNC-104.

Another important question is how the bidirectional movement of UNC-104 can be explained. Models include cooperative interactions between kinesins and dynein or by the so called tug-of-war model. To understand the role of dynein and dynactin we analyzed UNC-104::GFP motility in worms carrying either mutations in the dynein (DHC-1) or dynactin (DNC-1) gene. Interestingly, both, motor and cargo seemed to have reduced speeds in these mutants. We also analyze the distribution pattern of UNC-104 and SNB-1 along the neurons and found that both motor and vesicle tend to accumulate along the axons. We thus hypothesize a functional interaction between UNC-104 and dynein/dynactin. Indeed, yeast-two hybrid assays revealed interactions between UNC-104 and the dynein Tctex-1 light chain (Dylt-1), roadblock light chain (Dyrb-1), and p27 domains.

A final question is whether and how microtubule binding proteins affect UNC-104's axonal movement. Interestingly, in PTL-1(tau) knock-out worms, the motility of UNC-104 is critically affected: more motor reversals for retrograde movements are observed and at the same time more pausing events for retrograde movements can be seen (compared to wildtype). Moreover, UNC-104 and PTL-1 co-localize and even co-migrate in the nervous system of living animals, suggesting that PTL-1 might be a cargo of UNC-104.

Frataxin Suppression Reduces Fat Accumulation and Induces Autophagy in a p53-dependent Manner, Independently of a Caloric Restriction-like Response

Schiavi A¹, Torgovnick A¹, Megalou EV², Tavernarakis N², Testi R¹ and Ventura N¹

¹Department of Experimental Medicine and Biochemical Science, University of Rome Tor Vergata, Rome, Italy; ²IMBB, Foundation for Research and Technology, Heraklion 71110, Crete, Greece

Friedreich's Ataxia (FRDA), the most frequent inherited ataxia, is ascribed to severely defective expression of frataxin, a nuclear-encoded mitochondrial protein that plays a role in the biogenesis of Fe-S cluster containing proteins. Despite significant information have been gathered so far about frataxin structure and function, current therapy for patients affected by FRDA rely mostly upon the disputable use of antioxidants. Additional basic information particularly on the role of frataxin in cell survival is warranted to help in the design of more specific therapeutic approaches. Autophagy is a form of cellular self-digestion that has been associated both with prevention and causation of neurodegenerative diseases in human, and it is regulated by p53 [1, 2]. Autophagic genes are required in *C. elegans* for normal development and to increase lifespan in different genetic backgrounds, including *cep-1* (*C. elegans* p53 ortholog) mutant animals [3, 4]. We have now found that interfering with the *C. elegans* frataxin homolog, *frh-1*, prevents maximal induction of autophagy elicited in the p53/*cep-1* mutants.

Utilizing *C. elegans* as a model system, we had previously shown that *frh-1* RNAi prolongs animal lifespan in a p53/*cep-1* dependent manner [5, 6]. Of note, ablation or functional alterations of specific sensory neurons also extend *C. elegans* lifespan [7], and we have now found that *frh-1* RNAi animals display behavioral alterations ascribed to deficit of those same neurons. *frh-1* RNAi treated animals look smaller and paler than control untreated animals and, interestingly, we have now found that they also display increased autophagy and decreased fat accumulation. These features are independent from a caloric restriction-like response and require an intact p53/*cep-1* gene. It will be interesting to test whether we can pharmacologically exploit the autophagic pathway to prevent or postpone neuronal defect in *frh-1* suppressed animals.

1. Levine, B. and G. Kroemer, *Autophagy in the pathogenesis of disease*. Cell, 2008. **132**(1): p. 27-42.
2. Tasdemir, E., et al., *A dual role of p53 in the control of autophagy*. Autophagy, 2008. **4**(6): p. 810-4.
3. Melendez, A., et al., *Autophagy genes are essential for dauer development and life-span extension in C. elegans*. Science, 2003. **301**(5638): p. 1387-91.
4. Tavernarakis, N., et al., *The effects of p53 on whole organism longevity are mediated by autophagy*. Autophagy, 2008. **4**(7).
5. Ventura, N., et al., *Reduced expression of frataxin extends the lifespan of Caenorhabditis elegans*. Aging Cell, 2005. **4**(2): p. 109-12.
6. Ventura, N., et al., *p53/CEP-1 increases or decreases lifespan, depending on level of mitochondrial bioenergetic stress*. Aging Cell, 2009. **8**(4): p. 380-93.
7. Apfeld, J. and C. Kenyon, *Regulation of lifespan by sensory perception in Caenorhabditis elegans*. Nature, 1999. **402**(6763): p. 804-9.

Transgenic Nematodes as a Model for Parkinson's Disease

Rakesh K Bodhicharla, Jody Winter and David de Pomerai

University of Nottingham

Aggregation of the abundant neural protein α -synuclein contributes to cellular toxicity in Parkinson's disease. We have created transgenic nematodes carrying fusion constructs encoding human α -synuclein (S) tagged with YFP (V) and/or CFP (C) as a fluorescent marker. Using the *unc-54* myosin promoter, a synuclein-YFP (*unc-54::SV*) fusion construct is abundantly expressed in the body wall muscles of *Caenorhabditis elegans*. Permanent integrated lines were successfully generated for *unc-54::V*, *unc-54::S+unc-54::V*, *unc-54::SC+unc-54::SV*, *unc-54::C+unc-54::V*, and *unc-54::CV* using gamma irradiation. Transgenic synuclein strains are radiation sensitive and have a shorter life span and lower pharyngeal pumping compared to wild type N2 and *unc-54::V* worms. Fluorescence Resonance Energy Transfer (FRET) was measured for all the transgenic strains. The *unc-54::SC+unc-54::SV* worms showed FRET signals intermediate between the negative (*unc-54::C+unc-54::V*) and positive (*unc-54::CV*) control strains. FRET signals increase markedly during early adult life in *unc-54::SC+unc-54::SV* worms. RNA interference by feeding was done in *unc-54::SC+unc-54::SV* worms to knock out the HIP-1 co-chaperone function, thereby increasing the FRET signal. Pesticides such as chlorpyrifos and rotenone were tested with *unc-54::SC+unc-54::SV* fusion worms and both resulted in an increase in the size and intensity of fluorescent aggregates. Finally we have measured oxidative stress in *unc-54::SC+unc-54::SV* worms; chlorpyrifos induction of ROS was found to be very high whereas the effect of rotenone was smaller and not statistically significant. We are currently investigating apoptosis and immunohistochemistry in the fusion strains.

Identification of *Ascr1* as a Gender Specific Mate-finding Cue in the Free-living Nematode *Panagrellus redivivus*

Andrea Choe¹, Aaron T. Dossey², Tatsuji Chuman², Ramadan Ajredini², Hans Alborn³, Fatma Kaplan³, Frank C. Schroeder⁴, Arthur S. Edison² & Paul W. Sternberg¹

¹HHMI and Dept of Biology, Caltech; ²Dept of Biochemistry & Molecular Biology, University of Florida ³USDA Laboratory, ⁴Boyce Thompson Institute, Cornell University

Nematodes respond to various forms of stimuli in order to help them create an appropriate physiological or behavioral response to their environment. Chemical communication has been studied in the free-living nematode *Caenorhabditis elegans* and several plant-parasitic nematodes, helping us to understand how chemical cues relay information about population density and mate availability. The first pheromone identified in *C. elegans* is the ascaroside *ascr#1*, also known as “daumone” for its role in the entry into dauer, a stress-driven stage of developmental arrest. Since then, other ascarosides have been identified in the *C. elegans* and found to play a role in both dauer formation and mate-specific attraction.

