# POSTER PRESENTATIONS

| P-000: poster session A | P-000: poster session B | P-000: poster session C |
|---|---|---|

## Development

**P-001** NHR-40, a *Caenorhabditis elegans* Supplementary Nuclear Receptor Regulates the Development of Embryos and L1 Larvae
**E. Brozova,** K. Simeckova, Z. Kostrouch and M. Kostrouchova

**P-002** Elucidating the Role of MicroRNAs in *C. elegans* Development
**A. Clark** and E. Miska

**P-003** Understanding the Role of a Non-Peptidase Member of the ACE Family in Nematode Moulting
**H.L. Craig,** C. Hunton, D.R. Brooks and R.E. Isaac

**P-004** RNAi Screens in *C. elegans* to Identify Signalling Modulators in Vulval Development
**C. Crombie** and A. Fraser

**P-005** A GFP Reporter Based Screen for the Notch Target Genes during Vulval Development
**S. Farooqui,** I. Rimann and A. Hajnal

**P-006** Comprehensive Analysis of Gene Expression Patterns of Hedgehog-Related Genes
**L. Hao,** R. Johnsen, G. Lauter, D. Baillie, T.R. Bürglin

**P-007** GEI-16, a Putative Component of the Hemidesmosome, is Necessary for Tissue Stability and Epithelial Morphogenesis
**S.B. Hetherington,** F.I. Pellerone, C.A. Behm

**P-008** Valproic Acid Affects Gene Expression and Development in *Caenorhabditis elegans*
**M. Kostrouchova,** P. Liby, M. Kostrouchova and Z. Kostrouch

**P-009** Inhibition of *bir-1*, the Homologue of Human Survivin, Induces Changes of Expression of Developmentally Active Collagen Genes in L1 Larval Stage
**P. Liby,** M. Kostrouchova, M. Pohludka, J. Vohanka, E. Brozova, M. Kostrouchova and Z. Kostrouch

**P-010** NHR-25 Role in the Seam Cell Differentiation in *C. elegans*
**M. Machackova,** M. Silhankova, M. Jindra and M. Asahina

**P-011** Misexpression of Acetylcholinesterases in the *C. elegans pha2* Mutant Causes Post-Embryonic Pharyngeal Isthmus Deformation
C. Mörck, C. Axäng, M. Goksör and **M. Pilon**

**P-012** Role of *rad-5* in Development of *C. elegans*
**S. Moser,** A. Alpi, I. Büsing, R. Schnabel and A. Gartner

**P-013** Analyzing a Putative Acetyl-CoA Transporter Gene Involved in Development and Morphogenesis
**K.H. Nomura,** S. Mizuguchi, K. Dejima, D. Murata, Y. Matsuishi-Nakajima, N. Kawasaki, K. Gengyo-Ando, S. Mitani, Y. Hirabayashi and K. Nomura

**P-014** Structural and Functional Characterization of Aminopeptidase P in *Caenorhabditis elegans*
K.A.P. Payne, **H. Craig,** D.R. Brooks, A.J. Turner, M.R. Parsons, R.E. Isaac

**P-015** Analysis of Epithelial Genes during Postembryonic Development of *C. elegans*
J. Pilipiuk, G. Helbig and **O. Bossinger**

**P-016** Analysis of CHR-3 *(nhr-23)* Loss of Function in L1 Larval Stage Using Whole Genome Microarrays and Comparative Two Dimensional Protein Chromatography
**M. Pohludka,** J. Vohanka, M. Kostrouchova and Z. Kostrouch

**P-017** Role of the miR-52 Family of microRNAs in *Caenorhabditis elegans* Development
**W.R. Shaw,** I. Alvarez-Garcia and E.A. Miska

**P-018** NHR-60, a *Caenorhabditis elegans* Supplementary Nuclear Receptor Residing at the Nuclear Periphery, Regulates Embryonic Development in Connection with Acyl-Coenzyme A Binding Protein
**K. Simeckova,** E. Brozova, J. Vohanka, M. Pohludka, Z. Kostrouch and M. Kostrouchova

**P-019** Phenotypic Analysis and Mapping of a New *C. elegans* Mab Mutant, *mab-29*
**M. Simões** and A. Woollard

**P-020** CEN-1, a Nucleotidase Involved in the UPR Mechanism, is Required for Pharyngeal Morphogenesis in *C. elegans*
D. Uccelletti, **A. Pascoli,** P. Mancini, C.B. Hirschberg and C. Palleschi

**P-021** Identification of Genes required for Anchor Cell Attachment and Invasion during Vulval Development
**S. Vassalli** and A. Hajnal

**P-022** Distinct Roles of the Pumilio and FBF Translational Repressors During *C. elegans* Vulval Development
**C. Walser,** G. Battu, E. Fröhli Hoier and A. Hajnal

**P-161** The Role of Otx/otd Homeobox Genes in *C. elegans* Development
**Y.G. Tong,** T.R. Bürglin

## Germline and Reproduction

**P-023** Germline Totipotency and the "worm teratoma"
**B. Biedermann,** M. Senften and R. Ciosk

**P-024** Role of Short RNAs in *Caenorhabditis elegans* Germline Development
**P.P. Das,** H. Buhecha and E. Miska

**P-025** GLS-1, A Novel GLD-3 Interacting Protein Regulates Germ Cell and Embryonic Development in *C. elegans*
M. Harterink, **A. Kupinski,** J. Stamford and C. Eckmann

**P-026** Creation of Germ Cells: Characterization of Germline Cytokinesis
**T.M. Kachur** and D. Pilgrim

**P-027** Analysis of Genetic Interactions Affecting Germline Development Using Microarrays
**A.C. Nelson** and A.G. Fraser

**P-028** Moving Forward With Actin and MSP (Major Sperm Protein)
L. Trichet, S. Delrot, C. Sykes and **J. Plastino**

**P-029** TEN-1 is required for the Formation of the Somatic Gonad in *Caenorhabditis elegans*
**A. Trzebiatowska,** K. Drabikowski and R. Chiquet-Ehrismann

**P-030** Maintenance of Asymmetry during Germ Line Cell Cleavages
**S. von Elsner,** H. Schmidt and R. Schnabel

**P-031** Are Males Important? – An Experimental Approach
**V. Wegewitz**, H. Schulenburg and A. Streit

**P-032** Post-Transcriptional Control of Germline Immortality
**T.M. Zucchero,** M. Eckler, M. Lowden, Y. Liu, S. Ahmed

## Dauer Formation, Ageing and Senescence

**P-033** Characterization of the Long-Lived Mutant *liv-9(pv18)*
**A.M. Brokate-Llanos,** J.M. Monje, M.J. Muñoz

**P-034** *C. elegans* Thioredoxins Implicated in Nervous System Function and Aging
**J.C. Fierro González,** P. Swoboda, A. Miranda-Vizuete

**P-035** Transcriptional Changes Underlying Chemosensory Regulation of Lifespan
**M.M. Gaglia** and C. Kenyon

**P-036** The Role of $IP_3$ Signalling in the Regulation of Rhythms and Rates in *C. elegans*
**R. Gatsi** and H.A. Baylis

**P-037** Coenzyme Q Administration is not Sufficient to Revert *coq-1* Knockout Phenotype
**A. Gavilán,** J.C. Rodríguez-Aguilera, C. Asencio and P. Navas

**P-038** RNAi Screen for Genes that Regulate Stress Resistance and Life Span of *C. elegans*
**B. Gerisch,** A. Antebi, M-L. Yaspo and H. Lehrach

**P-039** Age-related Alterations in Proteasome Activity
**C.I. Holmberg,** D. Dowlatshahi, C. Voisin, S.G. Fox and R.I. Morimoto

**P-040** Modulated MicroRNA Expression during Adult Lifespan in *C. elegans*
**C. Ibañez-Ventoso,** M. Yang, G. Suzhen, H. Robins, R.W. Padgett, M. Driscoll

**P-041** A Potential DAF-16 Target Involved in Longevity
**V.L. Jensen,** D.L. Baillie and D.L. Riddle

**P-042** Analysis of Insulin-Like Peptides in *C. elegans* by Mass Spectrometry
**A. Kleinert,** D. Hess, J. Hofsteenge and J. Alcedo

**P-043** Identification of Genes Involved in the Neural Regulation of Lifespan
W. Maier, **M. Regenass,** M. Thomas and J. Alcedo

**P-044** The Role of pkc-1/ttx-4 in Dauer Formation
**J. M. Monje,** M.A. Fidalgo, M.J. Muñoz

**P-045** Using *C. elegans* to Model the Systems Biology of Stress Response and Age-Related Disorders
**J. Sämann,** E. Schmidt, M. Seifert and R. Baumeister

**P-046** Signaling Via eIF4E Regulates Ageing in *C. elegans*
**P. Syntichaki,** K. Troulinaki and N. Tavernarakis

## Neurobiology and Behavior

**P-047** Characterization of PQN-21, A Prion-Like Protein Involved in Learning and Memory in *C. elegans*
**D. Bazopoulou** and N. Tavernarakis

**P-048** The Function of Latrophilin in *C. elegans*
**D.R. Bell,** A. Ademola, J. Davy, S. van der Poel, D. Kendall, P.N.R. Usherwood, I.R. Duce, I.R. Mellor and D. de Pomerai

**P-049** Identification, by a Proteomic Approach, of the Signaling Networks Controled by UNC-73(Trio) in the Developing Nervous System of *C. elegans*
**J-M. Bellanger** and A. Debant

**P-050** Signalling in quinine avoidance
**C. Bergamasco,** G. Esposito, S. Arbucci and P. Bazzicalupo

**P-051** Adaptation to the Nicotinic Agonist Levamisole
**T. Boulin,** S. Eimer, J. Richmond and J-L. Bessereau

**P-052** The Potent Inhibitory Action of the Anthelmintic Emodepside in the *C. elegans* Pharynx Requires the Latrophilin-Like Receptor LAT-1 but not LAT-2
**K. Bull,** J. Wilson, L. Holden-Dye, R. Walker

**P-053** Involvement of DAF-19 in the General Development and Subtype Specification of Cilia
**J. Burghoorn,** S. Sahlin and P. Swoboda

**P-054** An Antagonistic Interaction between NPY-like Receptors *npr-1* and *npr-2* Regulates Pharyngeal Pumping
**A. Cook,** N. Kriek, E. Siney, N.A. Hopper and L. Holden-Dye

**P-055** Dissecting Gustatory Plasticity in *C. elegans* Using Mammalian Receptors
**M. Dekkers,** M. Teng, J. McCafferty and G. Jansen

**P-056** The Molecular, Cellular and Functional Organization of the *C. elegans* Metabotropic Glutamate Receptor, MGL-1
**J. Dillon,** N.A. Hopper, L. Holden-Dye and V. O'Connor

**P-057** The Integral Membrane Protein UNC-50 is required for Nicotinic Receptor Trafficking in *C. elegans*
**S. Eimer,** A. Gottschalk, J. Richmond, M. Hengartner, W.R. Schafer and J-L. Bessereau

**P-058** Analysis of the Role of npr-1 in Response to Noxious Stimuli in ASH
**M. Ezcurra,** S. Lee, P. Swoboda and W.R. Schafer

**P-059** Stimulating the Pharyngeal Nerve Ring: Characterisation of Putative Post-Synaptic Potentials
**C.J. Franks,** N.A. Hopper, V.M. O'Connor and L.M. Holden-Dye

**P-060** Ectopic Expression of DAF-19, a Transcription Factor that Controls Cilium Development
G. Gahmon and **P. Swoboda**

**P-061** LEV-9, a New Player in Acetylcholine Receptor Clustering at Neuro-Muscular Junctions
**M. Gendrel,** D. Williams, B. Schafer, E. Jorgensen, J. Richmond and J-L. Bessereau

**P-062** Lnp and Siah/Ring E3 Ubiquitin Ligase: Partners Associated to Multisubunit Ring Finger Complexes involved in Synaptogenesis?
**L. Ghila,** P. Latorre and M. Gomez

**P-063** Investigations into the Mode of Action of the Novel Anthelmintic Emodepside
**M. Guest,** K. Bull, K. Amliwala, J. Wilson, R. Walker, N.A. Hopper, L. Holden-Dye

**P-064** Electrophysiological Analysis of *C. elegans* Mutants in Levamisole-Receptor Associated Proteins
**J. Liewald,** R. Almedom, T. Schedletzky and A. Gottschalk

**P-065** The Physiological Role of Peptidergic Transmission in the Pharyngeal Nervous System
**S. Luedtke,** R.J. Walker, N.A. Hopper, V. O'Connor and L. Holden-Dye

**P-066** Evidence that Ethanol Equilibriates Rapidly Across *C. elegans* Cuticle
**P. Mitchell,** K. Bull, N.A. Hopper, S. Glautier, L. Holden-Dye, V. O'Connor

**P-067** Evaluating the Role of the Neuronal MAP Kinase JNK-1 in Orientation Behavior and Thermotaxis
**F. Nunes,** M. Voss, A. Henkel, M. Wolf and R.J. Paul

**P-068** Association of Two Lipid Kinases Involved in Neurotransmitter Release
**B. Pérez-Mansilla,** S. Nurrish

**P-069** The *xbx* Genes Dataset Uncovers *C. elegans* ciliary genes
P. Phirke, E. Efimenko, F. Yström and **P. Swoboda**

**P-070** Role of the Immunophilin FKBP-52 in the Nervous System of *C. elegans*
**N. Roudier,** K. Rajkowski, B. Chambraud and E.E. Baulieu

**P-071** Characterization of SOC-1 and PLK-2, Two Proteins Modulating Levamisole-Receptor Function in *C. elegans*
T. Schedletzky and **A. Gottschalk**

**P-072** Characterisation of Gene Deletion Mutants for Putative Neuropeptide Receptors
**E. Siney,** A Cook, N. Kriek and L. Holden-Dye

**P-073** Valproic Acid Acts on Both IP3 and DAG Regulated Behaviours in *C. elegans*
**S.M. Tokuoka** and S. Nurrish

**P-074** Identification of Novel Genes Mediating Pathfinding of the AVG Pioneer Axon and its Followers
**T. Unsoeld,** I. Wacker and H. Hutter

**P-075** ITR-1, the *C. elegans* $IP_3$ Receptor, Functions in ASH-Mediated Responses
**D.S. Walker,** E. Gregory, N.J.D. Gower and H.A. Baylis

**P-076** Regulation of *C. elegans* Behaviour by the Metabotropic Glutamate Receptors
T. Zeng, J. Dillon, N.A. Hopper, **V. O'Connor**

## Cell Biology and Metabolism

**P-077** Lysosomal Biogenesis and Function is Critical for Necrotic Cell Death in *C. elegans*
**M. Artal-Sanz,** C. Samara, P. Syntichaki and N. Tavernarakis

**P-078** Identification of Normal and Pathological Functions of Muscle Specific Proteins with RNA Interference in *C. elegans*
**L. Chambonnier** and L. Segalat

**P-079** A Partial Loss of Functional Allele of *klp-19* Causes Intestinal Nuclear Division Defects and Sterility
**M. Cundall,** C. Clucas and I.L. Johnstone

**P-080** The Rap-1 and Ral-1/Sec-5/Exo-84 Pathway Synergize in Hypodermal Cell Division and Migration during Embryogenesis
**E.W. Frische,** W. Pellis-van Berkel, J.L. Bos and F.J.T. Zwartkruis

**P-081** Identification of O-linked N-acetylglucosamine Modified Proteins in *Caenorhabditis elegans*
J. Grabitzki, M. Ahrend, B. Schmitz, R. Geyer and **G. Lochnit**

**P-082** Glycosidases of *Caenorhabditis elegans* involved in N-glycan processing
**M. Gutternigg,** D. Lubich, M. Hackl, K. Paschinger, U. Stemmer, V. Jantsch, G. Lochnit, R. Ranftl, P. Geier and I.B.H. Wilson

**P-083** Identification of Intermediate Filament Organizers in *Caenorhabditis elegans*
**K. Hüsken** and R.E. Leube

**P-084** The Atypical Calpains of *C. elegans*
**P.I. Joyce** and P.E. Kuwabara

**P-085** Identification of the Protein Kinase VRK-1 as an Essential Regulator of Nuclear Envelope Formation and BAF Phosphorylation
**E. Klerkx,** M. Gorjánácz, C. López-Iglesias, I.W. Mattaj and P. Askjaer

**P-086** Regulation of Cell Proliferation versus Differentiation in *C. elegans*
**J. Korzelius,** M. Boxem, J. Ceron, V. Portegijs, S. Christiaan and S. van den Heuvel

**P-087** Modulation of Ubiquitin-Dependent Pathways by Ataxin-3/ATX-3
**K. Kuhlbrodt,** R. Barikbin, C. Eckmann adn T. Hoppe

**P-088** Establishment of Apical Junctions in *C. elegans:* An Approach by RNAi Screening to Identify the Proteins Interacting with LET-413
**C. Lefebvre,** J. Mouysset, R. Legouis

**P-089** Characterization of the *C. elegans* Homologue of the Adaptor Protein EPSIN
**M.G. Malabarba,** E. Gerbino, A. Croce, H. Kloess, A.E. Salcini, G. Cassata and P.P. di Fiore

**P-090** Membrane Traffic and Development: A Genome-Wide RNAi Screen Looking for *unc-101* Enhancers
**G. Michaux**

**P-091** Functional Knocking Out of Glycome-Related Genes in *Caenorhabditis elegans*
**S. Mizuguchi,** K.H. Nomura, K. Dejima, T. Nagaisi, D. Murata, S. Mitani, K. Gengyo-Ando, A. Seko, K. Yamashita, T. Izumikawa, H. Kitagawa, K. Sugahara, Y-D. Kwon, H. Narimatsu, K. Nomura

**P-092** Characterization of *ced-8* and *ced-8* Interacting Genes
**J.K. Phelan,** K. Wong, S.M. Pinto, L. Falquet, H.H. Jung and M.O. Hengartner

**P-093** Sec61beta and the Exocyst in *C. elegans*
**J. Pispa** and J. Jäntti

**P-094** A Proteomic Approach to Decipher LIN-26 Function
**J. Polanowska,** S. Bamps, J-F. Dierick and J. Reboul

**P-095** Disulphide Bond Formation in Multicellular Organisms
**P. Psarrakos** and P. Klappa

**P-096** Investigation of Interactions between Mutant Collagens Using *C. elegans* as a Model Organism
**B. Roberts, G. Stapleton** and I. Johnstone

**P-097** Tissue Specific Regulation of Intestinal Proliferation through CDC-25.1
**A. Segref** and I.L. Johnstone

**P-098** The Loss of the Single APC Homolog *apr-1* Causes Hyperplasia in the *C. elegans* Intestine
R. Schneider, G. Helbig and **O. Bossinger**

**P-099** In Search of the Lost CAMs
**G. Shemer** and B. Goldstein

**P-100** The MAGI-1 Protein Functions as an Organizer Protein at the Adherens Junctions
**A. Stetak,** E. Fröhli-Hoier and A. Hajnal

**P-101** The Role of Endocytosis and Intracellular Trafficking in *C. elegans* Neurodegeneration
**K. Troulinaki** and N. Tavernarakis

**P-102** The Function of LIN-5 in Chromosome Segregation and Asymmetric Division
**M. van der Voet,** M. Wildwater, M. Boxem, J. Peters, C. Berends, M. Galli, I. The, A. Thomas and S. van den Heuvel

**P-162** Development Specific Collaboration between the E3 Ligases UFD-2 and CHN-01 Regulates the Myosin Assembly Chaperone UNC-45
**C. Janiesch,** J. Kim and T. Hoppe

## Signal Transduction and Gene Expression

**P-103** *cis*-acting Factors and Downstream Targets of the T-Box Transcription Factor *mab-9*
**P. Appleford** and A. Wollard

**P-104** Dysfunctional RRF-3 Up-Regulates Germ Line Specific Heterochromatin Factors in *Caenorhabditis elegans*
**S. Asikainen**, K. Reisner and G. Wong

**P-105** *C. elegans* Sumoylome
**S. Bamps,** J. Polanowska, J-F. Dietrick and J. Reboul

**P-106** RNA Degradation in the Nematode Worm *C. elegans*
**C. Bowen** and A. Wollard

**P-107** Searching for *rnt-1* Interacting Genes in *C. elegans*
**T. Braun** and A. Wollard

**P-108** Towards a Functional Map for Spliceosome Components in *C. elegans*
**J. Ceron** and S. van den Heuvel

**P-109** Screening for Negative Regulators of Grown Factor Signalling
**J. Dillon**, A.D. Wooller and N.A. Hopper

**P-110** Characterization of the Worm Homolog of *memo,* a Gene that Acts Downstream of the Mammalian EGF Pathway
**C. Ewald,** N. Hynes and J. Alcedo

**P-111** Identification and Characterization of Proteins Controlling the Intracellular Trafficking of LET-23 EGFR
**P. Gutierrez,** A. Stetak, E. Fröhli-Hoier and A. Hajnal

**P-112** Do *ark-1*, *gap-1* and *sli-1* Have synMuvB Activity?
**N.A. Hopper,** J. Lee, P.W. Sternberg and A.D. Wooler

**P-113** Identification of Upstream Factors Regulating the Expression of the T-box Gene mab-9
**G. Jafari** and A. Woollard

**P-114** Testing the *in vivo* Importance of Putative Regulatory Sites in $IP_3$ Receptors
J. Legg, R. Vazquez-Manrique and **H. Baylis**

**P-115** MicroRNAs: Subcellular Localisation of Silencing Complexes
**S.E.V. Linsen,** B. Tops, R.F. Ketting, R.H.A. Plasterk

**P-116** Characterization of *C. elegans* NuRD Complexes
**P. Moroni,** M. Passannante, K. Brunschwig, A-L. Chanez, V. Cerantola and F. Müller

**P-117** The Role of Presenilins in Calcium Signalling in *Caenorhabditis elegans*
**H. Peterkin** and H. Baylis

**P-118** A Candidate-Based Approach for Genetic Interaction with *gap-1* Identifies a WD40 Encoding Gene
**G. Poulin** and J. Ahringer

**P-119** Investigating the Role of rnt-1 and bro-1, and their Mammalian Orthologues, in Seam Cell Proliferation in *C. elegans*
**N. Saad,** H. Kagoshima and A. Woollard

**P-120** 3' End Processing and Transcription Termination in the Nematode *Caenorhabditis elegans*
**H. Sharpe,** C. Browne and A. Furger

**P-121** An Expression Study of Seven Putative Nuclear Receptors Organized in a Cluster on Chromosome V of *Caenorhabditis elegans*
**J. Vohanka,** Z. Kostrouch and M. Kostrouchova

**P-122** A Role of the *C. elegans* Mi-2 Protein LET-418 in LIN-12/Notch Signalling Pathway
Y. Zhang, F. Guerry, **C-O. Marti,** F. Müller

## Environment, Stress and Disease

**P-123** Characterizing APE-1
**E. Bogan,** E.A. Kritikou and M.O. Hengartner

**P-124** A Worm Model for Spinal Muscular Atrophy
**M. Briese**, B. Esmaeili, L.A. Brown, E. Burt, P. Towers and D.B. Sattelle

**P-125** The SLO-1 BK Channel of *C. elegans* is Critical for Muscle Function and is Involved in Dystrophin-Dependent Muscle Dystrophy
**M. Carre-Pierrat,** K. Grisoni, K. Gieseler, M-C. Mariol, E. Martin, M. Jospin, B. Allard and L. Ségalat

**P-126** Overexpression of Subunit C, the Main Component of the Storage Material in Juvenile Neuronal Ceroid Lipofuscinosis (JNCL), Causes Disruption of Mitochondria in *C. elegans* and Subsequent Death
G. de Voer, R.O.B. de Keizer, P. van der Bent, G-J. B. Van Ommen, D.J.M. Peters and **P.E.M. Taschner**

**P-127** Identifying Novel Genes Involved in DNA Damage Response Pathways in *C. elegans*
**C. Dittrich** and M.O. Hengartner

**P-128** Learning Lessons from *dys-1* and *snf-6* – Having Acetylcholine as a Teacher
**D. Faggionato,** O. Nyamsuren and R. Baumeister

**P-129** Optimization of a Screen for Molecules Slowing Muscle Degeneration in a *Caenorhabditis elegans* Model of Dystrophin-Dependent Myopathy
**J. Giacomotto** and L. Segalat

**P-130** Sugarland Express: Many *C. elegans* Genes Implicated in Glycosylation Affect Surface Adhesion to the Bacteria *Microbacterium nematophilum* and *Yersinia pestis*
**M.J. Gravato-Nobre,** D. O'Rourke, F.A. Partridge, D. Stroud, K.J. Yook, C. Darby, J. Hodgkin

**P-131** Identification of pH Regulated Genes in the Nematode *Caenorhabditis elegans:* New Potential Drug Targets for the Treatment of Pathogenic Nematodes
**R. Hall,** P. Klappa and F.A. Mühlschlegel

**P-132** Increased Sensitivity to Cisplatin by Depletion of ASNA-1 in *C. elegans*
**O. Hemmingsson,** M. Still, G. Kao, S. Tuck and P. Naredi

**P-133** Deciphering the Role of Serotonin Blocking Muscle Degeneration
**S. Jünemann** and L. Segalat

**P-134** Implication of the Dense Body Protein Dyc-1 in the Dystrophin Dependent Muscle Degeneration
**C. Lecroisey,** K. Gieseler and L. Ségalat

**P-135** Identification of Genes Affecting Resistance to Anoxia in *Caenorhabditis elegans*
**V. Menuz,** M. Formallaz, M. Gomez, M.O. Hengartner and J.C. Martinou

**P-136** PCB52 Induced Gene Expression in the Nematode *Caenorhabditis elegans*
**R. Menzel,** J. Kulas, H.L. Yeo, S. Li and S. Stürzenbaum

**P-137** The Role of SHC in Stress Response and Innate Immunity
**L. Moronetti Mazzeo,** J. Solinger, A. Sommer, G. Cassata

**P-138** A Knock-Out of the Small Non-Coding Y RNA in *C. elegans*
**M. Müller,** V. Jantsch, M. Jantsch, G. Steiner

**P-139** Bleomycin-Induced Fibrosis in *C. elegans?*
**A. Pasparaki,** N. Tavernarakis and E. Tzortzaki

**P-140** Genome-Wide Screen for Heat Shock Regulatory Networks in *C. elegans*
**K. Richter** and R.I. Morimoto

**P-141** Riboflavin Derivatives Improve Physiology and Growth of Frataxin Deficiency in *C. elegans* and *S. cerevisiae*
**S. Ros,** P. Gonzalez-Cabo, R. Vazquez-Manrique and F. Palau

**P-142** Use of Transgenic *Caenorhabditis elegans* (HS-1 line) for Detection of Heavy Metals in Aquatic Media
**A.I. Stormberg,** J.M. Barnes, S. Humphries, T. Glenn and P.L. Williams

**P-143** Mutations in a Predicted Galactosyl Transferase Affect the Ability of the Pathogen *M. nematophilum* to Infect *C. elegans*
**D. Stroud,** M. Gravato-Nobre and J. Hodgkin

**P-144** Environmental Genomics of Fluoranthene Exposure
S.C. Swain, J. Wren, M. Jonker, N. Redshaw, C. Svendsen, D.J. Spurgeon, A.J. Morgan, P. Kille and **S.R. Stürzenbaum**

**P-145** *Caenorhabditis elegans* is a model host for *Listeria monocytogenes*
**L.E. Thomsen,** B.M. Jensen, N.J. Faergeman, B.H. Kallipolitis and H. Ingmer

**P-146** KAL-1 Function in *C. elegans*
**C. Vicario,** E. Di Schiavi, G. Gelsomino, A. Sollo, S. Arbucci and P. Bazzicalupo

**P-147** The DOG-1 Helicase, Genomic Instability and Fanconi Anemia
**J.L. Youds,** N.J. O'Neil, L.J. Barber, S.J. Boulton and A.M. Rose

**P-148** Exploring Innate Immunity Signalling Networks
D. Wong, **K. Ziegler,** C.L. Kurz, N. Pujol and J. Ewbank

## Methods and Resources

**P-149** Towards the Development of a *Minos*-based Transposon Tool in *C. elegans*
**D. Bazopoulou** and N. Tavernarakis

**P-150** *C. elegans* Reporter Fusion Genes Generated by Seamless Modification of Large Genomic DNA Clones
**C.T. Dolphin** and I.A. Hope

**P-151** A French Functional Genomics Platform
Y. Duverger, J. Reboul, D. Wong and **J. Ewbank**

**P-152** Development and Optimisation of *Mos1* for Gene Tagging in *C. elegans*
**J. Gallagher** and P. Kuwabara

**P-153** Protocol Optimization to Localize Transposon Insertions in *C. elegans* Strains
**L. Granger,** E. Martin, E. Vallin and L. Segalat

**P-154** Screen for Synthetic Lethality with Tumor Suppressors and the Development of a DSB Repair Assay
**D.B. Pontier,** R.H.A. Plasterk and M. Tijsterman

**P-155** Genomic Engineering of Acetylcholine Receptor by *Mos*TIC
**G. Rapti,** V. Robert and J-L. Bessereau

**P-156** Progress of the Localization of Expression Mapping Project
**J. Shingles**, J. Reece-Hoyes, D. Dupuy, M. Vidal and I. Hope

**P-157** CGC and WormBase
**M.A. Tuli,** J. Hodgkin, T. Stiernagle, R. Herman and R. Durbin

**P-158** Molecular Characterization of MOS Transposon-Tag Strains Produced by the NemaGENETAG Project
**E. Vallin,** L. Granger, E. Martin and L. Segalat

**P-159** Curating Wormbase Gene Structures
**G. Williams,** P. Davis, A. Rogers, P. Ozersky, J. Spieth, T. Bieri, D. Blasiar

**P-160** Multi-Parameter Axial Profiling of Transgenic *C. elegans* Expressing Fluorescent Proteins from Various Cell-Specific, Tissue Specific and Developmentally Regulated Promoters
B. Wang, J. Thompson, Y. Zhang, M. Herman, M. Lomakina, B. Holcombe, **R. Pulak**

***P-001***

# NHR-40, a *Caenorhabditis elegans* Supplementary Nuclear Receptor Regulates the Development of Embryos and L1 Larvae

**Eva Brozova[1],** Katerina Simeckova[1], Zdenek Kostrouch[2], Marta Kostrouchova[1]
[1]Laboratory of Molecular Biology and Genetics and [2]Laboratory of Molecular Pathology, Institute of Inherited Metabolic Disorders, 1st Faculty of Medicine, Charles University, Prague

In this study, we functionally characterized NHR-40, a member of an extended group of supplementary nuclear receptors (Robisnon-Rechavi et al, J. Mol. Evol. 2005, 60: 577-586). We show that *nhr-40* is expressed in 3 isoforms that are regulated by two promoters organized in tandem. The function of NHR-40 was studied by RNA interference, by overexpression of *nhr-40* and using a mutant strain, RB840, which carries a deletion in the first intron. We show that NHR-40 regulates embryonic and early larval development. This phenotype is connected with defective movement and muscle cell development. This adds NHR-40 to the growing list of nematode NHRs involved in the regulation of development.

Acknowledgement: We thank Drs. A. Fire for vectors and host used in RNAi, M.W. Krause for support and advice, The *C.elegans* Gene Knockout Consortium for preparation of the deletion strain RB840. The work was supported by grants 303/03/1115 and 301/ 05/0859 from the Czech Science Foundation and by the grant 0021620806 from the Ministry of Education, Youth and Sports of the Czech Republic.

BACK

***P-002***

# Elucidating the Role of MicroRNAs in *C. elegans* Development

**Alejandra Clark,** Eric Miska
The Wellcome Trust/Cancer Research UK Gurdon Institute, University of Cambridge, UK

MicroRNAs (miRNAs) are small non-coding RNAs found in all metazoa studied so far. In animals miRNAs regulate gene expression by binding with incomplete complementarity to the 3'UTR of target mRNAs inhibiting their translation. Although a number of miRNAs have now been cloned and identified, in most cases their functions remain elusive. This is partly due to the difficulty in identifying target genes based on partial complementarity between miRNAs and target mRNAs. To investigate the biological roles of miRNAs in *C. elegans* development we are using an algorithm that predicts miRNA targets based on miRNA seed complementarity and conservation of the mRNAs 3'UTR between *C. elegans* and *C. briggsae* (Stark A. et al., 2005, Brennecke J. et al., 2003). To validate the targets proposed by this method and gain new insight into the biological roles of specific miRNAs, *C. elegans* miRNA deletion strains will examined for the expected up-regulation of a number of predicted targets (Miska E. et al., unpublished). Antibody stainings and GFP::Target 3'UTR reporter constructs in wild-type and miRNA deletion strains will be investigated. In addition, predicted target genes will be tested by RNAi knockdown screening for suppression of abnormal phenotypes of miRNA deletion strains.

BACK

# Structural and Functional Characterization of Aminopeptidase P in *Caenorhabditis elegans*

K. A. P. Payne[1], **H. Craig[1]**, D. R. Brooks[2], A. J. Turner[1], M. R. Parsons[1], R. E. Isaac[1]
[1]Faculty of Biological Sciences, Miall Building, University of Leeds, Leeds LS2 9JT, UK; [2]Biosciences Research Institute, School of Environment and Life Sciences, University of Salford, Salford M5 4WT.

Aminopeptidase P (APP) is a metallopeptidase that specifically removes amino acids from the N-terminus of peptides with a penultimate N-terminal proline residue. In humans at least two APP genes exist, coding for a soluble cytosolic enzyme (APP-1) and a membrane-bound form (APP-2). APP-2 has been the subject of much research due to it's role in the regulation of blood pressure and potential as a drug target in the treatment of hypertension. APP-1 however has, as yet, no clear role assigned to it. Recently, a potential third human homologue (APP-3) has been identified and is predicted to be targeted to the mitochondrion. Examination of the *C. elegans* Genome reveals that homologues of both APP-1 (W03G9.4) and APP-3 (R119.2) are present. With a view to understanding further the roles of aminopeptidase P, we are carrying out analysis of both APP-1 and APP-3.

The *C. elegans* homologue of APP-1 has been cloned, over-expressed and purified. Biophysical and biochemical analyses show recombinant APP-1 to form a homodimer and identifies $Zn^{2+}$ as the active site metal ion. APP-1 has been crystallized and diffraction data collected to 2.3 Å. Immunostaining with polyclonal antibodies that have been raised against recombinant APP-1 showed localization to the intestinal cells, the female germline and embryos. Preliminary investigation of an APP-1 deletion mutant (tm1715) indicates a likely involvement in embryo development

BACK

***P-004***

# RNAi Screens in *C. elegans* to Identify Signalling Modulators in Vulval Development

**Catriona Crombie** and Andrew Fraser
The Wellcome Trust Sanger Institute, Hinxton, Cambridge, CB10 1SA

In *C. elegans*, genome-wide RNAi screens are a powerful way to identify genes involved in any given process. We are seeking to use RNAi screens in the worm to find as comprehensively as possible the genes involved in different signalling pathways and the points of cross talk between the pathways.

*C. elegans* vulval development is well characterised: the 22 cells of the fully developed vulva are derived from 6 precursor cells and this is already known to require the EGFR-ras-raf-MAPK, NOTCH, and Wnt signalling pathways. Thus in this simple tissue we can examine multiple conserved signalling pathways, all of which can play major roles in cancer when deregulated. We have carried out multiple RNAi screens to identify new genes that modulate signalling in the vulva. These include screens in the *let-60 (n1046)* strain, which has a gain of function mutation in the ras ortholog *let-60*. ~50% of *let-60 (n1046)* worms are Muv and we have used RNAi to screen for both enhancers and suppressors of this phenotype. We have also screened for suppressors in strains that are 100% Muv such as *lin-15 (n765)* and *lin-1 (e1026)*. Finally we have screened for genes that have a Muv RNAi phenotype in wild type strains. We have found several novel genes that appear to repress ras signalling along with several downstream effectors of signalling including transcription factors. The human orthologues of genes identified in these screens may play a role in cancer. We will present these results.

BACK

***P-005***

# A GFP Reporter Based Screen for the Notch Target Genes during Vulval Development

**Sarfarazhussain Farooqui**, Ivo Rimann and Alex Hajnal
Institute of Zoology, University of Zurich, Winterthurerstr. 190, 8057 Zurich, Switzerland, sarfarazhussain.farooqui@zool.unizh.ch

During hermaphrodite vulval development, lateral signaling from the primary vulval precursor cell (VPC) P6.p activates the LIN-12 Notch pathway in the neighbouring secondary VPCs P5.p and P7.p. The LIN-12 Notch signal inhibits primary fate specification and promotes the secondary vulval fate by activating transcription factors of the CSL family, which turn on the expression of specific target genes.

LIN-12 Notch targets usually contain clusters of CSL binding sites (RTGGGAA) in their promoter/enhancer regions. To identify new target genes of the LIN-12 Notch pathway in a systematic way, we searched the *C. elegans* genome sequence for genes containing clusters of three or more CSL sites within 2 kbp upstream of the start codon. We further refined our list of candidate Notch targets by taking into consideration only those genes in which the putative CSL sites are conserved in the *C. briggsae* orthologs. Promoter-GFP fusion constructs for the 23 strongest candidate genes were made by fusion PCR and injected into wild-type worms to create transgenic lines. Details on the expression patterns of the reporter constructs generated so far will be presented at the meeting.

BACK

# Comprehensive Analysis of Gene Expression Patterns of Hedgehog-Related Genes

**Limin Hao[1],** Robert Johnsen[3], Gilbert Lauter[1, 2], David Baillie[3], Thomas R. Bürglin[1,2]
[1]Department of Biosciences and Nutrition, and Center for Genomics and Bioinformatics, Karolinska Institutet, [2]School of Life Sciences, Södertörns Högskola, Alfred Nobels Allé 7, SE-141 89 Huddinge, Sweden. [3]Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, B.C. Canada

The Caenorhabditis elegans genome encodes ten proteins that share homology with the Hedgehog signaling molecule through their C-terminal autoprocessing Hint/Hog domain. These proteins contain novel N-terminal domains, and C. elegans encodes dozens of additional proteins containing only these N-terminal domains. These gene families are called warthog, groundhog, ground-like and quahog, collectively called hedgehog (hh)-related genes. Previously, the expression pattern of sixteen genes was examined which showed that they are primarily expressed in the ectoderm. With the completion of the C. elegans genome sequence in October 2002, we examined and identified all 61 hh-related ORFs. ORF analysis revealed that 30% of the genes still had errors in their predictions that we corrected here. We performed a comprehensive expression analysis using GFP reporters. The hh-related genes are expressed in one or a few of the following tissues: hypodermis, seam cells, excretory duct and pore cells, vulval epithelial cells, rectal epithelial cells, pharyngeal muscle or marginal cells, arcade cells, support cells of sensory organs, and neuronal cells. Using time-lapse recordings, we discovered that some hh-related genes are expressed in a cyclical fashion in phase with molting during larval development. We also generated several translational GFP fusions, but they did not show any subcellular localization. In addition, we also studied the expression patterns of two genes with similarity to Drosophila frizzled, T23D8.1 and F27E11.3A, and the ortholog of the Drosophila gene dally-like, gpn-1, which is a heparan sulfate proteoglycan. The two frizzled homologs are expressed in a few neurons in the head, and gpn-1 is expressed in the pharynx. No bona-fide Hh signaling pathway is present in C. elegans. Given that the hh-related gene products have a predicted signal peptide for secretion, it is possible that they constitute components of the extracellular matrix (ECM). They might be associated with the cuticle or be present in soluble form in the body cavity. They may interact with the Patched or the Patched-related proteins in a manner similar to the interaction of Hedgehog with its receptor Patched.

BACK

# GEI-16, a Putative Component of the Hemidesmosome, is Necessary for Tissue Stability and Epithelial Morphogenesis

**SB. Hetherington**, FI. Pellerone, CA. Behm
School of Biochemistry and Molecular Biology, Australian National University, Acton, AUSTRALIA, 0200

The *Caenorhabditis elegans* hemidesmosome or fibrous organelle (FO) is a trans-epidermal attachment structure that spans the hypodermis, linking the basal hypodermal membrane to the cuticle, thereby transducing the force of muscle contraction to the exoskeleton and propagating movement. The hemidesmosome has a similar structure and the same localisation and function as the vertebrate hemidesmosome, but there is little direct homology between the components except for the intermediate filaments (IFs), and the plectin VAB-10A (Boscher, 2003; Hahn, 2001).

*gei-16* was isolated in a RNA interference (RNAi) screen for potential anthelminthic drug targets because it fulfilled the selection criteria of being nematode specific, and when knocked down results in lethality at many stages of development, sterility and paralysis. Specifically, RNAi of *gei-16* in *C.elegans* results in slow development, and muscle weakness before paralysis, arrest and death in the later larval and adult stages. It appears that *gei-16* is necessary for maintaining adhesion of the muscles and cuticle to the hypodermis as depletion of *gei-16* message causes 100% penetrance of a muscle detachment phenotype, and a 47% penetrance of a cuticular detachment phenotype. Epithelial morphogenesis is also affected with larvae and adults showing uneven excretory channels and abnormal vulval morphogenesis. RNAi affected worms often arrest or die before the maturation of the reproductive system. Worms that survive to adulthood produce few eggs, which are retained within the uterus and often fail to hatch. This appears to be due to the combined failure of vulval morphogenesis, detachment of the vulval muscles and a failure of the embryo to elongate. Localisation of *gei-16* mRNA to all muscle-apposed epithelia is consistent with a role in epithelial tissue stability and morphogenesis—an expression pattern which mirrors that of hemidesmosome components. Furthermore, RNAi in GFP reporter strains of *C. elegans* shows that components of the hemidesmosome are disorganised in *gei-16* RNAi worms. Results from the interactome data set show that *gei-16* binds to *mua-6*, an IF in the hemidesmosome (Li et al, 2000).

**References:**

BOSHER, et al. (2003) The Caenorhabditis elegans vab-10 spectraplakin isoforms protect the epidermis against internal and external forces. *J Cell Biol,* 161**,** 757-68.

HAHN, B. S. & LABOUESSE, M. (2001) Tissue integrity: hemidesmosomes and resistance to stress. *Curr Biol,* 11**,** R858-61.