Panagrellus redivivus is a free-living gonochoristic nematode that serves as a useful comparative model to the hermaphroditic species *C. elegans*. It has been studied for decades and is the first nematode species in which both male-specific and female-specific attraction has been observed, however the chemical nature of this interaction has been unknown. We have developed a robust bioassay in which nematode attraction and repulsion are scored with an automated program. In addition to this, we have used activity-guided fractionation of *P. redivivus* liquid cultures, in combination with NMR and LCMS analysis, to identify gender-specific sex pheromones.

Here we report the isolation of natural *ascr#1* from the free-living nematode *P. redivivus* by large-scale purification. This is the same pheromone that plays a small role in dauer-formation in *C. elegans*, but does not play any role in *C. elegans* male or hermaphrodite attraction. We have evidence that *ascr#1* not only attracts *P. redivivus* males, but also repels *P. redivivus* females, indicating that the same pheromone can be used across multiple genera for different purposes both between species and within species. Because it is becoming increasingly clear that ascarosides play a conserved role in nematode physiology/behavior, these findings may help to reveal novel approaches to parasitic control.

The Neuronal Circuit for the Thermal Avoidance Response in *Caenorhabditis elegans*

Shu Liu^{1,2,3}, Ekkehard Schulze^{1,2,3}, Ralf Baumeister^{1,2,3,4}

¹Bioinformatics and Molecular Genetics (Faculty of Biology), ²Center for Biochemistry and Molecular Cell Research (Faculty of Medicine), ³Center for Systems Biology (ZBSA), ⁴FRIAS Freiburg Institute of Advanced Studies, School of Life Sciences (LIFENET), Albert-Ludwigs-University Freiburg, Schänzlestr. 1, D-79104 Freiburg i. Brsg., Germany

Upon exposure to noxious heat, *Caenorhabditis elegans* executes an escape reflex. This response, termed thermal avoidance (Tav) response, is essential for protecting the worms from heat damage. We had previously shown that sensory neurons in the head and tail, but not in the midbody region, are involved in the perception of heat¹. Here we use behavioral analysis, neuron-ablation and Ca²⁺ imaging to demonstrate that *C. elegans* senses noxious heat stimuli using at least three different types of sensory neurons, namely the FLP neurons and the temperature-sensing AFD neurons in the head, and the PHC sensory neurons in the tail. These results together with the well-known function of AFD in thermotaxis² suggest that the AFD neurons may be polymodal neurons mediating the sensation of and response to distinct external stimuli including physiological and non-physiological temperatures.

Further, we want to address the question which interneurons are used to transmit the signal. It has been shown that the AFD thermosensory neurons and the AIY interneurons form the neuronal circuit that is needed for a functional thermotaxis behavior in *C. elegans*³. Our genetic and neuron-ablation data indicate that *C. elegans* uses a different neuronal circuit for the detection of noxious heat than for the sensation of temperatures within the physiological range.

Altogether, our data suggest that at least two different classes of head sensory neurons AFD and FLP act partially redundant in mediating the avoidance response upon noxious temperature. Moreover, the tail Tav response is mainly mediated by a single pair of tail neurons PHC. Therefore, in contrast to the response of *C. elegans* to physiological temperatures, which is mediated mainly by the AFD-AIY neuronal circuit⁴, the potentially life-saving reaction to noxious temperatures is much more complex and well backed-up.

1. Wittenburg, N. & Baumeister, R. Thermal avoidance in *Caenorhabditis elegans*: an approach to the study of nociception. *Proc Natl Acad Sci U S A* **96**, 10477-10482 (1999).
2. Kimura, K.D., Miyawaki, A., Matsumoto, K. & Mori, I. The *C. elegans* thermosensory neuron AFD responds to warming. *Curr Biol* **14**, 1291-1295 (2004).
3. Satterlee, J.S., *et al.* Specification of thermosensory neuron fate in *C. elegans* requires *ttx-1*, a homolog of *otd/Otx*. *Neuron* **31**, 943-956 (2001).
4. Kuhara, A., *et al.* Temperature sensing by an olfactory neuron in a circuit controlling behavior of *C. elegans*. *Science* **320**, 803-807 (2008).

A Computational Model of Associative Learning and Chemotaxis in the Nematode Worm *C. elegans*

Peter A. Appleby and Netta Cohen

School of Computing, University of Leeds, Leeds LS2 9JT, United Kingdom,

An important and fundamental challenge is to understand how the effectively hard wired circuitry in *C. elegans* generates the complex behaviors that the worm exhibits. This challenge is particularly interesting in the context of circuits that serve multiple functions (e.g. multi-modal sensory integration) and that undergo plasticity or learning in order to modulate its behavioral strategies in response to changing environments. One such circuit is the relatively well characterized chemotaxis circuit in the head. In chemotaxis *C. elegans* will move up or down a chemical gradient dependent on whether the chemical acts as an attractant or repellent. It does this by a combination of two navigational strategies: (i) gradually steering left or right until the worm points up or down the gradient and (ii) modulating the probability of so-called pirouettes and choosing the final orientation of the worm after the pirouette has finished. A large body of work has shown that the chemotaxis response is dynamic and that the degree of influence a particular chemical has on navigation can be changed, or even reversed depending on experience. Changes are reversible, specific to the chemical in question, and can be generated by classical conditioning experiments. All of these are hallmarks of associative learning, a sophisticated process that requires integration of multiple signals to produce a coordinated change in a behavioral response.

Despite the wealth of experimental data on learning in chemotaxis in *C. elegans*, comparatively little is known about how the known circuitry of *C. elegans* carries out the computations that underlie it. Even less is known about how that circuitry changes during associative learning. We focus on the worm's chemotaxis and the ability of *C. elegans* to learn associations between salt (NaCl) concentrations and food. We draw upon existing experimental data from a variety of sources including electrophysiological and anatomical data to construct a simplified NaCl chemotaxis circuit in *C. elegans*. The circuit include the two key NaCl sensory neurons ASEL and ASER, key chemotaxis interneurons, head motor neurons and locomotion command neurons AVB and AVA. The properties of individual neurons in this network are constrained by results from electrophysiological and calcium imaging experiments. We also define a set of experimentally observed behaviors we wish to reproduce, including gentle turning, modulation of reversals and pirouette frequency, control of final orientation following a pirouette, and associative learning. In particular, we are interested in the alteration in behavioral response to NaCl that arises due to the pairing of high concentrations of NaCl with food or starvation. We use this to derive a family of model networks with the capacity to generate the specified set of behaviors. We implement one of these models and show that when model worms are placed in a simulated environment we observe qualitatively realistic chemotaxis behavior and adaptation and demonstrate that our model is robust and tolerant to noise.

Our proposed chemotaxis circuit leads to a number of distinct predictions that could be used to test the model experimentally. This includes postulating the computational role of each neuron in the (simplified) network and the locus and nature of the plasticity underpinning the experimentally observed associative learning. Our model also suggests that this plasticity be expressed not by changes in synaptic strength but by changes in the sensory neurons themselves. Plasticity in our model of chemotaxis is therefore expressed at a neuronal rather than synaptic level.