LI, S, et al. (2004) A map of the interactome network of the metazoan C. elegans. *Science,* 303**,** 540-3.

BACK

***P-008***

# Valproic Acid Affects Gene Expression and Development in *Caenorhabditis elegans*

**Marketa Kostrouchova**[1], Petr Liby[1], Marta Kostrouchova[2] and Zdenek Kostrouch[1]
[1]Laboratory of Molecular Pathology, and [2]Laboratory of Molecular Biology and Genetics, Institute of Inherited Metabolic Disorders, 1st Faculty of Medicine, Charles University, Prague

Valproic acid (VPA, 2-propylpentanoic acid), a drug widely used in the treatment of epilepsy and bipolar disorder, has been shown to posses anti-cancer activity. VPA suppresses tumor growth, induces tumor cell differentiation and reduces the formation of metastases. Multiple mechanisms were shown to be part of VPA's mode of action, including inhibition of histone deacetylase activity, inhibition of glycogen synthase 3 and interaction with the regulation of nuclear hormone receptors. We studied the effect of VPA on the development and gene expression in *C. elegans*. VPA delivered to worms by microinjections to the ovarial syncytium or by incubating worms in various concentrations of VPA (1 to 10 mM) caused a dose dependent delay of larval development and lethality in two developmental stages: in late embryogenesis and in L1 larval stage. Surprisingly, most animals were able to overcome the effect of VPA, suggesting a compensatory or balancing mechanism. Microarray experiments show that genes affected by VPA can be divided to at least three different categories suggesting multiple mechanisms to be involved in VPA mode of action.

Acknowledgement: We thank Dr. M.W. Krause for support and advice. We thank the NIDDK Microarrays facility for performing the microarrays analysis. The work was supported by the grant 303/03/0333 from the Czech Science Foundation, by the grant NC-7554 from the Ministry of Health of the Czech Republic and by the grant 0021620806 from the Ministry of Education, Youth and Sports of the Czech Republic.

BACK

# Inhibition of *bir-1*, the Homologue of Human Survivin, Induces Changes of Expression of Developmentally Active Collagen Genes in L1 Larval Stage

**Petr Liby[1],** Marketa Kostrouchova[1], Michal Pohludka[1], Jaroslav Vohanka[2], Eva Brozova[2], Marta Kostrouchova[2] and Zdenek Kostrouch[1]
[1]Laboratory of Molecular Pathology and [2]Laboratory of Molecular Biology and Genetics, Institute of Inherited Metabolic Disorders, 1st Faculty of Medicine, Charles University, Prague

BIR-1 is a member of the Inhibitors of Apoptosis protein family that is involved in the regulation of microtubule organization, condensation of chromosomes and cell division in *C. elegans* as well as in vertebrates. In *C. elegans*, *bir-1* is organized in an operon together with SKIP, a transcription and splicing cofactor. *bir-1* inhibition induces developmental defects including dpy and egl phenotypes. Here we studied the effect of bir-1 inhibition by RNAi in L1 larval stage using whole genome microarrays (Affymetrix). Microarrays detected changes in expression of several developmentally active collagen genes in *bir-1* inhibited larvae. We further characterized the effect of *bir-1* inhibition on selected collagen gene expression and induction of dpy phenotype.

Acknowledgement: We thank Drs. A. Fire for GFP construct, vectors and host used in RNAi and M.W. Krause for support and advice. We thank the NIDDK Microarrays facility for performing the microarrays analysis. The work was supported by the grant 303/03/0333 from the Czech Science Foundation, by the grant NC-7554 from the Ministry of Health of the Czech Republic and by the grant 0021620806 from the Ministry of Education, Youth and Sports of the Czech Republic.

BACK

***P-010***

# NHR-25 Role in the Seam Cell Differentiation in *C. elegans*

**Martina Machackova**[1], Marie Silhankova[1], Marek Jindra[1,2] and Masako Asahina[1,2]
[1]Dept. of Molecular Biology, University of South Bohemia and [2]Biology Center, Czech Academy of Sciences, Budweis, Czech Republic 37005

Epidermal cell differentiation involves dynamic cell shape changes, important for animal development. During the postembryonic development of *Caenorhabditis elegans*, most of the stem epidermal seam cells asymmetrically divide and their anterior daughters fuse with the surrounding hyp syncytium, thus creating gaps between the seam stem cells. After each asymmetric division that separates them, the seam cells actively bridge over the gaps to renew their mutual contacts via adherens junctions. The seam cell contacts are important for further epidermal differentiation but the mechanism underlying their renewal is still unknown. We and others have previously shown that the conserved nuclear receptor NHR-25 is necessary for the seam cells to restore contacts after the asymmetric divisions occurring during each larval stage: attenuated NHR-25 function leaves the seam cells round and isolated. *nhr-25(RNAi)* adults consequently exhibit extra seam cells, indicating that the proper cell fate decision in these epidermal stem cells has been perturbed. NHR-25 might be required either in the seam cells or in the hyp syncytium. Since NHR-25 is highly expressed in the seam cells, its action could be autonomous to these cells. To test this hypothesis, we took an approach of a tissue-specific RNAi system. We have utilized the seam cell-specific promoter (SCM), which is typically used as a seam cell marker, to express a hairpin-loop of *nhr-25* RNA in transgenic worms. Independent lines of these transgenic adults show extra seam cells, similar to the effect of *nhr-25* RNAi delivered by injection. Although NHR-25 may play a role also in the hyp syncytium, this result suggests that NHR-25 is autonomously required in the seam cells.

Supported by projects Z60220518 (GAAV), 524/03/H133 (GACR) and 6007665801 from the Czech Ministry of Education.

BACK

# Misexpression of Acetylcholinesterases in the *C. elegans pha-2* Mutant Causes Post-Embryonic Pharyngeal Isthmus Deformation

Catarina Mörck[1], Claes Axäng[2], Mattias Goksör[3] and **Marc Pilon**[2]
[1]Dept. of Cell and Mol. Biol., Göteborg Univ., Box 462, SE-405 30 Göteborg, Sweden; [2]Dept. of Chem. and Biol. Eng., Chalmers Univ., Box 462, SE-405 30, Göteborg, Sweden; [3]Dept. of Physics, Göteborg Univ., SE-412 96, Göteborg, Sweden.

*pha-2* is the *C. elegans* homolog of the vertebrate homeobox gene *Hex*. Embryonic expression of *pha-2* is mostly pharyngeal and the only described mutant allele of *pha-2* results in a severe pharyngeal defect in which certain muscle cells (pm5 cells) and neurons are grossly deformed. Here we report a detailed characterization of the *pha-2* gene and phenotype using cell-type specific reporters, optical manipulation of the nuclei in pharyngeal muscle cells using optical tweezers, electron microscopy, staining of the actin cytoskeleton as well as phenotypic rescue and ectopic expression experiments. The main findings of the present study are: (i) The *pha-2 (ad472)* mutation specifically impairs the pharyngeal expression of *pha-2*; (ii) In the *pha-2* mutant, the cytoskeleton of the pm5 cells is measurably weaker and severely disrupted by large tubular structures and organelles; (iii) The pm5 cells of the *pha-2* mutant fail to express the acetylcholinesterase genes *ace-1* and *ace-2*; (iv) Ectopic expression of *pha-2* can induce ectopic expression of *ace-1* and *ace-2*; and (v) Inhibition of acetylcholinesterase in a mutant with mislocalized pm5 cell nuclei can reproduce the deformed isthmus phenotype of the *pha-2* mutant.

BACK

# Role of *rad-5* in Development of *C. elegans*

**Sandra Moser**[1], Arno Alpi[2], Ingo Büsing[3], Ralf Schnabel[3] and Anton Gartner[1]
[1]Welcome Trust Biocentre, University of Dundee, Dundee DD1 5EH, UK [2]Medical Research Council Laboratory of Molecular Biology, Cambridge CB2 2QH, UK; [3]Institut für Genetik, TU Braunschweig, D-38106 Braunschweig, Germany

DNA damage checkpoints have evolved to ensure genomic stability. Upon DNA damage these checkpoints transiently stop cell cycle progression which allows the repair of damaged DNA or if the damage is too severe the removal of compromised cells by apoptosis. *rad-5* is an evolutionary conserved DNA damage checkpoint gene that in C. elegans has been shown to be required for γ-irradiation induced cell cycle arrest and apoptosis. Genetic observations
revealed that rad-5 does not act in the conserved *mrt-2 hus-1* DNA damage pathway but its exact role in the DNA damage checkpoint pathway is still unknown. In addition to its role as DNA damage checkpoint gene *rad-5* is also required for embryonic development as *rad-5* mutants shifted to 25°C show embryonic lethality.

To assess the developmental phenotypes of *rad-5* and to potentially determine whether these are related to *rad-5* function in maintaining genome stability we aim to describe the developmental phenotypes of *rad-5*. To determine the precise embryonic defects we performed a lineage analysis for *rad-5* mutants that were shifted to 25°C. Interestingly, in contrast to other DNA damage checkpoint genes like *atl-1* or *chk-1* which when knocked out also result into embryonic lethality *rad-5* mutants exhibit a distinct lineage defect at the 12 cell stage but don't show any abnormalities earlier in development {Garcia-Muse et Boulton, 2005, Kalogeropoulos et al., 2004, Brauchle et al., 2003). Surprisingly, a *rad-5* deletion mutant *rad-5 (tm1528)* showed no apparent defect in embryonic development but worms homozygous for *rad-5 (tm1528)* are sterile and show a protruding vulva (pvl) phenotype indicating that *rad-5* might play a role in the vulva cell organization or in the cell lineages giving rise to the vulva. To further pin down these defects we are currently creating rad-5 (tm1528) lines carrying GFP makers that stain specific vulva cells. To determine whether the slow growth phenotype of some *rad-5* alleles is rescued by other checkpoint proteins we made the respective doubles and found that only *cep-1* leads to a partial rescue of the slow growth phenotype, whereas *atm* does not have such a defect whereas *mrt-2* and *hus-1* enhance the slow growth phenotype (Ahmed Alpi et al., 2001).

Finally to start addressing how *rad-5* might function at the molecular level, failing to get new insights from a suppressor screen that only lead to the identification of intragenic suppressors, we are now trying to identify interacting proteins of RAD-5 using immunoprecipitation and to assess *rad-5* localization and potential changes in its localization upon DNA damage by immunofluorescence in worm and tissue culture based systems.

BACK

# Analyzing a Putative Acetyl-CoA Transporter Gene Involved in Development and Morphogenesis

**Nomura, K. H.**[1,2], Mizuguchi, S.[1,2], Dejima, K.[1,2], Murata, D.[1,2], Matsuishi-Nakajima Y.[2,3], Kawasaki, N.[2,3], Gengyo-Ando, K.[2,4], Mitani, S.[4], Hirabayashi, Y.[2,5], Nomura, K.[1,2]

[1]Department of Biological Sciences, Kyushu University, Fukuoka, Japan; [2]CREST, JST, Saitama, Japan; [3]National Institute of Health Science, Tokyo, Japan; [4]Department of Physiology, Tokyo Women's Medical University School of Medicine, Tokyo, Japan; [5]RIKEN Brain Science Institute, Wako, Japan

In eukaryotes, acetylation is the most common covalent modification out of over two hundred types of modifications reported to date. Among various acetylation, acetylation of ε-amino group of lysine residues have been extensively studied in regard to "histone code" of epigenetic information transfer mechanism. Recently, lysine acetylation has been detected not only in nuclear histone/non-histone proteins but also in various proteins found in cytoplasm. One of the authors (Y. Hirabayashi) previously reported a cDNA cloning of mammalian acetyl-CoA transporter gene (AT-1; SLC33). The gene product is involved in O-acetylation of glycoconjugates in mammalian cells and its DNA sequence is well preserved from bacteria to human.

There is only one SLC33 orthologue (T26C5.3) in the nematode. We isolated intron deletion mutant (tm246) and exon deletion mutant (tm1317) of this gene by screening TMP/UV deletion mutant library. The mutant worm (tm1317) showed decreased brood size (1/3 of N2), poor gonadal development but mantained in our laboratory for over 300 generations. In transgenically rescued tm1317 mutant worm strains, *T26C5.3*::GFP fusion proteins are expressed in the head region, seam cells, spermatheca, vulva and the tail region.

In this paper, we extracted proteins from wild type and these mutant worms, and analyzed proteomes of these animals with two dimensional (2D) electrophoresis. We differentiated more than 2,000 spots, and the spot patterns were analyzed with image analysis software. Two dimensional differential in-gel electrophoresis (2D-DIGE) technique was also used to compare proteomes between wild type and the mutant worms. About 500 spots showed over 1.5-fold increase in deletion mutant, and about 300 spots showed over 1.5-fold decrease in the mutant. Over 100 spots were picked up from the gel, and sequenced with LC/MS/MS to identify gene networks involved in development of mutant phenotypes. In this paper, we describe the results of transgenic expression analysis of the gene and report the results of proteome analysis (2D PAGE and 2D-DIGE). Among the proteins identified are paramyosin, intermediate filament proteins and α-tubulin.

BACK

# Understanding the Role of a Non-Peptidase Member of the ACE Family in Nematode Moulting

**Hannah L. Craig[1],** Clare Hunton[1], Darren R. Brooks[2], R.Elwyn Isaac[1]
[1]Faculty of Biological Sciences, Miall Building, University of Leeds, Leeds LS2 9JT, UK; [2]Biosciences Research Institute, School of Environment and Life Sciences, University of Salford, Salford M5 4WT

Analysis of the large number of protease-like genes present in animal genomes has shown that a considerable proportion of these genes code for proteins that are structurally related to peptidases, but lack key catalytic residues. These proteins are classified as 'non-peptidase' family members. One such protein is the single member of the angiotensin-converting enzyme (ACE) family of zinc metallopeptidases found in *C. elegans*. ACN-1 (ACE-like non-metallopeptidase) shares considerable identity with ACE from other organisms, but lacks the conserved active site residues required for proteolytic activity. ACN-1 also differs from other ACEs in having a proline/glutamic acid-rich domain, and, towards the C-terminus, a cysteine-rich region, a proline and threonine/serine rich region and a potential GPI anchor attachment site. It has recently become apparent that an alternatively-spliced form of *acn-1* may be expressed at low levels. A single EST with 5' and 3' sequence identical to *acn-1*, but missing a major portion of the central ACE-like domain, has been identified. Sequences showing considerable similarity to *acn-1* are also present in EST and genomic datasets from several parasitic nematodes.

ACN-1 has an essential role in C. elegans development and morphogenesis. GFP-tagged ACN-1 expression was observed in the hypodermal cells and developing vulva of hermaphrodites and also the ray papillae of the male tail. Deletion of a 692 bp fragment (strain tm844, provided by Shohei Mitani, Tokyo Women's Medical College) comprising part of exon 7 and all of exons 8 and 9 resulted in arrested embryonic development and acn-1 RNAi resulted in larval arrest due to a moulting defect. Further examination showed that acn-1 RNAi-treated adults had disrupted alae, an incomplete seam syncytium and a protruding vulva. SEM images of arrested larvae showed a failure to shed the old cuticle after RNAi. Adult males displayed similar hypodermal defects, including a severely disrupted tail. The nuclear hormone receptors *nhr-23* and *nhr-25* were shown by RNAi to regulate expression of *acn-1::gfp*, hence positioning *acn-1* downstream of these nuclear hormone receptors in the genetic cascade controlling moulting.

BACK

# Analysis of Epithelial Genes during Postembryonic Development of *C. elegans*

Jennifer Pilipiuk, Gisela Helbig and **Olaf Bossinger**
Institut für Genetik, Heinrich-Heine-Universität Düsseldorf, D-40225 Düsseldorf, Germany

We wish to understand how epithelial polarity and tissue integrity is maintained. Here, we consider the role of DLG-1 (Discs large), LET-413 (Scribble), ERM-1 (Ezrin-Radixin-Moesin), and the catenin-cadherin complex (HMP-1–HMP-2–HMR-1) during postembryonic development of *C. elegans*. In the course of embryogenesis these genes play an important role in the establishment and maintenance of epithelial polarity, the formation of the lumen, and cell-cell adhesion. In contrast, during larval and adult development only "newly" established epithelia (e.g. the spermatheka or the vulva) show severe defects after bacterial RNAi against DLG-1, LET-413 and ERM-1, while the depletion of the catenin-cadherin complex seems not to cause visible defects. Nevertheless, how polarity of already established epithelia (e.g. the intestine or the hypodermis) is maintained during postembryonic development remains elusive. In the case of DLG-1, our results suggest that a small amount of protein is sufficient, but cannot fall below a certain threshold without causing defects. Intestinal and hypodermal polarity during larval and adult development of *C. elegans* might also depend on other, so far unidentified proteins. A detailed analysis of DLG-1, LET-413 and ERM-1 phenotypes during postembryonic epithelial development in *C. elegans* will be presented and discussed.

BACK

***P-016***

# Analysis of CHR-3 (*nhr-23*) Loss of Function in L1 Larval Stage Using Whole Genome Microarrays and Comparative Two Dimensional Protein Chromatography

**Michal Pohludka[1],** Jaroslav Vohanka[2], Marta Kostrouchova[2] and Zdenek Kostrouch[1]
[1]Laboratory of Molecular Pathology and [2]Laboratory of Molecular Biology and Genetics, Institute of Inherited Metabolic Disorders, 1st Faculty of Medicine, Charles University, Prague

CHR3 (*nhr-23*, NR1F4) is an evolutionarily conserved nuclear receptor involved in the regulation of molting and body morphology in larval development. We studied the role of CHR-3 (*nhr-23*) by RNAi in L1 stage and analysed the phenotype on mRNA and protein levels using whole genome microarrays (Affymetrix) and a comparative two dimensional protein chromatography by ProteomeLab™ PF 2D Protein Fractionation System. Protein profiling was achieved by Isoelectric chromatofocusing used as the first dimension separation and reversed phase chromatography separating proteins by hydrophobicity as the second dimension. The differentially expressed proteins were detected by ProteoVue and DeltaVue Software. Microarrays data subjected to computer analysis using GeneSpring program detected clusters of similarly regulated genes. Two dimensional chromatography revealed multiple fractions containing differentially expressed proteins in CHR3 (*nhr-23*) inhibited fractions suitable for analysis by mass spectroscopy. Genome and proteome wide analysis may help to further characterize phenotypes of gene inhibition by RNAi.

Acknowledgement: We thank Drs. A. Fire for GFP construct, vectors and host used in RNAi and M.W. Krause for support and advice. We thank the NIDDK Microarrays Facility for performing the microarrays analysis. The work was supported by the grants 301/05/0859 and 303/03/0333 from the Czech Science Foundation, and by the grant 0021620806 from the Ministry of Education of the Czech Republic.

BACK

***P-017***

# Role of the miR-52 Family of microRNAs in *Caenorhabditis elegans* Development

**W. R. Shaw,** I. Alvarez-Garcia, E. A. Miska
Wellcome Trust Cancer Research UK Gurdon Institute of Cancer and Developmental Biology, Cambridge, UK

MicroRNAs (miRNAs) are a class of small regulatory RNA that inhibit target gene expression post-transcriptionally. Several hundreds of miRNAs have been discovered in diverse organisms but their biological roles are unclear. *Caenorhabditis elegans* mutants with deletions in miRNA genes have been generated by a deletion screen (E. Miska *et al.*, Unpublished) to study their functions by observing abnormal phenotypes, which are seen in about 10% of mutants. Single mutants in the *miR-52* family of related miRNAs show no obvious abnormal phenotype but may act redundantly as embryos mutant in five members of the family arrest development during embryogenesis. Time-lapse microscopy and tissue-specific markers will be used to determine which tissues and/or processes are affected. Available miRNA target prediction algorithms provide lists of candidate targets mRNAs whose increased translation into protein is expected to cause the lethal phenotype. These targets will be tested using RNAi, fluorescent protein reporters and antibodies.

BACK

# NHR-60, a *Caenorhabditis elegans* Supplementary Nuclear Receptor residing at the Nuclear Periphery, Regulates Embryonic Development in Connection with Acyl-Coenzyme a Binding Protein

**Katerina Simeckova[1],** Eva Brozova[1], Jaroslav Vohanka[1], Michal Pohludka[2], Zdenek Kostrouch[2], Marta Kostrouchova[1]
[1]Laboratory of Molecular Biology and Genetics and [2]Laboratory of Molecular Pathology, Institute of Inherited Metabolic Disorders, 1st Faculty of Medicine, Charles University, Prague

NHR-60 is a *Caenorhabditis elegans* supplementary nuclear receptor that belongs to a subset of 18 of the 284 nuclear hormone receptors characterized by the P-box sequence CNGCKT. We show that NHR-60 is expressed ubiquitously and is predominantly localized at the nuclear periphery. The expression of NHR-60 is accented in seam cells, gland cells and in the germline. In seam cells, the expression of NHR-60 is dependent on CHR3 (NHR-23). Our research also focused on the possible interaction of Acyl-CoA-binding protein, which is known to interact with several nuclear hormone receptors, and NHR-60. The interaction in vivo between these two proteins was confirmed by co-immunoprecipitation and co-localization using confocal microscopy. *acbp-1* inhibition by RNAi induced developmental defects similar to *nhr-60* RNAi. These results indicate that regulation of development by nuclear hormone receptor NHR-60 is functionally linked to Acyl-CoA-binding protein and may constitute a connection with fat metabolism.

Acknowledgement: We thank Drs. A. Fire for vectors and host used in RNAi and M.W. Krause for support and advice. The work was supported by grants 303/03/1115 and 301/05/0859 from the Czech Science Foundation and by the grant 0021620806 from the Ministry of Education, Youth and Sports of the Czech Republic.

BACK

***P-019***

# Phenotypic Analysis and Mapping of a New *C. elegans* Mab Mutant, *mab-29*

**Mariana Simões** and Alison Woollard
University of Oxford

Mab (Male ABnormal) mutants have defects in male tail copulatory structures and therefore have a reduced ability to mate. Nonetheless, these mutations can be isolated without difficulties accompanying lethality since the normal population consists of hermaphrodites.

In a recent F1 screen for new Mab mutants, one such mutant was isolated, *mab-29*. Male tails have swollen bursa/ rays and reduced fan, suggesting a defect in morphogenesis. Extensive phenotypic analysis of the male tail using conventional microscopy and specific tissue markers shows abnormal attachment of the rays and phasmid to the hypodermal sheath, resulting in the striking appearance of the lumpy and amorphous rays and reduced fan.

By using recombination mapping, *mab-29* has been mapped to an interval on Linkage Group X. We are using a combination of three-factor crosses and SNP snip-mapping approaches to narrow the genomic region in which *mab-29* is found. Snip mapping is being carried out using snips in the interval of about three map units, between *lin-2* and *unc-9*.
Our mapping strategy and progress towards defining the *mab-29* map position will be presented. Further analysis depends on the nature of this gene.

BACK

***P-020***

# CEN-1, a Nucleotidase Involved in the UPR Mechanism, is required for Pharyngeal Morphogenesis in *C. elegans*

D.Uccelletti, **A. Pascoli**, P. Mancini[1],C.B. Hirschberg[2] and C. Palleschi
Dpt Developmental and Cell Biology, University of Rome "La Sapienza"; [1]Dpt Experimental Medicine and Pathology, University of Rome "La Sapienza"; [2]Dpt Molecular and Cell Biology, Boston University, MA, USA

We report the characterization of *cen-1*, a *C. elegans* gene involved in the unfolded protein response (UPR). Inactivation of UPR signaling has deleterious consequences on cell survival and accumulation of misfolded proteins in the ER may play an important role in human diseases. Therefore, cells have elaborated a phase of the UPR consisting of transcriptional activation of genes whose products play a role in protein processing in the secretory pathway. The mRNAs induced by the UPR encode ER chaperones, disulfide exchange factors and many components of the ER-associated protein degradation (ERAD) machinery. We found that the nucleotidase CEN-1, highly similar to a rat brain UDPase, was transcriptionally up regulated, through ire-1 activation, in the conditions that induced UPR. In total membrane fractions of *cen-1*(RNAi) worms a drastic reduction of UDPase activity was observed. The nucleotidase is probably required for the hydrolysis of UDP generated by the UDP-Glc:glycoprotein glucosyltransferase(GT), a key enzyme of UPR. The UDP is converted into UMP to relieve inhibition of the enzyme and to provide antiport substrates to couple the entrance of nucleotide sugars from the cytosol into the lumen of the secretory pathway with the exit of nucleoside monophosphates. RNAi experiments showed that, when *cen-1* was transiently knocked down, a delay in larval development occurred and a reduction of life span and a defect in pharyngeal morphogenesis could be observed. Although the tissue-specific expression of CEN-1 remain to be analyzed, this protein seems also to be important in the biosynthesis of cuticle and surrounding tissues; this imply a role in the functioning of the secretory apparatus.

BACK

# Identification of Genes required for Anchor Cell Attachment and Invasion during Vulval Development

**Sara Vassalli** and Alex Hajnal
University of Zurich, Institute of Zoology, 8057 Zurich, Switzerland

During vulval development in the hermaphrodite, three out of six equivalent vulval precursor cells (the VPCs P3.p to P8.p) are induced by a signal from the gonadal anchor cell (AC). The LIN-3 EGF growth factor produced by the AC activates the EGFR/RAS/MAPK pathway in the VPCs to specify the primary cell fate.

Prior to vulval induction, the gonad is separated from the VPCs by two basal laminas covering each tissue such that the position of the AC relative to the VPCs is variable when the animal moves. At the time of vulval induction in the early L3 stage, however, the AC attaches to the basal side of the future primary cell P6.p. After the first round of vulval cell divisions (Pn.px stage), the basal laminas separating the gonad from the vulval cells start to dissolve precisely under the AC. After the second round of divisions (Pn.pxx stage), the basal laminas between the AC and the four primary vulval cells are interrupted, and the AC invades the vulval tissue.

While the mechanism of vulval induction is well studied, less is known about genes regulating the attachment of the AC to P6.p and the following invasion step. To study these two processes, we performed a forward genetic screen for mutants with defects in AC positioning and/or invasion. Since worms with an abnormal or missing connection between the gonad and the vulva usually display a protruding vulva (Pvl) phenotype, we first screened for animals with a Pvl phenotype and then examined them for defects in the positioning or invasion of the AC. Among 5300 F1 clones, we identified 8 sterile mutants with a misplaced AC and 2 mutants, in which the AC is normally positioned but fails to dissolve the basal laminas and invade the vulval tissue. Details about the cloning and analysis of the genes identified in this screen will be presented at the meeting.

BACK

***P-022***

# Distinct Roles of the Pumilio and FBF Translational Repressors during *C. elegans* Vulval Development

**Claudia Walser**, Gopal Battu, Erika Fröhli Hoier and Alex Hajnal
Zoologisches Institut, Universität Zürich, Winterthurerstrasse 190, CH 8057, Zürich, Switzerland

Members of the conserved PUF (Pumilio and FBF repeat) protein family regulate various aspects of germ line development by selectively binding to the 3' untranslated region of their target mRNAs and repressing translation. FBF-1 and FBF-2 regulate the sperm/oocyte switch and mitosis versus meiosis decision by repressing *fem-3* and *gld-1* translation, respectively (Zhang et al., 1997; Crittenden et al., 2002). We found that *puf-8, fbf-1* and *fbf-2* also act in the soma where they negatively regulate vulval development.

Loss-of-function mutations in *puf-8* cause excess vulval differentiation when combined with mutations in negative regulators of the EGFR/RAS/MAPK pathway, and they suppress the Vulvaless phenotype caused by mutations that reduce EGFR/RAS/MAPK signaling. A PUF-8::GFP translational reporter is initially expressed in all six vulval precursor cells (the VPCs P3.p through P8.p) but gets restricted to the distal, uninduced (3°) VPCs after vulval fate specification. Moreover, the fusion of the distal VPCs to the hypodermal syncytium is retarded in *puf-8(lf)* mutants. Thus, PUF-8 acts cell-autonomously to limit the temporal competence of the vulval precursor cells to respond to the extrinsic patterning signals. *fbf-1* and *fbf-2*, on the other hand, function as redundant inhibitors of vulval cell fate specification in a distinct pathway that involves the repression of *gld-1* translation. *fbf-1 fbf-2* double mutants show ectopic 1° cell fate marker expression in adjacent VPCs. We conclude that the FBFs ensure that only one VPC (P6.p) is selected for the 1° cell fate.

Therefore, the PUF family translational repressors regulate various aspects of cell fate specification in the soma, and they may play a conserved role in modulating signal transduction during animal development.

BACK

# The Role of Otx/otd Homeobox Genes in *C. elegans* Development

**Yong Guang Tong**[1, 2], Thomas R Bürglin[1]
[1]Department of Biosciences and Nutrition, Karolinska Institue, Huddinge, 14189, Sweden; [2]Department of Natural Sciences, Södertörns Högskola, Hudding, 14152, Sweden

The Otx/otd homeodomain-containing transcription factors play essential roles in the formation and specification of the anterior brain in Drosophila and vertebrates. The C. elegans genome encodes three Otx/otd orthologues, TTX-1, CEH-36 and CEH-37. All C. elegans otd genes and rat Otx-1 can substitute for ceh-36 and ceh-37, but only ceh-37 functionally substitutes for ttx-1. The three genes specify distinct sensory neuron identities in C. elegans (Anne et al.2004). We have been characterizing the expression pattern of ceh-36, ceh-37 and ttx-1 during embryogenesis using GFP reporter constructs and 2-channel 4D-microscopy. The expression pattern of pceh-37::GFP consists of two phases of expression, one during gastrulation, and a later one at the end of gastrulation /beginning of morphogenesis. Expression of pceh-36::GFP is also in two phases, the first during gastrulation. The GFP signal fades after ventral cleft closure except for the sensory neurons AWC and ASE. For the third otd homeobox gene, ttx-1, 4D analysis shows only one phase of expression after gastrulation, in the excretory cell and the AFD sensory neurons. The observed expression patterns are evolutionarily consistent with the expression patterns observed for Otx/otd genes in other animals. For ceh-36, ceh-37 and ttx-1, there is possibly functional redundancy. So we continued to study the interaction of these genes using double mutant strains after double RNA-interference (RNAi) showed synergistic effects. We are analyzing ceh-37 deletion mutant strains (tm253, tm254) and ceh-36 deletion mutant strains (ok795 and tm251).We made ceh-37 (tm253) ttx-1 (p767) and ceh-36(ok795) ttx-1(p767) double mutant strains. The results show a severe L1 arrest phenotype, almost 100% of the worms are dead at the L1 stage. We also performed ceh-36(RNAi) experiments in the background of tm253, which results in about 40% lethality at the L1 stage. We continue to investigate the nature of the double mutant lethalities.

*[late coming abstract]*

BACK

***P-023***

# Germline Totipotency and the “worm teratoma”

**Bjoern Biedermann**, Matthias Senften and Rafal Ciosk
Friedrich Miescher Institute for Biomedical Research, 4058 Basel, Switzerland

Teratomas (derived from *teraton*, the Greek word for monster) are germ cell tumors that contain a mixture of somatic tissue such as hair or bone. These tumors are thought to result from abnormal somatic differentiation of germ cells, but very little is known about the molecular mechanisms that normally maintain germ cell totipotency. Two conserved translational repressors, MEX-3 and GLD-1, have been recently found to maintain totipotency in the *C. elegans* germline (Ciosk et al., Science 311, 851, 2006). In *mex-3 gld-1* mutants, germ cells transdifferentiate into various somatic cell types such as muscles or neurons. This “worm teratoma” requires the entry into meiosis and coincides with the disappearance of germline-specific P granules. To understand MEX-3/GLD-1 role in maintaining totipotency, we want to identify the proteins that normally need to be repressed by MEX-3 and GLD-1. Genetic and biochemical strategies will be discussed at the meeting.

BACK

# Role of Short RNAs in *Caenorhabditis elegans* Germline Development

**Partha Pratim Das,** Heeran Buhecha, Eric Miska
Wellcome Trust/ Cancer Research UK Gurdon Institute for Cancer and Developmental Biology, University of Cambridge, UK

Several hundreds of endogenous small RNAs, namely microRNAs (miRNAs), repeat- associated small interfering RNAs (rasiRNAs), small interfering RNAs (siRNAs) and tiny non-coding RNAs (tncRNAs) have been discovered in diverse organisms. These small RNAs are derived from different sources and they cause transcriptional gene silencing, translational repression and mRNA cleavage through effector complexes. Several functionally distinct RNA silencing effector complexes have already been isolated from different organisms. Effector complexes are composed of members of the Argonaute (AGO) protein family and single stranded small RNAs, along with other associated proteins. Members of the piwi subfamily Argonaute proteins have evolutionary conserved roles in germline development and stem cell maintenance. In *C. elegans*, PRG-1 and PRG-2 are the two members of piwi subfamily of Argonaute proteins. In order to study the role of PRG-1 and PRG-2 in *C.elegans* germline development, we generated *prg-1; prg-2* double mutant worms, which show severe germline defects. Additionally, microarray analysis showed several genes are up-regulated in the double mutant worms compared to wild type. Interestingly, these up-regulated genes are the predicted targets of some of newly cloned short RNAs. To understand the detailed mechanism of PRG-1 and PRG-2 and their role in germline development, we are currently isolating PRG-1 containing complexes and their components. Cloning of short RNAs associated with PRG-1/2. Potential components of the complexes will be investigated by *in vitro* (protein-protein interaction) as well as *in vivo* (RNAi) studies.

BACK

# GLS-1, A Novel GLD-3 Interacting Protein Regulates Germ Cell and Embryonic Development in *C. elegans*

Martin Harterink[1,2], **Adam Kupinski[1]**, Joanne Stamford[1] and Christian Eckmann[1]
[1]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany; [2]Dept. of Chemistry, Utrecht University, Netherlands

The development of all multi-cellular organisms relies upon the perfect balance between cellular growth and differentiation to generate and maintain tissues and organs. We have investigated the molecular controls of germ cell fate decisions in the *Caenorhabditis elegans* germ line. Germ cells can either divide mitotically or enter the meiotic cell cycle to differentiate as either sperm or oocytes. Strikingly, these decisions were found to be controlled by a network of conserved RNA regulators (1, 2, 3). In particular, the switch from mitosis to meiosis requires the translational repressors GLD-1, FBF, and NOS-3, and the translational activator complex GLD-2 / GLD-3. GLD-2 is a multi-subunit cytoplasmic poly(A) polymerase that is presumed to interact with target mRNAs through the putative RNA binding protein GLD-3, a Bicaudal C protein family member. *gld-2* and *gld-3* are required for normal germ cell entry and progression through meiosis and for proper early embryogenesis (2, 4).

However, *gld-3* has additional roles in other cell fate decisions independent of *gld-2*. Maternal *gld-3* ensures germ cell survival and zygotic *gld-3* controls the sperm/oocyte switch (4). In order to study the role of *gld-3* in detail for these functions, we performed a yeast 2-hybrid screen to identify interacting proteins. We isolated a novel gene, tentatively named *gls-1* (germline survival defective-1), which we also found to be involved in regulating germ cell survival and embryonic development. GLS-1 binds to GLD-3 specifically *in vitro* and is expressed in the cytoplasm throughout germline development and associates with P granules. We will present our molecular and genetic work on characterizing *gls-1* deletion mutations. This study not only supports a role for *gls-1* in germ cell survival and early embryogenesis, but suggests additional roles for *gls-1* in the mitosis/meiosis and sperm/oocyte decisions as well.

(1) Eckmann et al.; (2004) Genetics 168, pp147-60
(2) Eckmann et al.; (2002) Dev. Cell 3, pp697-710
(3) Hansen et al.; (2004) Development 131, pp93-104
(4) Wang et al.; (2002) Nature 419, pp312-6

BACK

***P-026***

# Creation of Germ Cells: Characterization of Germline Cytokinesis

**Torah M. Kachur** and Dave Pilgrim
University of Alberta, Edmonton, CANADA

The creation of germ cells as discrete cells is essential to the fecundity of metazoan species. The process of separating developing germ cells into distinct compartments requires an actomyosin based contractile apparatus. We have identified components of the germline cellularization mechanism that includes: UNC-45, a myosin chaperone, and its associated myosin, NMY-2. Lack of either myosin or its chaperone results in complete sterility with a failure to create oocytes in the proximal gonad. Partial defects in germline cellularization are apparent with different sized embryos that develop from *unc-45* or *nmy-2* partially depleted adults resulting from a defect in neighboring oocytes to complete cytokinesis. In the absence of UNC-45, there is a failure to organize the actomyosin cytoskeleton into a characteristic hexagonal pattern in the distal syncytial gonad and a subsequent failure in the proximal gonad to separate nucleii into oocytes. Both males and hermaphrodites require intact myosin function for cellularization in the germline where sperm formation fails in both hermaphrodites and sperm does not accumulate in males. We present a detailed analysis of germline cytokinesis in *C. elegans* indicating that cellularization requires an intact actomyosin cytoskeleton in order to properly cellularize both types of primordial germ cells.

BACK

# Analysis of Genetic Interactions Affecting Germline Development Using Microarrays

**Andrew C. Nelson,** Andrew G. Fraser
Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1HH, UK

All aspects of metazoan biology are governed by the interaction of multiple genes. Furthermore, most human genetic diseases result from the combined effects of multiple mutations. Identifying genetic interactions is therefore an important step towards understanding multigenic disease. We have focussed on identifying genetic interactions involved that affect the *C. elegans* germline, a complex tissue comprising mitotic and meiotic regions governed by multiple signalling pathways. We previously carried out RNAi screens in strains carrying mutations in core components of EGF, Notch, Wnt and other signalling pathways screens and identified multiple genetic interactions that affect the germline. These screens, however, do not give any information about the mechanism underlying each interaction nor any detailed view of the nature of the germline defect that results. We therefore propose to use microarrays to produce "molecular phenotypes" for each single gene and bi-genic defect produced in our RNAi screens. Genes may then be clustered into pathways based on comparison with array profiles from well-characterized mutants. Here we present the progress that has been made, the experimental plan to validate our use of arrays and how we intend to follow on from this.

BACK

***P-028***

# Moving Forward With Actin and MSP (Major Sperm Protein)

Léa Trichet, Sylvain Delrot, Cécile Sykes, **Julie Plastino**
UMR 168/CNRS, Laboratoire Physicochimie Curie, Institut Curie, Paris, France

Cell motility is the result of a complex coordination of plasma membrane extension, adhesion to the substrate and cell body traction. Since cell movements are at the root of processes such as wound healing, immune response and cancer cell metastasis, a precise understanding of motility represents a considerable challenge. In human cells, and more generally in most motile cells, lamellipodia extension is performed by actin filaments, which polymerize with their growing ends directed toward the plasma membrane. Due to the hydrolysis of ATP, actin filaments depolymerize from the ends directed toward the cell body, and the rear edge of the cell is subsequently brought forward by contraction of the actin cortex by myosins. Nematode sperm cells display a similar amoeboid movement, but interestingly, these cells contain no actin and no myosin. Instead, lamellipodia extension is powered by the polymerization of the Major Sperm Protein (MSP). Overall the nematode sperm cell represents a new system for studying cell motility, and a comparison of the MSP and actomyosin systems will provide insights as to the requirements for movement.

BACK

# TEN-1 is required for the Formation of the Somatic Gonad in *Caenorhabditis elegans*

**Agnieszka Trzebiatowska**[1], Krzysztof Drabikowski[2], Ruth Chiquet-Ehrismann[1]
[1]Friedrich Miescher Institute, Basel, Switzerland; [2]Institute of Biology III, University of Freiburg, Freiburg, Germany

TEN-1 is a member of a novel family of transmembrane proteins named teneurins that have been characterized in *Drosophila*, zebrafish, chicken, mouse and man. Teneurins were shown to be mainly expressed in the developing and adult nervous system and to play a role in morphogenesis, cell migration and at sites of pattern formation. C. elegans TEN-1 was proposed to act as a receptor that signals directly to the nucleus. Its intracellular domain is cleaved and translocates to the nucleus where it may influence gene expression. Little is known, however, about the cleavage mechanism and possible target genes.

*C. elegans* has a single teneurin orthologue, which is under control of alternative promoters. The upstream promoter is active in the somatic gonad, some muscle cells, the gut and in a number of neurons. The expression from the downstream promoter is mainly detected in the nervous system. TEN-1 is a type II transmembrane protein consisting of a short intracellular domain followed by a transmembrane domain and a long highly conserved extracellular part containing eight EGF-like repeats, a cysteine rich region and YD repeats.

In order to determine the function of TEN-1 in *C.elegans*, we investigated two loss-of-function mutants: *ten-1(ok641)* and *ten-1(tm651)*. The former carries an in frame deletion of four EGF-like repeats and a part of the cysteine rich region; the latter has a deletion that removes the transmembrane domain and introduces a frameshift mutation resulting in the loss of the entire protein except for the first 194 aminoacids. In both mutants transcripts for the predicted truncated proteins could be detected on the level comparable to the wild type form.

Both *ten-1* mutants show a pleiotropic phenotype, similar to the phenotype observed after reduction of *ten-1* expression by RNAi. Mutant worms are sterile due to various defects in the somatic gonad and ectopic germline formation in the proximity of the vulva. Time course analysis of germline development showed that these abnormalities may result from an early defect in the somatic gonad formation. We were able to partially rescue the phenotype of *ten-1(ok641)* mutant worms by injecting the cosmid F36A3 carrying the entire genomic region of the *ten-1* gene. Since such an extrachromosomal array is likely to be silenced in the germline, gonadal defects appear to be the result of impaired signaling in the somatic gonad.

BACK

# Maintenance of Asymmetry during Germ Line Cell Cleavages

**Sophie von Elsner**, Henning Schmidt and Ralf Schnabel
Institut fuer Genetik, TU Braunschweig, Spielmannstr. 7, D-38106 Braunschweig, Germany
s.von-elsner@tu-bs.de

In most organisms axis formation is one of the first processes taking place in the developing embryo. The establishment of the antero-posterior axis in the C. elegans zygote is a prime example with the PAR-proteins polarising the cell cortex after the sperm entry. The asymmetric distribution of these proteins around the cortex initiates the reorganisation of the cytoplasm with all included proteins like the P granules or the transcription factor PIE-1 previous to a division.

During the first division also the unequal distribution of *germ line* and *soma* begins which is maintained by only a minimal number of similar asymmetric cleavages.
In the *cib* mutants (***c***hanged ***i***dentity of ***b***lastomeres) *cib-1* to *cib-4* the maintenance of germ line identity during these divisions is defect. Depending on the gene activity the germ line cells P1 to P3 pause up to a full cell cycle and divide symmetrically. The division of the zygote P0 is never affected.

We will report on the function of *cib-3* which has no homology or described domain. In a Y2H screen we found that CIB-3 interacts with two proteins which were so far not known to be involved in embryogenesis. In *cib-3* RNAi experiments and in the *cib-3* ts mutant PAR-2::GFP is localised to the cortex like in WT embryos before a symmetric division but cytoplasmic factors like MEX-5 or nuclear like PIE-1 are aberrant.

Therefore we suggest that CIB-3 is involved in transmitting the cortical polarity to cytoplasmic factors and therefore maintaining the asymmetry.

BACK

# Are Males Important? – An Experimental Approach

**Viktoria Wegewitz**[1], Hinrich Schulenburg[2] and Adrian Streit[1]
[1]Max-Planck-Institute for Developmental Biology, Tuebingen, Germany; [2]University of Tuebingen, Germany

One proposed advantage of outcrossing is creating new favourable gene combinations by recombination, which possibly helps adaptation to changing environmental conditions. This model predicts two alternative strategies for reproduction in *Caenorhabditis elegans*: Selfing would be an advantage under constant conditions because favourable combinations of genes are preserved and the cost of male production is reduced. In contrast outcrossing would be an advantage under changing environmental conditions. If this hypothesis is true and there are genetic determinants for the abundance of males, constant conditions should select for low incidence of males and changing selective pressure for higher number of males. We plan to test this model in the following way: A genetically variable population with a high number of males will be produced by interbreeding the two wild type strains N2 and CB4856. Half of this population will be cultivated under changing selection pressure of different pathogenic bacteria. The other half of the population will be kept under standard laboratory conditions. We have already shown that in N2 and CB4856 males are maintained at different rates in growing cultures under standard laboratory conditions. While N2 males are lost from the population within a few generations, CB4856 males are maintained at significantly higher proportion over longer periods, especially in large populations. This indicates that male abundance is, at least in part, determined genetically and that the corresponding genetic component differs between N2 and CB4856. If the model outlined above is true, we expect that the populations from the two treatments in our selection experiment differ in male frequency. We are also testing parameters that might be the reason for the different maintenance of males, for example mating efficiency and brood size. First Results will be presented.

BACK

# Post-Transcriptional Control of Germline Immortality

**Theresa M. Zucchero**[1,2], Matt Eckler[1], Mia Lowden[1,3], Yan Liu[1], Shawn Ahmed[1,3]
[1]Department of Genetics, [2]SPIRE Postdoctoral Fellowship Program, [3]Department of Biology, University of North Carolina, Chapel Hill, NC, USA

We are interested in understanding how the germline achieves proliferative immortality, avoiding damage that accumulates over time. To investigate mechanisms involved in this phenomenon, large scale EMS-mutagenesis screens have been conducted in *C. elegans* to identify *mortal germline (mrt)* mutants, mutants that become sterile when propagated over multiple generations. From these screens, a total of 430 *mrt* mutants were found to be sterile under various conditions. Of these, 292 lines are temperature-sensitive and become sterile only at 25°C.

One temperature-sensitive *mrt* mutant displayed a germline tumor/proximal proliferation (Pro) phenotype near sterility. This mutant, *yp3*, was mapped to the center of chromosome I based on the locations of genes known to confer a tumorous phenotype when defective. The *yp3* mutation failed to complement two alleles of *gld-2*, *q497* and *h292*, for its temperature-sensitive Mortal Germline phenotype, suggesting that *yp3* may be a conditional allele of *gld-2*. *gld-2* affects the transition from mitosis to meiosis and encodes a poly(A) polymerase that may regulate RNA translation or turnover. All known alleles of *gld-2* are sterile and most alleles, including the null *q497*, display a low penetrance of symmetric Pro defects, whereas a higher frequency is observed for the hypomorphic *h292* allele. Interestingly, both symmetric and asymmetric Pro phenotypes are observed in *yp3*, which become progressively exacerbated as *yp3* lines are passaged at 25°C. Thus, this mutant may accumulate a heritable form of damage at 25°C that affects meiotic development. We conclude that the *yp3 mrt* mutation may be an allele of *gld-2* and, therefore, that a master regulator of germline development may be critical for maintenance of germline immortality.

BACK

# Characterization of the Long-Lived Mutant *liv-8(pv18)*

**Ana Mª Brokate-Llanos**, José M. Monje, Manuel J. Muñoz
Centro Andaluz de Biologia del Desarrollo, Universidad Pablo de Olavide, CSIC, Crta. De Utrera, Km1. 41013. Seville. Spain. mmunrui@upo.es

Using a positive selection for thermotolerant mutants (Munoz et al. Genetics 2003 163: 171-80) we have isolated *liv-8(pv18),* a thermotolerant and long-lived mutant. The phenotype of *liv-8(pv18)* is very pleiotropic, at 16ºC shows severe gonad migration defects and when incubated at 25ºC they are embryonic lethal.

Because thermotolerance it has been also observed in mutants related to the dauer formation pathway, we have studied the interactions with mutants of the TGF-B and the insulin/IGF dauer signalling pathways. At 20ºC *liv-8(pv18)* enhances the dauer formation phenotype of the TGF-B mutants *daf-1* and *daf-7* but does not affect this feature in mutants of the insulin/IGF pathway.

The insulin/IGF pathway is involved also in regulation of longevity. *daf-2(e1370)* mutants increase wild-type life span, and the double with *liv-8(pv18)* lives even longer, suggesting that *liv-8(pv18)* may be involved in a different pathway to *daf-2* regulating longevity. This is also supported by the evidence that, differently to *daf-2* mutants, *liv-8(pv18)* increases longevity without relocating the transcription factor *daf-16* into the nucleus. The interaction with *daf-16* is interesting because we showed that this gene is needed for the increase of longevity of *liv-8(pv18).*

Mapping analysis indicate that is located on the right arm of the chromosome I, actually we are actively working in its identification.

BACK

# *C. elegans* Thioredoxins Implicated in Nervous System Function and Aging

**Juan Carlos Fierro González**[1], Peter Swoboda[1], Antonio Miranda-Vizuete[2]
[1]Karolinska Institute, Department of Biosciences and Nutrition, Södertörn University College, School of Life Sciences, S-14189 Huddinge, Sweden; [2]Centro Andaluz de Biología del Desarrollo (CABD-CSIC), Universidad Pablo de Olavide, E-41013 Sevilla, Spain.

Thioredoxins constitute a large family of proteins present in all organisms that catalyze thiol-disulfide redox reactions. They are involved in various cellular processes, such as DNA synthesis and repair, antioxidant defense, regulation of transcription factor DNA binding activity or longevity. Moreover, thioredoxins are also implicated in many important pathologies such as cancer, cardiovascular diseases, diabetes or Alzheimer's and Parkinson's diseases.

The *C. elegans* genome encodes for two thioredoxin reductases and at least eight thioredoxins, many of which have clear orthologs in mammals. We report here the characterization of the first member of the thioredoxin family in metazoans that is mainly associated with neurons. The *C. elegans* gene B0228.5 encodes a thioredoxin (TRX-1) that is expressed in ASJ sensory neurons and, to some extent, also in the posterior-most intestinal cells. ASJ neurons regulate longevity and also the entry into and exit from the dauer stage, an endurance larval form triggered by adverse environmental conditions or by hormonal cues. A mutant worm strain carrying a null allele of the gene *trx-1* displays a reproducible decrease in mean lifespan as compared to wild type, which implicates *trx-1* in ASJ-dependent mechanisms that regulate longevity. On the other hand, the *trx-1* null mutant is neither dauer formation defective (Daf-d) nor dauer formation constitutive (Daf-c), suggesting either that TRX-1 is only involved in ASJ functions that specifically influence longevity, or that potential TRX-1-dependent dauer phenotypes might only be revealed under sensitized (e.g., double mutant) backgrounds.

In this context, we are currently combining the *trx-1* mutation with others in genes known to regulate longevity and/or dauer formation, such as *daf-2*, *daf-16*, *daf-11* and *daf-21*. Obtaining double mutants of *trx-1* with the last two genes is especially interesting with regard to searching for potentially synthetic phenotypes, as these genes have been reported to function in ASJ neurons. In parallel, by using GFP reporter fusion constructs, we are studying the expression patterns of all the thioredoxin and thioredoxin reductase genes in *C. elegans*, to identify genes associated with the nervous system and possibly interacting with *trx-1*. The outcome of these studies will help us clarify the implications of thioredoxins in *C. elegans* nervous system function and aging.

BACK

***P-035***

# Transcriptional Changes Underlying Chemosensory Regulation of Lifespan

**Marta Maria Gaglia** and Cynthia Kenyon
Graduate Program in Neuroscience and Department of Biochemistry and Biophysics, University of California, San Francisco

Mutations, ing thatStudies have shown that aging is a genetically-regulated process. The insulin/IGF pathway in particular is thought to regulate the aging process, as mutation in the insulin/IGF-1-like receptor *daf-2* can extend the lifespan of worms two-fold. is not clearesIInterestingly, mutants with altered chemosensation also have an extended lifespan (Apfeld and Kenyon, 1999). Both *daf-2* and chemosensory mutant longevity are dependent on the activity of the FOXO transcription factor DAF-16. This suggests that external inputs through the chemosensory system may regulate the insulin pathway and in turn affect lifespan.some of the cellular components of the sensory pathway of lifespan control (Alcedo and Kenyon, 2004). However, it is not known how themechanism by which the chemosensory system modulates lifespan remains still largely unknown and the insulin/IGF system interact.

To begin to elucidate the mechanisms of chemosensory regulation of lifespan, we have performed genome-wide expression analysis with microarrays on the long-lived mutant *daf-10*. We are in the process of characterizing the genes whose expression was altered with mutants and RNA interference in sensitized backgrounds. In particular, we are interested in those genes that affect lifespan without causing a chemosensory defect, as they may act downstream of the sensory neurons. In addition we are comparing the expression changes to those occurring in *daf-2* mutants, to determine whether the two pathways are in parallel or the chemosensory system feeds into the insulin pathwaymodulate.

BACK

# The Role of $IP_3$ Signalling in the Regulation of Rhythms and Rates in *C. elegans*

**R. Gatsi** and HA. Baylis
Department of Zoology, University of Cambridge, UK

Rhythmic processes are an integral part of life of most organisms. Biological rhythms of less than a day are called ultradian rhythms. In *C. elegans*, ultradian rhythms include pharyngeal pumping, defecation, and ovulation. There is a growing body of evidence which supports the hypothesis that these rhythmic functions share a common molecular mechanism[1]. A central component of this pathway is the second messenger $IP_3$. $IP_3$ is produced by the action of phospholipase C in response to external stimuli. $IP_3$ binds to $IP_3R$ located in the ER and regulates the release of calcium. $IP_3Rs$ are regulated by a broad range of molecules and proteins including $Ca^{2+}$ itself and ATP. Signalling through $IP_3$, *itr-1* (IP3R) and $Ca^{2+}$ are clearly involved in the control of rhythms in pumping, defecation, and ovulation [2,3,4,5]. Interestingly, genetic analysis also suggests that ultradian rhythms are linked to mechanisms that control the rates of growth and ageing. In particular *clk* and *gro* genes regulate both types of processes [6,7].

We are currently dissecting the role of $IP_3$ signalling in the regulation of timing in *C. elegans* by analysing the relationship between rhythmic processes, such as pharyngeal pumping, and rates such as the rate of growth and ageing. We have evidence which suggests that $IP_3$ signalling might play a central role in these processes. We have shown that, in addition to changes in ultradian rhythms, mutants of *itr-1* show alteration in growth rate (see also Dal Santo et al, 1999) and ageing. We have characterised these changes in more detail. In particular our results indicate that the ATP-binding regulatory site of the $IP_3Rs$ might play a significant role in these processes. In addition, phenotypic characterization of suppressor mutants of *itr-1 (sa73)* (ts l.o.f. mutant) suggest that growth rate may be regulated independently of other phenotypes.

1. Baylis HA. (2005) *Cell.* 123, 5-7.
2. Clandinin TR, DeModena JA & Sternberg PW (1998) *Cell.* 92, 523-533.
3. Dal Santo P et al (1999) *Cell.* 98, 757-767.
4. Walker DS., et al. (2002) *Mol Biol Cell.* 13, 1329-1337.
5. Yin X, Gower NJ, Baylis HA & Strange K. (2004) *Mol Biol Cell.* 15, 38-49.
6. Branicky R, Benard C and Hekimi S (2000). *Bioessays* 22, 48-56.
7. Hekimi S, Lakowski B, Barnes TM, Ewbank J (1998) *J. Trends Genet*. 14, 14-20.

BACK

***P-037***

# Coenzyme Q Administration is not sufficient to Revert *coq-1* Knockout Phenotype

**A. Gavilán,** J.C. Rodríguez-Aguilera,C. Asencio, P. Navas
Centro Andaluz de Biología del Desarrollo, Universidad Pablo de Olavide.41013 Seville, Spain

Coenzyme Q (CoQ) like some other antioxidants, influences aging in *C. elegans* probably by preventing the accumulation of oxidative damage. A family of genes involved in CoQ biosynthesis, named *coq* genes, has been identified.

We study the role of *coq-1* in *C. elegans*, focusing on relevant phenotypes associated with aging, using VC479 *coq-1* heterozygotic strain. Knockout *coq-1* mutants showed severe defects, such as development arrest at L1-like larval stage, abnormal movement, including paralysis from post-pharyngeal region to tail, abnormal organogenesis evident in muscle-based structures, minimal pharyngeal pumping and sterility.

Heterozygotic *coq-1* individuals (wild type phenotype) were fed with different Q isoforms, and only $Q_9$–producing bacteria were able to partially rescue phenotype in *coq-1* knockout F1 individuals. The positive impact of $Q_9$ supplementation increased median and maximal lifespan, whereas the rest of above-described mutant characteristics remained unchanged.

To analyze the effect of *coq-1* expression we obtained transgenic worms through the microinjection of *coq-1* wild type allele and describe morphological and physiological effects.

BACK

***P-038***

# RNAi Screen for Genes that Regulate Stress Resistance and Life Span of *C. elegans*

**Birgit Gerisch**[1], Adam Antebi[2], Marie-Laure Yaspo[1] and Hans Lehrach[1]
[1]MPI, Berlin, Germany; 2) Baylor College of Medicine, Houston, Texas

Oxidative stress appears to play major role in life span regulation. Long-lived mutants of daf-2/insulin-like receptor (insulin/IGF-1 signaling) also show increased resistance to environmental stress, such as high temperature and reactive oxygen species. Moreover most known long-lived mutants tested so far are resistant to various environmental stress.

We are using this correlation between longevity and stress resistance to identify genes that are involved in the process of life span regulation, and screened 275 RNAis corresponding to phosphatases for oxidative and heat stress resistance. Positive RNAis were tested in aging experiments.

Out of the 275 RNAis 15 result in strong resistance to oxidative stress, 3 in resistance to heat stress, and 5 in oxidative and heat stress resistance. At the moment these RNAis are being tested in aging experiments. The most interesting candidate we have obtained up to now is an evolutionary conserved phosphatase involved in cell division, which results in resistance to heat stress and shows a strong life span extension.

Further characterization and assignment of the identified genes to known pathways should yield insights into cellular processes involved in longevity and stress resistance.

BACK

# Age-related Alterations in Proteasome Activity

**Carina I. Holmberg**[1], Dara Dowlatshahi[2], Cindy Voisine[2], Sue G. Fox[2], and Richard I. Morimoto[2]

[1]Molecular and Cancer Biology Program, Biomedicum Helsinki, University of Helsinki, Helsinki, Finland and [2]Department of Biochemistry, Molecular Biology, and Cell Biology, Rice Institute for Biomedical Research, Northwestern University, Evanston, IL, USA

The proteasome is one of the major machineries involved in maintaining protein homeostasis in the cell. By selectively degrading key regulatory proteins and damaged proteins, the ubiquitin-proteasome system orchestrates cellular processes such as cell cycle, DNA repair, transcription, signal transduction, stress response, development, and apoptosis. Hence, it is not surprising that defects in this system are associated with severe diseases such as neurodegenerative disorders, inflammation, and various types of cancer. Many of these diseases are also linked to aging suggesting that proteasome activity plays a vital role in the aging process. However, it is still unclear as to how proteasome activity is regulated during the lifespan of an animal.

To gain molecular insights into the regulation of the proteasome we are developing a combination of *in vivo* and *in vitro* approaches to measure proteasome activity and the subsequent effects on protein homeostasis. Here, we report that the three proteasome peptidase activities characterized in yeast and vertebrate models are conserved in *C. elegans*. Surprisingly, aging resulted in a gradual increase in the chymotrypsin-like activity despite a decrease in the proteasome levels. In contrast, changes in the peptidyl-glutamyl-peptidase hydrolyzing activity and trypsin-like activity coincided with the proteasome levels revealing that aging has a differential effect on the proteasome peptidase activities. These age-related changes in proteasome activity are not mediated via the insulin-like signaling pathway, as demonstrated genetically. Our results suggest that cellular changes that occur during the aging process, whether mediated by genetic factors independent from the insulin-like signaling pathway or environmental factors, alter the animal's proteasome activity. These studies in combination with ongoing *in vivo* studies will provide a deeper understanding on the regulation of the proteasome during an animal's lifespan. This knowledge could translate into the development of therapeutic options for treatment of proteasome-associated human diseases.

BACK

***P-040***

# Modulated MicroRNA Expression during Adult Lifespan in *C. elegans*

**Ibáñez-Ventoso, Carolina**; Yang, Maocheng; Suzhen, Guo; Robins, Harlan; Padgett, W. Richard; Driscoll, Monica
Dept. of Mol. Biol. & Biochem.; Rutgers University; Piscataway; New Jersey, USA

MicroRNAs (miRNAs) are small (~22nt), abundant non-coding RNAs that regulate gene expression in multicellular organisms by binding to complementary sequences within the 3'UTR of specific mRNAs. MiRNAs have been shown to affect a broad spectrum of biological activities, including developmental fate determination, cell signaling and oncogenesis.

Little is known of miRNA contributions to aging. We have performed a genome-wide analysis of how expression of miRNAs changes over adulthood in aging *C. elegans*. We have also used bioinformatics analysis to predict miRNA targets encoded in the *C. elegans* genome and to identify candidate miRNA-regulated genes among *C. elegans* genes previously shown to affect longevity, genes encoding insulin-like ligands, and genes preferentially expressed in *C. elegans* muscle.

Our observations identify miRNAs as potential modulators of age-related decline and suggest a general reduction of message-specific translational inhibition during aging, a previously undescribed feature of *C. elegans* aging. Since many *C. elegans* age-regulated miRNAs are conserved across species, our observations identify candidate age-regulating miRNAs in both nematodes and humans.

BACK

# A Potential DAF-16 Target Involved in Longevity

**Victor L. Jensen**[1], David L. Baillie[2], and Donald L. Riddle[3]
[1]Medical Genetics, University of British Columbia; [2]Molecular Biology and Biochemistry, Simon Fraser University; [3]Michael Smith Laboratories, University of British Columbia

Genes differing in steady-state RNA levels between N2 and *daf-2* adults and N2 dauer larvae were identified using Serial Analysis of Gene Expression (SAGE) databases. One gene, a putative transcription factor, was isolated from a screen for genes involved in longevity. The outcrossed knockout allele was shown to have extended longevity, ~3% Him and ~2% Vab phenotypes. The 1 kb of genomic sequence 5′ of the confirmed transcription start site contains several possible DAF-16 binding sites. By using a series of truncated promoter sequences to drive GFP expression, possible *in vivo* activity for these DAF-16 binding sites was observed. One consensus site DBE (DAF-16 Binding Element) may act to repress transcription. This is in agreement with the SAGE data which shows down regulation of this transcription factor in the *daf-2* background. In *daf-2* mutants DAF-16 is activated and is required for extended longevity. Transcriptional targets and the DNA binding motif for the transcription factor will be elucidated, and its role in longevity will be characterized.

BACK

# Analysis of Insulin-Like Peptides in *C. elegans* by Mass Spectrometry

**Astrid Kleinert**, Daniel Hess, Jan Hofsteenge and Joy Alcedo
Friedrich Miescher Institute for Biomedical Research, Maulbeerstrasse 66, CH-4058 Basel, Switzerland

Genetic analyses in *C. elegans* have implicated an endocrine signaling pathway, the insulin/IGF-1 pathway, in regulating lifespan. Mutations in the gene *daf-2*, which encodes an insulin/IGF-1 receptor homologue, can increase worm lifespan by more than 100% (1). In addition, the insulin and IGF-1 pathways have been shown to influence fly and mouse lifespan (2-5).

Although downstream components of the DAF-2 pathway have been studied in detail, far less is known about the putative DAF-2 ligands predicted to be encoded by 38 insulin-like genes in the worm. It remains unknown which of the predicted insulin-like peptides are present in wild type or in longevity mutants. In order to determine this, we have initiated a project to analyze the polypeptide subfractions of the *C. elegans* proteome by mass spectrometry. Worm lysates will be prepared from mixed stage, wild-type worms, excluding membrane-bound proteins. Acid-ethanol extraction and gel filtration will be tested as methods to obtain a subfraction enriched in small polypeptides. Peptide identification will be done by liquid chromatography and tandem mass spectrometry (LC-MS/MS). Data analyses will be carried out using a MASCOT search database, which contains a subset of the UNIPROT database and a database generated of relevant *C. elegans* polypeptides. Finally, using the SILAC method for quantification (6), we plan to compare differences in the levels of polypeptides, *e.g.*, insulin-like peptides, between wild-type worms and longevity mutants.

References: (1) Kenyon et al., 1993. Nature 366: 461-4. (2) Clancy et al., 2001. Science 292: 104-6. (3) Tatar et al., 2001. Science 292: 107-110. (4) Holzenberger et al., 2003. Nature 421: 182-7. (5) Blüher et al., 2003. Science 299:572-4. (6) Krijgsveld et al., 2003. Nature Biotech. 21: 927-931.

BACK

***P-043***

# Identification of Genes Involved in the Neural Regulation of Lifespan

Wolfgang Maier, **Martin Regenass**, Monique Thomas and Joy Alcedo
Friedrich Miescher Institute for Biomedical Research, Maulbeerstrasse 66, CH-4058 Basel, Switzerland

Aging is influenced by several factors, which include the environment of the animal. For example, mutations that impair sensory function extend the lifespan of *C. elegans*, which suggests that lifespan, of at least this animal, is regulated by the perception of environmental cues (1). Specific sensory neurons appear to regulate lifespan, at least in part, through the insulin/IGF-1 pathway (1, 2). Since several putative insulin-like ligands are also expressed in these neurons (3), it is possible that an environmental signal(s) sensed by these neurons regulates the production of these ligands. To elucidate further the molecular mechanisms of the sensory influence on lifespan, we are carrying out an RNA-mediated interference screen for enhancers and suppressors of the lifespan phenotype of sensory mutant animals.
As we identify genes involved in the sensory influence on lifespan, we will study their expression patterns using GFP-fusion reporter constructs to determine in which cells they function. We also plan to measure the lifespans of strains in which the particular gene was knocked out or overexpressed. Furthermore, we will conduct pertinent studies on how the gene products function, such as testing if the functions of these genes require each other or involve the *daf-2* insulin/IGF-1 pathway or other signaling pathways in the worm known to influence its lifespan.
References: (1) Apfeld, J., and C. Kenyon. 1999. Nature (Lond.). 402: 804 – 809; (2) Alcedo, J., and C. Kenyon. 2004. Neuron 41: 45 – 55; (3) Pierce, S. B., et al. 2001. Genes Dev. 15: 672 - 686.

BACK

# The Role of pkc-1/ttx-4 in Dauer Formation

**José M. Monje,** Manuel A. Fidalgo, Manuel J. Muñoz
Centro Andaluz de Biología del Desarrollo. Universidad Pablo de Olavide. CSIC, Crta. De Utrera. Km.1. 41013. Seville. Spain. Email: jmmonmor@upo.es

The regulation of dauer formation is a complex process. Several genes and different pathways converge in the same point to decide between develop to adult or to the resistant stage known as dauer.

Previously, we have isolated the *aap-1(m889)*, a mutant in the regulatory subunit of PIP3 kinase, that enter to dauer constitutively at 27ºC (Wolkow et al J Biol Chem. 2002 Dec 20).

In this work we present the isolation of a suppressor of this phenotype. One of the mutants isolated encodes to PKC-1/TTX-4 a protein kinase C homologue to nPKC-epsilon. PKCs plays important roles in signal transduction.

Our allele of pkc-1 can suppress all the insulin pathway mutants we tested, except *daf-2 (e1370).* daf-16, a transcription factor downstream of daf-2, goes to the nucleus in the insulin mutants background, but when we look to the daf-16 localization in a double mutant *aap-1(m889) pkc-1(pv12*), is still nuclear, so our allele suppress the daf-c (dauer formation constitutive) phenotype without relocating daf-16 out of the nucleus, behaving differently to other daf-c suppressors reported. In regards to longevity, *pkc-1(pv12)* shows a decrease in the lifespan at 25ºC compare to wild type, but it cannot suppress the long-lived phenotype of all the daf-2 alleles. *pv-12* itself is not daf-d

Surprisingly, *pv12* enhances the dauer constitutive formation phenotype of the daf-1 and daf-7 mutants that belong to the TGF-b pathway (a parallel pathway to the IGF/ insulin for dauer formation control). *pv-12* itself is not daf-c.

We are investigating if the two antagonist effects of pkc-1 are due to pathway specific roles of this protein in different cell types.

BACK

# Using *C. elegans* to Model the Systems Biology of Stress Response and Age-Related Disorders

**Julia Sämann,** Enrico Schmidt, Mark Seifert and Ralf Baumeister
Bio3/Bioinformatics and Molecular Genetics, Center for Systems Biology, University of Freiburg, Germany

Our lab is interested in understanding the connection of stress response and disease. According to a prevailing theory, aging is characterized by the declining activity of genome maintenance and repair mechanisms that deal with the detrimental consequences of external and internal stress. This eventually results in the accumulation of DNA mutations and increases the risk of stroke, cancer, coronary heart diseases, and degenerative disorders (the four major causes of death in our society). One focus of our research is the generation of *C. elegans* model to study the biological role of the affected genes. We focus here on genes whose mutations segregate with hereditary cases of Parkinson's Disease to result in an early age-of-onset and rapid progression. *C. elegans* homologues of four respective genes were identified and mutants are available. These are the ubiquitin ligase parkin/pdr-1, the mitochondrial kinase PINK1/pink-1, two genes with strong similarities to human DJ-1 (pdr-2 and pdr-3: Parkinson's Disease-related gene 2 and 3), and the cGMP binding protein Dardarin/LRRK2/lrk-1. All of them have been linked to intracellular mechanisms of (oxidative and unfolded protein) stress response, suggesting that they can be functionally linked to one another. We have initiated genome-wide screens to characterize the "Parkinome", the interaction network of factors related to Parkinson's Disease. One purpose of this endeavour is the identifation of common targets and regulators of PD-related gene products, as well as the modeling of their roles in the onset of disease. Using the split-ubiquitin yeast two-hybrid interaction screen, we identified over 100 proteins as potential interactors for PD-related proteins. Using a medium throughput biochemical assay we are currently working on the confirmation of these interactions, and apply genetic and pharmacological assays to understand the physiological processes that are perturbed in mutants. In previous experiments, we generated screening models for parkin and human α-synuclein mutants in *C. elegans*. The expression of α-synuclein A53T in a *pdr-1* mutant background that behaves similar as the Parkin mutants found in PD resulted in temperature-sensitive toxicity that enabled us to screen for suppressors/enhancers of this phenotype. Until now, we identified more than 50 modulators of this toxicity. Results of the various screens support the model of a functional connection between PD-related factors.

BACK

***P-046***

# Signaling via eIF4E regulates ageing in *C. elegans*

**Popi Syntichaki**, Kostoula Troulinaki and Nektarios Tavernarakis
Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology Hellas, Heraklion, Crete, Greece

Although ageing is a complex and highly stochastic process, studies in model organisms, over the past decade have provided evidence that ageing can be regulated genetically. One example is the regulation of lifespan by the insulin/IGF-like signalling pathway. Current evolutionary theories of ageing suggest that the rate of ageing is primarily controlled through genes that regulate cellular maintenance and repair mechanisms in the soma. Regulation of protein synthesis is critical for cell growth and maintenance. Ageing in many organisms, is accompanied by marked alterations in both general and specific protein synthesis. Whether these alterations are simply a corollary of the ageing process or have a causative role in senescent decline remains unclear. We are studying the role of key regulators of protein synthesis in ageing using *C. elegans*.

The eukaryotic initiation factor 4E (eIF4E), which binds the 7-methyl guanosine cap at the 5' end of all nuclear mRNAs, strongly affects the rate of translation initiation, the rate-limiting step of protein translation. We find that loss of a specific eIF4E isoform, IFE-2 that functions in somatic tissues, extends the lifespan of *C. elegans*. Lifespan extension is independent of the forkhead transcription factor DAF-16, the downstream effector of the insulin-like signalling pathway on ageing. In addition, IFE-2 deficiency further extends the lifespan of long-lived *age/daf*, *clk* and dietary-restricted *eat* nematode mutants. Knockdown of the phosphatidyl inositol kinase TOR that controls protein synthesis in response to nutrient cues further increases the longevity of *ife-2* mutants. Thus, we propose that signalling via eIF4E is a novel pathway influencing ageing in *C. elegans* by adjusting the levels of protein turnover in the soma.

BACK

# Characterization of PQN-21, A Prion-Like Protein Involved in Learning and Memory in *C. elegans*

**Dafne Bazopoulou** and NektariosTavernarakis
Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology Hellas, Heraklion, Crete, Creece

Prions are infectious agents implicated in a variety of neurodegenerative diseases in mammals, generally referred to as transmissible spongiform encephalopathies. Prions contain a glutamine/asparagine (Q/N)-rich domain which appears to be responsible for the unusual capacity of these proteins to fold into structurally and functionally distinct conformations, one of which is self-perpetuating. Usually the self-perpetuating-'prion' form is organized in self-seeding polymers and can induce other proteins with similar sequences to acquire the 'prion' state, creating a chain reaction. Prion-like proteins are widespread (found in mammals, fungi, yeast), conserved and serve numerous diverse functions. In *Aplysia*, the prion-like neuronal protein CEPB functions as a positive regulator of mRNA translation in stimulated synapses and helps to maintain long-term synaptic changes associated with memory acquisition and storage.

PQN-21 is a *C. elegans* protein bearing a Q/N rich-'prion'domain. It also contains a zinc-finger domain, which is common among DNA binding proteins. We find that the *pqn-21* gene is expressed in neuronal and epithelial cells and the PQN-21 protein localizes in the nucleus. Deletion of the *pqn-21* gene results in defective gonadal and axonal outgrowth. Interestingly, while PQN-21-depleted animals are normal for chemotaxis to several soluble and volatile compounds, they show strongly impaired conditioning to chemicals, indicating a shortfall in associative learning and memory. Based on this observation, we hypothesize that prion roles in associative learning and memory are conserved from nematodes to mammals, thus, making *C. elegans* an attractive model in which to dissect the relevant mechanisms. To this end, we are characterizing the function of PQN-21 and its involvement in additional phenomena of learning and memory in *C. elegans*.

BACK

# The Function of Latrophilin in *C. elegans*

**David R. Bell**, A. Ademola, J. Davy, S. van der Poel, D. Kendall, P.N.R. Usherwood, I.R. Duce, I. R. Mellor and D. de Pomerai
School of Biology, University of Nottingham, University Park, Nottingham, NG7 2RD, UK

Black Widow spider venom (BWSV) contains latrotoxins that induce catastrophic neurotransmitter release in mammals, and is known to bind with high affinity to three neural proteins in mammals, including latrophilin. We have investigated C. elegans as a model system for studying the function of these proteins, and their role in regulating neurotransmitter release by latrotoxins.

We have shown that latrophilin is required for the lethality of BWSV in C. elegans by RNAi experiments. We now present data on the characterisation of null mutants of the C. elegans latrophilin, lat-1, illustrating the function of this gene in C. elegans, and show that lat-1 nulls are resistant to emodepside. The expression of latrophilin-GFP constructs is examined, and compared with the phenotypes seen in null mutants. Genetic interactions between endogenous signaling pathways and the lat-1 nulls have been examined by the construction of double mutant worms, revealing novel facets of lat-1 function.

BACK

# Identification, by a Proteomic Approach, of the Signaling Networks Controled by UNC-73(Trio) in the Developing Nervous System of *C. Elegans*

**Jean-Michel Bellanger** and Anne Debant
CRBM-CNRS, Montpellier, France

The development of the embryonic nervous system, which is remarquably conserved through evolution, requires that nascent axons extend across a complex extracellular environment to reach their specific targets. Guidance cues steer the axons towards the appropriate destination and must be relayed through the growth cone to the actin cytoskeleton. Rho-GTPases play a pivotal role in conveying signals to the actin cytoskeleton, causing morphological changes in many different cell types, and there is now compelling evidence that they transduce signals from membrane receptors to the actin cytoskeleton during axon guidance. These proteins are activated by the guanine-nucleotide exchange factors (RhoGEFs), that favor the active, GTP-bound state, of their GTPase targets. Trio is a unique RhoGEF because it contains two GEF domains with different specificity and numerous signaling motifs probably involved in protein-protein interactions. Genetic studies indicate that Trio has essential and evolutionary conserved functions in cell motility and axon guidance, by controling Rho-GTPases activity on actin cytoskeleton remodeling. Compeling evidence suggest that Trio is part of multimolecular complexes that are essential for the transmission and the integration of the intracellular signals elicited by the guidance cues.
However, the composition and the functional role of these complexes remain elusive.
Our goal is to contribute identifying such complexes in the context of a developing nervous system. Three main reasons lead us to consider *C. elegans* as the model of choice for our project. First, Trio is represented by a unique family member in the nematode, *unc-73*, which is very well described, both at the genetic and molecular levels, for its roles in cell migration and axon guidance. Second, due to its relative simplicity, the worm nervous system has been described at a level that is not reachable in other systems. Consequently, a wide panel of tools (both genetic, molecular and cellular) are now available for neurobiological analyses. Third, due to its short life cycle and its ability to be grown in liquid culture, large amounts of biological material can be obtained from this model organism.

Therefore, we propose: i) to identify, by a proteomic approach, the UNC-73-associated proteins *in vivo*, directly in the developing nervous system of *C. elegans* embryos or young larvae; ii) to analyze, by molecular and genetic approaches, the function of theses complexes *in vivo*; iii) finally, to determine whether the complexes identified in the nematode are conserved in mammals and if they are involved in the same signaling and/or developmental pathways. The poster will describe our strategy and its current experimental status.

BACK

# Signalling in quinine avoidance

**Carmela Bergamasco,** Giovanni Esposito, Salvatore Arbucci, Paolo Bazzicalupo
Istituto di Genetica e Biofisica, Adriano Buzzati Traverso CNR, Via Pietro Castellino 111, 80131 Naples Italy

Chemical sensitivity allows animals to identify and respond appropriately to the chemical composition of the environment. Noxious water-soluble compounds that are avoided by *C. elegans* are generally sensed as bitter by humans and are discarded in double choice test by mice. We have used *C. elegans* to focus on the molecular mechanisms involved in primary sensing of quinine, a molecule detected as bitter by humans. ASH is the main sensory neuron involved in sensing quinine. Two Gα subunits, GPA-3 and ODR-3 are necessary for the response of ASH to repellent stimuli (Hilliard et al., 2004 and 2005). In addition the TRPV channel proteins, OSM-9 and OCR-2, are also necessary for the ASH avoidance responses (Colbert et al 1997, Tobin et al 2002). Finally we identified a novel protein, QUI-1, as an essential components of the response to quinine (Hilliard et al., 2004).
With regard to the molecular function of QUI-1, we demonstrate that QUI-1 function is required in ASH for the response to quinine and, using specific antibodies, that the protein is localized to the sensory cilia. These results, together with the discovery that QUI-1 contains an RGS (Regulator of G protein Signaling) domain, strongly suggest that this novel protein might be involved in quinine signaling.
Are there other components of the quinine signal transduction pathway?
We are using a best candidate approach and a variety of behavioral assays to identify new molecules involved in sensing repellent chemicals and in particular quinine. We analyzed behaviorally loss of function and overexpression mutants in several molecules known to act in the G protein signaling pathways (Gα subunits, Gβ subunits, RGS proteins, etc.). The results obtained will be discussed.

**Colbert, H. A., Smith, T. L. and Bargmann, C. I.** (1997). OSM-9, a novel protein with structural similarity to channels, is required for olfaction, mechanosensation, and olfactory adaptation in Caenorhabditis elegans. J Neurosci **17**, 8259-69.
**Hilliard, M. A., Apicella, A. J., Kerr, R., Suzuki, H., Bazzicalupo, P. and Schafer, W. R.** (2005). In vivo imaging of C. elegans ASH neurons: cellular response and adaptation to chemical repellents. Embo J **24,** 63-72.
**Hilliard, M. A., Bergamasco, C., Arbucci, S., Plasterk, R. H. and Bazzicalupo, P.** (2004). Worms taste bitter: ASH neurons, QUI-1, GPA-3 and ODR-3 mediate quinine avoidance in Caenorhabditis elegans. Embo J **23**, 1101-11.
**Tobin, D., Madsen, D., Kahn-Kirby, A., Peckol, E., Moulder, G., Barstead, R., Maricq, A. and Bargmann, C.** (2002). Combinatorial expression of TRPV channel proteins defines their sensory functions and subcellular localization in C. elegans neurons. Neuron **35**, 307-18.

BACK

# Adaptation to the Nicotinic Agonist Levamisole

**Thomas Boulin**[1], Stefan Eimer[1], Janet Richmond[2], Jean-Louis Bessereau[1]
[1]Ecole Normale Supérieure-INSERM U789, 46 rue d'Ulm, 75005 Paris, France; [2]University of Illinois at Chicago

Chronic exposure to receptor agonists often leads to long lasting changes in synaptic function and adaptation to these drugs. Analysis of the adaptation process can provide interesting paradigms to get further insights into mechanisms of synaptic plasticity. We are using a genetic approach to characterize the adaptation behavior to levamisole, a nematode-specific nicotinic agonist.

In *C. elegans*, body-wall muscles are innervated by inhibitory GABAergic and excitatory cholinergic motoneurons. Fast excitatory transmission is mediated by nicotinic acetylcholine receptors. Levamisole activates acetylcholine receptors at neuromuscular junctions and causes paralysis and death of wild-type worms after acute exposure to high concentrations (1mM). However, when wild-type animals are chronically exposed to sublethal concentrations of levamisole (0.1mM), they still enter paralysis but 'adapt' and regain partial mobility after 6-12 hours. Adapted worms are uncoordinated and resemble mutants of levamisole-sensitive AChR subunits. Indeed, adapted worms gain resistance to 1 mM levamisole, suggesting that responsiveness to levamisole is reduced in muscle. Consistently, levamisole-specific currents recorded during bath application of levamisole are reduced by 50% in adapted worms. This effect is specific since the response to GABA remains unchanged in adapted animals.

The reduction of levamisole currents could be explained by a number of scenarios. First, receptor levels could be diminished. Immunofluorescence staining of UNC-29 and UNC-38, two subunits of the levamisole- sensitive AChRs, indicates that receptors remain visible and clustered after adaptation. Precise quantification of fluorescence signals are in progress to detect potential variations of receptor amounts at synapses. Second, subcellular redistribution of the levamisole receptor via endocytic mechanisms could be involved in adaptation. Indeed, immunofluorescence staining does not allow us to differentiate between receptor in the plasma membrane or in adjacent sub-cellular compartments. Finally, the quality of receptor present in the muscle membrane could be changed. For instance, post-translational modifications of the receptor subunits could modulate the sensitivity to levamisole.

In order to identify the molecular mechanisms involved in adaptation, we are testing candidate mutants affecting various physiological processes. So far, we have shown that the calcium-dependent protein-phosphatase calcineurin/*tax-6* is required in body wall muscle for adaptation to levamisole.

BACK

***P-052***

# The Potent Inhibitory Action of the Anthelmintic Emodepside in the *C. elegans* Pharynx Requires the Latrophilin-Like Receptor LAT-1 but not LAT-2

**Kathryn Bull**, James Willson, Lindy Holden-Dye, Robert Walker
Neuroscience Research Group, School of Biological Sciences, University of Southampton, Southampton SO16 7PX, UK

Parasitic helminth infections are a major global threat to the survival of humans, animals and plants. Heavy reliance on anthelmintic drugs to control infections has led to the emergence of parasite resistance. The novel compound emodepside possesses broad anthelmintic activity and a mechanism of action not utilized by currently available anthelmintics, suggesting that this compound could break resistance[1].

Electrophysiological analysis has revealed that emodepside potently paralyses the pharyngeal muscles of adult wild type (Bristol N2) worms ($IC_{50}$ 4.5nM). [1]Previous research has indicated that the *C. elegans* latrophilin-like receptors LAT-1 and LAT-2 function in the mechanism of action of emodepside in the pharynx[2,3]. Recent work using the electropharyngeogram technique has demonstrated that the gene deletion mutant *lat-1 (ok1465)* has reduced sensitivity to pharyngeal paralysis by emodepside. This is similar to the reduced sensitivity previously reported in *lat-1* RNAi treated animals and is consistent with the hypothesis that LAT-1 is involved in conferring emodepside susceptibility at the pharyngeal neuromuscular junction[2]. In contrast, the emodepside sensitivity of the pharynx in the gene deletion mutant *lat-2 (tm463)* is not significantly different to wild-type, suggesting that emodepside is highly specific in terms of the proteins targeted by the compound to exert its effects.