Microfluidic Devices for Neuronal Transport Imaging in *C. elegans*

Sudip Mondal¹, Sikha Ahlawat¹, V. Venkataraman², Sandhya P. Koushika¹

¹National Centre for Biological Sciences, Bangalore, India; ²Department of Physics, IISc, Bangalore, India

Microfluidic devices can provide important engineering solutions to address a number of biological questions. We have focused our attention on the problem of neuronal transport in the *C. elegans* model to develop an easily accessible platform of imaging multiple biological processes. We have validated the device and its utility to ensure that biologists interested in tracking sub-cellular high resolution processes can do so easily upto an hour. Axonal transport is an important process for neuronal function since most molecules and organelles are synthesized in the cell body and require transport to reach a major functional unit of neurons, 'synapses'. Conventionally transport has been studied using anesthetized or glued animals which have anecdotally been reported to have detrimental effects. Studying *in vivo* transport using live organism requires the ability to track sub-cellular organelles in immobilized organism. In this study we have developed an anesthetic free imaging assay using microfluidic devices.

PDMS devices, fabricated using soft-lithography techniques, have been used to image axonal, intraflagellar and intra dendritic transport in *C. elegans*. These devices are based on the principle of membrane deflection where an individual worm is immobilized using a thin layer of PDMS deflected using pressurized gas. We validated the use of the device in studying transport of three different cargoes GFP::RAB-3, OSM-6::GFP and ODR-10::GFP. In all three cases anesthetics have concentration dependent effects on transport. Further imaging early developmental stages was not possible in anesthetic preparations. Using microfluidic devices we overcome both these problems. Data generated using our microfluidic device show that synaptic vesicle flux increases throughout development and are more processive which is consistent with the increase in the microtubule numbers and density during the development. The net increase in the flux towards the synapse was found to scale with the accumulation of synaptic GFP::RAB-3 fluorescence.

In summary we have developed PDMS microfluidic device that allows us to perform anesthetic free imaging of *C. elegans* over all developmental stages and various neuronal processes. The device has also been demonstrated for fluorescence imaging of *Drosophila* larvae and can be extended to other transparent model systems.

In vivo* Optical Projection Tomography (OPT) Imaging in *Caenorhabditis elegans

Matthias Rieckher¹, Udo Birk², Jorge Ripoll² and Nektarios Tavernarakis¹

¹Institute of Molecular Biology and Biotechnology and ²Institute of Electronic Structure and Laser, ^{1,2}Foundation of Research and Technology-Hellas, Heraklion Crete, Greece

Optical projection tomography (OPT) is a recently developed technology for 3D imaging that has emerged as a powerful tool for 3D reconstruction and visualization. This is achieved by applying a filtered back projection algorithm on images taken from equidistant angles of a rotating small specimen 1-10 mm across, as previously shown for zebra fish, chick and mouse embryos.

We describe a highly adjustable microscopic OPT system for three-dimensional imaging of live *C. elegans* animals, which offers significant advantages over currently available methods when imaging dynamic developmental processes and animal ageing. We verify its capacity to image live transgenic *C. elegans*, carrying green fluorescent protein (GFP) reporter fusion expressed in pharyngeal muscle tissue or in the 6 mechanosensory neurons, respectively. We show the ability for high-resolution 3D reconstruction at the level of single neuronal cells including their processes. We demonstrate that combining absorption and fluorescence images reveal dependable anatomical information about gene expression patterns. Additionally, we reveal the power of *in silico* 2D sectioning of the specimen as well as for volumetric 3D rendering of specific parts or of the entire animal.

The presented OPT system is simple, cost effective and permits monitoring of spatio-temporal gene expression and anatomical alterations with single-cell resolution, it utilizes both fluorescence and absorption as a source of contrast. The setup is easily adaptable for other small model organisms and can readily be equipped with microfluidic systems that facilitate high throughput OPT of large amounts of live animals.

Abstracts
Poster Presentations

Mutagenic Screen for Rescue of Neurotransmission and Locomotion Defects in *unc-18 (e81)* Null Alleles

Victoria Martin and Jeff W. Barclay

Institute of Translational Medicine, University of Liverpool, Liverpool, UK

Neurotransmitters are released from vesicles in presynaptic nerve terminals by the process of exocytosis. Membrane-membrane fusion is driven by the formation of the SNARE complex of proteins; however, other accessory proteins can be essential for exocytosis. Munc18-1/UNC-18 is one such critical presynaptic regulator of exocytosis primarily characterised via biochemical interactions with the SNARE protein syntaxin (UNC-64) or the assembled SNARE complex (UNC-64:SNB-1:RIC-4). Current evidence points to a central, evolutionarily-conserved role for UNC-18 in stimulating SNARE-directed vesicle fusion, although precisely how this is achieved is unclear. Here we describe the results from an unbiased genetic screen for suppressors of *unc-18* loss-of-function.

The *unc-18 (e81)* null allele carries a premature stop codon in the coding domain resulting in a complete loss of UNC-18 protein and worms that are uncoordinated and paralysed. We treated *e81* worms with a standard EMS mutagenic protocol and 2nd generation, homozygous progeny were examined for suppression of locomotion defects. From this screen we generated two distinct lines of rescued worms which were quantitatively analysed for locomotion and aldicarb sensitivity. Subsequently the phenotype of one line (*e81::resc01*) was determined to have been the result of a reversion in the original *e81* mutation. The other line (*e81::resc02*) however had no reversion nor any additional mutations in the *unc-18* gene. Phenotypically *e81::resc02* alleles had a similar aldicarb sensitivity profile to *e81* worms transgenically expressing wild type *unc-18*. Quantitative analysis of locomotion showed that rescues exhibited a wild type rate of locomotion with respect to body-bends on agar whereas rate of thrashing was only slightly improved. Video analysis also showed that *e81::resc02* alleles had an increased tendency to reverse which acted to localise worms to a smaller area in bacterial lawns of OP50. We are currently in the process of determining the genetic origin for the phenotype of the *e81::resc02* allele.

GTL-1 together with TRPA-1 Mediates PVD's Response to Cold Temperatures

Emiliano Cohen¹, Marios Chatzigeorgiou², Bill Schafer², Howard Baylis³ and Millet Treinin¹

¹Department of Medical Neurobiology, Institute for Medical Research Israel Canada, Hebrew University-Hadassah Medical School, Jerusalem, Israel. ²Cell Biology Division, MRC Laboratory of Molecular Biology, Cambridge, UK. ³Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK

PVD is a multi-dendritic polymodal nociceptor that senses both noxious and innocuous signals to regulate *C. elegans* behavior. Thus, it combines the functions of multiple mammalian somatosensory neurons. Among the many genes enriched in PVD, the following members of the Transient Receptor Potentiation (TRPs) were found to be highly expressed-*trp-2*, *gon-2* and *gtl-1*. The TRP family has important roles in both sensory (Vision, olfaction, nociception, temperature reception, taste and more) as well as in non-sensory pathways. Recently, it was shown that *trpA-1* plays a role in mediating the response to acute cold pain in *C. elegans*. In mammals, the response to cold pain is mediated by two members of the TRP family-TRPM8 (30-25°C) and TRPA1 (<17°C). *gtl-1* belongs to the TRPM sub-family and is homologous to TRPM8 as well as to TRPM6 and 7. Calcium imaging of *gtl-1* null mutants reveals that there is a decrease over time in the calcium levels in PVD after the worms were transferred from 25°C to 15°C, while in the wt worms it remains stable. Thus, *gtl-1* is required to maintain the response to chronic cold pain. In order to test whether this change in calcium levels corresponds with a behavioral defect, we compared the locomotive behavior of the wild-type *C. elegans* and the null mutant when transferred to a cold environment. The most significant differences identified were the percent of time that the worms are not moving (pause) and the rate of pauses. Rescue experiment confirm that this defect is due to lack of GTL-1 within PVD. Functional GFP tagged GTL-1 was found to be localized in the cell body, the primary branch and some secondary branches. Thus GTL-1 functions together with TRPA-1 to enable the response to cold temperatures.