In conclusion, emodepside offers an exciting prospect as a new anthelmintic for combating parasitic nematode infection, but also provides a novel tool for investigating how the neuromuscular junction functions at a molecular level.

[1]Harder et al. (2003) J. Antimicrob. Agents, 22, 318-331. [2]Willson et al. (2004) Current Biology, 14, 1374-1379. [3]Willson et al. (2003) PhD. Thesis.

BACK

***P-053***

# Involvement of DAF-19 in the General Development and Subtype Specification of Cilia

**Jan Burghoorn**[1, 2]**,** Sofie Sahlin[1, 2], Peter Swoboda[1, 2]
[1]Department of Biosciences and Nutrition, Karolinska Institute, S-14157 Huddinge, Sweden; [2]School of Life Sciences, Södertörn University College, S-14189 Huddinge, Sweden

The highly conserved *C. elegans* DAF-19 protein, a regulatory factor X (RFX) transcription factor was originally identified as a key regulator for cilia development (Swoboda et al. 2000). Cilia are specialized subcellular organelles that in *C. elegans* are present on the endings of sensory neuron dendrites. They are functional compartments that mediate signal reception and transduction from the environment. In order to be able to receive many different signals from the environment, cell type specific expression of signal transduction and cilia structural genes have evolved. This resulted in, for example, rod-like cilia, which are involved in the perception of soluble chemotaxis compounds. By contrast, the wing-like cilia are involved in smell perception.

However, how different cilia subtypes develop is hardly known. Recent data suggests the involvement of developmental cascades in the promotion of cell specific neuron cilia subtypes (Lanjuin and Sengupta 2004). In this cascade well-conserved transcription factors through possibly multiple transcription events are thought to specify the identity of sensory neuron cilia subtypes. This is similar to the neuron cell (sub)type specification cascade.

We are interested whether DAF-19 is involved in this cilia subtype specification cascade. This would imply that DAF-19 does not only play a role in general ciliogenesis, but also plays a role in cilia subtype specification. In a genome-wide search for candidate genes with a DAF-19 recognition site in the promoter region, the X-box, many transcription factors were identified (Blacque et al. 2005; Efimenko et al. 2005). We are currently establishing the expression patterns of more than 20 selected transcription factors, for three of which we could already demonstrate to be expressed in ciliated neurons. In addition we will establish if those transcription factors, expressed in ciliated sensory neurons, are transcriptionally dependent on DAF-19 and determine if they play a role in cilia subtype specification.

BACK

# An Antagonistic Interaction between NPY-like Receptors *npr-1* and *npr-2* Regulates Pharyngeal Pumping

**A. Cook**, N. Kriek, E. Siney, N.A. Hopper, L. Holden-Dye
Neurosciences Research Group, School of Biological Sciences, University of Southampton, Bassett Crescent East, Southampton, SO16 7PX

NPR-2 is a *C. elegans* neuropeptide-Y like (NPY) receptor. Previous work has demonstrated that both gene deletion and RNAi targeting for NPR-2 results in defective locomotion (Keating *et al.* 2003 Curr Biol. **13:** 1715-20). NPR-2 is widely expressed in the nervous system, including the pharyngeal nervous system. We therefore hypothesised that NPR-2 may also play a role in the regulation of pharyngeal pumping. To investigate this possibility we have made both extracellular and intracellular electrophysiological recordings from the pharyngeal muscle of *npr* mutant and wild-type animals.

Comparison of N2 and *npr-2(ok419)* animals revealed a significantly lower basal rate of pumping in the mutant coupled with a reduced sensitivity to 5-HT. NPR-1 is the most closely related receptor to NPR-2 and is also expressed in the pharyngeal nervous system. The reduction in basal pumping and 5-HT sensitivity were suppressed in an *npr-2(ok419);npr-1(ky13)* double mutant. This has also been noted with regards to the locomotor defect of *npr-2(ok419)* and suggests an antagonistic interaction between the signalling pathways in which these genes are active. As the neuropeptides FLP-18 and FLP-21 have previously been identified as ligands for NPR-1 (Rogers *et al.* 2003 Nat Neurosci **6:** 1178-85) we tested the response of the *npr* mutants to exogenous addition of these peptides. Exogenous application of either FLP-18A or FLP-21 (1μM) inhibits pharyngeal pumping. The inhibitory response to FLP-21 but not FLP-18A, is absent in *npr-2(ok419)*. However, *npr-1*(*ky13*) responds to both peptides similarly to wild-type animals. The double mutant *npr-2(ok419);npr-1(ky13)* behaves similarly to *npr-2*(*ok419*) i.e. the inhibitory response to FLP-21 is absent. This indicates that although *npr-1*(*ky13*) suppresses the effect of *npr-2*(*ok419*) on pharyngeal pumping, it does not suppress the abolition of the response to FLP-21. Furthermore, it would appear that *npr-2*, but not *npr-1* is required for the pharyngeal response to exogenous FLP-21. Currently we are testing the hypothesis that FLP-21 directly activates NPR-2 in the pharyngeal nervous system.

*Funded by the BBSRC, UK. Thanks to Mario De Bono and the C. elegans knockout consortia (NBRP Japan and USA) for the provision of strains.*

BACK

# Dissecting Gustatory Plasticity in *C. elegans* Using Mammalian Receptors

**Martijn Dekkers**[1], Michelle Teng[2], John McCafferty[2] & Gert Jansen[1]
[1]Dept. of Cell Biology and Genetics, Erasmus MC Rotterdam, The Netherlands; [2]Wellcome Trust Sanger Institute, Hinxton, Cambridgeshire CB10 1SA, United Kingdom

We use *C. elegans* to study the molecular and cellular mechanisms of salt perception, using behavioural assays and calcium imaging. We discriminate three distinct responses to NaCl: First, attraction to NaCl concentrations ranging from 0.1 to 200 mM. Second, avoidance of higher concentrations. Third, avoidance of an otherwise attractive NaCl concentration after prolonged exposure. We call this latter behaviour gustatory plasticity. Previous studies have shown that chemo attraction to NaCl is mediated primarily by ASE, and to a lesser extent by ASI, ADF and ASG, and avoidance of high concentrations of NaCl is mediated by ASH (Bargmann & Horvitz, 1991). In our lab we have identified 85 proteins and five pairs of gustatory neurons that mediate gustatory plasticity. Based on our results we propose a model in which prolonged exposure to 100 mM of NaCl, elicits a signal from the ASE neurons, leading to sensitisation of the avoidance signalling ASI, ADF, ADL and ASH neurons. This results in avoidance of low concentrations of NaCl.

In an effort to identify the roles of the individual cells in gustatory plasticity we expressed either a TRP channel or a G-Protein Coupled Receptor (GPCR) in the neurons that have been implicated in gustatory plasticity. This allows us to specifically activate those cells. The TRP channel that we use is the mammalian capsaicin receptor VR-1. Normally *C. elegans* does not respond to capsaicin. Previously it has been shown that expression of VR-1 in the ASH neurons results in avoidance of capsaicin (Tobin et al 2002). We have generated animals that express the VR-1 receptor in the ASE, ASI, ADL and ADF neurons. We are currently testing their responses to capsaicin and the effects of preexposure to NaCl on this response, using behavioural assays.

The GPCR's that we have chosen are the mouse SSTR-2 somatostatin receptor and the human CCR-5 chemokine receptor. We have expressed these receptors in the ASH cells, and tested the responses in a novel avoidance assay. We found that the transgenic animals display specific avoidance behaviour to the ligands of the receptors, indicating that these GPCR's are integrated into the endogenous *C. elegans* signalling machinery, which is remarkable, given the evolutionary distance between the species. We are now making constructs to express these GPCR's in the other cells to assess their role in gustatory plasticity.

BACK

# Screening for Negative Regulators of Growth Factor Signalling

**James Dillon,** Anne D. Wooller and Neil A. Hopper
School of Biological Sciences, University of Southampton, Bassett Crescent East, Southampton, SO16 7PX, UK

The Egfr/Ras/Map kinase pathway functions several times during *C. elegans* development to specify various hypodermal specialisations including the excretory duct cell and hermaphrodite vulva. Animals severely compromised for Egfr/Ras/Map kinase pathway signalling fail to produce the excretory duct cell and die as vacuolated L1s. The Fgfr/Ras/Map kinase pathway regulates the activity of the excretory system and certain cell migrations, including that of the sex myoblasts. Animals homozygous for the *sem-5(n1619)* mutation are maternal effect lethal due to a failure to induce the excretory duct cell and/or due to effects upon excretory system activity. We screened for suppressors of the *sem-5(n1619)* lethality and identified 13 suppressors. Nine of these suppressors also suppressed the vulvaless phenotype of *sem-5(n1619)* and were given allele names (*pd8-pd16*). All suppressed animals are egg laying defective, suggesting that the sex myoblast migration defect of *sem-5(n1619)* is not suppressed. Candidates for suppressors include loss/reduction of function alleles in *ark-1, clr-1, gap-1* and *sli-1* and gain of function alleles of components of the Ras/Map kinase pathway. The four suppressing mutations that have been cloned were such candidates and include two gain of function *sos-1* alleles. We hypothesise that *sos-1(pd9gf)*, which produces a C662Y substitution, increases the rate of SOS catalysed nucleotide exchange on RAS and are performing biochemical experiments to test this.

In order to test whether the suppressors of *sem-5(n1619)* lethality we isolated were specific to the Egfr we also tested them for suppression of a weak *daf-2*/InsR allele, *m577*, using the dauer assay. The majority of *sem-5(n1619)* suppressors also suppressed the *daf-2(m577)* Daf-c phenotype. This included the *sos-1gf* alleles and also reference alleles of *ark-1, clr-1* and *sli-1*, but not *gap-1*. However, *gap-2* was found to weakly suppress the *daf-2(m577)* Daf-c phenotype. These findings may be explained by the finding that *let-60*/Ras is a positive regulator of DAF-2 signalling during dauer formation (see also Nanji, Hopper and Gems (2005) Aging Cell **4**, 235-245). In a related screen, we sought to look for suppressors of the *daf-2(m577)* Daf-c phenotype. To distinguish those that may suppress due to the enhancement of Ras signalling from other suppressors, we performed the screen in a *daf-2(m577); gap-1(n1691)* background, with the logic that any suppressors that enhanced Ras signalling would also produce a multivulva phenotype in this backgound. From a 10, 000 genome screen we isolated 16 suppressors of *daf-2(m577)* Daf-c, none of which produced a multivulva phenotype in combination with *gap-1(n1691).* Preliminary data from this screen will also be presented.

BACK

# The Integral Membrane Protein UNC-50 is required for Nicotinic Receptor Trafficking in *C. elegans*

**Stefan Eimer**[1], Alexander Gottschalk[2], Janet Richmond[3], Michael Hengartner[4], William R Schafer[2], and Jean-Louis Bessereau[1]
[1]Ecole Normale Superieure, Paris, France; University of California at San Diego, San Diego, USA; [3]University of Illinois at Chicago, Chicago, USA; [4]University of Zürich,Zürich, Switzerland

Nicotinic acetylcholine receptors (nAChRs) are pentameric ligand-gated ion channels that undergo multiple assembly, modification and transport steps prior to insertion into the plasma membrane. However, little is known about the molecules that are specifically participating in these processes. In *C. elegans* a nAChR sensitive to the nematode-specific cholinergic agonist levamisole has been well characterised. Mutants in all of its four subunits confer resistance to muscle hyper-contraction and death upon exposure to levamisole.

Similarly, *unc-50* mutant animals exhibit the same resistance to paralysis and death upon exposure to levamisole. By antibody staining and electro-physiological recording of body-wall muscles we show that levamisole receptors are not detectable at cell surface in *unc-50* mutants. However, a second type of muscle nAChR, which is insensitive to levamisole, and the GABA receptor are normally transported to the synapse.

*unc-50* encodes an integral membrane protein conserved from yeast to man. In *C. elegans, unc-50* is ubiquitously expressed and localized to the Golgi system. We showed that UNC-50 is required in body wall muscles for the transport of the assembled levamisole receptor to neuromuscular junction. In the absence of UNC-50 the receptor is sorted within Golgi to the lysosomal system and rapidly degraded. Therefore, UNC-50 might be part of/constitute a Golgi resident check point during levamisole receptor transport. UNC-50 interacts with the GBF1 class of large Arf GEFs which are involved in retrograde transport within the Golgi system. Models how retrograde transport and sorting would required for levamisole receptor trafficking will be discussed.

BACK

***P-058***

# Analysis of the Role of npr-1 in Responses to Noxious Stimuli in ASH

**Marina Ezcurra,** Sunkyung Lee, Peter Swoboda and William R. Schafer
Karolinska Institute, Department of Biosciences and Sodertorn University College, Section of Natural Sciences, S-14189 Huddinge, Sweden. Division of Biological Sciences, University of California, San Diego, La Jolla, California 92093, USA

Discrimination of favorable and noxious substances is fundamental for survival. The *C.elegans* polymodal amphid neuron ASH is the primary neuron for detection of aversive stimuli, and mediates avoidance of water-soluble repellents, high osmolarity and mechanical stimuli such as touch on the nose.

*npr-1* encodes a seven transmembrane receptor related to mammalian neuropeptide Y receptors. As shown by de Bono et al, natural variation at a single amino acid residue of NPR-1 determines whether the nematodes exhibit solitary or social feeding. Solitary strains such as N2 bear the NPR-1 215 V receptor, while social strains bear NPR-1 215F. Previous studies have shown that ASH neurons express the NPR-1 receptor, and since social feeding is mediated by the nociceptive neuron ASH, we investigated whether how the npr-1 genotype might affect ASH sensory responses. Preliminary behavioral experiments performed in the lab show that *npr-1* mutants have higher sensitivity to the repellents glycerol and copper, indicating that the lower NPR-1 activity in 215F might result in higher sensitivity to noxious stimuli. We are carrying out additional behavioral experiments to explore the role of *npr-1* in response to noxious stimuli, and using calcium imaging to investigate the role of *npr-1* in aversive detection in ASH.

In addition to ASH, ASK and ADL also play roles in detection of chemical repellents. ASK is particularly interesting as it mediates both attractive and repulsive behaviors in *C. elegans*, but it is not yet know how this polymodality is mediated. Several genes expressed in ASK, such as the TRPV channel *osm-9*, the soluble cyclic nucleotide gated channel subunit *tax-4*, and the GPCR alpha subunits *gpa-3* and *odr-3*, are known to be involved in sensory transduction. To test the role of these and other genes in ASK, we have generated a transgenic cameleon line expressed under an ASK specific promoter. This line will be used for calcium imaging to monitor the responses of ASK in mutants of the candidate genes.

BACK

# Stimulating the Pharyngeal Nerve Ring: Characterisation of Putative Post-Synaptic Potentials

**Franks, C. J.,** Hopper, N. A., O'Connor, V. M. & Holden-Dye L. M.
School of Biological Sciences, University of Southampton, Bassett Crescent East, Southampton, SO16 7PX, U.K

The pharynx of *C. elegans* contains 20 neurons. Based on their morphology these can be subdivided into 14 different types. All of the neurons send processes through the posterior part of the corpus where the majority are involved in the formation of a radial nerve ring. The neurons that contribute to this structure contain a number of neurotransmitters, which can include acetylcholine, glutamate, 5-HT and neuroactive peptides.

We have developed a new dissection protocol that enables us to reproducibly expose the corpus in adult hermaphrodite worms in order to attach an extracellular stimulating electrode. By selectively applying an electrical stimulus to the nerve ring and making an intracellular recording from the pharyngeal muscle we have been able to record putative post-synaptic potentials (PSPs).

Four tests have been employed to characterise the PSPs.

1. Manipulation of stimulating parameters, e.g. pulse width, voltage and polarity.
2. Reduction of $[Ca^{2+}]_o$ and increase of $[Mg^{2+}]_o$.
3. Reduction of $[Cl^-]_o$.
4. Application of the nicotinic acetylcholine receptor (nAChR) antagonist, d-tubocurarine.

The aim of these tests is to distinguish between PSPs associated with pre-synaptic release of a neurotransmitter and passive spread of stimulus current causing direct activation of voltage-gated ion channels in the muscle. The latter is of particular concern because of the very close association of nerve and muscle in the pharyngeal preparation. We can also distinguish between chloride mediated PSPs, associated with glutamatergic signaling on glutamate-gated chloride channels and cholinergic activation of nAChRs. It is unlikely that PSPs will be recorded in response to evoked 5-HT release as exogenous application of this transmitter does not result in a change of membrane potential. A large number of neuropeptides are found in the pharynx. For example, at least 10 RFamide-like peptide genes are expressed (Li *et al.,* 2004) However, the contribution of neuropeptides to evoked PSPs remains unclear.

Reference: Li et al., 2004. *J. Comp. neurol.* **475**:540-550.
Acknowledgment: Funding for this project was provided by the BBSRC. UK.

BACK

# Ectopic Expression of DAF-19, a Transcription Factor that Controls Cilium Development

Gabriele Gahmon and **Peter Swoboda**
Karolinska Institute, Dept. of Biosciences and Nutrition, Södertörn University College, School of Life Sciences, S-14189 Huddinge, Sweden

DAF-19 is an RFX-transcription factor (**R**egulatory **F**actor binding to the **X**-box), which has been shown to be essential for the development of cilia on 60 sensory neurons in *C. elegans*. Mutations in *daf-19* lead to the complete loss of all ciliated structures, but leave the cell bodies and neurites of these neurons intact. DAF-19 has been shown to regulate a large number of cilium-specific genes encoding structural and functional cilium components as well as other transcription factors. This finding suggests a very central role of DAF-19 during ciliogenesis. Since DAF-19 is essential for the formation of cilia, we hypothesize that it could also be sufficient. We try to answer this question by expressing the *daf-19* gene from heterologous promoters in cells that usually do not carry cilia with the aim of inducing ectopic cilia. When expressed in tail hypodermis cells, *daf-19* leads to morphological changes of the hermaphrodite tail and to the ectopic expression of direct DAF-19 target genes. Several of these cells do not show hypodermal cell characteristics anymore, but display more neuron-like features (e.g. processes). Currently we are characterizing in detail to what extent the ectopic expression of *daf-19* can transform hypodermis cells into neuronal or even cilium-carrying cells.

BACK

# LEV-9, a New Player in Acetylcholine Receptor Clustering at Neuro-Muscular Junctions

**Marie Gendrel**, Dan Williams[1], Bill Schafer[2], Erik Jorgensen[1], Janet Richmond[3], Jean-Louis Bessereau

Ecole Normale Supérieure-INSERM U789, 46 rue d'Ulm, 75005 Paris, France ; [1]University of Utah at Salt Lake City; [2]University of California at San Diego; [3]University of Illinois at Chicago

Clustering neurotransmitter receptors at the synapse is critical for efficient neurotransmission. Most of the proteins involved in receptor clustering function through cytoplasmic interactions with a sub-synaptic scaffold. However, we previously demonstrated that clustering acetylcholine receptors (AChRs) at *C. elegans* neuromuscular junctions (NMJs) requires LEV-10, a type I transmembrane protein that contains protein-protein interaction domains in its extracellular region. Expression of the LEV-10 ectodomain in muscle cells rescues the AChR clustering defects of *lev-10* mutants. In the wild type, LEV-10 is localized in post-synaptic membranes at NMJs. In mutants that do not express AChRs, LEV-10 is expressed but no longer clustered at the synapse. These results suggest that AChRs and LEV-10 are part of a complex that interacts with additional synaptic components to be recruited or stabilized at the synapse.

*lev-10* mutants are weakly resistant to levamisole, a nematode specific nicotinic agonist. To identify additional components of the AChR clustering machinery, we immuno-stained AChRs in weak levamisole-resistant mutants. We observed that AChR clusters are no longer detected in *lev-9* mutants. Electrophysiological recording of body-wall muscle cells in *lev-9* mutants indicated that the overall response to levamisole was the same as in wild type. These data demonstrate the presence of functional receptors at the muscle cell surface. Interestingly, LEV-10 clusters are no longer detected in *lev-9* mutants, suggesting that this gene might be required to localize both AChR and LEV-10 at the synapse.

SNP mapping experiments localized *lev-9* in a region that contained a *Mos1* insertion previously isolated in a screen for resistance to the acetylcholinesterase inhibitor aldicarb. We demonstrated by genetic complementation that *ox177::Mos1* is a *lev-9* allele. In this strain, *Mos1* is inserted in the first exon of a gene that encodes a putative secreted protein with multiple protein-protein interaction domains. *lev-9* identity was confirmed by single ORF rescue and identification of mutations in three independent *lev-9* alleles. Mosaic experiments demonstrated that *lev-9* was required in muscle. Experiments are in progress to localize LEV-9 at the subcellular level and to test possible physical interactions between LEV-9, LEV-10 and AChRs. LEV-9 might be part of a putative extracellular scaffold involved in AChR clustering at the synapse.

BACK

# Lnp and Siah/Ring E3 Ubiquitin Ligase: Partners Associated to Multisubunit Ring Finger Complexes involved in Synaptogenesis?

**Luiza Ghila,** Patrizia Latorre and Marie Gomez
Department of Zoology and Animal Biology, University of Geneva, Switzerland

In *C. elegans*, two multisubunit Ring E3 complexes, the anaphase promoting complex/cyclosome (APC/C) and the Skp1/Cullin/Lin-23 complex ($SCF^{LIN-23}$) are involved in the regulation of the GLR-1 (glutamate receptors) abundance in ventral cord nerve cells. The E3 ubiquitin ligase SIAH (the mammalian homolog of the drosophila seven in absentia/SINA) binds to the C-terminal part of APC and interacts with SCF through the Siah-interacting protein SIP. Moreover it has been shown that Siah interacts with the C-terminal part of the GLR-1 and the ubiquitin ligase activity of Siah through this interaction is required for GLR-1 degradation.

In C. *elegans*, a gene coding for a new protein named LNP was shown to interact in a yeast two hybrid screen with SIAH, the C. *elegans* homolog of SINA. *lnp* expression pattern is highly related to that of *siah*. They are widely expressed during embryogenesis and in later stages, their expression seems to be enriched in neural tissues, e.g. the ventral nerve cord and the nerve ring. We have shown that LNP enhances Siah E3 ubiquitin ligase activity. Two *lnp* loss-of-function mutants showed a significant increase in the resistance to the aldicarb, an acetylcholine esterase inhibitor, having egg laying defects and shorter life span. These suggest that Lnp has a function in the nervous system, probably connected to synaptogenesis. These three major E3 ligases (APC/C, SCF complexes and Siah) are also involved in the cell cycle regulation, being responsible for the periodic proteolysis of many cell-cycle regulators. We noticed that Lnp cellular localisation is regulated during cell cycle. During interphase, LNP protein presents a “vesicular” cytoplasmic pattern in all cell lines we tested. During M phase, LNP concentrates on not yet characterized structures underlying the plasma membrane reminiscent to cell adhesion structures. LNP is over-expressed all along the M phase, but in confluent monolayer cells (mitotic inactive) its expression is drastically reduced. We do not know yet the functional aspect of this regulation during cell cycle.

We are currently investigating whether in worms SIAH together with LNP are required for the regulation of the GLR-1 receptors and/or for the regulation of the cell cycle and what are the connections of LNP and SIAH with the APC/C and the SCF complexes in these two biological processes.

BACK

# Investigations into the Mode of Action of the Novel Anthelmintic Emodepside

**Marcus Guest,** Kathryn Bull, Kiran Amliwala, James Wilson, Robert Walker, Neil A. Hopper, Lindy Holden-Dye
University of Southampton

Emodepside is a novel cyclo-octadepsipeptide that paralyses nematodes (1). A number of different lines of evidence indicated that the effect of emodepside may involve an effect on neurotransmitter release including the following: it causes de-staining of neurones labelled with FM4-64; *goa-1(n1134)* and *egl-30(tg26)*, both of which are predicted to have increased ACh release at the neuromuscular junction, are hypersensitive to emodepside in two assays, locomotion and pharyngeal pumping; strains with mutations in various synaptic proteins involved in vesicular-mediated release of neurotransmitter are less susceptible to the paralytic actions of emodepside (2).

A mutagenesis screen has been performed to isolate highly resistant animals. These resistant animals show at least a hundred-fold less sensitivity to emodepside than wild-type and intriguingly show hyper-sensitivity to the acetylcholine esterase inhibitor aldicarb. This suggests that emodepside may reduce acetylcholine release at the neuromuscular junction, in contrast to the previous model of increased transmitter release. Aldicarb is being used as a tool to address the effects of emodepside on acetylcholine release and to resolve these conflicting results. Recent data indicate that treatment of wild-type animals with a low concentration of emodepside increases the time to paralysis in the presence of aldicarb. Currently we are testing whether sensitivity to levamisole is also affected by pretreatment with emodepside.

(1) Harder *et al*., 2005 Parasitol Res 97, S1-S10
(2) Willson *et al*., 2004 Current Biology 14, 1374-1379

*Supported by BBSRC CASE and Bayer. We are grateful to the C. elegans knockout consortium (USA and Japan) for the provision of strains*.

BACK

# Electrophysiological Analysis of *C. elegans* Mutants in Levamisole-Receptor Associated Proteins

**Jana Liewald**, Ruta Almedom, Thorsten Schedletzky and Alexander Gottschalk
Institute for Biochemistry, Johann Wolfgang Goethe-University, Max-von-Laue-Straße 9, D-60438 Frankfurt, Germany

We have previously identified proteins associated with the levamisole receptor, one of two nicotinic acetylcholine receptors (nAChR) acting at the neuromuscular junction of *Caenorhabditis elegans*, after tandem affinity purification, mass spectrometry, and subsequent RNAi-based functional screening (Gottschalk *et al.*, 2005). For proteins causing altered nicotine-sensitivity *in vivo*, genomic (deletion) mutants were obtained.

Currently, we are analyzing these mutants electrophysiologically for the effects on the functional properties of nAChRs (and $GABA_A$ receptors) present at the neuromuscular junction. Therefore, body wall muscle cells of *C. elegans* are whole cell patch-clamped and inward currents are determined, induced by application of neurotransmitters (ACh, GABA) and other cholinergic agonists (levamisole, nicotine).

Preliminary data indicate that the *nra-2(tm1453)* mutant shows significantly reduced currents mediated by ACh and levamisole, while GABA-induced currents are like in wild-type animals. This is in agreement with other functional assays for sensitivity to such agonists carried out in our group: *nra-2(tm1453)* mutants show moderate resistance to paralysis induced by levamisole and nicotine, as well as significantly reduced levamisole receptor levels at the neuromuscular junction (found after *in vivo* fluorescence labeling). Sensitivity to muscimol, a $GABA_A$ receptor agonist, however, was not affected in *nra-2(tm1453)* animals.

Loss of the POLO-like kinase PLK-2, which had caused weak nicotine resistance in our paralysis assays (see also abstract by T. Schedletzky), did not show significantly reduced inward currents in body wall muscle cells after application of cholinergic agonists, though a trend towards reduced currents became apparent.

Other mutants studied are a membrane-binding Copine (NRA-1) and the receptor-tyrosine kinase signalling protein SOC-1. Loss of either protein causes reduced synaptic levamisole receptor levels, while *soc-1* mutants are also affected for synaptic $GABA_A$ receptor expression. Their electrophysiological evaluation is in progress and results will be presented at the meeting.

References: Gottschalk, A., Almedom, R. B., Schedletzky, T., Anderson, S. D., Yates III, J. R., and Schafer, W. R. Identification and characterization of novel nicotinic receptor-associated proteins in Caenorhabditis elegans. EMBO J. 24 (2005), 2566-2578.

BACK

# Evidence that Ethanol Equilibriates Rapidly Across *C. elegans* Cuticle

**P. Mitchell**, K. Bull, N.A. Hopper, S. Glautier[1], L. Holden-Dye, V. O'Connor
Neurosciences Research Group, School of Biological Sciences, [1]School of Psychology, University of Southampton, Bassett Crescent East, Southampton, SO16 7PX

Ethanol has concentration-dependent effects on neural function. At concentrations in the low mM range, equivalent to the drink-driving limit, it has an intoxicating action, which is often associated with a transient excitability, followed by inhibition. At higher concentrations, greater than 100 mM, it has an anesthetic action and elicits muscle paralysis. *C. elegans* has been used to define the genetic basis for behavioural responses to ethanol exposure. In these studies intact animals have been exposed to 500 mM ethanol with the prediction that the internal concentration is more than ten-fold lower and therefore equivalent to an intoxicating, rather than an anesthetic, dose (Davies and McIntire, 2004). In order to underpin further studies, we have analysed the relationship between the external and internal ethanol concentration of *C. elegans*.

Immersion of *C. elegans* in a sealed chamber containing ethanol (range 100 mM to 500 mM) elicits an inhibition of thrashing rate. Inhibition reaches a steady-state value within 5 min. This effect is concentration-dependent (half-maximal effect at 300 mM). At all concentrations of ethanol a steady-state inhibition is observed within 5 min. The animals rapidly recovered when removed from ethanol. Notably, this recovery was complete within 2.5 min. This suggests that ethanol may rapidly equilibriate across the cuticle and that the internal concentration that is required to inhibit motility approximates to the external concentration. In order to directly assay the effects of ethanol on muscle we used the pharyngeal muscle preparation. In this assay the muscle is exposed and therefore the concentration of ethanol is known. This muscle is inhibited by ethanol at concentrations in excess of 100 mM. Although it could be argued that the pharyngeal muscle behaves in a different fashion to body wall muscle in terms of ethanol sensitivity, the results support the contention that concentrations in excess of 100 mM are required to inhibit muscle activity. Internal ethanol concentrations in *C. elegans* following exposure to ethanol have been estimated using a biochemical assay kit. However, this procedure requires that the animals are briefly washed in cold buffer prior to the measurement. Our observation that animals exposed to ethanol fully recover from the inhibition of motility within 2.5 min suggests that a significant amount of ethanol may be lost from the inside of the animals during the protocol. We tested this by determining the influence of the wash volume on the apparent estimate in internal ethanol concentration. This shows that the estimate of internal ethanol concentration increases as the volume of the wash buffer decreases. This suggests that the measurement of internal ethanol concentration using this method underestimates the true internal concentration.

Davies, A.G. and McIntire, S.L. 2004, Biol. Proced. Online 6, 113-119.
*Funded by the BBSRC, UK*

BACK

# The Physiological Role of Peptidergic Transmission in the Pharyngeal Nervous System

**S. Luedtke,** R.J. Walker, N.A. Hopper, V. O'Connor and L. Holden-Dye
Neurosciences Research Group, School of Biological Sciences, University of Southampton, Bassett Crescent East, Southampton, SO16 7PX

We are interested in the peptidergic regulation of neuronal function. As the pharyngeal muscle potently responds to a number of flp neuropeptides, we are determining whether peptidergic signalling is involved in the physiological regulation of pumping activity. In a first approach we wish to identify genes that are involved in pharyngeal pumping activity and peptidergic signalling. To achieve this we have described the pumping activity on and off food in wild-type and mutant animals. Here we show data for several different mutants with a dysfunctional feeding phenotype and how this might help in understanding the interactions in the neuronal circuit underlying pharyngeal pumping.

In another approach, we are developing an optical assay for peptidergic signalling. The neuropeptide we are focusing on initially is FLP-13, as this is expressed in pharyngeal neurones and has been shown to have a potent inhibitory effect on pharyngeal pumping. We have generated a *flp-13::dsRed2* fusion construct in which the optical marker is inserted within the coding sequence of one of the FLP-13 peptides. This construct has been injected into animals using the *pha-1* protocol as a selection for transgenic animals. Stable lines that were rescued for *pha-1* were recovered, PCR confirmed they had the *flp-13*::*dsRed* transgene and RT-PCR confirmed that this construct was expressed. However, none of the animals exhibited fluorescence. Currently we are experimenting with other fluorophores that may be more suitable for use with the dense core secretory pathway.

*Funded by the Gerald Kerkut Charitable Trust. We are grateful to the C. elegans Genetics Center for the provision of strains.*

BACK

***P-067***

# Evaluating the Role of the Neuronal MAP Kinase JNK-1 in Orientation Behavior and Thermotaxis

**Frank Nunes**, Melanie Voss, Arne Henkel, Marc Wolf, Rüdiger J. Paul
Institut für Zoophysiologie, Westfälische Wilhelms-Universität Münster, Hindenburgplatz 55, D-48143 Münster, Germany

The *C. elegans* MAP kinase JNK-1 is a homolog of human stress activated protein kinases (SAPK). *jnk-1* deletion mutants are short-lived and more susceptible to heavy metal stress. On the other hand, If JNK-1 was overexpressed, worms showed an increased resistance to oxidative stress and a prolonged lifespan (Oh *et al.*, 2005, Villanueva *et al.*, 2001). As shown by GFP reporter gene assays, JNK-1 is exclusively expressed in the worm's nervous system (Kawasaki *et al.*, 1999). By western blot analyses we could demonstrate an increasing activation of JNK-1 with rising but moderate temperatures, which should not be a harmful stress to *C. elegans*. Therefore we started to investigate the role of JNK-1 in thermotaxis behavior. Indeed *jnk-1* deletion mutants [VC8 (gk7)] have an altered temperature preference compared to the wildtype (Bristol N2) when placed on a linear temperature gradient. The wildtype prefers temperatures between 15°C and 20°C whereas *jnk-1* deletion mutants are predominantly found between 15°C and 24°C, thus having a broader temperature preference range. *C. elegans* explores its environment by modulating the frequency of occasional turns and reversals. When the environmental conditions are unfavorable reversals or sharp turns and consequently reorientation become more frequent (Gray et al., 2005). We have found that *jnk-1*-deletion mutants show significantly lower rates of orientation movements at 20°C and 25°C, which could be the reason for the observed altered temperature preference. Thus it is possible that cellular stress response as well as stress avoidance behavior are both activated in a coordinated manner through JNK-1.

BACK

# Association of Two Lipid Kinases Involved in Neurotransmitter Release

**Borja Pérez Mansilla**, Stephen Nurrish
Medical Research Council-LMCB, University College London

We have previously reported the ability of the small G protein Rho to modulate the release of synaptic vesicles at the neuro-muscular junction. One way for Rho to achieve this is by inhibiting the DAG-metabolising enzyme diacylglycerol kinase (DGK-1) presynaptically with the consequent accumulation of DAG at release sites and an increase in the rate of neurotransmitter release. But there appears to be other Rho-regulated mechanisms independent of DGK-1 that can influence the locomotion behaviour in *C.elegans*. With this in mind, we attempted to determine whether another lipid metabolizing enzyme, which is known to be a Rho effector in mammalian systems and activates neurotransmitter release in mammals, phosphatidylinositol 4-phosphate 5-kinase, may also be involved in the modulation of animal behaviour. *C. elegans* only possesses one type I PI(4)P5K that phosphorylates PI(4)P to generate $PI(4,5)P_2$. called PPK-1, which is an essential gene. We could confirm that the interaction between RHO-1 and PPK-1 is conserved in *C.elegans*.

By using the mammalian counterparts of both lipid kinases, DGKθ and PI(4)P5Kα, in mammalian cell culture we could determine that both enzymes interact with each other. Furthermore, this interaction seems to be dependent on the conditional activation of some cellular protein kinase C. Previous reports in mammalian systems have shown that DGKθ can be phosphorylated by at least two PKCs, the ε and η isoforms (van Baal *et al*., 2005), and in the case of PI(4)P5K, it appears that PKC activation leads to the dephosphorylation of the enzyme via activation of the phosphatase PP1 (Park *et al*., 2001).

All this taken together suggests that lipid modifying enzymes involved in production and removal of DAG exist in a multi-protein complex, and that activites of these enzymes are modified by DAG signalling. Thus the production and perdurance of DAG in the membrane is likely to be highly regulated both spatially and temporally.

BACK

# The *xbx* Genes Dataset Uncovers *C. elegans* ciliary genes

Prasad Phirke[1,2], Evgeni Efimenko[1,2], Filip Ystrom[1,2], **Peter Swoboda[1,2]**
[1]Karolinska Institute, Dept of Biosciences & Nutrition, Huddinge, Sweden; [2]Södertörn University College, School of Life Sciences, Huddinge, Sweden

Cilia are widespread eukaryotic subcellular organelles that function in cell motility, movement of extracellular fluids and sensory reception. In *C. elegans*, the expression of many ciliary genes is regulated by DAF-19, an RFX-type transcription factor that recognizes DNA sequence motifs (X-boxes) in promoters of its targets, which include genes of ciliary structure and transport machinery, receptors and other factors.

Using a genome-wide search approach for X-box containing genes (*xbx* genes) we identified a list of about 750 *xbx* genes (candidates). This list comprises some already known ciliary genes as well as new genes, many of which we hypothesize to be important for cilium structure and function, among them the *C. elegans* gene *xbx-2*, which encodes a dynein light chain (DLC) (Tctex-1 family). Previously, we have shown that XBX-2 protein is involved in intraflagellar transport (IFT), an important ciliogenic process. A recently obtained mutant allele for *xbx-2* will allow us to investigate at which point XBX-2 molecules are necessary for proper IFT to occur. To find the possible role of *xbx-2* in IFT, we will apply experimental approaches like cilia length measurements in *xbx-2* mutants, expression of XBX-2::GFP in *bbs* and in various IFT mutant backgrounds, as well as expression of various other IFT proteins in an *xbx-2* mutant background.

In parallel, we are performing global expression analysis of various cilia mutants at different developmental stages. First expression analyses of embryonic stages of cilia versus no-cilia mutants have revealed a list of candidate *xbx* and ciliary genes that displayed statistically significant down-regulation in their expression patterns. Using the above approaches together with stringent bioinformatic analyses and comparisons with other datasets, we will be able to identify the complete set of genes that is involved in ciliogenesis and functional maintenance of cilia.

BACK

***P-070***

# Role of the Immunophilin FKBP-52 in the Nervous System of *C. elegans*

**N. Roudier**, K. Rajkowski, B. Chambraud, E.E. Baulieu
Inserm U788, Hopital du Kremlin-Bicetre, 94276 Kremlin-Bicetre Cedex, France

In mammals, immunophilins were first described in immune cells as targets for immunosupressive drugs, such as rapamycin and FK506, which bind to the group of FK506-binding proteins (FKBPs). All FKBPs share a peptidyl-prolyl cis-trans isomerase (PPIase) domain, whose activity is inhibited by the binding of FK506. The FKBP of 52 kDa (FKBP-52) was discovered as a component of the unliganted steroid receptor heterocomplex through an interaction with the HSP90 protein. The N-terminal region of FKBP-52 contains the PPIase domain and include a tetratricopeptide repeat (TPR) domain which interacts with HSP90. Immunophilins are abundant in neurons, and it has been suggested that some immunophilin ligands may be used as therapeuthic agents for accelerating neuroregeneration and for neuroprotection.

In order to better understand the function of FKBP-52 in the nervous system, we have started studies in the whole living organism *Caenorhabditis elegans*, whose genome contains eight putative FKBP homologs (FKB-1 to FKB-8). Among those, FKB-6 is the FKBP-52 homolog. Strains containing the promoter and coding sequence of *fkb-6* fusioned to GFP were obtained. FKB-6 is localized preferentially in the neurons, excretory cell, pharynx, muscles, distal tip cell, vulva and spermatheca. Western-blot experiments using a polyclonal antibody directed against two peptides of the rat FKBP-52 (Chambraud, unpublished data) show that it cross-reacts with FKB-6. Immunocytochemical experiments using this antibody confirm the localisation. RNAi experiments by feeding on the N2 wild-type worms did not show any visible phenotype, but RNAi injection in the sensitive *rrf-3* strain gives early embryonic lethality. Future studies will help us to know more about the role of FKBP-52 *in vivo*.

BACK

# Characterization of SOC-1 and PLK-2, Two Proteins Modulating Levamisole-Receptor Function in *C. elegans*

Thorsten Schedletzky and **Alexander Gottschalk**
Institute for Biochemistry, Johann Wolfgang Goethe-University, Max-von-Laue-Straße 9, D-60438 Frankfurt, Germany

We have previously identified novel proteins associated with the levamisole receptor, one of two nicotinic acetylcholine receptors (nAChRs) acting at the neuromuscular junction (NMJ) of *Caenorhabditis elegans*, using a tandem affinity approach, mass spectromety and RNAi-based functional screening (Gottschalk *et al.*, 2005). Two of the proteins that show altered nicotine-sensitivity after knock-down are the multisubstrate adaptor protein SOC-1 and the polo-like kinase PLK-2.

*soc-1(n1789)* mutants show resistance to nicotine and levamisole in paralysis assays. As it is well known that SOC-1 acts downstream of the EGL-15 fibroblast growth factor (FGF) receptor tyrosine kinase (RTK), we tested *egl-15* as well as other positive regulators of the FGF pathway. We could show that *soc-1* is connected not only to EGL-15 signalling in clustering of levamisole receptors at the NMJ, but also to a second RTK, CAM-1, and that it also affects clustering of $GABA_A$ receptors, potentially through a third, as yet unknown RTK. A SOC-1::GFP fusion is strongly expressed in amphid sheath and socket cells, but nevertheless rescues the *soc-1* phenotype in *clr-1; soc-1* double mutants. We try to detect SOC-1 in other cells, using a specific antibody.

Currently, we are screening for other receptor tyrosine kinases in *C. elegans* causing a nicotine or levamisole resistance phenotype, to identify novel upstream regulators of SOC-1, that may be involved in nAChR and/or $GABA_A$ receptor clustering at the NMJ.

PLK's are kinases generally involved in cell-division and chromosome segregation, however, vertebrate PLK-2 homologues (Fnk and Snk) also affect synaptic plasticity. *plk-2(tm1395)* mutants are weakly nicotine and levamisole resistant. Characterization of *plk-2(tm1395)* by electrophysiology, however, indicated only a trend towards reduced currents induced by cholinergic agonists in muscle (see abstract by J. Liewald). *plk-2* mutants also show a "high incidence of males" (*him*) phenotype, indicating indeed a role in centrosome duplication and chromosome segregation. Consistently, PLK-2::GFP appears to be expressed both in dividing and non-dividing cells, including muscle.