Emodepside is a Resistance Breaking Anthelmintic Drug with Selective Toxicity to the Parasite's SLO-1 Calcium-activated Potassium Channel

Anna Crisford^A, Lindy Holden-Dye^A, Achim Harder^B, Vincent O'Connor^A, Robert Walker^A

^ASchool of Biological Sciences, University of Southampton, UK; ^BBAYER Healthcare AG, Monheim, Germany

The cyclooctadepsipeptide emodepside is a resistance breaking compound, effective against a wide range of gastrointestinal nematodes including *Haemonchus contortus* and *Trichostrongylus colubriformis* in sheep, *Cooperia oncophora* in cattle, *Toxocara cati*, *Toxascaris leonina*, *Ancylostoma tubaeforme* and cestodes in cats.

In the model genetic animal, the nematode *Caenorhabditis elegans*, emodepside inhibits neuromuscular function and thus impairs feeding, locomotion and egg-laying in dose dependent manner. Mutagenesis screening for worms resistant to the paralytic actions of emodepside identified a calcium-activated potassium channel SLO-1 as a mediator of the paralytic effects of emodepside. A reference allele for *slo-1*, *js379*, a predicted functional null, is highly resistant to emodepside. Transgenic *slo-1(js379)* animals expressing wild type copy of *slo-1* behind the native *Pslo-1* promoter have the same sensitivity to emodepside as wild type *C. elegans* with IC50 of 20nM.

slo-1 belongs to a family of highly conserved potassium channels that regulate cell excitability throughout animal phyla. Bioinformatic analysis revealed a human *kcnma1* channel as a closest mammalian homologue to nematode *slo-1*. SLO-1 and KCNMA1 share 55% identity and 69% similarity in primary amino acid sequences. To investigate selective toxicity of emodepside to the parasites we expressed a closest mammalian homologue *kcnma1* in *slo-1(js379)* mutants.

Behavioural assays and electropharyngeogram (EPG) recordings from the pharyngeal muscle of wild type and transgenic *C. elegans* confirmed that *kcnma1* is a functional homologue of *slo-1*. Conversely, transgenic worms carrying the mammalian homologue are not affected by 1µM and only slightly sensitive to 10µM emodepside in behavioural assays. In EPG assays these worms are slightly affected by 1µM emodepside. This suggests that emodepside is at least 10-100 folds more selective to the nematode SLO-1 channel.

Furthermore, to elucidate a mode of action of emodepside we characterised effects of known activators of mammalian BK channels, NS1619 and Rottlerin on locomotion of wild type and transgenic *C. elegans*. In behavioural assays *C. elegans* carrying *kcnma1*, but not *slo-1* or wild type were sensitive to NS1619 after 3 hours exposure and exhibited uncoordinated movement. Rottlerin slowed locomotion of all transgenics and wild type worms after 24 hours exposure. *slo-1(js379)* mutants were not sensitive to any of the drugs tested.

Taken together these data are most parsimoniously explained by a mode of action in which emodepside directly interacts with the nematode calcium-activated potassium channel *slo-1* and has very low affinity to its mammalian homologue *kcnma1*.

The Role of the *Caenorhabditis elegans* Globin Family in the Response to Hypoxia

De Henau Sasha, Braeckman Bart and Vanfleteren Jacques

Department of Biology and Center for Molecular Phylogeny and Evolution, Ghent University, B-9000 Ghent, Belgium

Globins are small globular proteins, usually consisting of about 140–150 amino acids that comprise eight α -helical segments (named A-H), displaying a characteristic 3-over-3 α -helical sandwich structure that encloses an iron-containing heme group. Besides a conventional O₂ storage and transport function, a wealth of diverse functions has been described for invertebrate globins.

Caenorhabditis elegans contains a surprisingly high number of globins, which are all expressed. These globins feature a remarkable diversity in gene structure, amino acid sequence and expression profiles.

C. elegans frequently encounters severe hypoxic conditions in its natural environment. We used real-time quantitative PCR (qPCR) to analyze the expression patterns of this globin family following a hypoxic stress condition. We found that oxygen shortage (0,1%, 0,5% and 1% O₂) modulates the expression of several globin-like genes.

We are currently analyzing the expression pattern of these hypoxia-modulated globin genes using transcriptional reporters. They are expressed in neuronal cells in the head and tail portions of the body, and the nerve cord. More detailed analysis showed that the majority occur in only a very limited number of neurons, and there is little overlap in expression between the different reporters.

In addition, we tested whether the sensitivity to hypoxia of this subset of globin genes is regulated by the transcription factor Hypoxia-Inducible Factor 1 (HIF-1), one of the key regulators of oxygen homeostasis. By using *hif-1* knockout mutants and *vhl-1* and *egl-9* mutants, which have continuously active HIF-1, we were able to demonstrate that several hypoxia sensitive globin genes are HIF-1 regulated. The remaining hypoxia-sensitive globin genes were still induced following hypoxia in *hif-1* mutants, suggesting a HIF-1 independent regulation of these globin genes during oxygen deprivation.

A Worm Model of Tauopathy

Chronis Fatouros¹, Jeelani Pir Ghulam², Eckhard Mandelkow², Eva-Maria Mandelkow², Enrico Schmidt¹, Ralf Baumeister¹

¹Institute of Biology III, ZBSA, Albert Ludwig University of Freiburg, Germany, ²Max Planck Unit for Structural Molecular Biology, 22607 Hamburg, Germany

Tau is a neuronal microtubule associated protein and is found mutated in a variety of neurodegenerative diseases. Under pathological conditions, Tau gets hyper-phosphorylated and forms intracellular aggregates. The deletion of K280 is a mutation that enhances the aggregation propensity of Tau moieties and is present in patients with Frontotemporal Dementia with Parkinsonism linked to chromosome 17 (FTDP-17). On the other hand, the substitution of 2 Isoleucines with beta-sheet breakers Prolines in the hexapeptide motifs confers the Tau molecules anti-aggregation properties. In this study, we established a *C. elegans* model of Tauopathy by expressing pro- or anti- aggregation Tau fragments from a panneuronal promoter, along with full length human Tau. This resulted in accelerated Tau pathology in the pro-aggregation transgenics, manifested by impaired motility already by one-day adults, neuronal defects such as axonal gaps and varicosities, as well as less pre-synaptic termini along the dorsal cord. In addition, preliminary data point to the fact that axonal transport of mitochondria is impaired in the pro-aggregation transgenics as well. The anti-aggregation animals show some mild defects only at older age. RNAi against Tau can revert these defects and a compound that was tested to have dissolving activity on Tau aggregates in vitro, has also a partial effect in our in vivo model. Having the thrashing behavior as a read-out, we set out on a genome-wide RNAi screen for suppressors of the Tau induced phenotype. We uncovered some novel potentially interesting genes that when knocked-down alleviate the Tau pathology. Further validation and investigation of these candidates is under way and a cross-test in a mammalian system will be essential.

Investigating the Role of UNC-31 in Glutamatergic Signaling in the Pharynx

Chris Franks, Caitriona Murray, David Ogden[¶], Vincent O'Connor and Lindy Holden-Dye

Institute of Life Sciences, University of Southampton, Life Sciences Building 85, Highfield campus, Southampton, SO17 1BJ, UK. [¶]Laboratoire de Physiologie Ce'ere'brale, UMR8118 Universite' Paris Descartes, 75006 Paris, France

We have expressed channelrhodopsin-2 (ChR2) in a subset of glutamatergic pharyngeal neurons (I5, M3, NSM, I2 expressing *peat-4::ChR2;mRFP*). They form part of a larger network of some 20 neurons and most of them synapse directly onto the muscle (I5, NSM, M3). However, they contain several different transmitters, including glutamate, 5-HT and neuropeptides. They also synapse with other cells in the network, including cholinergic motoneurons. Photo-activation of these cells would be likely to evoke compound post-synaptic potentials (PSPs) in the muscle. But, we reasoned that the glutamatergic contribution would likely form a significant part of the earliest phase of the PSP. We made voltage recordings from the muscle and our light stimulus (473nm, 1 ms) did indeed evoke compound PSPs which often reached threshold to elicit a superimposed action potential. Because of this we measured the initial slope of each event rather than amplitude for a readout of transmitter release.

To dissect out the glutamatergic element we used genetic and pharmacological approaches. We used benzoquinonium (50µM) to block muscle nicotinic receptors and manipulated external chloride concentration ($[Cl^-]_{out}$) to change the magnitude of chloride mediated events. The pharyngeal glutamate response is chloride mediated. In wild-type worms (N2) a decrease of 60% increased PSP slope from 0.32 ± 0.09 to 0.67 ± 0.15 , $n=10$). This confirmed the presence of a chloride-mediated event in the initial phase of the PSP. *eat-4* worms, lack the vesicular glutamate transporter and therefore glutamatergic transmission. In this mutant, reducing ($[Cl^-]_{out}$) did not significantly affect PSP slope (0.05 ± 0.03 in normal $[Cl^-]_{out}$ and 0.06 ± 0.04 in low $[Cl^-]_{out}$, $n=8$). Furthermore, the latency of PSPs was significantly longer in *eat-4* (4.73 ± 0.31 ms in N2 and 25.66 ± 1.05 ms in *eat-4*). PSPs in *eat-4* were not chloride sensitive and occurred at a different time indicating that they were not glutamatergic. We are currently assessing PSPs in *avr-15* worms which lack one subunit of the muscle glutamate-gated chloride channel. UNC-31 encodes the *C. elegans* ortholog of human CADPS/CAPS (calcium-dependent activator protein for secretion). It is thought to sub serve a role in regulating dense-core vesicle and therefore neuropeptide release. PSPs recorded from *unc-31* worms were qualitatively similar to those recorded in N2. *unc-31* PSP latency was 4.22 ± 0.30 ms ($n=9$) similar to N2 and reducing $[Cl^-]_{out}$ increased the PSP slope from 0.14 ± 0.03 to 0.35 ± 0.06 ($n=10$, $P < 0.05$). We conclude therefore that a chloride mediated PSP was present in *unc-31* although the control PSP slope was shallower than in N2. We are currently investigating PSPs recorded in *egl-3* pharynx which lacks the majority of neuropeptides.

This work was funded by the BBSRC.

Characterizing the Role of Prolyl Hydroxylation during Neuronal Development

Torpe, N and Pocock, R

BRIC – Biotech Research and Innovation Centre, University of Copenhagen, Ole Maaløes Vej 5, 2200 Copenhagen N, Denmark

In the developing nervous system of both vertebrates and invertebrates the guidance of the neuronal axonal growth cone is regulated by several signaling mechanisms and interactions with the extracellular matrix (ECM). We are interested in the identification of novel ECM modifiers that regulate axonal guidance. Through a screen of enzymes that modify the ECM we identified the collagen prolyl 4-hydroxylase (P4H) -subunit, DPY-18, as a new protein required for neuronal development in *C. elegans*. P4Hs are enzymes known to be involved in the synthesis of collagen and the *dpy-18* mutant is characterized by a short and fat (dumpy) phenotype. In preliminary studies, we have found that *dpy-18* is also required for axonal guidance of the PVQ and HSN neurons in *C. elegans*.

We hypothesize that the DPY-18 protein interacts and modifies other proteins to regulate neuronal development. We propose to characterize the role(s) of DPY-18 at the molecular and cellular level and to identify molecular targets of DPY-18 to enable us to better understand how prolyl 4-hydroxylation regulates neuronal development.

Investigating the Effects of Compounds with Anthelmintic Potential: Amidantel, Bay d 9216 and Tribendimidine

Michelle Joyner^A, Sophie Kittler^A, Vincent O'Connor^A, Robert Walker^A, Achim Harder^B, Lindy Holden-Dye^A

^ASchool of Biological Sciences, University of Southampton, UK; ^BBAYER Healthcare AG, Monheim, Germany

Amidantel (Bay d 8815) and its deacylated derivative (Bay d 9216), have been shown to act as agonists at the nicotinic acetylcholine receptor in electrophysiological studies using muscle strips of the parasitic nematode, *Ascaris suum*, and neurones from the *Helix aspersa* snail. These compounds and a further derivative, tribendimidine, have been found to have anthelmintic activity against a range of noteworthy parasitic nematode infections *in vivo*, including hookworm and ascariasis. The efficacy of amidantel and its derivatives was comparable to currently available drugs.

The model genetic organism *Caenorhabditis elegans* has been employed to determine the effects of amidantel, Bay d 9216, and tribendimidine. The predominant effect of these compounds in wild type *C. elegans* is an inhibition of motility. This effect has been observed in body bend and thrashing assays, inhibition is equivalent to that seen with levamisole. Egg laying behaviour is increased on exposure to each of the compounds, both effects that are associated with disruption of neuromuscular transmission. No effect on pharyngeal pumping or developmental timing was observed with any of the compounds. Exposure of wild type worms from the first larval stage through to adulthood, to tribendimidine or levamisole, did not have any effect on the timing of development. Motility was notably affected at the L4 stage.

The kinetic profile of these compounds is currently being explored to reveal any differences between the actions of amidantel, Bay d 9216, tribendimidine or levamisole. Such differences will give clues as to the molecular target(s) of these compounds.

In conjunction with investigations using wild type *C. elegans*, a reverse genetic screen using transgenic strains mutated in genes expressing acetylcholine receptor subunits or their associated proteins is being undertaken with a focus on those strains with reported resistance to levamisole. Preliminary results suggest an inhibition of motility in some of the levamisole resistant strains. Susceptibility to any of these compounds in levamisole resistant strains would imply a resistance breaking mode of action.