Reference: Gottschalk A, Almedom R, Schedletzky T, Anderson SD, Yates JR, and Schafer WR (2005) *EMBO J* **24**: 2566-2578

BACK

# Characterisation of Gene Deletion Mutants for Putative Neuropeptide Receptors

**E. Siney**, A. Cook, N. Kriek, L. Holden-Dye
Neurosciences Research Group, School of Biological Sciences, University of Southampton, Bassett Crescent East, Southampton, SO16 7PX, United Kingdom

*C. elegans* is predicted to express approximately 250 neuropeptides. Many of these have potent biological activity. A subset of more than forty G-protein-coupled receptors (GPCR) are candidates for mediating these effects. As part of a long-term goal to pair neuropeptide receptors with their cognate ligands, we have been carrying out a systematic analysis of GPCR gene deletion mutants. Gene deletion mutants have been supplied by the *C. elegans* knockout consortia for C10C6.2 *(npr-3)*, C16D6.2, C25G6.5, C39E6.6 *(npr-1)*, C53C7.1, C56G3.1, F35G8.1, F41E7.3, T05A1.1 *(npr-2)*, T22D1.12, Y58G8A.4, ZC412.1, C30F12.6, C38C10.1, C43C3.2, C48C5.1, F57H12.4, Y54E2A.1, ZK455.3. The deletions have been sequenced and the strains out-crossed four or more times. As neuropeptides have been shown to have potent effects on the body wall and pharyngeal muscle of nematodes, we have assayed these mutants for defects in thrashing and pharyngeal pumping on food. Animals which have defects in thrashing have been further tested for aldicarb sensitivity in order to determine whether there is altered cholinergic signalling at the neuromuscular junction. Mutants with altered pharyngeal pumping were further analysed using electropharyngeogram recordings. These mutants were also tested for altered responses to neuropeptides which have previously been shown to have potent effects in this preparation. The majority of the GPCR mutants do not show any overt phenotype. The thrashing assay has identified two mutants with defects in this behaviour, *npr-2* (*ok419*), and a deletion in C10C6.2, *npr-3 (tm1583)*. So far, none of the mutants exhibit altered pharyngeal pumping behaviour.

*Funded by the BBSRC, UK. Thanks to the C. elegans knockout consortia (NBRP Japan and USA) for the provision of strains.*

BACK

# Valproic Acid Acts on Both IP3 and DAG Regulated Behaviours in *C. elegans*

**Suzumi M. Tokuoka** and Stephen Nurrish
MRC Cell Biology Unit, University College London

Phosphatidylinositol-4,5-bisphosphate (PIP2) is hydrolysed by Phospholipase C into Inositol tri phosphate (IP3) and diacylglycerol. Recent work has suggested that defects in inositol phophate signalling may underlie behavioural defects such as bi-polar disorder (manis depression). In support of this model commonly used drugs to treat bipolar disorder, such as Lithium and Valproic acid (VPA) (Nature 417:292 2002). Lithium is known to inhibit enzymes involved in recycling of inositol phosphates but the targets of VPA are unclear. We are searching for VPA targets using the model system *C. elegans*.

Addition of VPA or $Li^+$ to *C. elegans* affects many behaviours, one of which, defecation, is known to be regulated by IP3 inositol phosphates. Thus, both $Li^+$ and VPA affect inositol signalling in both mammals and *C. elegans*. Other effects of VPA on *C. elegans* are: a retarded growth rate, altered levels of acetylcholine release, reduced egg laying, increased locomotion rate at low concentrations, and paralysis at high concentrations. All of these behaviours are known to be regulated by DAG, suggesting that VPA affects both IP3 and DAG signalling pathways.

To identify VPA targets we have carried out genetic screens for VPA-resistant mutants. Some of the mutants isolated from locomotion or growth assays were also resistant for the effects of VPA on defecation. Those mutations are currently being mapped. We have also tested a number of candidate mutants defective for neurotransmitter release for their response to VPA: protein kinase C mutants (*pkc-1*) are resistant to VPA, whereas phospholipase C beta mutants (*egl-8*) and diacylglycerol kinase mutants (*dgk-1*) are hypersensitive in the paralysis assay at high concentrations. The similar phenotype of *egl-8* and *dgk-1* is a surprise as they have opposite effects on DAG levels. EGL-8 produces DAG and DGK-1 phosphorylates DAG to phosphatidic acid (PA) and PA is the first step for the resynthesis of PIP2 from DAG thus both mutants might be expected to have lowered PA levels and defective resynthesis of PIP2. However, a DAG analog (PMA), which can be neither phosphorylated by DGK-1 nor be used to resynthesize PIP2, caused hypersensitivity to VPA. Thus, VPA appears to mediate at least some of it's effects by altering levels of DAG.

BACK

# Identification of Novel Genes Mediating Pathfinding of the AVG Pioneer Axon and its Followers

**Thomas Unsoeld**[1,2], Irene Wacker[2], Harald Hutter[1,2]
[1]Simon Fraser University, Burnaby, BC, Canada; [2]Max Planck Institute for Medical Research, Heidelberg, Germany

In order to form a correct neuronal network outgrowing axons face a challenging navigational problem due to the large numbers of neurons involved. The complexity of this problem is reduced by sequential outgrowth. The first single or few early outgrowing axons, the so called 'pioneers' path the way. Later outgrowing axons then frequently follow these pre-made pathways. In *C. elegans* the right ventral cord axon tract is pioneered by the axon of the AVG neuron.

In a screen for axonal guidance defects, using EMS mutagenesis, we isolated two mutants, *ast-4(rh311)* and *ast-6(rh313)*, that cause navigation defects of the AVG pioneer itself and of follower axons. In both mutants AVG axons grow out into the left axon tract or switch from right to left axon tract. The penetrance of these defects is low but significant. Axons of followers like motoneurons and interneurons are affected in various ways and show ventral cord midline crossing defects or disturbances in commissure outgrowth. Therefore *ast-4* and *ast-6* possibly encode signals of a axon guidance system used by AVG as well as other axons. Using SNPs we mapped *ast-4* to the left end and *ast-6* close to the center of chromosome IV. Identification and characterization of these genes will provide further insights to the molecular basis of the AVG pioneer – follower relationship.

In an independent approach to identify novel AVG-expressed genes important for the guidance of follower axons we try to identify *cis*-regulatory elements that mediate expression in AVG. Therefore we are subfragmenting promoters of AVG-expressed genes like *odr-2* and *inx-18* to define the minimal region required for AVG expression. Minimized reporter fragments will be screened for a consensus sequence which will be used subsequently to find putative AVG-expressed genes. After validation of AVG expression interesting candidates e.g. genes encoding putative cell surface receptors will eventually be tested for a potential role in the guidance of AVG follower axons.

BACK

# ITR-1, the *C. elegans* $IP_3$ Receptor, Functions in ASH-Mediated Responses

**Denise S. Walker**, Elizabeth Gregory, Nicholas J.D. Gower and Howard A. Baylis
Department of Zoology, University of Cambridge, Downing Street, Cambridge, CB2 3EJ, U.K

Inositol 1,4,5 trisphosphate receptors ($IP_3Rs$) are ligand-gated $Ca^{2+}$ channels that control $Ca^{2+}$ release from intracellular stores. They are central to a wide range of cellular responses. $IP_3Rs$ in *C. elegans* are encoded by a single gene, *itr-1*, which is widely expressed, and for which a wide range of functions have been identified. To determine the role of $IP_3$ receptors in the nervous system, we have used the neuronal *unc-119* promoter[1] to direct expression of an $IP_3$ sponge[2]. In a parallel approach we have knocked down *itr-1* by expressing an RNA inverted repeat[3] under the control of the *unc-119* promoter. We describe the role of *itr-1* in the aversive response to nose touch, and we present evidence that both *egl-8* (encoding phospholipase C beta) and *plc-3* (encoding phospholipase C gamma) function upstream of *itr-1* in this response. The response to nose touch is mediated by the ASH polymodal sensory neurons, which also mediate avoidance of high osmolarity and volatile and water-soluble repellents[4,5]. We demonstrate that while *itr-1* does not function in the majority of these responses, it does function in avoidance of benzaldehyde. We are currently investigating the cellular basis of these functions, and the role of signalling pathway components that may function upstream.

1. Maduro, M. & Pilgrim, D. (1995) *Genetics* 141, 977-988.
2. Walker *et al.* (2002) *Mol. Biol. Cell* 13, 1329-1337.
3. Tavernarakis *et al*. (2000) *Nat. Genet*. 24, 180-183.
4. Hart *et al.* (1999) *J. Neurosci*. 19, 1952-1958.
5. Hilliard *et al.* (2004) *EMBO J*. 23, 1101-1111.

BACK

# Regulation of *C. elegans* Behaviour by the Metabotropic Glutamate Receptors

Ting Zeng, James Dillon, Neil A. Hopper, **Vincent O'Connor**
School of Biological Sciences, University of Southampton, SO16 7PX, UK

Glutamate is a major neurotransmitter in the mammalian brain, signalling via ionotropic glutamate receptors, which act as ion channels, and metabotropic glutamate receptors (mGluRs), which couple to G proteins. Glutamate was first implicated as a neurotransmitter in *C. elegans* through the discovery of glutamate gated chloride channels. More recently, *glr-1* and *nmr-1*, which encode AMPA and NMDA class ionotropic glutamate channels respectively in *C. elegans*, have been shown to regulate *C. elegans* behaviours such as the forward versus backing locomotory behaviour. Three mGluR-like genes [*mgl-1, mgl-2* and *Y4C6A.2a* (referred as *mgl-3*)] have been identified in *C. elegans*. Our aim is to understand the role of *mgl* receptors in regulating *C. elegans* behaviour and we sought to test whether they are involved in behaviours known to be dependent upon glutamatergic signalling.

We have obtained deletion mutants of each of the three *mgl* genes from the knock-out consortium. All three backcrossed mutant strains appear superficially wildtype (N2) and display apparently normal locomotion on agar and thrashing behaviour in liquid. However, *mgl-1(tm1811)* shows a defect in backing and moves forward for longer than the wild type when placed on unseeded NGM plates. Mutants deficient in ionotropic glutamate receptors, which back more, are known to be deficient in their ability to move towards a point food source. We hypothesized that the decreased frequency of backing in *mgl-1* mutants might impact on this behaviour. However, we were surprised to note that both *mgl-1(tm1811)* and *mgl-3(tm1766)* behaved normally in this foraging assay. In contrast the *mgl-2* mutants, which move normally on unseeded plates, were significantly retarded in their ability to locate and/or move towards a point food source. Further experiments in which the backward and forward movement was assayed in individual N2 and mutant strains placed on plates with or without a point food source helped delineate sub-behavior underlying this deficiency. As expected we observed that N2 and *mgl-3(tm1766)* worms indeed backed less as they directed themselves towards the food. This change in backing behaviour was apparent even when the worms were some distance (>5cm) away consistent with worms readily detecting food. *mgl-1(tm1811)* back infrequently even in the absence of food and the presence of food had little effect on this rate. Finally we found that *mgl-2(tm355)* worms, which backed at the same frequency as N2 worms in the absence of food, failed to alter this behavior in the presence of food. This result implies that *mgl-2(tm355)* worms are deficient in their ability to sense food or in their ability to integrate the detection of food to modify locomotory behaviour. We are currently using modality specific assays to understand at what level the *mgl-2(tm355)* phenotype might occur, as the preliminary expression patterns of *mgl-2* are consistent with both hypotheses. Taken together our analysis highlights distinct neuromodulatory functions of *mgl-1* and *mgl-2* and provides a platform to investigate the molecular basis of the circuits and behaviours that utilize glutamatergic transmission in the worm.

BACK

***P-077***

# Lysosomal Biogenesis and Function is Critical for Necrotic Cell Death in *C. elegans*

**Marta Artal-Sanz,** Chrysanthi Samara, Popi Syntichaki and Nektarios Tavernarakis
Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology Hellas, Heraklion, Crete, Greece

Necrotic cell death is defined by distinctive morphological characteristics, displayed by dying cells. The cellular events that transpire during necrosis to generate these necrotic traits are poorly understood. Recent studies in our lab show that cytoplasmic acidification develops during necrosis and is required for cell death in *C. elegans*. However the origin of cytoplasmic acidification remains elusive.

We are investigating the role of lysosomes in necrotic cell death. We observed that alkalization of endosomal and lysosomal compartments ameliorates necrotic cell death triggered by diverse stimuli. In addition, mutations in genes that result in altered lysosomal biogenesis and function markedly affect neuronal necrosis. We used a genetically encoded fluorescent marker to follow lysosome fate during neurodegeneration *in vivo*. Strikingly, we find that lysosomes fuse and localize exclusively around a swollen nucleus. In advanced stages of cell death, the nucleus condenses and migrates towards the periphery of the cell, while GFP-labeled lysosomal membranes fade, indicating lysosomal rupture. Our findings demonstrate a prominent role for lysosomes in cellular destruction during necrotic cell death that is likely conserved in metazoans.

BACK

# Identification of Normal and Pathological Functions of Muscle Specific Proteins with RNA Interference in *C. elegans*

**Chambonnier L.** & Segalat L.
CGMC, CNRS-UMR 5534, Université Lyon 1, France

During the evolution, nematodes and mammals diverged but nematodes muscles preserved many structural and molecular features similar to mammal muscles. Therefore *C. elegans* is an excellent model to study muscle function.

Since 2005, our laboratory is part of MYORES European research network. This project, financed for 5 years by the European Commission, focuses on basic issues of muscle physiology and eventually the development of therapies for muscle pathologies. MYORES aggregates 6 technical platforms, which utilize different model organisms (Nematodes, Drosophila, Zebra fish, chick and mouse) to accelerate studies on normal and aberrant muscle development. All data about muscular development assembled by MYORES will be integrated into the MYORES database: Myobase.

Our laboratory is in charge of the *C. elegans* RNAi platform. We use RNA interference in *C. elegans* to study normal and pathological functions of muscle specific proteins. The purpose of this platform within MYORES is to give access to *C. elegans* to researchers familiar with other models.

In collaborative projects, we are currently doing gene inactivation experiments on *MyoD*, *Pax-3* and *Six* genes, which are important developmental switches for the development of vertebrate muscles. We will present data obtained in this work.

BACK

***P-079***

# A Partial Loss of Function Allele of *klp-19* Causes Intestinal Nuclear Division Defects and Sterility

**Cundall M**, Clucas C, Johnstone IL
Division of Molecular Genetics, Institute of Biomedical and Life Sciences, University of Glasgow, UK

Chromosomes must be replicated and segregated accurately to ensure the successful completion of mitosis. During embryonic development of the C. elegans E cell lineage 20 daughter cells are derived from a single progenitor cell to form the intestine. Post-embryonically there are no further cell divisions in the intestine, although many adult intestinal cells are bi-nucleate as a result of nuclear division without cytokinesis that occurs at the end of the L1 larval stage.

ij51 is a recessive allele that was isolated from a forward genetic screen for mutants with an altered E-lineage (Clucas and Johnstone, 1999). The L1 nuclear division fails in a variable number of intestinal cells in ij51 homozygotes, causing abnormal elongated mono-nuclei in normally bi-nucleate cells. ij51 homozygotes are sterile, producing few embryos that arrest before morphogenesis.

We have identified a missense mutation in *klp-19*, a member of the kinesin-4 family of chromokinesins, in ij51 mutants. KLP-19 is a plus-end directed microtubule motor that localizes to chromatin between kinetochores and generates antipoleward forces on chromosomes during meiosis, preventing merotelic chromosome attachments (Powers *et al*, 2004). We suggest that ij51 is a partial loss of function allele, as homozygous animals are viable unlike another allele, bn126, which causes homozygous animals to arrest at the L1 stage (Powers *et al*, 2004). Data will be presented on the further characterization of *klp-19*(ij51).

Clucas C, Johnstone IL. (1999)
GFP transgene expression pattern based mutant screens to identify genes involved in endoderm development in Caenorhabditis elegans. *International C. elegans Meeting* 241

Powers J, Rose DJ, Saunders A, Dunkelbarger S, Strome S, Saxton WM. (2004)
Loss of KLP-19 polar ejection force causes misorientation and missegregation of holocentric chromosomes. *J Cell Biol*. 166(7):991-1001.

BACK

***P-080***

# The Rap-1 and Ral-1/Sec-5/Exo-84 Pathway Synergize in Hypodermal Cell Division and Migration during Embryogenesis

**Ester W. Frische**, Wendy Pellis-van Berkel, Johannes L. Bos and Fried J.T. Zwartkruis
Department of Physiological Chemistry and Center for Biomedical Genetics, Universiteitsweg 100, 3584 CG Utrecht, the Netherlands

The small GTPase Rap1 has been shown to be involved in processes requiring polarized protein transport like integrin mediated adhesion and targeting of E-cadherin to adherens junctions. Deletion of Rap1 in the fly is lethal due to defects in cell migration, but *rap-1* null worms only show a mild phenotype. Feeding RNAi of another small GTPase, RAL-1, results in embryonic lethality. RAL-1 also regulates processes requiring polarized protein transport of which filopodia formation and exocyst mediated transport are examples. A *C. elegans* genome wide RNAi screen was performed based on the observed synthetic lethality of RAL-1 knockdown in a *rap*-1$^{-/-}$ strain. >From this screen, the known Ral effectors *exo-84* and *sec-5* were found to cause synthetic lethality, whereas the others did not show an effect.

RNAi phenotypes for *ral-1*, *sec-5* and *exo-84* in the *rap-1* nulls becomes progressively more severe: the first progeny hatched normally, but later abnormal embryos appeared and finally egglay completely stopped. To analyze the phenotypes, we visualized the adherens junctions of hypodermal and intestinal cells using an integrated *dlg-1*::GFP transgene. Two classes of phenotypes were observed. Class I embryos are completely surrounded by hypodermal cells, but the pattern of hypodermal adherens junctions is clearly abnormal, suggesting cell division defects. In contrast, intestinal cells have a normal appearance, In class II embryos, hypodermal cells are even more disorganized and have a low DLG::GFP staining, suggesting migration defects. In these embryos intestinal cells are present but mislocalized. In all cases AJM-1 staining colocalized with DLG::GFP. To study individual cells in the hypodermis, we made use of the seam cell marker SCM::GFP. As expected on the basis of DLG-1::GFP staining, it was found that in a fraction of Class I embryos the number and location of this type of hypodermal cell was abnormal. In more affected embryos a complete loss of SCM::GFP staining was found.

From the above results it is clear that that loss of Rap-1 sensitizes worms to decreased signaling via Ral, resulting in cell division and migration defects of hypodermal cells. The exocyst complex targets vesicles to sites of cell-cell contact and is required for transport of proteins to the basolateral membrane. Although loss of RAP-1 does not lead to overt defects in cell-cell contacts, these contacts may have lost the capacity to efficiently serve as docking landmarks for the exocyst complex.

BACK

# Identification of O-linked *N*-acetylglucosamine Modified Proteins in *Caenorhabditis elegans*

Julia Grabitzki[1], Michael Ahrend[2], Brigitte Schmitz[2], Rudolf Geyer[1] and **Günter Lochnit**[1]

[1]Justus-Liebig University Giessen, Medical Faculty, Institute of Biochemistry, Friedrichstrasse 24, D-35392 Giessen, Germany; [2]University of Bonn, Institute of Animal Sciences, Katzenburgweg 9a,D-53115 Bonn, Germany

The posttranslational modification *N*-acetylglucosamine O-glycosidically linked (O-GlcNAc) to serine and threonine residues of proteins has been shown to be ubiquitous amongst eukaryotic proteins of the nucleus, cytoskeleton, cytoplasm, and has also been detected on cytosolic tails of membrane proteins [1].

O-GlcNAcylated proteins can form reversible multimeric complexes with other polypeptides or structures. The modification is often accompanied by phosphorylation/ dephosphorylation. O-GlcNAc can act either simultaneously or in a reciprocal fashion with phosphorylation. According to the "Yin-Yang" hypothesis, the phosphorylation/ dephosphorylation regulates O-GlcNAc-modified protein function (z.B. signal transduction and protein-protein interaction) in concert with phosphorylation [2-4]. The addition of O-GlcNAc to and the removal from the protein backbone is dynamic with rapid cycling in response to cellular signals or cellular stages.

Despite the fact, that *Caenorhabiditis elegans* is the best studied model organism, there have been no studies on O-GlcNAcylation in this organism so far. Therefore, to elucidate the role of O-GlcNAcylation, we investigated the proteome of a *C. elegans* mixed-stage population by two-dimensional gelelectrophoresis and subsequent western-blotting with the O-GlcNAc-specific antibody CTD 110.6 for the occurrence of this modification and identified the modified proteins by mass-spectrometry. We detected and identify several O-GlcNAc-modified proteins in *C. elegans*. Most of the identified proteins are involved in metabolic pathways. The prediction of the cellular localisation of the identified proteins revealed a predominant cytosolic occurrence of the O-GlcNAc modification.

References:

[1] Rex-Mathes, M., J. Koch, Werner, S., Griffith, L. S and B. Schmitz. 2002. Methods Mol Biol **194**: 73-87.
[2] Zachara, N.E. and G.W. Hart, Chem Rev, 2002. **102**(2): p.431-8.
[3] Griffith, L. S. and B. Schmitz. 1999. Eur J Biochem **262**(3): 824-31.
[4] Wells, L. and G. W. Hart. 2003. FEBS Lett 546(1): 154-8.

BACK

# Glycosidases of *Caenorhabditis elegans* involved in N-glycan processing

**Martin Gutternigg**, Dorothea Lubich, Matthias Hackl, Katharina Paschinger, Ute Stemmer, Verena Jantsch[1], Günter Lochnit[2], Ramona Ranftl, Petra Geier and Iain B. H. Wilson

Department für Chemie, Universität für Bodenkultur Wien, A-1190 Austria; [1]Vienna Biocenter II, Universität Wien, A-1030 Austria; [2]Institut für Biochemie, Justus-Liebig Universität Gießen, D-35292, Germany

Recent data indicates that in addition to the Golgi α-mannosidases, the model nematode *Caenorhabditis elegans* also possesses, like insects, an N-acetylhexosaminidase activity putatively involved in N-glycan processing in the Golgi. The presence of such an activity is invoked, not just on the basis of the detected enzyme activity, but also to explain the absence of terminal *N*-acetylglucosamine residues on structures which require the prior action of *N*-acetylglucosaminyltransferase I during their biosynthesis. In order to understand the genetic basis for these activities, we have cloned cDNAs encoding members of both glycohydrolase families 20 and 38 from the worm. The encoded glycosidases were expressed in the yeast *Pichia pastoris* as soluble forms lacking putative cytoplasmic and transmembrane domains. Four glycohydrolase family 20 members were shown to cleave *p*-nitrophenyl-β-*N*-acetylglucosaminide and/or *p*-nitrophenyl-β-*N*-acetylgalactosaminide, but showed contrasting specificities with regard to N-glycan substrates. On the other hand, one glycohydrolase family 38 member was shown to be active using *p*-nitrophenyl-α-mannoside as a substrate and, in addition, had mannosidase II activity. Analysis of the glycans of the relevant mutant showed large-scale changes in the N-glycosylation spectrum. These, therefore, are the first data on the activity of *Caenorhabditis* glycosidases towards N-glycan substrates and should aid the further elucidation of N-glycan processing in this organism.

BACK

# Identification of Intermediate Filament Organizers in *Caenorhabditis elegans*

**Katrin Hüsken** and Rudolf E. Leube
Department of Anatomy and Cell Biology, Johannes Gutenberg-University, Mainz

Besides actin filaments and microtubules, intermediate filaments (IFs) constitute the third fibrous component of the cytoskeleton. Functionally, IFs play a role in maintaining mechanical integrity of cells. It is believed that their phosphorylation state is essential for correct assembly and network maintenance. Alterations of IF assembly are pathological features of several diseases (e.g. epidermolysis bullosa simplex, amyotrophic lateral sclerosis).

Compared to the 65 genes coding for human IFs, the cytoplasmic IF cytoskeleton in *C. elegans* consists of only 16 differentially expressed polypeptides, several of which fulfil essential functions. *C. elegans* IFs are expressed mainly in epithelial organs. They occur as three dimensional networks in the uterus, as polarized networks in the terminal web of intestinal cells or as dense bundles in the marginal cells of the pharynx.

To examine molecular mechanisms that are responsible for these different assembly types we designed an optical screen. To this end we established transgenic strains with distinct and easily discernible fluorescence patterns. They are being subjected to a genome wide RNAi screen to identify gene products altering these patterns. We will further assess the consequence of altered IF organization on cell functions in the nematode.

BACK

# The Atypical Calpains of *C. elegans*

**Peter I. Joyce** and Patricia E. Kuwabara
Department of Biochemistry, School of Medical Sciences, University of Bristol, University Walk, Bristol, BS8 1TD, United Kingdom

Regulated proteolysis of receptors, cytoskeletal proteins and transcription factors is an important process that modulates cell growth and development. A family of calcium-regulated thiol proteases, known as calpains, has been shown to perform such processes in mammals, and other eukaryotes. Loss of function mutations in calpains are linked to certain pathological conditions including limb-girdle muscular dystrophy 2A (LGMD2A); whereas, gain of function mutations are associated with the formation of cataracts.

We are investigating the biochemical and functional role of atypical calpains in *C. elegans*. We have identified seven atypical calpains and eight calpain-like sequences within the *C. elegans* genome. To gain an understanding of their possible roles in *C. elegans* development we have created calpain promoter transcriptional expression constructs for the atypical calpains 1 to 7 using monomeric RFP. The transcriptional expression patterns were analysed by co-localisation with the following tissue specific GFP markers: *unc-119*::GFP (all neurons), *unc-47*::GFP (GABAergic neurons within the ventral nerve cord), *myo-3*::GFP (body wall muscle), *exc*::GFP (excretory cell) and seam::GFP (seam cells). Five out of the seven atypical calpains show promoter transcriptional expression activity; *clp-1, clp-2, clp-4, tra-3* and *clp*-7. Two of the atypical calpain promoter constructs fail to show detectable transcriptional expression activity; *clp-3* (lacks all catalytic residues) and *clp-6* (possible pseudogene). *clp-1* shows the highest expression throughout the animal, specifically in neurons and body wall muscle.

In parallel with expression studies we have taken a global approach into studying calpain function using RNAi, calpain mutant analysis and ectopic over-expression. Preliminary studies have revealed no obvious effects. Because calpains require calcium for proteolytic activity, we are investigating ways in which calcium levels can be perturbed within *C. elegans*, as a way to activate calpains ectopically. We are also undertaking biochemical studies to characterise the proteolytic properties of the atypical calpains.

BACK

# Identification of the Protein Kinase VRK-1 as an Essential Regulator of Nuclear Envelope Formation and BAF Phosphorylation

**Elke Klerkx**[1], Mátyás Gorjánácz[2], Carmen López-Iglesias[3], Iain W. Mattaj[2], Peter Askjaer[1]
[1]Institute of Biomedical Research IRB-PCB, Barcelona, Spain, [2]EMBL, Heidelberg, Germany, [3]University of Barcelona, Barcelona, Spain

The nuclear envelope (NE) consists of an inner and outer nuclear membrane and separates the genome from the cytoplasm. Spanning both membranes, nuclear pore complexes comprised of multiple copies of approximately 30 different nucleoporins mediate transport between the nucleus and the cytoplasm. In many cellular processes, protein kinases play essential regulatory roles by specific phosphorylation of substrates. Vaccinia Related Kinase-1 (VRK-1) is a highly conserved protein kinase in metazoa. Our aim for a better understanding of NE dynamics has shown an important function of *C. elegans* VRK-1 during mitosis. We found that VRK-1 is a highly dynamic protein that localises both to the NE and to chromatin in a cell-cyle dependent manner. Downregulation of VRK-1 expression by RNAi in *C. elegans* embryos results in a defective mitotic division with undefined transmission of chromatin and impaired NE formation. Our data show a failure of nucleoporins Nup35, Nup96 and Nup107 to localise to the nuclear rim, and severe mispositioning of integral nuclear membrane proteins MAN1 and emerin upon VRK-1 downregulation. Currently, we are analysing the consequences of VRK-1 depletion on nuclear membrane structure by transmission electron microscopy. We identified BAF protein as a substrate for VRK-1 and found that depletion of VRK-1 causes an abnormal association of BAF with chromatin. We propose that phosphorylation of BAF by VRK-1 plays a crucial role in the association of BAF with chromatin and nuclear membrane proteins during NE formation.

BACK

# Regulation of Cell Proliferation versus Differentiation in *C. elegans*

**Jerome Korzelius[1],** Mike Boxem[2], Julian Ceron[2], Vincent Portegijs[1], Sigmarlis Christiaan[1] and Sander van den Heuvel[1,2]

[1]Dept. of Developmental Biology, Padualaan 8 3584CH Utrecht University, The Netherlands; [2]MGH Cancer Center, Charlestown, MA 02129 U.S.A.

The relation between cell proliferation and differentiation is one of the most fundamental questions in developmental biology and cancer research. We use *C. elegans* as a model system to study the balance between proliferation and differentiation in the context of animal development. Previous work has demonstrated that evolutionarily conserved mechanisms control cell-cycle progression and differentiation processes in *C. elegans*. Our lab has shown that simultaneous inactivation of the $G_1$/S regulators *lin-35* Rb and *cki-1* Cip/Kip causes substantial extra division in multiple cell lineages (Boxem and van den Heuvel 2001). However, many lineages that are completed during embryogenesis are not affected by double inactivation of *lin-35* and *cki-1*, and the differentiated cells formed in such mutants generally do not re-enter the cell cycle (our unpublished data). This suggests that additional mechanisms control cell-cycle exit and prevent differentiating cells from continuing proliferation. Our goal is to define such mechanisms and identify the most critical players that limit proliferation of differentiated cells.

In this project, we target the intestine and body wall muscles as cell types susceptible and resistant, respectively, to cell-cycle deregulation. These lineages use well-characterized differentiation cues (Maduro and Rothman 2002, Krause 1995) and are easily accessible to microscopic observation, expression of reporter genes and RNAi. Our approach is to interfere with cell-cycle and differentiation control in both lineages through a variety of methods, combing overexpression of transgenes, RNAi and mutant alleles. We have constructed lineage-specific expression constructs for CYD-1 and CDK-4 and are currently testing their effect in both lineages. Interestingly, we observed S phase induction in developmentally arrested L1 larvae upon CYD-1/CDK-4 expression in the intestine, but not in the body wall muscle. We will present our recent findings at the meeting. Ultimately, this work aims to identify molecular mechanisms that can be targeted to shift the balance between proliferation and differentiation, both to promote differentiation of hyper-proliferating cells and promote regeneration of differentiated cell types.

BACK

# Modulation of Ubiquitin-Dependent Pathways by Ataxin-3/ATX-3

**Kirsten Kuhlbrodt**[1], Roja Barikbin[1], Christian Eckmann[2] and Thorsten Hoppe[1]
[1]Centre for Molecular Neurobiology (ZMNH), University of Hamburg, Falkenried 94, 20251 Hamburg, Germany; [2]Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstrasse 108, 01307 Dresden, Germany

The ubiquitin-proteasome system is responsible for selective proteolysis of intracellular proteins in eukaryotic cells. In this system, protein substrates are marked for degradation by attachment of multiubiquitin chains. The process of multiubiquitylation requires a cascade of enzymes, additional proteins ensure correct targeting to the 26S proteasome. As has been shown for a variety of genes of the ubiquitin pathway, also the disease pathology of spinocerebellar ataxia type 3/Machado-Joseph Disease (SCA3/MJD) is attributed with alterations in ubiquitin-pathway functions. This disease is one of at least nine neurodegenerative polyQ (polyglutamine) diseases including Huntington´s disease caused by the pathological expansion of a polyQ region in the disease protein, which leads to a misfolding process and subsequent accumulation of insoluble protein aggregates. One protein implicated in SCA3/MJD is Ataxin-3/ATX-3, which comprises two distinct catalytic activities functionally related to the ubiquitin system: a deubiquitinating Josephin domain at the N-terminus and C-terminal UIM domains which mediate binding to ubiquitin.

Currently, we are interested in the molecular function of the *C. elegans* homolog of mammalian Ataxin-3. We were able to show the de-ubiquitylation activity with recombinantly expressed protein *in vitro* and are now investigating the binding properties of Ataxin-3/ATX-3 to ubiquitin or to ubiquitylated proteins. With the yeast two hybrid assay we identified new interactors of Ataxin-3/ATX-3. One of the identified interactors shows an overlapping expression pattern with Ataxin-3/ATX-3 in the spermatheca. Moreover, our genetic analysis with two *atx-3* loss of function mutants revealed a genetic interaction: brood size defects of a mutant allele for this interactor is suppressed in the *atx-3* mutant background. Our current model suggests, that Ataxin-3/ATX-3 in conjunction with additional components of the ubiquitin-proteasome system modulates ubiquitin-dependent pathways which might play a role throughout sex-determination.

BACK

***P-088***

# Establishment of Apical Junctions in *C. elegans*: An Approach by RNAi Screening to Identify the Proteins Interacting with LET-413

**Christophe Lefebvre**, Julien Mouysset, Renaud Legouis
CGM, CNRS, Avenue de la terrasse, 91190 Gif-sur-Yvette

Epithelial cells are polarized, with apical and basal compartments demarcated by tight and adherens junctions. Specialized subapical junctions play a critical role in maintaining epithelial cell polarity and tissue integrity and provide a platform for intracellular signaling.

We have identified, LET-413, a member of the protein's family called LAP (PDZ and LRR domains), which localizes to the basolateral membrane and maintain the polarity of epithelial cells in *C.elegans*. More precisely, LET-413 is involved in the assembly of the *C.elegans* apical junction (CeAJ) and the correct positioning of CeAJ components at discrete subapical position during epithelialization. Indeed, in *let-413* mutants, the lost of LET-413 leads to an embryonic lethality, the formation of discontinuous CeAJ and strong adhesion and polarity defects.

We previously carried out a structure/function study of LET-413 and showed that the LRR domain is essential for the localization to the basolateral membrane and is necessary for LET-413 function during embryogenesis. LRR domains are involved in protein-protein interaction, but no interaction is known for LRR of LAP proteins. We hypothesize that LET-413 is localized to the membrane through an interaction of it's LRR with another protein which are not yet identified.

To better understand the role of LET-413 in the formation of CeAJ, we decided to look for physiological partners of LET-413 protein in *C.elegans*. We initiated a genomic RNAi screen based on the fact that the delocalization of LET-413 from basolateral membrane results in an embryonic lethality. LET-413::GFP transgenic worms were fed with RNAi bacteria from Julie

Ahringer's library and checked for embryonic lethality and delocalization of LET-413::GFP. We would like to analyze the totality of the *C.elegans* genes leading to lethality, which represent around 920 genes. The screen is in process and chromosome I and II and 66% of chromosomes III have been screened. The preliminary data allowed us to identify genes candidates, which inactivation results in the delocalization of LET-413. The specificity of the phenotype is checked using various markers of epithelial polarity. Progress in the screen and last results about the potential partners of LET-413 will be presented.

This approach should be helpful to understand how an epithelial cell acquires its morphological and physiological characteristics.

BACK

# Characterization of the *C. elegans* Homologue of the Adaptor Protein EPSIN

**M.G. Malabarba**[1], E. Gerbino[1], A. Croce[1], H. Kloess[1], A. E. Salcini[2], G. Cassata[1] and P. P. di Fiore[1]

[1]IFOM, Istituto FIRC di Oncologia Molecolare, Via Adamello 16, 20134 Milan, Italy; [2]BRIC, Biotech Research & Innovation Centre, Fruebjergvej 3, Box 1, 2100 Copenhagen, DK

Epsin belongs to a class of endocytic adaptors proteins whose function is not yet completely elucidated. *C. elegans* has proved to be a good model system to study endocytosis and it has the great advantage of studying the role of a protein in the contest of a whole organism. EPN-1 is the only *C. elegans* homologue of the mammalian family of Epsin and it retains the same modular organization. From the N-terminal, it displays the ENTH domain, two UIM domains, several AP2 binding motives (DPW) as well as two clathrin binding consensus, and at the C-terminal portion four EH binding motives (NPF).

As initial approach to study the function of Epsin in the nematode, we downregulated the expression of the gene by RNA*i*. Interfered worms displayed retardation in growth, uncoordinated movements and egg laying defects. Endogenous EPN-1 protein is ubiquitously expressed with higher level of expression in the pharynx, vulva epithelial cells, and some unidentified neurons, judged both by the generation of transgenic worms, expressing *epn-1p*::EPN-1::GFP fused protein and by immunofluorescence using specific antisera. To better analyze EPN-1 function, we generated a deletion mutant by EMS mutagenesis: *epn-1(ng5)*. *epn-1(ng5)* display a lethal phenotype occurring in L1/L2 stage. The larvae accumulate vacuoles and have a general sick aspect until they die. The phenotype is recessive. The study of the phenotype associated to loss of *epn-1* gene function was approached by two main lines of experiments: the investigation of a possible cell specific requirement of the protein and a structure–function analysis of the different EPN-1 domains. At present, we established that 1) *unc-119p::EPN-1::GFP*, which drives the expression of the protein specifically in neurons, is the only array able to rescue larval lethality (besides arrays containing the endogenous promoter), while, when EPN-1 is expressed only in the pharynx or in the intestine the lethality persist; 2) the ENTH-UIM domains are the minimal region of the EPN-1 protein capable to restore viability. Rescued animals, although viable, show peculiar phenotypes that may contribute to identify the function(s) of EPN-1 in *C. elegans*. Preliminary results indicating a role for EPN-1 at the neuromuscular junctions and in neurotransmission will be presented and discussed. In conclusion, we determined that *epn-1* is an essential gene in *C. elegans*, as in yeast and *D. melanogaster*, moreover, we generated a valuable set of reagents, which will allow us to study its function in the context of the different molecular and genetic pathways in which the protein acts.

BACK

# Membrane Traffic and Development: A Genome-Wide RNAi Screen looking for *unc-101* Enhancers

**Grégoire Michaux**
MRC LMCB – University College London – UK

Membrane traffic is a central problem in cell biology: how does the cell manage to control the spatial and temporal addressing of proteins? This is crucial not only for cell survival, but also for the correct development of an organism. For instance, the intracellular trafficking of Notch is regulated by Rab5 and Vps25, two proteins implicated in endosome formation. To better understand the interconnections between membrane traffic and development, I choose to look at the function of the adaptor complex AP-1.

AP-1 is an adaptor which recruits clathrin during post-Golgi vesicle formation. It has been identified as important for secretion and endosome trafficking in various organisms from yeast to human cells, but it is also implicated in development as shown by the embryonic lethality induced in mice and worms when AP-1 is mutated.

In *C. elegans*, AP-1 total loss of function leads to an embryonic arrest at the 2-fold stage. However, there are two copies of the μ subunit, UNC-101 and APM-1, and the total loss of function of only one of these subunits leads to a partial AP-1 loss of function. In particular, the null allele *unc-101(sy108)* induces only a partial larval lethality; but a synthetic embryonic lethality occurs when RNAi against *apm-1* is done is an *unc-101(null)* background (Shim *et al*, MBC, 11, 2743).

Based on these results, I decided to look for RNAi inducing a synthetic lethality in the *unc-101(sy108)* background by conducting a genome-wide RNAi screen. Four chromosomes (I-IV) have been screened and over 50 genes displayed a genetic interaction with *unc-101*, as defined by an enhancer phenotype. So far, about 15% of the genetic interactors are known to be directly implicated in membrane traffic, including a small GTPase of the Rab family, a GEF (guanine exchange factor) and several proteins implicated in endosome trafficking. An other set of candidates (10%) are linked to known signalling pathways, including the Notch and Wnt/Wingless pathways. I am currently screening the remaining two chromosomes, and characterising the AP-1 loss of function phenotype. Progress will be reported at the meeting.

BACK

***P-091***

# Functional Knocking Out of Glycome-Related Genes in *Caenorhabditis elegans*

**Souhei Mizuguchi[1,2],** Kazuko H. Nomura[1,2], Katsufumi Dejima[1,2], Takayuki Nagaisi[1,2], Daisuke Murata[1,2], Shohei Mitani[3], Keiko Gengyo-Ando[2,3], Akira Seko[2,6], Katsuko Yamashita[2,6], Tomomi Izumikawa[7], Hiroshi Kitagawa[2,7], Kazuyuki Sugahara[2,7], Yeon-Dae Kwon[8]. Hisashi Narimatsu[8], Kazuya Nomura[1,2]

[1]Department of Biological Sciences, Faculty of Science, Kyushu University, Fukuoka, Japan; [2]CREST, JST, Saitama, Japan; [3]Department of Physiology, Tokyo Women's Medical University, Japan; [4]National Institute of Health Science, Tokyo, Japan; [5]Department of Pharmacology and Toxicology, Kyorin University, School of Medicine, Tokyo, Japan; [6]Department of Biochemistry, Sasaki Institute, Tokyo, Japan; [7]Department of Biochemistry, Kobe Pharmaceutical University, Kobe, Japan; [8]National Institute of Advanced Industrial Science and Technology, Japan

To understand the roles of carbohydrates in development and morphogenesis of multicellular organisms, we are performing systematic knocking out of genes involved in glycome formation. Many orthologs and homologs of glycome-related genes can be found in the *C. elegans* genome. By using sophisticated bioinformatics techniques we found 145 predicted orthologs of human glycosyltransferase (GT) genes in the nematode. We depleted 108 of these GTs involved in glycoprotein and/or glycolipid synthesis using RNAi or TMP/UV deletion mutagenesis. In the present paper, we describe various important functions of carbohydrate chains in the nematode, and report several examples of our functional analysis of glycome-related genes. RNAi of at least 10% of these GT genes resulted in severe phenotypes including growth defects, abnormal morphogenesis and lethality. Special emphasis is placed on functions of proteoglycan-related proteins including chondroitin synthase, heparan sulfate synthase and glycosaminoglycan related factors. Inhibition of chondroitin synthase activity resulted in abnormal cytokinesis in early embryos, and inhibition of heparan sulfate synthesizing enzymes resulted in embryonic lethal and egg laying defects with abnormal neuronal wiring. RNAi of several genes involved in carbohydrate modification and glycolipid synthesis also resulted in severe phenotypes including embryonic lethality, abnormal morphogenesis and growth defects. These findings shows clearly that carbohydrates are playing various critical roles in multicellular organisms. By using the nematode as a model system, we would like to crack the glycocodes hidden in living organisms and would like to understand the general roles of carbohydrates in multicellular organisms including humans.