This work is funded by Bayer Healthcare AG, Monheim, Germany.

Cell Fate Determination of Oxygen and Carbon Dioxide Sensing Neurons in *C. elegans*

Kasper Langebeck-Jensen, Vaida Juozaityte, Roger Pocock

Master students in Biochemistry, BRIC, Ole Maaløes Vej 5, University of Copenhagen

C. elegans in environments that have substantial local fluctuations in gases such as oxygen and carbon dioxide. The BAG neurons in *C. Elegans* are able to generate rapid behavioral responses to decreases in oxygen and increases in carbon dioxide.

However, the molecular factors that determine BAG cell fate, and therefore their behavioral role, are not known. The aim of this project is to identify the molecular factors that are required for BAG cell differentiation. We are taking two approaches to identify these molecular factors:

Systematic promoter dissection

- Identification of elements in the promoters of genes expressed in the BAG neurons that are required for BAG expression
- Search for putative transcription factors required for BAG differentiation

Forward genetic screen

- Forward genetic screen for loss of BAG cell fate using a BAG-specific GFP reporter
- Characterization and identification of genetic lesions by SNP mapping and deep sequencing

Investigating the Functional Roles of the FLP-8 Neuropeptide in Dauer-induced Stress Responses

Petersen, J G and Pocock R

BRIC – Biotech Research and Innovation Centre, University of Copenhagen, Ole Maaløes Vej 5, 2200 Copenhagen N, Denmark, E-mail: jakob.petersen@bric.ku.dk

Specific neuronal circuits are responsible for orchestrating behavioral responses to environmental cues. A previous study in the laboratory has also shown that animals overcome stress by reprogramming specific neuronal circuits (Pocock and Hobert 2010). We want to further investigate if reprogramming of neuronal circuits is a general response mechanism used by animals that have been subjected to stress.

The expression of FLP-8, a neuropeptide belonging to the FMRF neuropeptide family, was shown to be induced in the mechanosensory neurons of dauer animals (Kim and Li 2004). To identify molecular factors that control dauer-induced FLP-8 expression we performed an EMS mutagenesis screen. We have conducted a high-throughput screen (~200.000 F2 animals) using a COPAS Biosort system, which has provided us with several mutants that exhibit suppression of dauer-induced FLP-8 expression. Analyzing these mutants with a combination of deep sequencing and SNP mapping will enable us to identify essential genes that are involved in the induced expression of FLP-8. In parallel to this work, we are characterizing the biological role of FLP-8 in general stress responses in addition to dauer formation and behaviour. We hope that this work will identify novel molecules and pathways required for stress-induced neuroplasticity.

Kim, K. and C. Li (2004). "Expression and regulation of an FMRFamide-related neuropeptide gene family in *Caenorhabditis elegans*." J Comp Neurol **475**(4): 540-550.

Pocock, R. and O. Hobert (2010). "Hypoxia activates a latent circuit for processing gustatory information in *C. elegans*." Nat Neurosci **13**(5): 610-614.

The Golgi Protein SQL-1/GMAP210 Modulates Intraflagellar Transport in *C. elegans*

Suzanne Rademakers, Joost Broekhuis, Martijn Dekkers, Jan Burghoorn, Gert Jansen

Dept of Cell Biology, Erasmus MC Rotterdam, The Netherlands

The cilia of *C. elegans*' amphid channel neurons are assembled and maintained by intraflagellar transport (IFT), which uses microtubule-dependent motor proteins to move IFT particles bi-directionally along the axoneme of the cilium. The amphid channel cilia can be divided into a middle and distal segment. Anterograde IFT in these cilia is mediated by two kinesin-2 complexes, kinesin II and OSM-3. Both kinesins enter the cilia middle segments and move together, but only OSM-3 enters the distal segments. In the middle segments OSM-3 and kinesin II move together at a speed of 0.7 $\mu\text{m/s}$, and in the distal segments OSM-3 moves alone at 1.2 $\mu\text{m/s}$. In the absence of OSM-3 kinesin II moves alone at 0.5 $\mu\text{m/s}$. We have recently shown that the sensory G protein GPA-3 plays a role in regulating IFT, and that a dominant active mutant of *gpa-3* (*gpa-3QL*) affects cilia morphology, resulting in a dye filling defect. To identify new genes in the same pathway we performed a genetic screen for mutants that suppress the *gpa-3QL* Dyf phenotype. After SNP mapping, sequencing and rescue experiments, we found *sql-1* (suppressor of *gpa-3QL*), which encodes the homologue of the mammalian Golgi protein GMAP-210. GMAP-210 has been shown to interact with IFT-20, a complex B protein. GMAP-210 mutant cells have shorter cilia and reduced polycystin-2 in their cilia. Immunofluorescence staining and western blotting showed that no protein is expressed in *sql-1(lf)* animals. SQL-1 is ubiquitously expressed, including expression in sensory neurons and is localized to the Golgi. Using IFT protein GFP fusion constructs and cell specific GFP markers we have shown that the morphology and length of the cilia is not affected in *sql-1(lf)* mutants. Speed measurements showed that IFT is perturbed in *sql-1(lf)* animals: in the middle segments OSM-3 moves faster (0.88 $\mu\text{m/s}$) and kinesin II moves slower (0.59 $\mu\text{m/s}$), suggesting that the two kinesins are at least partially uncoupled. Speeds of the OSM-3 and kinesin-2 motors *per se* are not affected. We are currently investigating how *gpa-3* and *sql-1* interact in the regulation of IFT. Our results suggest that *sql-1* and *gpa-3* function in the same genetic pathway. Surprisingly, in the distal segments of *gpa-3QL sql-1(lf)* double mutants OSM-3::GFP speed is significantly reduced.

Molecular Mechanisms of Salt Taste

Oluwatoroti O Umuerrri, Renate Hukema, Martijn Dekkers, Gert Jansen

Department of Cell Biology, Erasmus MC, P.O. Box 2040, 3000CA, Rotterdam, The Netherlands

NaCl is essential for salt and water homeostasis and it is important for physiological functions in many organisms. However, the molecular mechanism of NaCl detection is not well known. In mammals, the epithelial Na⁺ channel (ENaC) has been shown to be involved in NaCl detection. But there is also an ENaC channel independent salt taste mechanism. Several genes involved in NaCl chemoattraction have been identified. These include *tax-2* and *tax-4* (cyclic nucleotide gated (CNG) channel subunits), *tax-6* and *cnb-1* (calcineurin A and B subunits) and *ncs-1* (neuronal calcium sensor). Analysis of these mutants in our assay, in which we expose the animals to a very steep NaCl gradient, showed reduced chemotaxis to NaCl. However, we found that these mutants still showed significant attraction to higher NaCl concentrations. By analyzing the behaviour of double mutants, we found that chemotaxis to NaCl involves two genetic pathways. The first pathway involves two mitogen activated protein (MAP) kinases, *nsy-1* and *sek-1*, and three genes that have been previously characterized, *tax-2*, *tax-4* and *tax-6*. The second pathway involves *tax-2*, another CNG channel subunit, *cng-3*, the G α protein *odr-3*, the TRPV channel subunit, *osm-9*, and the guanylate cyclase, *gcy-35*. We use cell specific rescue of the mutant genes, laser ablation of specific neurons and neuronal calcium imaging to find out where in the neuronal circuit of *C. elegans* these genes function. Thus far, the involvement of the main salt sensing neurons, ASE, has been confirmed. In addition, we found that the ADF neurons also play a role in chemoattraction. Finally, we have performed a synthetic genetic screen in an *odr-3* mutant background to identify additional genes that play a role in NaCl chemotaxis. We found 6 mutants that have completely lost all NaCl taste response and 23 mutants that showed reduced chemoattraction to NaCl. We are using SNP-mapping and deep sequencing to identify the genes affected in three mutants.

***Caenorhabditis elegans* as a Model of Homocystinuria: Characterization of Nematode Cystathionine Beta-synthase**

Roman Vozdek, Ales Hnizda, Jakub Krijt, Marta Kostrouchova, Viktor Kozich

Institute of Inherited Metabolic Disorders, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague

Cystathionine beta-synthase (CBS) is a PLP-dependent enzyme, which converts homocysteine into cystathionine in the transsulfuration pathway. Deficiency of CBS is the most common cause of inherited metabolic disorder homocystinuria affecting three major systems - connective tissue, vascular system and central nervous system. In this work we aimed at developing a new animal model of homocystinuria due to CBS deficiency using *Caenorhabditis elegans*.

By blastp searching we identified gene orthologous to CBS in *C. elegans* – *ZC373.1* (CeCBS). Purified recombinant protein CeCBS expressed in prokaryotic system exhibited CBS activity with K_M values for serine and homocysteine of $3,96 \pm 1,09$ mmol/l and $1,75 \pm 0,18$ mmol/l, respectively. We have shown that the CeCBS, unlike mammalian CBS, cannot be further activated by S-adenosyl methionine and that it did not contain heme as determined by UV/VIS spectrometry. The GFP signal in transgenic animals carrying translational vector *CeCBS::GFP* occurred in hypodermis, intestine, body wall muscle cells and some nonstriated pharyngeal muscles. Subcellular distribution of the GFP signal indicated cytoplasmic localization. Inhibition of CeCBS by RNA-mediated interference showed developmental delay of nematodes. Affected worms exhibited slow movement and abnormal morphology of intestine and pharynx. Quantitative analysis of aminothiols in crude extracts of interfered worms compared to wild type worms showed homocysteine concentration of 10.9 nmol/mg and 1.1 nmol/mg of total protein, respectively, with no significant differences in other aminothiols.

We report here that the gene *ZC373.1* encodes CBS in *C. elegans* and that is necessary for its normal development. Our data suggest that nematode *C. elegans* may be a useful model for some features of homocystinuria due to CBS deficiency.

This study was supported by Grant No. 21709 from Grant Agency of Charles University, Prague, Czech Republic and by Research Project of Charles University No. VZMSM0021620806

A High-throughput whole Genome Sequencing Pipeline to Identify Genes Involved in *C. elegans* Aggregation Behaviour

Katherine P. Weber, Felix Baier and Mario de Bono

MRC Laboratory of Molecular Biology, Cambridge, UK

The nematode *C. elegans* provides a powerful model to study the neural networks that underlie behaviour. The connectivity of its 302 neurons is known, and each of these neurons can be manipulated transgenically, as well as imaged in live animals with genetically encoded calcium sensors.

The *C. elegans* reference strain, N2, feeds in isolation, but most wild-caught *C. elegans* aggregate to form groups on food. This difference is caused by a polymorphism in the neuropeptide receptor *npr-1*. When this gene is knocked out in N2 animals, they strongly aggregate. To identify molecules that promote aggregation in *C. elegans*, we mutagenized *npr-1(null)* animals and isolated mutants that no longer feed in groups. We have identified a series of genes involved in aggregation behaviour, e.g. soluble guanylate cyclases that function as O₂ sensors (1), a neural globin, *glb-5* (3), and others. We have gone on to characterize the role of these genes in the neural networks underlying aggregation behaviour. However, traditional genetic mapping and gene cloning is time-consuming.

Recent studies have shown that Next Generation Sequencing technologies can be used extensively to identify the molecular identities of *C. elegans* mutants (4). In order to increase throughput for cloning behavioural mutants, we have established a pipeline to sequence individual mutants using Illumina sequencing by synthesis (SBS) technology. Many of these mutants have been mapped roughly (usually to ~1 Mb); examining their genome sequences thus allows us to rapidly identify the lesion responsible for their phenotype. Using a paired-end, non-amplified library preparation (2), we have obtained >25x coverage of the *C. elegans* genome from one lane of sequencing on an Illumina Genome Analyzer II (GAII), and we are now increasing this throughput using the HiSeq 2000. Our downstream analysis can be completed in approximately 2 days by using the BWA (Burrows Wheeler Algorithm) program (5) to align altered sequence reads to the reference genome. It then implements a custom pipeline to highlight exon changes, indicate genes affected and predict coding changes. This method has greatly accelerated our ability to molecularly characterize mutants isolated in our screens.

Cheung, B.H.H., F. Arellano-Carbajal, I. Rybicki & M. de Bono (2004): Soluble Guanylate Cyclases Act in Neurons Exposed to the Body Fluid to Promote *C. elegans* Aggregation Behavior. - *Current Biology* **14**: 1105-1111.

Kozarewa, I., Z. Ning, M.A. Quail, M.J. Sanders, M. Berriman & D.J. Turner (2009): Amplification-free Illumina sequencing-library preparation facilitates improved mapping and assembly of (G+C)-biased genomes. - *Nature Methods* **6**(4): 291-295.

Persson, A., E. Gross, P. Laurent, K.E. Busch, H. Bretes & M. de Bono (2009): Natural variation in a neural globin tunes oxygen sensing in wild *Caenorhabditis elegans*. - *Nature* **458**: 1030-1033.

Sarin, S., S. Prabhu, M.M. O'Meara, I. Peer & O. Hobert (2008): *Caenorhabditis elegans* mutant allele identification by whole-genome sequencing. - *Nature Methods* **5**(10): 865-867.

Li, H. and Durbin, R. (2010) Fast and accurate long-read alignment with Burrows-Wheeler Transform. *Bioinformatics*, Epub. [PMID: 20080505].

List of Participants

Artemis ANDREOU

Institute of Molecular Biology and
Biotechnology, FORTH, Heraklion, Greece
andreou@imbb.forth.gr

Marta ARTAL-SANZ

Albert Ludwig Univ. Freiburg, Inst. Fuer
Biology Iii/ZBSA, Freiburg, Germany
marta.artal@biologie.uni-freiburg.de

Felix BAIER

MRC Laboratory of Molecular Biology
Cambridge, UK
felixbaier@yahoo.uk

Jeff BARCLAY

University of Liverpool, School of
Biomedical Sciences, Liverpool, UK
barclayj@liverpool.ac.uk

Ralf BAUMEISTER

Albert Ludwig Univ. Freiburg, Inst. Fuer
Biology Iii/ZBSA, Freiburg, Germany
ralf.baumeister@frias.uni-freiburg.de

Viktor BILLES

Eötvös Lorand University, Dept. of
Genetics, Budapest, Hungary
billesviktor@gmail.com

Rakesh BODHICARLA

University of Nottingham, School of
Biology, Nottingham, UK
plxrbk2@nottingham.ac.uk

Antonio CABALLERO REYES

Kings College London, MRC Centre for
Developmental Neurobiology, London, UK
antonio.caballero_reyes@kcl.ac.uk