BACK

# Characterization of *ced-8* and *ced-8* Interacting Genes

**James K. Phelan**[1], Kelvin Wong[1], Sergio M. Pinto[1,2], Laurent Falquet[3], Hans H. Jung[4] and Michael O. Hengartner[1]
[1]Institute of Molecular Biology, and [4]Department of Neurology, University of Zurich; [2]Graduate Program in Areas of Basic and Applied Biology (GABBA), University of Porto, Porto, Portugal, and [3]Swiss Institute of Bioinformatics, Lausanne, Switzerland;
e-mail: james.phelan@molbio.unizh.ch

Mutations in *ced-8* were first identified in a screen for genes required for the timely engulfment of developmental cell corpses (Ellis, Jacobson, & Horvitz, 1991). Subsequent work clarified that the persistent corpses were the result of a delay in their generation, rather than a failure of engulfment (Stanfield & Horvitz, 2000). With ten predicted hydrophobic regions, CED-8 is anticipated to be a transmembrane protein and has been proposed to be a transporter. The function of *ced-8*, however, remains undetermined. Our goals are to clarify the role of *ced-8* in cell corpse generation and clearance, and to utilize the mutation as a tool to identify additional genes with which *ced-8* interacts. We are in the process of mapping the spatiotemporal expression pattern of *ced-8* during the *C. elegans* lifecycle, and of experimentally determining the membrane topology of CED-8. Potential candidate interacting genes are being tested by double mutant and RNAi analysis. The resulting data will allow educated hypotheses to be formulated as to the function(s) of CED-8. In addition, *ced-8* is now known to be the sole *C. elegans* representative of a gene family with multiple members in mammals. The founding member of the gene family, *XK*, is mutated in McLeod syndrome, a neuroacanthocytosis disease in which a neuromuscular degeneration is found in association with altered red blood cell morphology. We are attempting to rescue the *ced-8* mutant phenotype by expression of representative mammalian cDNAs in order to ascertain which, if any, represent functional orthologs of CED-8.

BACK

# Sec61beta and the Exocyst in *C. elegans*

**Johanna Pispa** and Jussi Jäntti
Institute of Biotechnology, University of Helsinki, Finland

Correct regulation of protein secretion is required for many cellular processes, e.g. for the formation of cellular polarity. Consequently it needs to be tightly linked with other cellular signalling pathways. Very little is known about the molecular mechanisms that regulate this crosstalk. We are examining the interaction between the exocyst and the ER-associated protein translocation machinery in *C. elegans*. The exocyst is an eight-subunit complex that is required during the last stage of secretion. Its role is to tether secretory transport vesicles onto the plasma membrane. Previous work by us and others has shown that a subunit of the protein translocation complex, Sec61beta (or Seb1 in yeast) interacts both genetically and physically with the exocyst (Toikkanen et al, 2003 JBC 278:20946, Lipschutz et al., 2003 JBC 278:20954). In bacteria and yeast Sec61beta is not required for viability but in *Drosophila* it is (Valcárcel et al., 1999 J Cell Sci 112:4389). However, the precise function of Sec61beta is not clearly understood, and nothing is known about the significance of its interaction with the exocyst in multicellular organisms.

Our initial aim is to characterise the *C. elegans* Sec61beta (Y38F2AR.9). We have obtained a deletion mutant from Shohei Mitani, the National Bioresource Project, Japan. Homozygous *Sec61beta* worms are non-viable indicating that in multicellular animals Sec61beta is essential. Sec61beta RNAi by bacterial feeding reduces growth rate during larval development, possibly by defects in secretion of the cuticle. We have also generated worms expressing GFP-reporter constructs using Sec61beta promoter fragments of two different sizes. The longer 1.5kb promoter drives GFP expression mainly in the intestine and the pharynx. Our next goal is to analyse the Sec61beta-exocyst interactions both genetically and biochemically. For this aim we are generating antibodies against Sec61beta, and two subunits of the exocyst, Sec3 (F52E4.7) and Sec15 (C28G1.3).

BACK

***P-094***

# A Proteomic Approach to Decipher LIN-26 Function

**Jolanta Polanowska**, Sophie Bamps, Jean-François Dierick[1] and Jérôme Reboul
INSERM UMR599; Centre de Recherche en Cancérologie de Marseille; Institut Paoli Calmettes; Université de la Méditerranée; 27 Bd Lei Roure 13009 Marseille- France ; [1]Biovallée-Protéomics Rue des Pr. Jeener et Brachet, 12 - 6041 Gosselies - Belgium

The main aim of our studies is to map the molecular network implicated in the establishment of epithelial identity (polarity, adhesion..). We have chosen a set of over 100 proteins described as involved in the establishment of cell polarity or containing domains present in polarity related proteins and subjected them to a stringent yeast two hybrid screening of *C. elegans* cDNA and normalized AD-ORF libraries. The analysis of the obtained network revealed an interesting cluster around LIN-26 nod. It was previously shown that ectopic expression of *lin-26* can reprogram the normal fate of blastomeres to induce the expression of three markers that play differents roles in maintaining epithelial cell polarity (*jam-1*,*dlg-1* and *che-14*) (1). Several observations including the DNA sequence of the gene coding for a putative zinc-finger protein led to the proposition that one of the aspects of *lin-26* function is to act as a transcriptional regulator. The yeast two-hybrid cluster brought the hints for possible *lin-26* interactors. In order to further enhance the confidence of these findings and therefore understand how *lin-26* is involved in epithelial differentiation we have directed ourselves towards a second proteomic approach implicating tandem affinity purification of protein complexes associated with epitope-tagged (HA-6xHis-Myc) LIN-26 from stable transgenic animals. Complexes purification has been carried on the protein extracts covering all developmental stages and all tissues of the animal. Extraction was performed in a way to distinguish the soluble portion of LIN-26 from the one linked to chromatin. Complexes obtained from both fractions were resolved by one and two dimension electrophoresis and analyzed by LC-MS/MS. Combination of yeast two hybrid with TAP approaches let us put to evidence novel interactors of LIN-26,
Progress and preliminary results will be reported.

(1) den Boer *et al.* Development 125, 3213 (1998)

BACK

# Disulphide Bond Formation in Multicellular Organisms

**Panagiotis Psarrakos,** Dr. Peter Klappa
Department of Biosciences, University of Kent, Canterbury, Kent CT2 7NJ

Proteins are vitally important macromolecules that perform diverse roles in all organisms. A common feature of any protein is its requirement for a specific three-dimensional structure for biological function. In many secreted and membrane-associated proteins this three-dimensional structure is stabilised by intra- or intermolecular disulphide bonds, which are usually formed post-translationally in the endoplasmic reticulum (ER). Research currently indicates that disulphide bond formation can occur via multiple parallel pathways where a large number of protein families may play a role in supplying redox equivalents. Furthermore, late-stage isomerization reactions, where disulphide bond formation is commonly linked to conformational changes in protein substrates that possess significant secondary structures, are thought to be catalysed by members of the protein disulphide isomerase (PDI) family.

Several proteins with similarity to PDI have been described in the ER of higher eukaryotes. The precise *in vivo* functions of these proteins remain elusive and the question arises why there are different members of the PDI family present in the same intracellular compartment. One inevitable speculation is that they have overlapping biological functions with their distinct roles only coming into effect under specific conditions, but this has not to date been systematically examined. *Caenorhabditis elegans* contains a full set of proteins with high homology to the mammalian members of the PDI family and to members of other protein families actively involved in disulphide redox reactions and thus, is invaluable as a eukaryotic model system for their biological and biochemical characterisation.

We report here on the RNAi analysis of members of the PDI family and of other proteins involved in disulphide bond formation in a multicellular organism. In addition, biochemical characterization of the major members of the PDI family in *C. elegans*, coupled with analysis of loss-of-function phenotypes (either RNAi-induced or based on genomic knockouts), strongly indicates an overlap in substrate specificities and, potentially, functions.

*I would like to thank the Alexander S. Onassis Public Benefit Foundation for awarding me with a scholarship for the duration of this project.*

BACK

# Investigation of Interactions between Mutant Collagens Using *C. elegans* as a Model Organism

**Brett Roberts, Genevieve Stapleton** and Iain Johnstone
Division of Molecular Genetics, IBLS, University of Glasgow, Glasgow G11 6NU, Scotland

Extracellular matrix (ECM) provides the structural basis of tissues and organs in animals. Collagen proteins form a diverse range of supramolecular components in ECM and mutations in various human collagen genes which result either in a reduction or loss of a collagen species from the ECM, or in some cases insertion of mutant collagen, are responsible for a wide variety of inherited diseases, including osteogenesis imperfecta, Ehlers-Danlos Syndrome and some forms of osteoporosis, osteoarthritis and invertebral disc disease. Excessive collagen synthesis is also detrimental resulting in fibrosis.

In *C. elegans* the cuticle is an ECM comprised primarily of collagens, encoded by a large gene family, which interact to form distinct substructures within the cuticle. Previously we have demonstrated specific requirements for collagen partners during assembly of cuticle collagen sub-structures. We observe distinct differences between the behaviour of certain classes of mutant collagen. The most common class, Gly substitution mutants within the repetitive Gly-X-Y domains cause retention of the mutant collagen in the ER. However null mutations in some cuticle collagen genes result in neither assembly nor ER retention of obligate partner collagens. We are interested in studying the fate of this "partnerless" collagen; various approaches will be presented.

We are also interested in the retention of Gly sub collagen in the ER. We have performed a mutant screen in a *dpy-7*(e88) glycine substitution mutant background to identify modifiers of the *dpy-7*(e88) phenotype. Two classes of mutants have been isolated: those that significantly enhance the severity of the *dpy-7*(e88) phenotype, resulting in shorter, fatter animals; and those that display a distinct Roller modifier phenotype. Our preliminary analyses of these new modifier alleles will be described.

BACK

# Tissue Specific Regulation of Intestinal Proliferation through CDC-25.1

**Alexandra Segref** and Iain L. Johnstone
University of Glasgow, Glasgow, Scotland, UK

The *cdc-25.1(ij48)* hypermorphic allele has been previously identified in our lab in a mutant screen to detect animals with altered numbers of intestinal cells [1]. The *ij48* lesion comprises a single S to F mutation (S46F) in a highly conserved putative N terminal DSG consensus site, which may act as a regulatory binding site for an F-box protein. Consistent with this notion, the CDC-25 protein is supplied maternally to all embryonic cells, but its abundance decreases and is undetectable after the 100 cell stage of embryogenesis [1]. Intriguingly, in *cdc-25.1 ij48* mutants, proliferation of other tissues is unaffected, but removal of CDC-25 by RNAi results in reduced cell divisions in most or all lineages [1, 2]. Thus, there is general requirement for CDC-25 function in all embryonic blastomeres. Until now no obvious difference has been observed for the localisation or abundance of CDC-25 compared to CDC-25 S46F. Deciphering the mechanism that controls the site of the *ij48* lesion will provide significant insights into the tissue specific regulation of the *C. elegans* cell cycle.
We have performed an RNAi screen for candidates that negatively regulate CDC-25 and identified an F-box protein, which mimics the *ij48* intestinal specific cell proliferation phenotype when depleted from the embryo by RNAi.
Knock-down of the F-box protein by RNAi in an *ij48* background does not result in a synergistic effect, implying it may function through S46. In accordance with this result, CDC-25 protein levels are significantly increased in embryonic extracts derived from the *ij48* mutant as compared to wild type and knock-down of the F-box protein does increase CDC-25 but not CDC-25 S46F protein levels.
Surprisingly, the increased abundance of CDC-25 S46 is not restricted to intestinal cells suggesting the intestinal proliferation is more sensitive to CDC-25 protein levels than other cell types. We are currently examining whether the RNAi effect observed is the result of a direct physical interaction between CDC-25 and the F-box molecule.

1. Clucas, C., et al., Oncogenic potential of a C.elegans cdc25 gene is demonstrated by a gain-of-function allele. EMBO J, 2002. **21**(4): p. 665-74.
2. Ashcroft, N.R., et al., RNA-Mediated interference of a cdc25 homolog in Caenorhabditis elegans results in defects in the embryonic cortical membrane, meiosis, and mitosis. Dev Biol, 1999. **206**(1): p. 15-32.

BACK

***P-098***

# The Loss of the Single APC Homolog *apr-1* Causes Hyperplasia in the *C. elegans* Intestine

Robin Schneider, Gisela Helbig and **Olaf Bossinger**
Institut für Genetik, Heinrich-Heine-Universität Düsseldorf, D-40225 Düsseldorf, Germany

In humans APC is a powerful tumour suppressor that is mutated in most cases of sporadic or congenital colorectal cancer. In these forms of cancer, cells lose their ability to control the cell cycle and cell migration along the crypt-villus axis is disturbed. The *C. elegans* intestine is a simple epithelial tube that consists of only 20 cells (E-cells). Depletion of the *C. elegans* APC homolog APR-1 (Hoier et al., 2000) by bacterial RNAi causes hyperplasia of E-cells. In contrast, *apr-1(RNAi)* embryos contain up to 40 E-cells, which in most cases achieve to arrange into a tube-like structure. The use of gut-specific RNAi against APR-1 and the gain-of-function phenotype of the cycline dependent kinase CDC-25.1 (Clucas et al., 2002) suggests that intestinal cell proliferation is under organ-specific control. Preliminary data indicate that *C. elegans* homologs of the vertebrate WNT pathway are also involved in the regulation of endodermal cell proliferation. A detailed analysis of gut-specific hyperplasia phenotypes will be presented and discussed with regard to the involved pathways.

BACK

# In Search of the Lost CAMs

**Gidi Shemer** and Bob Goldstein
Biology Department, University of NC - Chapel Hill, Chapel Hill, NC; USA

From the very first division and throughout embryogenesis cells contact and adhere to each other via cell adhesion molecules (CAMs). Known CAMs are classified into various groups; among those are the Cadherins, the Ig superfamily CAMs and the Selectins.

Although cell adhesion had been investigated in depth in various systems, we are interested in developing *C. elegans* as a unique model system in which cell manipulation strategies can be combined readily with the advantages of an excellent genetic system to study the mechanisms and functions of cell adhesion. In the absence of sufficient knowledge about adhesion during early worm embryogenesis, we started a comprehensive analysis of this critical process. To focus on adhesion between cells without external forces and constraints, we utilized blastomere isolation techniques whereby egg shells are removed from 2-cell embryos and blastomeres are separated and then re-adhered using a mouth pipette. We found that 15 seconds after the cells make first contact, this “kiss-like" contact zone starts to expand until establishing full and stable contact after 3-5 min. We treated isolated cells with EDTA and Trypsin, known inhibitors of adhesion in cell cultures. Surprisingly, such treatment did not abolish adhesion in worm blastomeres. Rather, even upon exposure to high concentrations of EDTA, these cells were still able to re-adhere despite the fact that their development was arrested. Further, more sensitive adhesion assays showed that this adhesion is less stable than normal, implying that more than one mechanism may be involved in this process.

In *C. elegans,* the identity of the molecules that act to adhere blastomeres is still an enigma. Various candidates, based on expression pattern and homology to known CAMs have been shown to be active in late embryo morphogenesis, but showed no detectable functions in adhesion events prior to this stage. We constructed *lad-1/*L1CAM*; hmp-2/*beta-catenin double mutants and tested these animals for adhesion defects. Preliminary results show that blastomere adhesion is intact in these mutants but the gastrulation cleft, normally enclosed by migrating neuroblasts, is not sealed properly, leading to embryonic lethality. We are currently testing whether these defects are due to lack of adhesion between the neuroblasts.

In a quest for components of the early adhesion machinery and in view of possible redundancy between different CAMs, we are currently conducting an RNAi screen, injecting pools of candidate dsRNAs and testing the animals for adhesion defects. Different adhesion assays established in the course of this work will be discussed.

BACK

# The MAGI-1 Protein Functions as an Organizer Protein at the Adherens Junctions

**Attila Stetak**, Erika Fröhli-Hoier and Alex Hajnal
Institute of Zoology, University of Zurich, Winterthurerstrasse 190, 8057 Zürich, Switzerland

The *C. elegans* hermaphrodite vulva is formed by the descendents of three out of six equipotent vulval precursor cells (the VPCs P3.p through P8.p). The gonadal anchor cell produces the LIN3 EGF signal that activates the conserved EGFR/RAS/MAPK signaling pathway in the adjacent VPCs and specifies the primary cell fate in P6.p. The basolateral localization of LET-23 EGFR in the VPCs is essential for the efficient receptor activation and consequently for proper vulval induction. A ternary complex consisting of the PDZ-domain proteins LIN-7, LIN-2 and LIN-10 is required for the localization of the EGFR to the basolateral compartment. Interestingly, PDZ-domains are often found in adaptor proteins that are control the subcellular localization of other proteins such as receptors.

Using a forward genetic approach, we have identified the *magi-1* gene (K01A6.2) as a suppressor of the *let-60(gf)* Multivulva phenotype. MAGI-1 is a multi-PDZ domain protein consisting of an N-terminal guanylate kinase domain with two overlapping WW repeats followed by five PDZ domains. Mammalian MAGI is expressed in neurons and epidermal cells and is localized at the cell junctions where it interacts with several proteins including PTEN and β-Catenin. The *C. elegans magi-1* gene encodes two isoforms transcribed from alternative promoters. Using translational MAGI-1::GFP reporters, we found that the two MAGI-1 isoforms are expressed during embryogenesis, larval development and adulthood in epidermal cells, neurons, muscle cells, the gut and the somatic gonad. However only the isoform encoded by the shorter transcript is expressed in the VPCs. Furthermore, the MAGI-1::GFP fusion protein is localized at the adherens junctions of the epidermal and gut cells. MAGI-1 interacts in GST-pull-down experiments with HMP-2 ß-Catenin via its fifth PDZ domain and with both LET-23 EGFR and LIN-7 via its guanylate kinase or WW domains. Surprisingly, neither a deletion of the fifth PDZ domain in MAGI-1 nor RNAi against components of the adherens (HMR-1, HMP-2, APR-1) or septate junctions (AJM-1, DLG-1) causes a loss of junctional MAGI-1 localization. Moreover, *magi-1* deletion mutants display no obvious developmental phenotypes, but *magi-1(lf)* animals exhibit reduced basolateral LET-23 EGFR staining in the VPCs and enhanced embryonic lethality in combination with *apr-1* RNAi. Thus, MAGI-1 might serve as a junctional organizer protein during embryogenesis and vulval development.

BACK

# The Role of Endocytosis and Intracellular Trafficking in *C. elegans* Neurodegeneration

**Kostoula Troulinaki** and Nektarios Tavernarakis
Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology Hellas, Heraklion, Crete, Greece

There are numerous studies that implicate necrotic cell death in many devastating human pathologies, such as stroke and neurodegenerative diseases. Recent findings demonstrate that under extreme circumstances, normal cellular activities are destabilized and lead the cell to death. The earliest detectable morphological abnormality in a dying cell is the formation of small, tightly wrapped membrane whorls that seem to originate at the plasma membrane. These whorls are then internalized and seem to coalesce into large, electron-dense membranous structures, indicating that endocytosis and intracellular trafficking might contribute to degeneration. Interestingly, disrupted intracellular trafficking has been implicated in several neurodegenerative diseases in humans, as Alzheimer's disease, Huntington's disease and ALS.

We are investigating the possible role of endocytosis and intracellular trafficking in the execution of necrotic cell death inflicted by genetic and environmental insults. To this end, we examine the effects of genetic knockdown of genes involved in key steps of endocytosis on neurodegeneration. Our preliminary findings demonstrate that elimination or dysfunction of specific proteins involved in endocytosis ameliorates neurodegeneration in *C. elegans*. These observations implicate endocytosis and intracellular trafficking in the process of necrosis. We anticipate that further studies in this direction will reveal novel aspects of the cell biology of necrotic cell death.

BACK

# The Function of LIN-5 in Chromosome Segregation and Asymmetric Division

**Monique van der Voet**[1], Marjolein Wildwater[1], Mike Boxem[1,2], Justin Peters[1], Christian Berends[1], Matilde Galli[1], Inge The[1], Adri Thomas[1] and Sander van den Heuvel[1,2]

[1]Department of Developmental Biology, Kruytbuilding, Padualaan 8, Utrecht University, 3584 CH Utrecht, The Netherlands; [2]MGH Cancer Center, Harvard Medical School, Bld 149 13th Street, Charlestown, MA 02129, USA

The position of the spindle apparatus determines the plane of cell division. During asymmetric division, proper positioning of the spindle is needed in the generation of daughter cells that differ in size, cytoplasmic determinants and developmental fate. We have focused our attention on the *C. elegans* gene *lin-5* to identify novel regulatory mechanisms and molecules that control spindle-mediated functions.

Our previous studies demonstrated that *lin-5* is generally required for chromosome segregation and spindle positioning during meiotic and mitotic M phase. *lin-5* encodes a coiled-coil protein that localizes to the cell cortex as well as spindle asters, and recruits the G protein regulators GPR-1/GPR-2 to the same locations. Results from our lab as well as others support that LIN-5 and GPR act in concert with the GOA-1/GPA-16 $G\alpha_{i/o}$ subunits of heterotrimeric G proteins in an evolutionarily conserved pathway for spindle positioning.

Many aspects of this process remain poorly understood. In particular, it is unclear how polarity determinants at the cortex affect LIN-5/GPR/$G\alpha_{i/o}$ function to create asymmetry in the pulling forces that act on the spindle asters. In addition, the downstream targets of the LIN-5/GPR/$G\alpha_{i/o}$ pathway remain unknown. To better understand the molecular mechanisms of these processes, we continue to use a combined genetic and biochemical approach. We have identified novel candidate partners of LIN-5 through immunoprecipitation from embryonic lysates followed by mass spectrometry. In addition, we identified multiple residues of LIN-5 and GPR that are phosphorylated *in vivo*. We are currently testing the *in vivo* relevance of these interactions and modifications and hope to present our results at the meeting.

BACK

# Develoment Specific Collaboration between the E3 Ligases UFD-2 and CHN-1 Regulates the Myosin Assembly Chaperone UNC-45

**Christoph Janiesch**, Johnny Kim and Thorsten Hoppe
Centre for Molecular Neurobiology Hamburg (ZMNH), University of Hamburg, Falkenried 94, 20251 Hamburg, Germany

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

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

*[late coming abstract]*

BACK

# *cis*-acting Factors and Downstream Targets of the T-Box Transcription Factor *mab-9*

**Pete Appleford** and Alison Woollard
Genetics Unit, Department of Biochemistry, University of Oxford, OX1 3QU, UK

The *C. elegans* T-box gene *mab-9* was originally isolated in a screen for worms with mating defects. Loss of correct MAB-9 expression results in a defect in cell-fate specification of the hindgut cells B & F. In male worms, this leads to loss of the B-cell lineage which forms the larger part of the male-specific tail structures. *mab-9* mutants also have axon guidance defects which result in poor backward movement.

We are interested in identifying *cis*-acting elements important for the regulation of MAB-9 expression. Deletion of sections of the endogenous 5' sequence from a promoter-only *mab-9* expression construct has revealed regions required for VNC, hindgut and hypodermal components of the wild-type expression pattern. Cloning of the sequence required for VNC expression into a *pes-10* minimal promoter vector confirms that this part of the promoter is sufficient to drive expression in these nerve cells. Further dissection of the VNC promoter is in progress.

Sequence comparison of the promoters of *C. elegans mab-9* and its nearest *C. briggsae* homologue identified several areas of conserved sequence which could contain important regulatory elements. Deletion of 2 of these from a *mab-9::gfp* rescuing construct resulted in complete loss of tail hypodermal expression. Furthermore, insertion of these elements into the *pes-10* minimal promoter vector proved sufficient to drive expression in posterior hypodermis. Worms rescued with a *mab-9::gfp* rescuing construct lacking these 2 regions displayed neomorphic tail defects which suggest a previously unknown role of *mab-9* in hypodermal morphogenesis.

Downstream targets of *mab-9* were identified by a microarray-based approach. We used RNA from synchronized L1/L2 populations of *him-8* and *mab-9; him-8* worms to probe Affymetrix chips. Targets which were downregulated/upregulated in a *mab-9* mutant were confirmed by semi-quantitative RT-PCR. Promoter-only gfp fusions are being made to selected targets to compare expression levels in *him-8* and *mab-9; him-8* worms.

BACK

# Dysfunctional RRF-3 Up-Regulates Germ Line Specific Heterochromatin Factors In *Caenorhabditis elegans*

**Suvi Asikainen**[1], Kaja Reisner[1], Garry Wong[1]
[1]Department of Neurobiology, A. I. Virtanen Institute for Molecular Sciences, University of Kuopio, P.O.B. 1627, FIN-70211 Kuopio, Finland

RNA –directed RNA polymerase (RdRP) family members have been shown to have variegated roles in RNA -mediated gene silencing processes in *Caenorhabditis elegans* (*C. elegans*). Efficient gene silencing at post-transcriptional level is promoted by RRF-1 by amplification of small interfering RNAs (siRNAs), triggers of RNA interference (RNAi). Another member of RdRP family, RRF-3 has shown a role as a suppressor for post-transcriptional gene silencing. In addition, recent reverse genetics studies have indicated connection of RRF-3 with chromatin silencing. Third member, EGO-1 functions as a component in a chromatin silencing in *C. elegans* germ line.

We performed gene expression analysis using oligonucleotide arrays (Affymetrix) for *rrf-3* mutant *C. elegans* to study the role of RRF-3 in RNAi related pathways in more detail. Because *rrf-3* mutant *C. elegans* exhibits enhanced susceptibility for exogenous RNAi, we first expected up-regulation of components of basic cytoplasmic RNAi machinery. However, a broad amount of up-regulated genes were germ line specific factors and nuclear components associated with chromatin. In fourth larval stage *rrf-3* mutants, we observed up-regulation of germ line specific chromatin remodelling proteins, RNAi –related factors, oocyte-, sperm- and nuclear pore associated factors.

These results suggest that RRF-3 regulates heterochromatin formation in *C. elegans* germ line. In addition, our analysis provides a list of suggested genes involved in heterochromatin formation in *C. elegans* germ line for further investigations.

Keywords: RNA –directed RNA polymerase, RRF-3, gene expression analysis, RNA interference, RNAi, post-transcriptional gene silencing, PTGS, transcriptional gene silencing, TGS, heterochromatin, germ line.

BACK

# *C. elegans* Sumoylome

**Sophie Bamps,** Jolanta Polanowska, Jean-François Dierick and Jérôme Reboul
INSERM UMR599; Centre de Recherche en Cancérologie de Marseille; Institut Paoli Calmettes; Université de la Méditerranée; 27 Bd Lei Roure 13009 Marseille- France; [1]Biovallée-Protéomics Rue des Pr. Jeener et Brachet, 12 - 6041 Gosselies - Belgium

Ubiquitin-like proteins are believed to act as post-translational modifiers in a manner analogous to ubiquitin. SUMO (small ubiquitin-related modifier) has a potential to alter the conformation of the target or to convey a new surface resulting in the ability to interact with a new partner. This kind of modification can affect the nuclear targeting, stability of the protein and modulation of transcription factor activities. The list of sumo targets remains to be elucidated although the number of known targets continues to grown substantially in recent years.

The goal of the presented study is to address at least in part few of the key questions such as: what are the sumo targets, where sumoylation specificity comes from and what are the consequences of this modification; all still poorly defined. We aim to establish a SUMOylome, an exhaustive protein-protein interaction network of protein candidates implicated from close or far in sumoylation processes in *C.elegans.*

In this effort we are performing a middle scale yeast two-hybrid screening using 25 ORFs as baits. Evident candidates are proteins already described to be involved in the enzymatic pathways of sumoylation such as SMO-1, UBC-9, AOS-1, UBA2, potential sumo ligases (GEI-17, NPP-9), and others. Until now, our yeast two hybrid screens allowed identifying new potential substrates and actors of the sumoylation pathways. In regard to our initial results we feel the need to enlarge the obtained network complementing the yeast two-hybrid screen with a second proteomic approach, that is a tandem immunoaffinity purification of chosen proteins. This will first show the relevance *in vivo* of the interactions found *in vitro* and second, as the complexes retain the biological activity, will allow us to test *in vitro* the sumoylation activity of the co-purified proteins. The first candidate is UBC-9, a sumo conjugase for which we are at present purifying the complex of associated proteins and analyzing the identity of interactors by MS/MS.
Advancements of this work will be discussed.

BACK

# RNA Degradation in the Nematode Worm *C.elegans*

**Caroline Bowen** and Alison Woollard
University of oxford, Department of Biochemistry

RNA turnover is necessary to maintain basal levels of proteins, through mRNA regulation, and to remove aberrant mRNA transcripts. Here we examine processes of RNA degradation in *C.elegans***.** This approach is based on a functional investigation of the *C.elegans* components of the RNA degradation pathways, identified from homology to other organisms.

The 5'-3' RNA degradation pathway in *C.elegans* has been previously identified (Newbury and Woollard 2004). Here we investigate the 3'-5' pathway in *C.elegans***,** starting with the identification of its core and accessory components. 3'-5' exoribonucleic acid decay, first identified in *S.cerevisae,* is brought about by a complex of exoribonucleases, helicases and associated factors, known as the exosome, (Mitchell et al 1997). The exosome was hailed as a 'proteosome' for RNA, due to the degradation properties of the heteromeric complex.

We have used RNAi and an available mutant to show that disruption of the exosome complex, through the targeted destruction of individual components, results in worms with severe developmental problems including slowed growth rates. A full length GFP tagged exosome component, has shown the component to be largely confined to the nucleus of all cells, including the germline. Phenotypes associated with depletion of the exosome components include slow growth and developmental problems. These are attributed to effects on rRNA processing. When exosome components are silenced, there is a failure of the degradation of specific cleaved rRNA precursors, which in turn effects ribosome function and subsequently protein synthesis. We will present our findings on the nature of these rRNA processing defects, which will help to clarify the molecular basis of the phenotypes we observe.

BACK

# Searching for *rnt-1* Interacting Genes in *C. elegans*

**Toby Braun** and Alison Woollard
Genetics Unit, Department of Biochemistry, University of Oxford

RNT-1 is the only *C. elegans* member of the Runx family of transcriptional regulators, which are postulated to act both as oncogenes and tumour suppressors in mammalian cells. Work in our lab has shown that *rnt-1* is a rate-limiting regulator of seam cell proliferation during *C. elegans* development (see abstract by Nimmo et al).

Apart from this control of seam cell proliferation, rnt-1 shows a partial embryonic lethality in RNAi experiments. This suggests that rnt-1 may act together with other, as yet unknown factors, to regulate some essential aspect of embryonic development.

To uncover such redundant factors, we are currently performing two synthetic lethal screens in a *rnt-1* mutant background, using a genome wide RNAi feeding library and a random mutagenesis screen. Our approach allows us to easily discriminate between factors that are lethal during development in their own right and factors that are only lethal in conjunction with the absence of *rnt-1* (synthetic lethality).

We are convinced that this genome-wide screen will elucidate further factors that are involved in the control of *C. elegans* development.

BACK

# Towards a Functional Map for Spliceosome Components in *C. elegans*

**Julian Ceron**[1] and Sander van den Heuvel[1,2]
[1]Massachusetts General Hospital Cancer Center and Harvard Medical School, Charlestown, 02129 MA, USA; [2]Developmental Biology, Padualaan 8, Utrecht University, The Netherlands

We have initiated a functional genomics approach aimed at defining an interaction network for splicing-related components. The spliceosome contains a highly conserved collection of snRNPs (snRNAs and associated proteins) and many other protein factors recruited to pre-mRNA to carry out splicing. In addition, recent reports have implicated splicing factors in regulating gene expression through mechanisms different from splicing. How the different factors interact to carry out the various functions remains largely unknown.

To create a *C. elegans* dataset of spliceosome proteins, we used a collection of 254 human spliceosome proteins recently reviewed (Barbosa-Morais et al, 2006) to search for *C. elegans* orthologs in the InParanoid database**.** As a result, we found 164 *C. elegans* genes orthologous to human spliceosome genes that were further used in our functional genomics analysis.

Combining genomics information available in web-based resources, we are generating a platform to assemble a "Spliceosome functional map" in *C. elegans*. This information includes phenotypes observed in genome-wide RNAi screens (Phenome), large-scale identification of protein-protein interactions (Interactome), extensive characterization of expression profiles (Localizome) and transcriptional profiles obtained in microarray-based mRNA expression studies (Transcriptome). As splicing factors are evolutionarily highly conserved, this approach will provide a valuable resource for a better understanding of how molecules and pathways interrelate in the control of gene expression, and how alterations in the splicing system may lead to disease.

BACK

# The Molecular, Cellular and Functional Organization of the *C. elegans* Metabotropic Glutamate Receptor, MGL-1

**James Dillon,** Neil A. Hopper, Lindy Holden-Dye and Vincent O'Connor
School of Biological Sciences, University of Southampton, SO16 7PX

Metabotropic glutamate receptors (mGluRs) are G-protein coupled receptors that are responsible for the 'fine-tuning' of glutamatergic signalling within neural networks that subserve complex brain functions and behaviours. The *C. elegans* mGluR, *mgl-1*, is selectively expressed in the nervous system, where it is distributed in neurons of the tail, the nerve ring and the pharynx. We have defined a role for MGL-1 in the modulation of pharyngeal function and feeding behaviour. In the presence of the mammalian mGluR agonists, *trans*-ACPD (500μM) and L-CCG-I ($EC_{50}$: 3μM) pharyngeal pumping is inhibited. The basal pharyngeal pumping frequency of the *mgl-1* mutant strain *mgl-1(tm1811)* is not significantly different to wild-type levels in the absence of drug and is not inhibited by drug application (*trans*-ACPD 500μM and L-CCG-I in the range 1-12.5μM). The inhibition by L-CCG-I was rescued in *mgl-1(tm1811)* by the re-introduction of the *mgl-1* gene, suggesting MGL-1 is responsible for the agonist-mediated regulation of pharyngeal function.

The intracellular C-terminals of the eight mammalian mGluR subtypes have been shown to be responsible for directing protein-protein interactions with accessory proteins that scaffold the receptors function. Scaffolding entails the targeting of the receptor to specific compartments of the neuron, anchoring within specialized subcellular domains and the organised assembly of the receptors intracellular signalling pathway. A yeast-2-hybrid screen was performed with the MGL-1 C-terminal to identify proteins that scaffold MGL-1 function. The multi-PDZ domain protein MPZ-1a was identified as a strong interactor in yeast. MPZ-1a is encoded by the gene *mpz-1*, which we have identified as being co-expressed with *mgl-1* in neurons of the pharyngeal nervous system and the nerve ring.

The inhibition of pharyngeal pumping by MGL-1 in response to the agonist L-CCG-I was used as a behavioural assay to assess the functional significance of the interaction between MGL-1 and MPZ-1 in this neural network. Pharyngeal pumping was recorded from the available *mpz-1* mutant animals *mpz-1(tm1136)*, *mpz-1(gk273/+)* and *mpz-1(tm1136/gk273)* trans-heterozygotes. In each case a wild-type response was recorded in the presence of L-CCG-I, suggesting the MGL-1 signalling complex is intact. Although our study provides the first evidence for the function of MGL-1 in worm behaviour the significance of the PDZ-domain dependent interaction of MGL-1 and MPZ-1a requires further investigation.

BACK

***P-110***

# Characterization of the Worm Homolog of *memo*, a Gene that Acts Downstream of the Mammalian EGF Pathway

**Collin Ewald**, Nancy Hynes and Joy Alcedo
Friedrich Miescher Institute for Biomedical Research, Maulbeerstrasse 66, CH-4058 Basel, Switzerland

The ErbB2 receptor tyrosine kinase has been shown to play an important role in cancer metastases. This receptor is often overexpressed in human tumors of diverse origins, which include mammary and ovarian tissues. Two of the five major autophosphorylated tyrosine residues of the cytoplasmic tail of ErbB2--Tyr1201 and Tyr1227--are sufficient to restore the migratory phenotype of breast carcinoma cells, whereas the receptor lacking all of the 5 major autophosphorylation sites is impaired in stimulating cell migration. A novel protein called MEMO (Mediator of ErbB2-driven cell motility) has been identified and found to interact with the phosphorylated Tyr1227 residue (1). MEMO controls cell migration by relaying extracellular signals to the microtubule cytoskeleton (1). Interestingly, *memo* is highly conserved through evolution and its homolog in *C. elegans* has a sequence identity of 53%. The aim of this study is to investigate the role of *memo* in *C. elegans*. It is possible that *memo* is involved in cell migration and/or in the signal integration of the worm EGF pathway.

(1) R. Marone et al. (2004) Memo mediates ErbB2-driven cell motility. *Nature Cell Biol* 6: 515 - 522.

BACK

# Identification and Characterization of Proteins Controlling the Intracellular Trafficking of LET-23 EGFR

**Peter Gutierrez**, Attila Stetak, Erika Fröhli-Hoier and Alex Hajnal
Institute of Zoology, University of Zurich, Winterthurerstrasse 190, 8057 Zürich, Switzerland

The C. elegans hermaphrodite vulva is formed by the descendents of three out of six equipotent vulval precursor cells (VPCs, P3.p-P8.p). The gonadal anchor cell (AC) produces LIN-3 EGF that activates in P6.p the conserved LET-23 EGFR/RAS/MAPK pathway to specify the primary cell fate. In order to receive the AC signal, LET-23 has to be kept on the basolateral surface of the VPCs facing the AC. The basolateral localization of LET-23 is essential for the efficient activation of RAS/MAPK signalling pathway and consequently for efficient vulval induction. A ternary complex formed by the PDZ domain proteins LIN-7, LIN-2 and LIN-10 is required for LET-23 localization to the basolateral compartment of the VPCs. Interestingly, PDZ domains are often found in adaptor proteins that regulate the subcellular localization of plasma membrane proteins. Furthermore, mammalian PDZ-domain proteins have been reported to bind to FERM (band 4.1/Ezrin/Radixin/Moesin)-proteins that link plasma membrane proteins to cytoskeletal structures.

In our first approach to find additional regulators of LET-23 localization, we downregulated by RNAi all 34 genes encoding uncharacterized PDZ-domain proteins as well as the 16 genes encoding FERM-domain proteins, and monitored the localization of LET-23 by immunostaining. RNAi against *frm-8* and *tag-60* suppressed the *let-60(gf)* Multivulva phenotype and caused a mislocalization of LET-23 to the junctional region and into intracellular punctae.

Our second approach is based on the observations that single mutants in *lin-7*, *lin-2* or *lin-10* show a vulvaless phenotype due to the apical mislocalization of LET-23, but when either of these mutations is combined with a loss-of-function mutation in a negative regulator of vulval induction such as gap-1, lip-1 or sli-1, this results in a strong Multivulva phenotype. We are thus performing a forward genetic screen in a sensitized *gap-1(lf)* background to isolate Multivulva mutants displaying mislocalized LET-23. Besides the possibility to find novel factors regulating LET-23 localization this screen may also identify negative regulators of the Ras/MAPK pathway.

BACK

# Do *ark-1*, *gap-1* and *sli-1* Have synMuvB Activity?

**Neil A. Hopper**[1], Junho Lee[2,3], Paul W. Sternberg[2] and Anne D. Wooller[1]
[1]School of Biological Sciences, University of Southampton, Bassett Crescent East, Southampton, SO16 7PX, UK; [2]HHMI/Division of Biology, California Institute of Technology, Pasadena, CA91125, USA; [3]present address: Department of Biological Sciences, Seoul National University, Seoul, 151-747, South Korea.

During wild-type hermaphrodite development, the gonadal anchor cell induces 3 from 6 vulva precursor cells (VPCs) to form the vulva. This inducing signal activates the Egfr/Ras/Map kinase pathway in a dose dependent manner in the proximal VPCs. Lateral signalling refines this to ensure that the VPC closest to the anchor cell adopts the primary fate and the two adjacent VPCs adopt the secondary fate. Activity of the synthetic multivulva (synMuv) genes ensures that the remaining VPCs adopt the tertiary (non-induced) fate. The synMuv genes broadly fall into two classes: class A and class B. Mutations in either class alone are superficially silent with respect to vulval induction. However, in animals carrying mutations in both classes, all six VPCs adopt vulval fates. A third class, the synMuvC genes act redundantly with both synMuv A and B genes. The synMuvA genes are novel. Many class B synMuv genes encode components of the Rb/Histone deacetylase complex signaling pathway that represses transcription and conversely the synMuvC genes encode transcriptional co-activators that include a histone acetyl transferase. At least some of the synMuv genes have been shown to act outside the VPCs.

*ark-1*, *gap-1* and *sli-1* were identified as negative regulators of Egfr/Ras/Map kinase signalling and interact with each other to produce a partially penetrant excessive vulval induction phenotype. We recovered *lin-15A(pd3)* in a screen for mutations that interact with *ark-1* to produce excessive vulval induction. *lin-15A(pd3)* R27stop interacts with *ark-1*, *gap-1* and *sli-1* to produce a temperature sensitive Muv phenotype. This result is confirmed with additional *lin-15A* alleles and with a second synMuvA gene, *lin-8(n111)*. Moreover, the *ark-1; lin-15A* Muv phenotype is partially gonad independent. In contrast, no interaction is seen between *ark-1*, *gap-1* and *sli-1* and the synMuvB genes. This suggests that *ark-1*, *gap-1* and *sli-1* may have weak synMuvB activity. An alternate possibility is that the synMuvA genes have a non-redundant activity that weakly antagonises Egfr/Ras/Map kinase signalling in the VPCs, and this synergises with *ark-1*, *gap-1* and *sli-1*. Consistent with this latter possibility, null alleles of *lin-15A* display a partially penetrant excessive vulval induction phenotype at 25°C by themselves and along with *lin-8(n111)* enhance the *sos-1(pd10gf)* and *let-60(n1046gf)* Muv phenotype. However, unlike the enhancement of *ark-1*, *gap-1* and *sli-1* by synMuvA genes, the enhancement of *sos-1(pd10gf)* and *let-60(n1046gf)* Muv is not synMuvA specific, as it is also seen by a sub-set of synMuvB genes. These and other related findings will be presented.

BACK

# Identification of Upstream Factors Regulating the Expression of the T-box Gene mab-9

**Gholamali Jafari,** Alison Woollard
Genetics Unit, Department of Biochemistry, Oxford University, Oxford, Oxon, OX1 3QU, UK

T-box genes encode transcription factors involved in morphogenesis and organogenesis of invertebrates and vertebrates. The mab-9 gene was the first member of the C. elegans T-box gene family to be identified. mab-9 mutants are slightly uncoordinated (Unc) for backward movement, suggesting a role for mab-9 in the nervous system, and they are defective in hindgut and male tail development. Ectopic, or mis-expression of mab-9 is deleterious, hence this gene must be tightly regulated.

In order to identify upstream regulators of mab-9, we have established a high-throughput genome-wide RNAi screen. We have focused on transformation in the pattern of mab-9::GFP by RNAi silencing of 657 transcription factors, assuming that silencing of some transcription factors leads to a change of GFP expression in a strain of worms carrying an integrated mab-9::GFP reporter.

Our study suggests that the following genes have a strong effect in controlling mab-9 expression: nhr-23, ldp-2, nhr-22, unc-4, Y45F3A.4, nob-1, nhr-112, and ZK1193.5.

In addition, we have identified 62 other candidate genes with a possible role in mab-9 regulation. One striking finding is that we have detected a lot of nuclear hormone receptor (NHRs) genes with a potential role in regulating mab-9::GFP expression. By confirming those genes which are upstream of mab-9, we hope to elucidate the genetic network in which mab-9 operates.

BACK

# Testing the *in vivo* Importance of Putative Regulatory Sites in $IP_3$ Receptors

James Legg, Rafael Vazquez-Manrique and **Howard Baylis**
Department of Zoology, University of Cambridge, Downing Street, Cambridge, CB2 3EJ, UK

Signalling through the second messenger inositol trisphosphate ($IP_3$) and its receptor ($IP_3R$) is a central mechanism by which extracellular signals regulate intracellular calcium signals. $IP_3$ signalling plays roles in a wide range of processes in *C. elegans*. For example, $IP_3$ signalling is widely involved in non-neuronal ultradian rhythmic processes; in particular feeding, defecation and ovulation (see 1 and references therein). $IP_3$ signalling is also involved in developmental processes; embryos with disrupted $IP_3$ signalling have defects in differentiation and in morphogenesis (2,3).

The ability of $IP_3Rs$ to function in such a diverse range of processes is likely to require complex differential regulation. This is likely to be achieved, in part, through modulation of $IP_3R$ activity by other signalling molecules. A substantial number of potential and putative regulatory interactions have been identified in $IP_3Rs$ from a range of sources. These include interactions with other proteins and with small ligands such as ATP. Although much of this work has been performed with mammalian $IP_3Rs$, the binding sites for many of these interactors are conserved in the *C. elegans* receptor. We have recently established a system that allows us to modify the *itr-1* genomic DNA using homologous recombination in *E. coli* and then reintroduce modified *itr-1* genes into *C. elegans*, in particular into *itr-1 l.o.f* mutants. This allows us to test the effects of modifying particular binding sites in the $IP_3R$ on its ability to rescue particular functions. We have used this system to test the importance of two sites (a) a putative ATP binding site and (b) the predicted binding site for FKBP12. Disruption of the ATP binding site appears to have modest effects on *itr-1* function whereas disruption of the FKBP12 site appears to have severe effects on function. Therefore this system allows us to test the importance of regulatory sites, identified using biochemical and molecular approaches, in whole animals.

1. Baylis HA (2005) Cell **123**, 5-7
2. Walker DS et al. (2002) Mol Biol Cell **13**, 1329-1337
3. Thomas-Virnig CL et al. (2004) Curr Biol **14**,1882-1887

BACK

***P-115***

# MicroRNAs: Subcellular Localisation of Silencing Complexes

**Linsen S.E.V.,** Tops B., Ketting R.F., Plasterk R.H.A.
Hubrecht Laboratory, Uppsalalaan 8, 3584CT Utrecht, the Netherlands

MicroRNAs (miRNAs) form an abundant, well-conserved class of post-transcriptional gene silencing effectors. It is estimated that ~$10^2$-$10^3$ different miRNAs regulate the expression of ~ 30% of the genes. Recent work has identified the processing (P-)bodies as the subcellular regions where various RISC–associated components localise –which seems essential for effective silencing by miRNAs.

In contrast to a continuously growing list of *in silico* predicted and cloned miRNAs, biologically relevant miRNA targets are still hard to identify. Full complementarity to the seed sequence (i.e. nt 2-8) of the miRNA has become a golden standard, but does not explain all the observed interactions, including target recognition of let-7, the founding member of miRNAs in animals.

The presented work will describe a C. elegans model in which the subcellular organisation of silencing complexes is visualised. The used line expresses MS2::NLS::GFP and LacZ::let-7 complementary sites::MS2 binding sites::unc54 3'UTR, specifically in the seam cells. In case of silencing by let-7, GFP accumulates in cytoplasmic foci, indicating local accumulations of silenced messenger RNA. These foci are presumably P-bodies and if silencing deficiency is introduced by e.g. RNAi foods, they reduce in number. The model will be used to trap the miRNAs that target their complementary sites *in vivo*, to complement *in silico* miRNA-target predictions.

BACK

# Characterization of *C.elegans* NuRD Complexes

**Paolo Moroni**[*], Myriam Passannante[*], Karin Brunschwig, Anne-Laure Chanez, Vanessa Cerantola and Fritz Müller
Department of Biology, University of Fribourg, Switzerland
[*] *contributed equally to the work*

The NuRD (nucleosome remodeling and histone deacetylase) complex is thought to be involved in the establishment of repressive chromatin structures during development and in the negative control of gene expression. In vertebrates, this complex comprises at least seven polypeptides, including the SWI2/SNF2 helicase/ATPase Mi-2, the histone deacetylases HDAC1/2, the histone-binding proteins RbAp46/48, the metastasis-associated proteins MTA1/2, the methyl-CpG binding protein MBD3 and the potent transcriptional repressor p66. Orthologues of these NuRD subunits are also encoded by the genome of *C.elegans*, among them the two Mi-2 homologues LET-418 and CHD-3.
Mutations in *let-418* show a pleiotropic phenotype, including sterility and larval arrest (von Zelewsky and al., 2000), whereas *chd-3* mutants exhibit no obvious defects. However, a null mutation of *chd-3* enhances the *let-418* phenotype. This suggests that the two proteins have partially redundant functions during development (von Zelewsky et al., 2000).
To find proteins interacting with LET-418 and CHD-3 and to characterize putative NuRD complexes in *C.elegans*, we take different approaches. We use standard biochemical techniques, such as co-immunoprecipitation, tandem affinity purification (TAP) and yeast two-hybrid screens.
Our preliminary data suggest that LET-418 and CHD-3 may not be interchangeable members of the same complex, but rather are part of different NuRD or NuRD-like complexes.

von Zelewsky and al., Development 127: 5277-5284 (2000)

BACK

# The Role of Presenilins in Calcium Signalling in *Caenorhabditis elegans*

**Helen Peterkin** & Howard Baylis
Department of Zoology, University of Cambridge, CB2 3EJ

Presenilins are transmembrane proteins implicated in familial Alzheimer's disease. The primary function of presenilins is as a subunit of the γ secretase protein cleavage complex, which is implicated in many signalling pathways. Presenilins have recently been suggested to play a role in calcium signalling, possibly through interactions with $IP_3$ and ryanodine receptors[1]. The mechanism by which presenilins interact with $Ca^{2+}$ signalling is unclear and it is this that we aim to dissect. Three presenilin genes are present in *C. elegans*, *sel-12*, *hop-1* and *spe-4*. *sel-12* and *hop-1* show greatest homology and display a degree of functional redundancy[3]. We are using *sel-12* and *hop-1*mutants to investigate the relationship between presenilin activity and calcium signalling. *sel-12* is reported to be expressed in all tissues except the intestine[2] and we have shown, by GFP tagging, that *hop-1* is expressed in the nervous system, pharynx and vulva in worms carrying a mutation which upregulates *hop-1* expression. Alterations in signalling pathways can affect physiology and behaviour in *C. elegans* and these changes can be assayed. Both $IP_3$ and ryanodine receptors function in the pharynx and we have shown *sel-12* mutant animals have reduced pharyngeal pumping on food. Both chemosensory and mechanosensory abilities appear to be impaired in *sel-12* mutant animals. In addition all *sel-12* mutants show a reduction in locomotion in a number of dispersion and thrashing assays. *sel-12(ty11)*, a reported loss of function mutant, has reduced overall speed when measured on food and this defect can be rescued by expression of the full length *sel-12* protein. We have also crossed animals carrying the *sel-12(ty11)* mutation with animals carrying gain and loss of function mutations in *itr-1*, the $IP_3$ receptor gene in *C. elegans*, to identify any genetic interactions.

1. LaFerla FM. (2002) *Nature*. 3, 862-872
2. Levitan DJ, Doyle TG, Brousseau D, Lee MK, Thinakaran G, Slunt HH, Sisodia SS, Greenwald IS**.** (1996) *Proceedings of the National Academy of Sciences USA.* 931, 4940-14944
3. Xiajun L & Greenwald I. (1997) *Proc. Natl. Acad. Sci. USA.* 94, 12204-12209

BACK

# A Candidate-Based Approach for Genetic Interaction with *gap-1* identifies a WD40 Encoding Gene

**Gino Poulin** and Julie Ahringer
The Gurdon Institute, University of Cambridge, Tennis Court Road, Cambridge CB2 1QN, United Kingdom

Vulval fate induction is an established readout to identify regulators of multiple signalling pathways including the canonical RTK/RAS/RAF/MPK signalling pathway. Vulval fate adoption requires an inductive signal (LIN-3 EGF) that emanates from the anchor cell (AC) and activates Ras signalling in three of the six vulval precursor cells (VPCs). The VPC closest to the source of LIN-3 EGF adopts the primary fate and produces the lateral signal that activates LIN-12 NOTCH signalling in the neighbouring secondary VPCs. In these cells, negative regulators of Ras ensure down regulation of Ras signalling in a Notch dependant manner, an important process to maintain vulval cell patterning. The last three VPCs adopt a non-vulval fate, because of the Ras inhibiting activity of the genetically redundant synMuv genes.

GAP-1 is a GTPase activating protein that directly regulates LET-60 RAS by stimulating its GTPase activity and therefore increases the rate of LET-60 RAS bound to GDP, which is the inactive form. In absence of *gap-1* animals develop a normal vulva, however vulval fate adoption becomes more susceptible to elevated Ras signalling. Hence, additional loss of a negative regulator of Ras causes extra vulval precursor cells to adopt the vulval fate and produces the Muv phenotype. We have previously performed genome-wide RNAi screens for new synMuv genes. RNAi of about half of our initial candidates caused a synMuv independent low percentage Muv phenotype. We reassessed these and the synMuv interacting genes (100 genes) in a *gap-1* background and found seven where RNAi caused a high percentage Muv phenotype. We identified the members of the sumoylation pathway, two components of the NuRD complex, *sys-1*, and a novel WD40 encoding gene, that we named *gin-1*, for gap interactor-1. We focused our analysis on *gin-1* and found that it inhibits Ras signalling upstream of *sem-5* (Grb2). We also found that RNAi of gin-1 suppresses a *let-23* EGFR loss-of-function *mutant*, suggesting that the inhibition occurs at or downstream of the receptor. In addition, using *egl-17::cfp* as a marker for primary cells, we found that *gin-1(RNAi)* animals show persistent expression in the normally non-expressing secondary cells, showing that *gin-1* plays a role in Ras signalling inhibition during vulval fate adoption. To rule out the possibility that anchor cell duplication is responsible for our observations, we used *zmp-1::yfp* as an anchor cell marker and found no defect at this level. Taken together, our candidate-based approach has identify seven novel *gap-1* interactors; one of these, *gin-1*, appears to regulate Ras signaling between the receptor and *sem-5*.

BACK

# Investigating the Role Of rnt-1 and bro-1, and their Mammalian Orthologues, In Seam Cell Proliferation in *C. elegans*

**Nicole Saad**, Hiroshi Kagoshima, and Alison Woollard
Genetics Unit, Dept. of Biochemistry, University of Oxford, South Parks Road, OX1 3QU, UK

*rnt-1* is the *C. elegans* orthologue of the Runx family of transcriptional regulators implicated in various human cancers. There are three Runx genes in humans; Runx1 is associated with acute myeloid leukaemia and is thought to act both as an oncogene and as a tumour suppressor. Runx2 is associated with cleidocranial dysplasia and Runx3 is postulated to be associated with gastric cancer.

Previous work in the lab has identified *rnt-1* as the defective gene in *mab-2* mutants. Mab mutants have defects in the formation of the male tail, arising from seam cell lineages. *mab-2/rnt-1* mutants have missing rays because there is a reduction in the number of ray precursor cells. The reduction in ray precursor cell number is a direct consequence of a reduction in the number of seam cells. The reduction in seam cell number in *rnt-1* mutants is usually the result of a failure in particular cell divisions. Overexpressing *rnt-1* results in an increase in seam cell number. This is compelling evidence that *rnt-1* is a rate limiting regulator of cell proliferation.

The CBFbeta orthologue *bro-1* has a similar function to *rnt-1* in regulating seam cell proliferation. *bro-1* deletion alleles have reduced numbers of seam cells and are missing rays in the male tail. *bro-1* animals have similar cell proliferation defects to *rnt-1* animals. CBFbeta has also been linked with leukaemia, and been shown to act as a binding partner for Runx factors. We are currently investigating *bro-1* further. We would now like to explore whether over expression of *bro-1* results in seam cell hyperplasia, and whether a synergistic effect is seen if *rnt-1* and *bro-1* are co-overexpressed. We are also testing whether *rnt-1*-induced seam cell hyperplasia is dependent on *bro-1* function.

In order to assess the conservation of the Runx genes we are currently pursuing complementation experiments using mouse Runx cDNAs. Expression will be driven in worms using both the endogenous worm promoter and the hsp16.2 heatshock promoter to see if it is able to complement the function of the worm orthologue. We are also making transgenic worms expressing mammalian CBFbeta along with Runx1, Runx2 or Runx3, and testing these combinations for rescue of a *rnt-1* mutant and induction of hyperplasia. *C. elegans bro-1* is much more highly diverged from CBFbeta than *rnt-1* is from Runx genes, therefore it is possible that mammalian Runx proteins will not function in *C. elegans* in the absence of CBFbeta. Therefore, we will also test interactions between *bro-1* and mammalian Runx genes in yeast two-hybrid assays. We hope to present results from these experiments at the meeting.

BACK

# 3' End Processing and Transcription Termination in the Nematode *Caenorhabditis elegans*

**Helen Sharpe**, Cathy Browne and André Furger
Genetics Unit, Department of Biochemistry, University of Oxford, UK

We are using *C. elegans* to study the relationship between the recognition of the poly(A) sites and transcription termination in a chromosomal context. The compact genome, *trans*-splicing and the arrangements of genes in operon-like structures make *C. elegans* an interesting system to study RNA polymerase II transcription termination. In both mammals and yeast the recognition of a functional poly(A) site is instrumental in directing transcription termination. However, when operons in *C. elegans* are transcribed as polycistronic pre-mRNAs, several poly(A) sites are transcribed and recognized without inducing transcription termination. This implies that the two processes in the nematode can either be uncoupled, or that specific mechanisms are in place to prevent termination despite the recognition of the poly(A) sites. In addition, *trans*-splicing may require a more stringent transcription termination control in order to prevent expression of downstream located genes that are 'accidentally' transcribed.

To address this issue, we began to analyse the relationship between the recognition of poly(A) sites and transcription termination in three nematode genes. We have used a RT-PCR based technique to analyse how far transcription continues into the 3' flanking region of three *C. elegans* genes before cleavage at the processing site occurs.

We first investigated the transcription profile at the 3' end of *vit-2*, a gene that undergoes 'conventional' splicing (removal of intronic sequences) but is not *trans*-spliced. We detected uncleaved contiguous pre-mRNA transcripts that contained sequences located at least 900 bases downstream of the poly(A) site. As has been shown in a mammalian system, these extended pre-mRNAs still contain the terminal intron sequences. Transcription of the *vit-2* gene results in a contiguous pre-mRNA that extends into a downstream positioned predicted ORF located on the anti-sense strand and extends into promoter sequences of a downstream positioned predicted gene on the sense strand.

We are currently comparing the *vit-2* transcription profile to transcriptional events in several *trans*-spliced genes. Additionally, we are developing nuclear run-on analysis to confirm our transcription profiles.

BACK

# An Expression Study of Seven Putative Nuclear Receptors Organized in a Cluster on Chromosome V of *Caenorhabditis elegans*

**Jaroslav Vohanka[1],** Zdenek Kostrouch[2] and Marta Kostrouchova[1]
[1]Laboratory of Molecular Biology and Genetics and [2]Laboratory of Molecular Pathology, nstitute of Inherited Metabolic Disorders, 1st Faculty of Medicine, Charles University, Prague

Chromosome V contains a group of seven sequences recognized by the computer program GeneFinder as potential nuclear hormone receptors (NHRs). In order to identify whether genes from this cluster may be functional NHRs, we studied their expression during *C. elegans* development. RT-PCR identifies transcripts of all seven genes. The expression of all members of this cluster starts in embryos. Six of these genes are expressed throughout development while one member of this cluster is only expressed in embryos and early larval stages. We prepared constructs of GFP fusion genes containing various regions of genomic sequences. At least 2 different fragments containing putative promoters were prepared for each gene of the cluster; one consisting of approximately 500 bp and a second of approximately 2000 bp. The longer constructs contain parts of the coding sequences of the preceding genes. Our results show that members of this gene cluster have a diverse expression pattern and are likely to be functional NHRs.

Acknowledgement: We thank Drs. A. Fire for GFP constructs, vectors and host used in RNAi, M.W. Krause for support and advice. The work was supported by grants 303/03/1115 and 301/ 05/0859 from the Czech Science Foundation and by the grant 0021620806 from the Ministry of Education, Youth and Sports of the Czech Republic.

BACK

***P-122***

# A Role of the *C. elegans* Mi-2 Protein LET-418 in LIN-12/Notch Signalling Pathway

Y. Zhang, F. Guerry, **C-O Marti,** F. Müller
Departement of Biology, University of Fribourg, Switzerland

The NuRD (*Nu*cleosome *R*emodeling and histone *D*eacetylase) complex has been proposed to repress gene expression in eukaryotic cells through chromatin remodelling and histone modification. Mi-2, a central component of the NuRD complex, has two orthologs in *C. elegans*, LET-418 and CHD-3.

Interestingly, mutant animals defective for *let-418* show defects that resemble those of *lin-12(lf)* worms. It is therefore tempting to speculate that LET-418 is functionally linked to the LIN-12/Notch signalling pathway.

Here we present evidence suggesting that LET-418 interferes with the LIN-12/Notch lateral signalling by negatively regulating the expression of the ligand LAG-2/Delta. Quantitative RT-PCR demonstrated an up-regulation of *lag-2* mRNA in *let-418* depleted animals. This regulation seems to be direct, since LET-418 binds to the promoter of *lag-2*.

BACK

# Characterizing APE-1

**Erica Bogan**[1,2], Ekaterini A. Kritikou[1], Michael O. Hengartner[1]
[1]Institute of Molecular Biology and [2]PhD Program in Molecular Life Sciences, Universitty of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland

Apoptosis, or programmed cell death, is a complex process of cellular self-destruction. Programmed cell death has been extensively studied in *C. elegans* because it is an intrinsic, reproducible part of the developmental program in both the soma and the germ line of the worm. Several different, genetically separable pathways that lead to germ cell apoptosis have so far been described. These include a DNA damage pathway, induced by genotoxic stress, which results in the induction of germ cell apoptosis and mitotic germ cell cycle arrest.

The tumor suppressor protein p53 has a key role in the integration of cellular responses to genotoxic stimuli, by inducing either cell cycle arrest or apoptosis following DNA damage. Like its mammalian homolog, the *C. elegans* p53 protein, CEP-1, is required for DNA damage-induced germ cell apoptosis, but it appears to be dispensable for cell cycle arrest after exposure to ionizing radiation.

*ape-1* encodes a p53/CEP-1-binding protein conserved from worms to humans, which apparently inhibits p53/CEP-1 in the absence of genotoxic stress. *ape-1(lf)* mutants have increased germline apoptosis (Gla phenotype) that is p53/CEP-1-dependent. These worms also have other defects associated with DNA damage checkpoints: although the mitotic germ cells have a normal cell cycle arrest response induced by IR, the apoptotic response is defective. We are currently pursuing the biochemical characterization of APE-1 and its interaction with CEP-1, as well as genetic studies with *ape-1(lf)* mutants that will hopefully shed light on the mechanism through which APE-1 functions and how it interacts with the *C. elegans* p53 homologue CEP-1. We also hope this study will help us unravel the responses triggered by DNA damage that have been conserved from worms to humans.

BACK

# A Worm Model for Spinal Muscular Atrophy

**Michael Briese**[1], Behrooz Esmaeili[2], Laurence A. Brown[1], Emma Burt[1], Paula Towers[1] and David B. Sattelle[1]

[1]MRC Functional Genetics Unit, Department of Physiology, Anatomy and Genetics, Le Gros Clark Building, University of Oxford, South Parks Road, Oxford OX1 3QX, UK; [2]Genetics Unit, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder in which lower motor neurons in the spinal cord degenerate causing progressive muscle wasting. The predominant form of SMA shows childhood-onset and is associated with deletions or mutations of the *Survival Motor Neuron 1* (*SMN1*) gene. Its *C. elegans* orthologue *smn-1* is expressed in neurons, muscles and other cells and knockdown of *smn-1* by RNA interference (RNAi) results in embryonic lethality and sterility.

We have investigated the *smn-1(ok355)* deletion mutant which is sterile and arrests at larval stage L2-L3. Mutant worms show defects in pharyngeal pumping, movement and defecation. The movement defect becomes apparent at the late L1 stage in the form of an abnormal backward motion. As development proceeds, mutant animals become uncoordinated for both forward and backward movement. Using GFP reporters expressed pan-neuronally (*F25B3.3::GFP*) or in cholinergic neurons (*unc-17::GFP*), we could not detect any gross morphological abnormalities of the nervous system architecture. We are currently in the process of scoring *smn-1(ok355)* worms for synaptic defects using synaptic markers, including *unc-25::SNB-1::GFP*.

We are also investigating the neuromuscular functions of SMN-1 in *C. elegans* by attempting to knock down *smn-1* through promoter-driven hairpin RNAi. To this end we are using the plasmid pWormgatePro which we have previously tested on genes expressed in muscle cells and neurons. Additionally, *C. elegans* embryonic cell cultures are being deployed to investigate cell-autonomous functions of *smn-1* in neurons and muscles.

BACK

# The SLO-1 BK Channel of C. elegans is Critical for Muscle Function and is involved in Dystrophin-Dependent Muscle Dystrophy

**Maité Carre-Pierrat[1],** Karine Grisoni[1], Kathrin Gieseler[1], Marie-Christine Mariol[1], Edwige Martin[1], Maelle Jospin[2], Bruno Allard[2] and Laurent Ségalat[1]
[1]CGMC, CNRS-UMR 5534, Université C. Bernard Lyon-1, 69622 Villeurbanne, France; [2]Physiologie Intégrative, Cellulaire et Moléculaire, CNRS-UMR 5123, Université C. Bernard Lyon-1, 69622 Villeurbanne, France.

Duchenne Muscular Dystrophy (DMD) is a human disease caused by the absence of dystrophin in the skeletal muscles. It is characterized by a progressive muscular degeneration that leads to paralysis and death. Mutations in the *Caenorhabditis elegans* homologue of the dystrophin gene, *dys-1*, lead to a peculiar phenotype of head-bending and hyperactivity. By mutating *dys-1* in a sensitized background *hlh-1(cc561)*, we have developed a model of progressive muscular degeneration in *C. elegans*. We use this model to investigate the mechanisms of dystrophin-dependent muscular degeneration.

*slo-1* encodes a large conductance BK calcium-activated potassium channel, SLO-1, that is expressed in *C. elegans* neurons and muscle cells. It was previously shown to be involved in neurotransmitter release. New *slo-1* alleles were isolated in our lab in a search for *dys-1*-like mutants. When put in the *hlh-1(cc561)* background, *slo-1* mutations also lead to a progressive muscle degeneration.

We showed that it is the loss of the muscular form of SLO-1, and not the neuronal form, that leads to *dys-1*-like phenotypes. SLO-1 localization was observed by *gfp* reporter in *C. elegans* body wall muscle cells, where it seems to co-localize partly with dystrophin in dense bodies.

Finally, we recorded SLO-1 activity on body wall muscle cells using the inside-out configuration of the patch clamp technique. As previously established, SLO-1 channels were selective for K+ and their opening was reversibly inhibited by removal of internal Ca2+. Neither the abundance nor the conductance of SLO-1 were significantly changed in *dys-1* mutants compared to wild-type animals.
These results indicate that SLO-1 function in *C. elegans* muscle cells is related to dystrophin. Our working model is that the inactivation of *dys-1* causes a misregulation of SLO-1, which in turn may contribute to the pathogenecity of *dys-1* mutations.

BACK

# Overexpression of Subunit C, the Main Component of the Storage Material in Juvenile Neuronal Ceroid Lipofuscinosis (JNCL), Causes Disruption of Mitochondria in *C. Elegans* and Subsequent Death

Gert de Voer, Ronald O.B. de Keizer, Paola van der Bent, Gert-Jan B. van Ommen, Dorien J.M. Peters, and **Peter E.M. Taschner**
Department of Human Genetics, Center for Human and Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands

Subunit c of the mitochondrial ATP synthase (subunit c) normally is present in the F0 part of the ATP synthase complex in mitochondria. The very hydrophobic subunit c also forms the main component of the lysosomal storage material found in many forms of the hereditary neurodegenerative disorders called Neuronal Ceroid Lipofuscinosis (NCL, Batten disease). The juvenile form of NCL is caused by mutations in the CLN3 gene, which has three homologs in *C. elegans*. In order to obtain more insight in the etiology of juvenile NCL and how the mitochondrial subunit c protein ends up in large quantities in lysosomes, we have constructed *cln-3* triple mutants, in which all three *cln-3* genes have been mutated. The *cln-3* triple mutants had a decreased life span and brood size, but no lysosomal storage was observed, probably due to the relatively short life span of the worm.

We hypothesized that overexpression of subunit c might directly or indirectly induce lysosomal storage in *C. elegans* and potentially lead to a phenotype useful for genetic screens. Therefore, we identified the *atp-9* gene, which encodes the *C. elegans* subunit c protein, and generated transgenic worms carrying an *hsp-16.2* promoter-*atp-9* fusion construct. Induction of subunit c overexpression by a 2-hr heat shock causes the nematodes to disintegrate, presumably as a result of disrupted mitochondria. Electron micrographs of transgenic worms show altered mitochondria after induction of subunit c overexpression, but no lysosomal storage was detected irrespective of a wildtype or *cln-3* triple mutant background. Milder induction of overexpression affects the reproduction and the morphology of the worms.

This work was financially supported by the Center for Biomedical Genetics, the Batten Disease Support and Research Association, and the European Union, EU project NCL models (EU LSHM-CT-2003-503051).

BACK

# Identifying Novel Genes Involved in DNA Damage Response Pathways in *C. elegans*

**Christina Dittrich**[1,2] and Michael O. Hengartner[1]
[1]Institute of Molecular Biology, University of Zurich, Zurich, Switzerland; [2]MLS PhD program

Upon DNA damage, a cell responds by arresting its cell cycle, changing its transcription patterns, recruiting the repair machinery or, most severely, by executing its own death by apoptosis. Malfunction of any of these pathways allows cells to replicate in spite of DNA lesions, and thus their genomes accumulate mutations and becomes unstable, a hallmark of tumor cells. Therefore, it is not only of paramount biological, but also of high medical interest, to understand the molecular pathways underlying DNA damage responses. Genotoxic stress induces in the germ line of *Caenorhabditis elegans* a spectrum of DNA damage responses similar to that observed in humans. Because important genes are often conserved throughout evolution, studies in *C. elegans* might improve our understanding of the molecular programmes safeguarding the genome of human cells.

Wild-type *C. elegans* animals carry out proper DNA damage responses following exposure to ionizing radiation (IR), thus most of their progeny survive. In contrast, radiation-sensitive (Rad) mutants, which are defective in these pathways, show high levels of embryonic lethality upon IR. Taking advantage of this phenotype, we performed a forward genetic screen and identified 17 mutants whose embryos show reduced viability following IR treatment. Two of those mutants, *op442* and *op444*, display a strong Rad phenotype. We are currently mapping the affected genes taking advantage of molecular polymorphisms between the Bristol and the Hawaii isolate of *C. elegans*. Additionally, we are characterising those two mutants further in order to learn more about the molecular nature underlying the observed defects.

BACK

# Learning Lessons from *dys-1* and *snf-6* - Having Acetylcholine as a Teacher

**Davide Faggionato**, Oyunbileg Nyamsuren, Ralf Baumeister
Bio-3/Bioinformatics and Molecular Genetics, Albert-Ludwigs-University, Freiburg, Germany

We study the function/dysfunction of mutations of genes that in humans have been associated with degenerative disorders. Recently, we identified components of an ubiquitylation complex as regulators of muscle assembly and turnover. Specifically, mutational inactivation of ubiquitylation suppressed paralysis of *unc-45* mutant animals that show a severe myofibril disorganization [1].

In order to get an idea whether this is a generally applicable way to reduce the phenotypic consequences of mutations in muscle genes, we focused on other genes affecting muscle assembly and function.

*dys-1* encodes the C. elegans orthologue of human dystrophin, mutations of the latter being responsible for Duchenne muscular dystrophy. One of the available mutants in *dys-1*, cx18, displays a surprisingly mild impairment of movement, that, however, is strongly enhanced in the background of *hlh-1*/*MyoD*, generating a synthetic paralysis. However, *dys-1*(cx18) animals also display subtle phenotypes like increased sensitivity to aldicarb, suggesting an additional neuronal function of this gene [2].

In a previous screen for ethanol resistance, mutations in the muscular sodium-acetylcholine cotransporter gene *snf-6* were identified. Surprisingly, *snf-6* mutations strongly resemble the *dys-1* mutant phenotype in an *hlh-1* background, suggesting a link between cholinergic signalling and dystrophin function.

In order to understand *dys-1* dysfunction, we performed a detailed comparative analysis of both mutants and will present the data in our poster.

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

[2]Bessou C, Giugia JB, Franks CJ, Holden-Dye L, Segalat L.
Mutations in the *Caenorhabditis elegans* dystrophin-like gene dys-1 lead to hyperactivity and suggest a link with cholinergic transmission. Neurogenetics. 1998 Dec;2(1):61-72

BACK

# Optimization of a Screen for Molecules Slowing Muscle Degeneration in a *Caenorhabditis elegans* Model of Dystrophin-Dependent Myopathy

**Giacomotto J** & Segalat L
CGMC, CNRS-UMR 5534, Universté Lyon 1, France

Duchenne muscular dystrophy (DMD) is a degenerative muscular disease caused by mutations in the dystrophin gene. In the model animal *Caenorhabditis elegans*, mutations of the *dys-1* dystrophin-like gene lead to a muscular degenerative phenotype when they are associated with a mild *MyoD* mutation. This cheap and fast-growing model of dystrophinopathy may be used to screen chemical libraries for compounds able to slow muscle degeneration. In a blind screen of approximately 100 compounds covering a wide spectrum of targets, we found that prednisone is beneficial to the *C. elegans* dystrophin-deficient muscles. Prednisone reduces by 40% the number of degenerating cells in this animal. This result is a proof-of-principle for the use of *C. elegans* as a tool in the search for molecules active against the effects of dystrophin-deficiency. The subsequent test of 800 bioactive molecules lead us to identify 20 hits which are currently being further tested.

Recently, we have worked on speeding up the testing protocol which permits to assay the effect of chemical compounds on the *C. elegans* model of myopathy. The main problems to overcome were to obtain a large number of synchronised worms and to define a semi-solid medium that allow muscle degeneration as well as recovery of worms by pipetting. The new protocol speeds up the screening process 7 times. With this optimized protocol, we could positively detect our positive control (prednisone) in blind tests. We have used this new protocol to test a library of 2,000 chemical compounds. We have already found 26 news molecules (1,3%) that show a statistically significant effect on muscle degeneration. These drugs have been retested in an additional test to quantify their effect.

The best 10 molecules will eventually be tested on the mouse model of DMD.

BACK

# Sugarland Express: Many *C. elegans* Genes Implicated in Glycosylation Affect Surface Adhesion to the Bacteria *Microbacterium nematophilum* and *Yersinia pestis*

**Maria J. Gravato-Nobre**[1], Delia O'Rourke[1], Frederick A. Partridge[1], Dave Stroud[1], Karen J. Yook[1], Creg Darby[2], Jonathan Hodgkin[1]

[1]Department of Biochemistry, University of Oxford, Oxford, OX1 3QU, U.K; [2]Department of Microbiology and Immunology, University of Alabama at Birmingham, AL 35294-2170, U.S.A

The pathogens *M. nematophilum* and *Y. pestis/Y. pseudotuberculosis* are able to cause disease in *C. elegans* by adhesion to the cuticle surface, either in the rectum *(M. nematophilum)* or in the head *(Yersinia).* Nematode mutants that are resistant to either kind of infection are easily obtained, and we find that there is a substantial overlap between those that are uninfectable by *M. nematophilum* (Bus, or Bacterially Un-Swollen mutants) and those that do not support head biofilm formation by *Yersinia* (Bah, or Biofilm Absent on Head mutants). Five genes with this common phenotype have now been defined and cloned: *srf-3, bus-2, bus-4, bus-12, bus-17*. These encode either predicted sugar transporters (SRF-3, BUS-12) or predicted galactosyltransferases (BUS-2, BUS-4, BUS-17).

Mutants in two of these genes have pleiotropic phenotypes and exhibit altered lectin staining *(srf-3, bus-17),* but the other three *(bus-2, bus-4, bus-12)* show little obvious alteration in surface properties or other phenotypes. Nevertheless, the fact that mutations in these genes abrogate bacterial adhesion to both head and rectum suggests that they result in altered surface coat over the entire surface of the worm.

At least one other glycosyltransferase gene, *bus-8*, affects general surface properties but is resistant only to *M. nematophilum*, not to *Yersinia* (see presentation by Partridge et al.). Conversely, some Bah mutants are not resistant to *M. nematophilum*. Therefore, bacterial surface adhesion appears to involve both general and pathogen-specific host surface factors.

BACK

# Identification of pH Regulated Genes in the Nematode *Caenorhabditis elegans*: New Potential Drug Targets for the Treatment of Pathogenic Nematodes

**Rebecca Hall**, Peter Klappa and Fritz A.Mühlschlegel
Department of Biosciences, University of Kent, Canterbury, Kent CT2 7NJ, UK

Human pathogenic nematodes infect over 160 million people per year. *Brugia malayi* and *Onchocerca volvulus* are the causative agents of elephantiasis and river blindness, respectively. Treatment options are restricted and the most commonly used drug, Ivermection, is stage specific and has significant side effects. Furthermore, treatment failures due to drug resistance have been reported.

*Brugia malayi* and *O. volvulus* exhibit an indirect life cycle where an incubation period within an intermediate host vector is essential for development of the infectious stage. During host-vector transition pathogenic nematodes are exposed to extreme environmental changes including variations in pH. We decided to exploit the different environmental pH conditions encountered, in order to identify new therapeutic targets.

Using *C. elegans* as a model we designed a survival assay and were able to show that *the* nematodes are extremely resistant to environmental pH changes. This result was shown to be independent of the cuticle by the use of collagen cuticle mutants. Microarray experiments identified a set of pH-regulated genes. These genes were up regulated at pH9, an environment encountered by pathogenic nematodes residing in the vector gut. The genes were silenced using RNA mediated interference to establish their requirement for survival. There were no significant phenotypic changes observed after five days of treatment, and exposure of the silenced nematodes to acidic and alkaline environments did not significantly reduce survival. However, *in vivo* studies are currently being carried out using carbonic anhydrase inhibitors to identify whether the candidate genes have potential for drug targeting.

BACK

# Increased Sensitivity to Cisplatin by Depletion of ASNA-1 in *C. elegans*

**Hemmingsson O[1],** Still M[1], Kao G[2], Tuck S[2] and Naredi P[1]
[1]Department of Surgical and Perioperative Sciences, Umeå University Hospital, Sweden; [2]Umeå Center for Molecular Pathogenesis, Umeå University, Sweden

Background: Cisplatin is a widely used platinum-based drug against solid tumors in humans. However, the cytotoxic effect is limited by aquired resistance. Cross-resistance is reported between cisplatin and other metal salts, including arsenite and antimonite. Reduced accumulation of cisplatin and arsenite is found in resistant cells, suggesting a shared transport mechanism. In bacteria, the ars-pump is responsible for efflux of arsenite and antimonite. The catalytic subunit of the Ars-pump is an ATPase termed ArsA. ASNA-1 is the eukaryotic homologue of ArsA. Reduced ASNA1 expression in human cells results in increased sensitivity to cisplatin and arsenite. *ZK637.5*, the *C. elegans* counterpart of human *ASNA1,* encodes a predicted 342 residue protein with an ATP binding site and 53% homology to human ASNA1. The purpose with this study is to establish *C. elegans* as a model for toxicity trials and to study the effect of depleted ASNA-1 expression on nematode sensitivity to cisplatin.
Methods: Double stranded *C. elegans asna-1* RNA is injected into the gonads of adult *C. elegans* worms and their progeny is studied. In addition, two *asna-1* deletion mutants, *sv42* and *ok938* are used. A NheI/KpnI cassette containing the full length *C. elegans asna-1* cDNA is used when placing the gene in front of the P elt-2, P unc-119 and P daf-28 promoters. Worms are transferred to metal containing plates and grown at 20°C for 24 hours. The number of living and dead worms is determined. A binary logistic regression model is used for statistical analysis.
Results: The ASNA-1 null phenotype in RNAi treated L1 worms includes a statistically significant increase in sensitivity to cisplatin, arsenite and antimonite but not to zinc chloride or cadmium chloride. Cisplatin $IC_{50}$ for ASNA-1 null phenotype L1 worms is 197µg/ml compared to 360µg/ml in wild type L1's ($p<0.001$). Adult wild type worms are resistant to cisplatin at the maximal concentration of 500µg/ml cisplatin in agar. Adult *asna-1* deletion mutants *sv42* and *ok938* are sensitive to cisplatin, displaying $IC_{50}$ 242µg/ml and 248µg/ml respectively. The sensitivity to cisplatin at 300µg/ml in *sv42* is completely rescued by expression of *C. elegans asna-1* ($p<0.001$). Three tissue specific promotors for *asna-1* (intestinal, neuronal and daf-28) were tested and all rescued the phenotype. Expression of human *ASNA1* in the *sv42* background improved the survival from 56% to 86% at 300µg/ml cisplatin ($p<0.05$). We conclude that depletion of ASNA1 results in a phenotype of increased sensitivity to cisplatin, arsenite and antimonite. This phenotype is rescued by expression of *C. elegans* ASNA-1 or human ASNA1. These findings are consistent with our earlier observations that cells selected for resistance to cisplatin exhibit cross resistance to arsenite and antimonite. We propose that ASNA-1 can be a target to increase cisplatin sensitivity and that *C. elegans* can be a suitable model to identify proteins involved in cisplatin toxicity.

BACK

***P-133***

# Deciphering the Role of Serotonin Blocking Muscle Degeneration

**Stefan Jünemann** and Laurent Ségalat
University of Lyon, CGMC CNRS UMR5534, 43 bd du 11 Novembre, 69100 Villeurbanne, France

Duchenne muscular dystrophy (DMD) is a progressive myopathy which is caused by the disruption of the dystrophin gene. Until today, there is no effective cure of the disease. Neither do we understand completely the function of the dystrophin protein. In C. *elegans*, homologues for dystrophin and most of its associated proteins have been identified. In addition, a dys - 1/CeMyoD double mutation displays an activity - dependent, progressive degeneration of muscle fibres resembling the DMD. We therefore use C. *elegans* as a model system to get insights in both the function of dystrophin and in possible treatments of DMD. In a screen of biochemical compounds in the dys - 1/CeMyoD$^{-/-}$ genetic background, the neurotransmitter Serotonin was found to reduce significantly the degeneration of muscles. In order to decipher the pathway involved in this muscle - specific Serotonin function, we performed an EMS mutagenesis to screen for supressor mutants of the Serotonin effect in the dys – 1/CeMyoD background. Of 5000 F1 animals tested in our assay, we obtained 13 candidates which reduce the Serotonin effect to different degrees and at different stages, respectively. In particular, the Serotonin induced protection from muscle cell disorganisation, which apparently precedes cell degeneration and that is characterized by the loss of nuclei and perturbation of mitochondria pattern, is blocked in almost all supressor mutants examined. Future work will reveal the identity of the mutated genes and hopefully shed more light on the role of Serotonin in muscle function.

BACK

# Implication of the Dense Body Protein Dyc-1 in the Dystrophin Dependent Muscle Degeneration

**Claire Lecroisey,** Kathrin Gieseler and Laurent Ségalat
CGMC, CNRS-UMR 5534, Université Claude Bernard Lyon-1, 16 rue Raphaël Dubois, 69622 Villeurbanne cedex, France

The molecular mechanism underlying muscle necrosis in dystrophinopathies remains elusive. Our group addresses this question by using the genetically amenable animal model *Caenorhabditis elegans,* which has the same overall sarcomere composition and architecture as those of vertebrates.

In *C. elegans*, mutation of the dystrophin homologue, *dys-1(cx18)*, produces a peculiar behavioural phenotype (hyperactivity, a tendency to hypercontract). In a sensitized *hlh-1(cc561ts)* background, which is a mild mutation of the myogenic factor MyoD, the *dys-1(cx18)* mutation also leads to a progressive muscle necrosis.