Marios CHATZIGEORGIOU

MRC Laboratory of Molecular Biology
Cambridge, UK
mariosc@mrc-lmb.cam.ac.uk

Andrea CHOE

CALTECH University
Pasadena, USA
ac@caltech.edu

Emiliano COHEN

Hebrew University, Hadassah Medical
School, Jerusalem, Israel
emilianoc@ekmd.huji.ac.il

Netta COHEN

University of Leeds
Leeds, UK
n.cohen@leeds.ac.uk

Anna CRISFORD

University of Southampton, School of Biological
Sciences, Southampton, UK
mj105@soton.ac.uk

Sasha DE HENAU

Vanfleteren Lab. Dept. of Biology
Ghent, Belgium
sasha.dehenau@ugent.be

Matthew EDMONDS

University of Liverpool, School of Biomedical
Sciences, Liverpool, UK
M.Edmonds@liverpool.ac.uk

Muna ELMI

University College London, MRC Laboratory of
Molecular Cell Biology, London, UK
muna.elmi@ucl.ac.uk

Chronis FATOUROS

Albert Ludwig Univ. Freiburg,
Inst. Fuer Biology Iii/ZBSA, Freiburg, Germany
polychronis.fatouros@biologie.uni-freiburg.de

Anthony FLEMMING

Syngenta
Berkshire, UK
anthony.flemming@syngenta.com

Christopher FRANKS

University of Southampton, School of Biological
Sciences, Southampton, UK
mj105@soton.ac.uk

Steven HUSSON

Goethe University Frankfurt, Institute of
Biochemistry, Frankfurt, Germany
husson@biochem.uni-frankfurt.de

Gert JANSEN

Erasmus MC, Dept. of Cell Biology
Rotterdam, The Netherlands
g.jansen@erasmusmc.nl

Jennifer JERMY

University College London, MRC, Lab. of
Molecular Cell Biology, London, UK
ucbtjrw@ucl.ac.uk

James JOHNSON

University of Liverpool, School of
Biomedical Sciences, Liverpool, UK
J.Johnson@liverpool.ac.uk

Nanna Torpe JORGENSEN

University of Copenhagen
BRIC, Copenhagen, Denmark
nanna.joergensen@bric.ku.dk

Michelle JOYNER

University of Southampton, School of
Biological Sciences, Southampton, UK
mj105@soton.ac.uk

Vaida JUOZAITYTE

University of Copenhagen
BRIC, Copenhagen, Denmark
vaida.juozaityte@bric.ku.dk

Konstantinos KAGIAS

University of Copenhagen
BRIC, Copenhagen, Denmark
konstantinos.kagias@bric.ku.dk

Nikos KOURTIS

Institute of Molecular Biology and
Biotechnology, FORTH, Heraklion, Greece
kourtis@imbb.forth.gr

Sandhya KOUSHIKA

National Centre for Biological Sciences
TIFR, Bangalore, India
koushika@ncbs.res.in

Kasper LANGEBECK-JENSEN

University of Copenhagen
BRIC, Copenhagen, Denmark
kasper.langebeck@bric.ku.dk

Shu LIU

Albert Ludwig Univ. Freiburg,
Inst. fuer Biology Iii/ZBSA, Freiburg,
Germany
shu.liu@biologie.uni-freiburg.de

Wolfgang MAIER

Friedrich Miescher Institute for Biomedical
Research, Basel, Switzerland
wolfgang.maier@fmi.ch

Maria MARKAKI

Institute of Molecular Biology and
Biotechnology, FORTH, Heraklion, Greece
stemark@imbb.forth.gr

Evgenia MEGALOU

Institute of Molecular Biology and
Biotechnology, FORTH, Heraklion, Greece
emeg@imbb.forth.gr

Sudip MONDAL

National Centre for Biological Sciences
TIFR, Bangalore, India
sudipm@nebs.res.in

Hilde NILSEN

University of Oslo
The Biotechnology Centre, Oslo, Norway
hilde.nilsen@biotek.uio.no

Konstantinos PALIKARAS

Institute of Molecular Biology and
Biotechnology, FORTH, Heraklion, Greece
palikarask@imbb.forth.gr

Aggela PASPARAKI

Institute of Molecular Biology and
Biotechnology, FORTH, Heraklion, Greece
pasparak@imbb.forth.gr

Dhaval PATEL

Kings College London
London, UK
dhaval.patel@kcl.ac.uk

Mikael PEDERSEN

University of Copenhagen
BRIC, Copenhagen, Denmark
mikael.pedersen@bric.ku.dk

Jakob Gramstrup PETERSEN

University of Copenhagen
BRIC, Copenhagen, Denmark
jakob.petersen@bric.ku.dk

Roger POCOCK

University of Copenhagen
BRIC, Copenhagen, Denmark
roger.pocock@bric.ku.dk

Benjamin PODBILEWICZ

Technion-Israel Institute of Technology
Kyriat Ha Technion, Dept. of Biology, Haifa, Isreal
podbilew@technion.ac.il

Suzanne RADEMAKERS

Erasmus MC, Dept. of Cell Biology
Rotterdam, The Netherlands
j.rademakers@erasmusmc.nl

Matthias RIECKHER

Institute of Molecular Biology and
Biotechnology, FORTH, Heraklion, Greece
matthias@imbb.forth.gr

Hanne SKJELDAM

University of Oslo
The Biotechnology Centre, Oslo, Norway
h.k.skjeldam@biotek.uio.no

Peter SWOBODA

Karolinska Institute, Center for Biosciences
at NOVUM, Huddinge, Sweden
peter.swoboda@ki.se

Makerlla KATIDOU

Institute of Molecular Biology and
Biotechnology, FORTH, Heraklion, Greece
mkatidou@imbb.forth.gr

Nektarios TAVERNARAKIS

Institute of Molecular Biology and
Biotechnology, FORTH, Heraklion, Greece
tavernar@imbb.forth.gr

Millet TREININ

Hebrew University, Hadassah Medical
School, Jerusalem, Israel
millet_t@cc.huji.ac.il

Oluwatoroti UMUERRI

Erasmus MC, Dept. of Cell Biology
Rotterdam, The Netherlands
o.olusanya@erasmusmc.nl

Tibor VELLAI

Eötvös Lorand University, Faculty of Natural
Sciences, Dept. of Genetics, Budapest, Hungary
vellai@falco.elte.hu

Natascia VENTURA

University of Rome, Tor Vergata
Rome, Italy
venturan@uniroma2.it

Roman VOZDEK

Charles University in Prague, Inst. of Inherited
Metabolic Disorders, Prague, Czech Republic
roman.vozdek@lf1.cuni.cz

Oliver WAGNER

National Tsing Hua University, Institute of
Molecular, and Cellular Biology, Hsinchu, Taiwan
owagner@life.nthu.edu.tw

Kate WEBER

MRC Laboratory of Molecular Biology
Cambridge, UK
kweber@mrc-lmb.cam.ac.uk

Manuel ZIMMER

Research Institute of Molecular Pathology (IMP)
Vienna, Austria
mzimmer@rockefeller.edu

Secretary

Mrs. Georgia Houlaki

Foundation for Research and
Technology – Hellas
Institute of Molecular Biology and
Biotechnology
Heraklion, Crete
info@ewnm2010.gr