The *dyc-1* gene was previously identified in a genetic screen because its mutation leads to a phenotype similar to that of *dys-1(cx18)* mutation, which suggests that the two genes are functionally linked. Like *dys-1(cx18); hlh-1(cc561ts)*, the double mutant *dyc-1(cx32); hlh-1(cc561ts)* also shows a progressive muscle degeneration. Moreover, Dyc-1 overexpression partially suppresses the *dys-1(cx18); hlh-1(cc561ts)* phenotype.

In the sarcomere, Dyc-1 is localized at the edge of the dense body, the nematode muscle adhesion structure functionally equivalent to vertebrate Z disc, where actin filaments are anchored. Two hybrid and immunocytochemistry experiments indicate that Dyc-1, Deb-1 (the vinculin homologue and main component of dense bodies), Zyx-1 (a focal adhesion protein), and Atn-1 (alpha-actinin homologue) may form a complex at the dense body. Our results suggest that the dense body might be the site of early pathological events occurring in the absence of dystrophin.

We are currently trying to demonstrate that dystrophin impairs the function of dense bodies proteins.

BACK

# Identification of Genes Affecting Resistance to Anoxia in *Caenorhabditis elegans*

**V. Menuz[1],** M. Fornallaz[1], M. Gomez[1], M.O. Hengartner[+] and J.C. Martinou[1]
[1]Department of Cell Biology, Science III, 30 quai Ernest Ansermet, 1211 Geneva 4, Switzerland; [2]Institut für Molekularbiologie, Uneversität Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland

In obligate aerobe organisms, molecular oxygen is necessary for the production of ATP through the electron transport chain in mitochondria. Adult *C.elegans* have evolved the capacity to survive more than 24 hours in the complete absence of oxygen (anoxia). We found that up to 90% of young adults (72 hours post L1 stage) could tolerate 2 days of anoxia whereas the number of survivors dropped to less than 5% at 3 days of anoxia. Our goal is to understand the mechanisms that underlie resistance of this animal to anoxia. For this, we screened for mutants that either survive longer than 3 days or that are more sensitive that controls.

A random screen of a mutant library allowed us to identify eight mutants which were unable to survive anoxia. Among those mutants, the strongest phenotype was displayed by the *srf-3(yj10)* mutant whose survival did not exceed 3% after 2 days of anoxia. The *srf-3(yj10)* mutated allele comes from a random EMS mutagenesis. After analysis, we found that the cause of the sensitivity was due to another mutation in the *srf-3(yj10)* background that we called *Sensitivity to Anoxia 1* (*sta-1*).

Mutants able to survive anoxia were isolated by screening synchronous population of F2 progeny animals mutagenized with ENU. We identified a mutant which we called *Resistant to Anoxia 1* (*rta-1*) whose survival is more than 70% after 3 days of anoxia.
Identification of both *sta-1* and *rta-1* is undergoing.

BACK

# PCB52 Induced Gene Expression in the Nematode *Caenorhabditis elegans*

**Ralph Menzel**[1], Jana Kulas[1], Hui Ling Yeo[1], Shuang Li[1], and Stephen Stürzenbaum[2]
[1]Leibniz-Institut of Freshwater Ecology and Inland Fisheries, Müggelseedamm 301, 12587 Berlin, Germany; [2]School of Biomedical & Health Sciences, King's College London, 150 Stamford Street, London SE1 7NH, United Kingdom

Polychlorinated biphenyls (PCBs) are ubiquitous organic chemicals which can affect numerous biological responses. Because PCBs are toxic to man, recalcitrance to degradation and persist in the environment, they are listed in the inventory of priority pollutants compiled by numerous Environmental Protection Agencies.

Here we present an investigation of PCB52, an *ortho*-substituted, non-coplanar 2,2',5,5'-tetrachlorbiphenyl. PCB52 affected the reproductive capacity of *C. elegans* in a strict concentration dependent manner, thereby highlighting its toxicological significance. Based on the calculated $EC_{20}$ (5 mg/L), whole genomic DNA microarray experiments were performed to identify differentially expressed genes. Using a two-fold increase/decrease cut-off, 1158 genes were up-regulated and 560 down-regulated by PCB52. Up-regulated genes constituted family members of small heat shock proteins, chemoreceptors, nuclear hormone receptors, lipases as well as cytochromes P450 (CYP) and short-chain dehydrogenases/reductases (SDR), which are known to be involved in the biotransformation of persistent organic pollutants. Significant enriched classes of down-regulated genes are involved in organelle biogenesis, cell integrity and further general physiological processes, such as macromolecule metabolism and nucleotide binding.

The induction of five highly differentially regulated gene classes was confirmed by semi-quantitative RT-PCR experiments. Using a multiple RNAi approach the consequences of gene knockdown of single genes as well as entire gene subfamilies was determined. The efficiency of RNAi was proven by RT-PCR. Whilst GST and SDR gene expression knockdown intensify the toxicity of PCB52 (reflected in a reduced reproductive output) the knockdown of several CYP forms increased the reproductive capacity. This may indicate that the lack of CYP gene expression prevents the generation of toxic metabolites.

BACK

***P-137***

# The Role of SHC in Stress Response and Innate Immunity

**L. Moronetti Mazzeo**, J. Solinger, A. Sommer, G. Cassata
IFOM, Istituto Firc di Oncologia Molecolare, Milan, Italy

Shc proteins are molecular adaptors, involved in several signal transduction pathways. The conserved amino-to-carboxy terminal order of domains is: PTB to SH2 (both are protein-protein interaction domains). Two *C. elegans* orthologs with the same modularity have been identified: F54A5.3a and T27F7.2. Since, in all other species, this modularity is the signature of Shc-like proteins, Luzi et al. proposed that F54A5.3a and T27F7.2 correspond to nematode homologs of Shc. We termed these two genes *shc-1* and *shc-2* respectively. 3 deletion alleles are available until now: *shc-1(ok198)*, *shc-2(tm028)* and *shc-2(tm030)*. The first one is a putative null-mutant, the second and third, abolish only the PTB domain.

In mammals SHC was shown to be involved in several functions such as growth, apoptosis, stress resistance and lifespan. In order to understand if Shc-like proteins play a similar role in worms, we focused our attention on two evolutionarily conserved pathways: a) the Daf-2/Insulin-like pathway, that in worm is responsible for lifespan and stress response, and b) the MAPK/JNK pathway, that among other roles is important for stress resistance from nematodes to mammals. The *C. elegans* Interactome project has revealed that SHC-1 physically interacts with MEK-1, the homolog of mammalian MKK7, which regulates pathogen resistance through the phosphorylation of PMK-1(p38) and KGB-1(JNK).

As a first approach we wanted to know whether *shc-1* and *shc-2* are involved in one of these two pathways. Lifespan analysis revealed that the single mutants of *shc-1* or *shc-2* or double mutants, resulted in a shorter lifespan compared to the control. Both *shc-2* alleles result in a less severe phenotype than *shc-1*, but did not enhance the phenotype in the double mutant. Testing the *shc* mutants for "enhanced susceptibility to pathogens" (*esp* phenotype) we performed feeding experiments with Pseudomonas aeruginosa. The results showed that *shc-1* (similar to mutants of the MAPK/JNK pathway) was more sensitive to pathogen induced stress than wt. In addition, the absence of SHC-1 in *daf-2* loss of function alleles suppressed their enhanced stress resistance phenotype, but still remained more resistant to pathogens than wt worms, implying the existence of other pathways conferring resistance to pathogens. These could be also controlled by the *daf-2* signaling pathway.

Furthermore we have generated transgenic lines expressing full length SHC-1 under its own putative promoter. This transgene was able to rescue the *esp* phenotype. Expression analyses revealed that *shc-1* is ubiquitously expressed and downregulated in a daf-16 mutant background. Our preliminary results indicate that *shc-1* and *shc-2* may play a critical role in lifespan regulation; possibly through regulation of stress response and innate immunity.

BACK

# A Knock-Out of the Small Non-Coding Y RNA in *C. elegans*

**Michael Müller**[1], Verena Jantsch[2], Michael Jantsch[2], Günter Steiner[1]
[1]Department of Medical Biochemistry, Medical University of Vienna, Austria; [2]Department of Chromosome Biology, University of Vienna, Austria

Ro ribonucleoproteins (RNPs) are small cytoplasmic particles of unknown function that have been found in all eukaryotes except yeasts. The core structure of human Ro RNPs is composed of one molecule of Y RNA to which the 60 kD Ro protein (Ro60) and La protein are stably bound. Ro RNPs are predominant target structures in the autoimmune diseases Systemic Lupus Erythematosus and Sjögren's syndrome and autoantibodies to Ro60 and La, can be frequently found in the sera of patients suffering from these diseases. The Ro60 protein is highly conserved in higher eukaryotes and the phenotype of a disruption of the gene encoding the *C. elegans* Ro60 homologue (*rop-1*) was characterized (Labbé et al. *Genetics* 1999, *PNAS* 2000). A decrease in Y RNA levels and a higher frequency of misfolded ribosome-associated 5s rRNAs was observed in *rop-1* mutant worms. *Rop-1* mutants were also shown to be defective in the formation of *dauer* larvae. Recently the structure of Ro60 was established (Stein et al. *Cell* 2005) and it was shown that the Y RNA binding site of Ro60 overlaps with the binding site for misfolded RNAs. These results suggest a regulatory role the Y RNA for the binding of misfolded RNAs to Ro60.

In humans and most other vertebrates four different Y RNAs are expressed, while in the nematode *C. elegans* only one Y RNA species has been found. The *C. elegans* Y RNA is highly conserved in its structure and most closely related to human Y3 RNA. Yet it differs from other eukaryotic Y RNAs as it is missing a 3' extension to which La is binding (van Horn et al. *RNA* 1995).

To learn more about the function of Y RNAs, we took advantage of the fact that the *C. elegans* genome contains only a single Y RNA gene (*yrn-1*). We generated a knock-out line of the *C. elegans* Y RNA by the method of gene targeting by biolistic transformation. *Yrn-1* is located within an intron of an uncharacterized, protein-coding gene. We modified a method for gene disruption (Berezikov et al. *Nucleic Acids Res.* 2004) in our approach, as we deleted *yrn-1* in our knock-out construct rather than disrupting the locus, which could have resulted in a double knock-out.

*Yrn-1* mutant worms do not display any obvious morphological phenotype. However, preliminary results indicate that Y RNA deficient worms show a delayed response to chemoattractants. Future experiments are planned to elucidate the role of the Y RNA in damage repair upon UV induced damage, in coping with various forms of stress and in the *dauer* formation pathway.

BACK

# Bleomycin-Induced Fibrosis in *C. elegans*?

**Aggeliki Pasparaki**[1] Nektarios Tavernarakis[1] and Eleni Tzortzaki[2]
[1]Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology Hellas, Heraklion, Crete, Greece; [2]Department of Thoracic Medicine, Medical School, University of Crete, Heraklion, Crete, Greece

Bleomycin is an antibiotic drug isolated from a strain of *Streptomyces verticillus*, which has anticancer properties and acts by induction of DNA strand breaks. Due to its cytotoxic properties, the drug has been used to treat many malignant tumors. Bleomycin is also used to induce interstitial pulmonary fibrosis in rodents. Pulmonary fibrosis is a life-threatening condition of unknown molecular etiology in humans. Pulmonary fibrosis affects the alveolar epithelium, the capillary endothelium and the pulmonary basement membrane, where gas exchange is taking place during respiration, and results in abnormal collagen deposition in these tissues. In *C. elegans*, respiration is achieved by diffusion of oxygen via the intestinal tract, and through the aqueous film surrounding the body, resembling gas exchange in lung alveoli.

We are investigating the effects of bleomycin on *C. elegans* survival and physiology. Exposure of worms to bleomycin results in abnormal locomotion, feeding, egg-laying and morphological defects, and lethality. These phenotypes range in severity in a dose dependent manner. The most severe phenotypes are not reversed after removal of the drug. Interestingly, extended exposure to low doses of bleomycin alters the appearance of the cuticle and hypodermis, indicating that similarly to mammals, bleomycin may affect the collagenous exoskeleton of worms by modifying collagen deposition. Thus, genetic analysis in *C. elegans* may reveal conserved bleomycin targets relevant to the pathogenesis of pulmonary fibrosis in humans.

BACK

# *Genome-wide Screen for Heat Shock Regulatory Networks in* C. elegans

**Klaus Richter**, Richard I. Morimoto
Northwestern University, Evanston, USA

The response of organisms to heat results in the induction of a ubiquitous set of proteins, termed heat-shock proteins (Hsps). The critical transcription factor in this response has been found in all eucaryotic species to be the protein Hsf-1. While molecular concepts have been developed in single-cell systems, like yeast and mammalian cell culture, the thorough understanding of the heat-shock repsonse is still lacking in multi-cellular systems. To address questions like tissue-specific expression and regulation of the heat-shock response, we used an Hsp70-reporter strain in combination with genome-wide RNAi and identified modifiers of the response based on differences in the tissue-distribution of the expressed GFP. We identified 54 genes that upon knock-down caused constitutive expression of the Hsp70-reporter, many of which belong to genes involved in protein folding, secretion and degradation. In addition we identified about 150 genes required for the induction of the heat-shock response. Most of these proteins were found to be involved in mRNA and protein production processes and a striking overlap has been found to genes known to supress polyQ-induced protein aggregation. The combination of these results allows to obtain a working model of the systems involved in the induction and regulation of this apparently highly complex cellular response.

BACK

# Riboflavin Derivatives Improve Physiology and Growth of Frataxin Deficiency in *C. elegans* and *S. cerevisiae*

**Ros S[1],** Gonzalez-Cabo P[1], Vazquez-Manrique R[1,2], Palau F[1]
[1]Laboratory of Genetics and Molecular Medicine, Instituto de Biomedicina, CSIC, Valencia, Spain.[2]Department of Zoology, University of Cambridge, Cambridge, UK.

Friedreich ataxia is an autosomal recessive neurodegenerative disorder caused by mutations in the *FRDA* gene. The product of the gene is frataxin, an 18 kDa soluble mitochondrial protein. Frataxin function in mitochondria has not been well explained yet. Lack of frataxin homologues in yeast and mice leads to iron accumulation in mitochondria, increased sensitivity to oxidative stress, depletion of proteins with iron-sulfur clusters like respiratory chain complexes, and altered oxidative phosphorylation.

*S. cerevisiae* frataxin orthologue Yfh1p interacts physically with succinate dehydrogenase complex subunits Sdh1p and Sdh2p of the mitochondrial electron transport chain and also with Etfα and Etfβ subunits from the electron transfer flavoprotein complex (Gonzalez-Cabo P et al. Hum Mol Genet 2005;14:2091-8)
Knock-down model of the *C. elegans* frataxin deficiency by RNAi show slow growth, eggs laying defects, reduced brood size, altered defecation and reduced lifespan. Genetic synthetic studies show interaction between *frh-1* and *mev-1* (gene encoding the succinate dehydrogenase cytochrome b subunit of complex II in mitochondria) that again suggest a role of frataxin in the mitochondrial respiratory chain (Vazquez-Manrique RP et al. FASEB J 2006;20:172-4).

Therapeutic strategies in Friedreich ataxia are focused on antioxidants treatment with coenzyme $Q_{10}$ or its short-chain variant idebenone. We have addressed the analysis of alternative treatments with riboflavin. Two derivatives, riboflavin 5' phosphate (flavin mononucleotide [FMN]) and riboflavin 5' adenosine diphosphate (flavin adenine dinucleotide [FAD]), are cofactors in flavoprotein enzymes. Most of the flavin coenzyme systems help to regulate cellular metabolism.

After microinjection, *frh-1* (RNAi) worms were grown in plates with FAD (0.1μM, 5 μM, 20 μM and 40 μM), FMN (0.5 μM) or riboflavin (0.5 μM). We compared *frh-1* (RNAi) worms treated with drugs and *frh-1*(RNAi) worms without drugs. We analysed defecation, brood size of F2, lifespan and sensitivity to oxidative stress. Lifespan is recovered with these cofactors ($p<0.005$) and defecation and brood size of F2 is recovered depending on the cofactors concentration. The sensitivity to oxidative stress is not reduced with these drugs. On the other hand, we also observed growth recovery of the yeast frataxin mutant *Yfh1Δ* when grown in complete YPD medium and ethanol/glycerol medium in the presence of FAD.

BACK

# Use of Transgenic *Caenorhabditis elegans* (HS-1 line) for Detection of Heavy Metals in Aquatic Media

**Stormberg A. I.**[1], Barnes, J. M.[1], Humphries S.[2], Glenn T.[3], and Williams P. L.[4]
[1] Idaho National Laboratory, MS 2213, PO Box 1625, Idaho Falls, Idaho 83415, USA; [2]Stratus Consulting, Inc, Environmental and Energy Research, P.O. Box 4059, Boulder, CO 80306-4059, USA; [3]Savannah River Site, Drawer E, Aiken, South Carolina 29802, USA; [4]Department of Environmental Health Science, University of Georgia, Athens, Georgia 30602-2102, USA.

Detection and quantification of contaminants in water and soil continues to be of paramount importance in all areas of human activities, especially in those where waste is generated, treated and disposed-of. Bioassays have become the standard method for monitoring hazardous waste, and numerous approaches exist in the literature describing the use of invertebrates as sentinels for environmental health. In this study we describe a rapid, inexpensive assay of contaminant bioavailability. We used a transgenic line (HS-1) of the nematode *Caenorhabditis elegans* to develop dose-response assays for aqueous solutions of cadmium, zinc, mercury, lead, and nickel. This transgenic line carries an integrated promoter from the *C. elegans* metallothionein-2 gene (mtl-2), which controls the transcription of the green fluorescence protein (GFP). This line provides a very fast and sensitive assay for heavy metal exposure since the response can be directly detected with a fluorimeter and does not require additional reagents or co-factors. At high metal concentrations, the system requires only 4 hours of exposure and at lower levels, up to 24 hours.. This study shows that there is a positive correlation between the concentration of the specific metal used in the assay and the fluorescent emission. Work in progress will focus on testing the transgenic line under a mix of metals. In addition we are interested in determining if there is a linear correlation between GFP emission and metal concentration. The ultimate goal is to design a qualitative indicator system for the presence of heavy metals in aquatic samples.

BACK

# *Mutations in a Predicted Galactosyl Transferase Affect the Ability of the Pathogen* M. nematophilum *to Infect* C. elegans

**Dave Stroud**, Maria Gravato-Nobre, Jonathan Hodgkin
**University of Oxford, Department of Biochemistry, South Parks Road, OX1 3QU, Oxford**

*Microbacterium nematophilum* has previously been found to be pathogenic in *C. elegans*, causing a Distorted Anal Region (Dar) phenotype, post infection.

Mutants have been generated which convey a bacterially unswollen (Bus) phenotype when grown in the presence of the pathogen. One of these mutations *(bus-2*) was localised to a 1.7Mb region of chromosome IV, between *dpy-9* and *egl-4*. When exposed to the pathogen, no tail swelling and no rectal colonization by the bacteria was observed, suggesting that the cuticle surface has been altered such that the pathogen can no longer adhere.

In order to clone *bus-2*, we examined the area in question by SNP mapping and were able to localise a candidate gene. We narrowed down the *bus-2* gene to a single ORF, which encodes a galactosyl transferase within family 31. Sequence alterations in four EMS alleles and three *mut-7* alleles have been determined. Current work involves investigating the expression pattern of the gene, further phenotype characterizations, and interactions with other *bus* genes.

BACK

# Environmental Genomics of Fluoranthene Exposure

Swain, S.C.[1], Wren, J.[1,2], Jonker, M.[2], Redshaw, N.[1], Svendsen, C.[2], Spurgeon, D.J.[2], Morgan, A.J.[1], Kille, P.[1] and **Stürzenbaum S.R**[1,3]
[1]Cardiff School of Biosciences, Cardiff University, PO Box 915, CF10 3TL, U.K; [2]Centre for Ecology & Hydrology, Monks Wood, Abbots Ripton, PE28 2LS, U.K; [3]School of Biomedical & Health Sciences, King's College London, SE1 7NH, UK

Benzo(j)Fluoranthene, is a 5-ring polycyclic aromatic hydrocarbon (PAH), known to occur as a product of plant biosynthesis, but also as a byproduct of incomplete combustion of organic material. Fluoranthene has been identified in cigarette smoke, char-broiled foods, drinking water, lake sediments and ambient air. It is thought to interact with DNA, cause heritable genetic disorders and has been associated with lung, intestinal and pharyngeal cancer. Information regarding the toxicity of fluoranthene in *C.elegans* still remains scarce, a shortfall we are aiming to redress. An elaborate toxicity test (using the embryonic lethal TS mutant cib-1) revealed a dose dependant "slippery pharynx" phenotype and effects on specific demographic endpoints, such as a reduction of egg-hatchability, brood size, volumetric growth and life span. In addition, whole genome oligo array analysis identified stage-specific (L1, L4 and adult) genes and pathways that are modulated at the transcriptional level in a dose-responsive manner. Here we present a summary of fluoranthene induced toxicosis ranging from the molecular and cellular level to effects on life history parameters.

BACK

# *Caenorhabditis elegans* is a model host for *Listeria monocytogenes*

**Line Elnif Thomsen[1],** Bente M. Jensen[2], Nils J. Færgeman[2], Birgitte H. Kallipolitis[2] and Hanne Ingmer[1]
[1]Department of Veterinary Pathobiology, The Royal Veterinary and Agricultural University; [2]Department of biochemistry and Molecular Biology, University of Southern Denmark

Several bacterial pathogens, both Gram-positive and Gram-negative, kill the nematode *Caenorhabditis elegans* when supplied as a food source, and a variety of bacterial virulence factors, known to play a role in mouse-models, have been shown also to play a role in nematode pathogenesis.

The gram-positive facultative intracellular food-borne pathogen *Listeria monocytogenes* is associated with serious invasive infections in humans and animals. We have investigated the possibility of using *C. elegans* as a model to analyse the virulence of various *L. monocytogenes* strains. We found that *L. monocytogenes* kill *C. elegans* over the course of several days, as a consequence of an accumulation of bacteria in the worm intestine, and that there is an overlap between *L. monocytogenes* virulence factors required for mammalian and nematode pathogenesis.

*C. elegans* is therefore an attractive model to study the virulence of this pathogen and potentially to identify new virulence factors and further work might reveal the genetic repertoire required for infection of the nematode by *L. monocytogenes*.

BACK

# KAL-1 Function in *C. elegans*

**C. Vicario**, E. Di Schiavi, G. Gelsomino, A. Sollo, S. Arbucci, P. Bazzicalupo
IGB-ABT,CNR, Via P: Castellino 111,80131, Naples, Italy

*kal-1* is the *C. elegans* homolog of *Kal1* the gene responsible in human for X-linked Kallmann Syndrome (KS), an inherited disorder defined by the association of hypogonadism and anosmia. The gene is expressed in a subset of *C. elegans* neurons. The phenotypes presented by loss of function and overexpression mutants indicate that *kal-1* modulates neurite branching *in vivo* and that is part of a redundant mechanism by which neurons influence epidermal cells morphogenesis (Rugarli et al., 2002; Bulow et al., 2002).

To investigate the molecular function played by KAL-1 *in vivo* we have produced specific antibodies and we have followed a structure-function relationship approach.
*Polyclonal antibodies have been produced against the N-terminal and the C-terminal part of the protein, to analyse the* in vivo *localization of KAL-1.*

To test the role played by each of the domains that form KAL-1 (cystein rich, WAP and Fibronectin domains) we assembled different constructs coding for truncated forms of the protein. KAL-1 full length protein is able to rescue the epidermal morphogenesis defects of *kal-1* loss of function mutants. In addition when overexpressed in a wild type background the KAL-1 full length produces highly penetrant ventral enclosure defects and male tail alterations. On the contrary the N-terminal part of KAL-1 protein, containing only the cystein rich and WAP domains, is neither sufficient to rescue the epidermal morphogenesis defects nor to cause any visible phenotype. The use of the antisera suggests that the protein is correctly produced and is not simply degraded.

In order to identify *kal-1* interactors we are setting up an RNAi enhancer/suppressor screen. To this end we have improved the phenotypic description of the male tail phenotypes and of the low penetrant axonal defects presented by *kal-1* loss of function mutants. Thus we have established new cells affected and novel neuronal functions played by *kal-1*.

The understanding of the cellular and molecular mechanisms of *kal-1* action and the identification of other genes involved in the processes in which KAL1 is involved might help elucidate the still elusive mechanism of action.

BACK

# Exploring Innate Immunity Signalling Networks

Daniel Wong, **Katja Ziegler**, C. Léopold Kurz, Nathalie Pujol & Jonathan Ewbank
Centre d'Immunologie de Marseille-Luminy, INSERM/CNRS/Université de la Méditerranée, Case 906, 13288 Marseille cedex 9, France

Using cDNA microarrays developed by Yuji Kohara, we previously showed that infection of worms by the bacterial pathogen *Serratia marcescens* and the fungus *Drechmeria coniospora* provoke the upregulation of distinct sets of antimicrobial genes (1, 2). We have now extended this analysis, using whole genome long oligo microarrays, to a number of other bacterial pathogens. We found that each pathogen elicits a specific transcriptional response.

We have also used microarrays to analyse the transcription response of *nipi-1* and *nipi-3* (see abstract by Pujol et al.) to fungal infection. In addition to an abrogation of induction of the *nlp-29* gene that encodes an antimicrobial peptide (AMP), a number of other AMP genes (*cnc* and *nlp*, as well as novel putative AMP genes) show an altered expression in the *nipi* mutants.

We have taken a candidate gene approach to investigate signalling pathways involved in AMP regulation, using the IG274 strain and the Union Biometrica COPAS sorter (see abstract by Pujol et al.). Full induction of p*nlp-29::GFP* requires the NSY-1/SEK-1/PMK-1 MAPK pathway that has been shown to act in the intestine to protect worms from infection by *Pseudomonas aeruginosa* (3). It does not require *kgb-1* or *jkk-1* activity, but is partially dependent on the MAP2K *mek-1*. Induction of p*nlp-29::GFP* is also strongly dependent upon the TIR-domain adapter protein TIR-1 that is upstream of NSY-1 (2,4). The TIR-1/MAPK pathway also has a role in neuronal cell fate determination, acting downstream of *unc-43* (5), but *unc-43* mutants exhibit normal expression of p*nlp-29::GFP*. It is therefore not known what lies upstream of *tir-1* in the AMP-activation pathway, nor how infection is recognised in *C. elegans*. We will report on our ongoing bioinformatics analyses of the transcriptional response of worms to infection that we hope will reveal the underlying signalling networks required for innate immune defence.

**1.** Mallo et al. Curr Biol 12, 1209 (2002). **2.** Couillault et al. Nat Immunol 5, 488 (2004).
**3.** Kim et al. Science 297, 623 (2002). **4.** Liberati et al. PNAS 101, 6593 (2004). **5.** Chuang & Bargmann Genes Dev 19, 270 (2005).

BACK

# The DOG-1 Helicase, Genomic Instability and Fanconi Anemia

**Jillian L. Youds**[1], Nigel J. O'Neil[1], Louise J. Barber[2], Simon J. Boulton[2] & Ann M. Rose[1]

[1]Department of Medical Genetics, University of British Columbia, Vancouver, Canada; [2]DNA Damage Response Laboratory, London Research Institute, South Mimms, England

In *Caenorhabditis elegans*, DOG-1 is required for the maintenance of polyG/polyC-tracts (G-tracts). In the absence of DOG-1, it is thought that G-tracts form secondary structures that block replication, leading to deletions that initiate in the G-tracts. Using our assay for deletions forming in the absence of DOG-1, we have assayed the *in vivo* contribution of various repair genes to the maintenance of these tracts. We show that DOG-1 and the BLM ortholog, HIM-6, act synergistically during replication; simultaneous loss of function of both genes results in replicative stress and an increase in the formation of small deletions that initiate in G-tracts. Similarly, we show that genes implicated in homologous recombinational repair and trans-lesion synthesis are required to prevent G-tract deletions in the *dog-1* background. However, genes essential to the non-homologous end-joining and nucleotide excision repair pathways do not appear to be involved in deletion prevention or formation. By investigating the mechanisms that maintain genomic stability in *dog-1* mutants, we might better understand the genomic instability associated with Fanconi anemia, as the human gene most similar to *dog-1*, *BRIP1*/*FANCJ*, was recently shown to be mutated in a subset of patients with Fanconi anemia, implicating it in interstrand cross-link (ICL) repair. In collaboration with the Boulton lab, we are currently investigating the possibility that DOG-1 and BRIP1 have functionally conserved roles in DNA repair. Our preliminary data indicate that *dog-1* mutants are sensitive ICL-inducing agents and we are assessing the relationship to *fcd-2* and other genes involved in ICL repair.

This research is funded by the Natural Sciences and Engineering Council and the Michael Smith Foundation for Health Research.

BACK

# Towards the Development of a *Minos*-based Transposon Tool in *C. elegans*

**Dafne Bazopoulou** and NektariosTavernarakis
Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology Hellas, Heraklion, Crete, Greece

We aim to develop a heterologous transposon system in *C. elegans*, based on the well-characterized *Minos* transposable element from *Drosophila hydei*, which has already been mobilized in several distinct species. The major advantage of *Minos* resides in its capacity to transpose while carrying long fragments of exogenous DNA. Therefore, *Minos* can be used as a vehicle to introduce foreign sequences in the genome. This feature can be used to establish enhancer-trap systems which would permit among other applications, genome-wide screens for gene regulatory regions in *C. elegans*. *Minos* could also be used for genome-wide insertional mutagenesis, as an alternative to the already established *Mos* trasnposon system.

We are developing a binary system for *Minos* transposition in the worm, similar to the one in use for the *Mos* element. We have generated separate lines expressing the transposase under the heat-inducible promoter and also lines carrying the *Minos* transposon on different extrachromosomal arrays. Furthermore, in order to test the capacity of *Minos* to carry exogenous DNA, we have generated lines harboring a modified *Minos* element, comprised of *Minos* ITRs along with an insert of ~2kb, encompassing a GFP reporter expressed in body wall muscles, driven by the *unc-54* promoter. We obtained double-transgenic lines by crossing animals carrying the transposase source array with animals carrying either the native *Minos* element or the one engineered to harbor the GFP reporter gene. We confirmed proper expression of the transposase in these lines after heat shock by RT-PCR. While, we have observed mobilization of *Minos* in somatic cells of *C. elegans*, we have yet to achieve germline transposition. We are currently in the process of optimizing various parameters of our procedure in an effort to detect transposition of *Minos* in the germline. We are also developing alternative methods for screening such, likely rare transposition events, based on fluorescent marker expression.

BACK

# *C. elegans* Reporter Fusion Genes Generated by Seamless Modification of Large Genomic DNA Clones

**Colin T. Dolphin**[1] & Ian A. Hope[2]
[1]Pharmaceutical Science Research Division, King's College London, 150 Stamford St, London SE1 9NH, U.K; [2]Institute of Integrative & Comparative Biology, Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT, U.K

By determining spatial-temporal expression patterns, reporter constructs can provide significant insight into the function of *C. elegans* genes. An improved reporter gene fusion would contain significant stretches of the 5' and 3' sequence flanking the protein-coding region to maximize the chances of including all associated regulatory elements such that the resulting expression pattern would be highly likely to recapitulate that of the endogenous gene. Furthermore, it would also be desirable, for various reasons, to be able to insert the reporter sequence, seamlessly and in-frame, at any specific position within the target gene. Lastly, the construction method should not be overly complicated and be potentially adaptable for high-throughput construct generation. We have developed such a method.

Reporter genes were inserted precisely, by homologous recombination in *E.coli*, into *C. elegans* genes cloned in fosmids. The homologous recombination, commonly referred to as recombineering, is mediated by the bacteriophage lambda Red system. This system is coupled with a simple and robust two-step counter-selection protocol; first a cassette with both positive and negative selectable marker genes is inserted at the target location and the cassette is then replaced with the reporter gene. This procedure allows straightforward, flexible and precise construction of translational reporter gene fusions directly from *C. elegans* fosmid clones with minimal alterations to the target gene.

We have used the approach to insert either *gfp* or *cfp* precisely at the *C*-termini of three *C. elegans* target genes, each located centrally within the approx. 35-40kb inserts of different fosmid clones and examined previously using conventional reporter approaches. Recombineered fosmids were used in transformation of *C. elegans* by microinjection. Resulting transgenic lines revealed reporter expression consistent with previously published data for the tagged genes and also provided additional information including subcellular distributions. This simple and straightforward method generates reporter gene fusions highly likely to recapitulate endogenous gene expression and thus represents an important addition to the functional genomics toolbox. Its flexibility means the procedure will have many applications.

BACK

# A French Functional Genomics Platform

Yohann Duverger[1], Jérôme Reboul[2], Daniel Wong[1] and **Jonathan Ewbank[1]**
[1]Centre d'Immunologie de Marseille-Luminy, INSERM/CNRS/Université de la Méditerranée, Case 906, 13288 Marseille cedex 9, France ; [2]INSERM UMR 599, Institut Paoli Calmette, 13009 Marseille, France

For the last three years, we have offered a service of worm sorting based around the Union Biometrica COPAS platform. The COPAS machine is equipped with a Zymark twister robot, allowing automated analysis of multiple 96 well plates. We recently upgraded the machine to include the Profiler II that generates >1000 individual measurements per worm simultaneously for up to 4 channels (including 2 fluorescent). This equipment has been applied to a wide range of biological questions. They include quantifying the level of fluorescent reporter gene expression, large-scale RNAi and genetic screens and combinatorial library drug screening. Examples of each will be presented.

This platform is part of a fully integrated functional genomics facility open to the academic community (see http://www.ciml.univ-mrs.fr/EWBANK_jonathan /RIO.html). Other resources include the ORFeome (developed in Marc Vidal's laboratory), and Julie Ahringer's RNAi library, together with whole-genome microarrays. For the latter, through a collaboration with the Genome Sequencing Center at Washington, and the transcriptome platform at Nice, we have spotted the Illumina long oligo set onto glass slides and provide microarrays free of charge to the French *C. elegans* community and at cost price to academic researchers in Europe.

This French functional genomics platform has been made possible through funding from the National Genopole® network, Marseille-Nice genopole®, the CNRS and support from Union Biometrica.

BACK

# Development and Optimisation of *Mos1* for Gene Tagging in *C. elegans*

**Joseph Gallagher** and Patricia Kuwabara
University of Bristol, Department of Biochemistry, United Kingdom

The *Drosophila* transposon, *Mos1*, has been used successfully to generate insertional mutants in *C. elegans*[1]. *Mos1* can be mobilised in *C. elegans* worms, which carry two independent extrachromosal arrays, one encoding the *Mos1* transposase under the control of a heat shock promoter, and the other carrying copies of the *Mos1* transposase substrate. Heat induction of the transposase leads to the mobilisation of the transposon and its integration within the *C. elegans* genome. Stable *Mos1* insertions are detected by PCR after animals have been clonally passaged for a number of generations.

As a member of the NemaGENETAG Consortium, which is funded through EU fp6, we are working with our partners to optimise the already existing *Mos1* tools and to develop new strains to facilitate the high-throughput screening of *C. elegans Mos1* insertional mutants. The generation of *C. elegans* transposon tagged genes will enhance our understanding of gene function, especially those that are associated with human disease, and provide an invaluable resource to the research community.

Here, we present our preliminary results, which were obtained after screening a library consisting of almost 1000 strains. So far, from this and subsequent screens, over 300 *Mos1* strains have been identified by PCR. Each of these strains has been frozen at –80 C in triplicate, test thawed and re-tested to validate the presence of a *Mos1* insertion. Many of the characterised strains carry multiple copies of *Mos1* inserted at different sites, so the actual number of tagged loci is greater than the number of strains frozen. Steps are now being taken to improve the rate of transposition and the recovery of mutants.

1. Bessereau, J. L. et al. Mobilization of a Drosophila transposon in the *Caenorhabditis elegans* germ line. *Nature* **413**, 70-4 (2001).

BACK

# Protocol Optimization to Localize Transposon Insertions in C. *elegans* Strains

**L. Granger,** E. Martin, E.Vallin and L. Segalat
CGMC, CNRS-UMR 5534, University Claude Bernard-Lyon I, France

The NemaGENETAG project contributes to the constitution of a genome-wide collection of identified C. *elegans* mutants. The transposable element MOS is used to generate these insertions.

We determine the location and characterize these insertions by inverse-PCR and sequencing. The goal is to create a database which includes all MOS-tagged strains of C. *elegans* for the international scientific community.

The Inverse PCR is used to clone amplify DNA flanking a known sequence. DNA is first digested and ligated. PCR primers directed against the known sequences are used to amplify the flanking sequences and permit their sequencing. Percentages obtained by treating strains with two enzyme (HaeIII and MboI) are inferior to predictions based on the distribution of restriction sites (80%). We are investigating the causes of this discrepancy.

As part of the optmization of protocols, we have tested alternative protocols to the current SWIP (Single Worm Inverse PCR) method. The three modifications tested are the TAIL-PCR, a new set of restriction enzyme and the suppression of the re-amplifiaction step.

TAIL (Thermal Asymatric InterLaced) PCR, is a simple tool used for the recovery of DNA fragments adjacent to known sequences. With this method amplified products can be sequenced directly after the treatment. The number of samples which gave a PCR product by TAIL-PCR was similar to the number obtained by SWIP. The genome positions obtained by TAIL-PCR and by SWIP match in only 70% of the cases. Fewer insertions could be recovered with sequence-specific primers with TAIL-PCR than with SWIP, suggesting that some TAIL-PCR products do not correspond to real insertions.

In addition, we try other restriction enzymes for inverse PCR protocol, which would increase the proportion of PCR bands obtained from the strains. MseI, BfaI, DraI/EcoRV have been tested.
Finally, we wondered whether PCR products could BE sequenced without re-amplification.

Results of these tests will be presented at the meeting.

BACK

# Screen for Synthetic Lethality with Tumor Suppressors and the Development of a DSB Repair Assay

**Daphne B. Pontier**, Ronald H.A. Plasterk and Marcel Tijsterman
Hubrecht Laboratory, Institute for Biomedical Research, Utrecht, The Netherlands

Synthetic lethality is the phenomenon that a mutation in gene X or gene Y is viable, whereas inactivation of both X and Y simultaneously is lethal. Recently, the breast cancer susceptibility gene BRCA-2 was found to be synthetic lethal with PARP-1 in human cell cultures and in mice, possibly making PARP a potent and specific drug target for breast cancer in humans (Bryant *et al*, 2005; Farmer *et al*, 2005). Using *C. elegans*, we aim to identify additional synthetic lethal interactions with several tumor suppressor genes, such as *dog-1* (BACH1/FANCJ), Y41E3.9 (FANCD2), *pme-1* (PARP-1), *pme-2* (PARP-2), *cep-1* (p53) and *atm-1* (ATM). At least two alleles will be used for each gene to prevent nonspecific interactions due to background mutations. These alleles are used in a genome-wide liquid RNAi screen for synthetic lethality, by comparing the presence of offspring in mutant alleles to N2. Positive hits will be further validated and checked for homology in humans.

For this screen, we will isolate new mutant alleles from our EMS library. We use LIMSTILL software (available at http://limstill.niob.knaw.nl) for amplicon selection, primer design, sequence analysis and mutation annotation, facilitating high-throughput sequencing. Up to 3072 sequences can be processed and aligned simultaneously and can therefore be easily screened for mutations.

In addition, we are optimizing a reporter assay for double strand break (DSB) repair. We have developed transgenic animals that carry a reporter LacZ sequence interrupted by the restriction site of the yeast rare-cutting endonuclease I-SceI. Animals also carry a *hsp::I-SceI* array that allows the introduction of a localized DSB in the LacZ reporter sequence by heatshock treatment. Inadequate repair of the DSB can be detected by LacZ expression or by PCR of the sequence flanking the DSB. This assay can be used to study localized DSB repair and may also be used for genome-wide screens for additional DSB-repair and signaling genes.

BACK

# Genomic Engineering of Acetylcholine Receptors by *Mos*TIC

**Georgia Rapti**, Valérie Robert and Jean-Louis Bessereau
Ecole Normale Supérieure-INSERM U789, 46 rue d'Ulm, 75005 Paris, France

Acetylcholine receptors (AChRs) sensitive to levamisole, a nematode-specific nicotinic agonist, are hetero-pentameric ligand-gated ion channels that mediate fast excitatory neurotransmission at *C. elegans* neuromuscular junctions. Immunostaining demonstrates that these receptors are clustered in small post-synaptic domains. However, tagged AChR subunits expressed from transgenes are often not properly assembled into functional receptors or tagged receptors fail to cluster, most probably due to inappropriate expression levels. To generate tagged AChRs that would recapitulate the expression and the subcellular localization of the endogenous protein in wild-type and mutant backgrounds, we have started to engineer chromosomal loci encoding AChR subunits. We are using *Mos*TIC (*Mos*1 induced transgene-instructed gene conversion), a technique recently developed to create custom alleles in the *C. elegans* genome. During *Mos*TIC, a *Mos1* transposon insertion is excised following the expression of the Mos transposase. *Mos1* excision creates a double strand break (DSB) that can be repaired by homologous recombination using a transgene as a repair template. During this repair process, custom mutations contained in the repair template will be copied into the genome near the DSB site.

We undertook *gfp* knock-in in *unc-63,* a locus coding for an α-subunit of the levamisole-sensitive AChR. A mutagenic *Mos1* insertion was recovered in the region encoding the M3-M4 cytoplasmic loop of UNC-63. GFP or Venus, a YFP variant, has been introduced in-frame in a genomic *unc-63* rescuing fragment at a position close to the *Mos1* insertion point. Tagged proteins have been expressed from extra-chromosomal arrays to test their functionality. We demonstrated that these UNC-63 fusions were able to rescue the *unc-63* mutant phenotype and were detected at neuromuscular junctions. We next designed repair templates that contain *gfp* flanked by *unc-63* sequence. Flanking sequences are long enough to enable recombination but cannot rescue the mutant phenotype. *Mos*TIC events will reconstitute a functional *unc-63* locus and will be identified based on reversion of the mutant phenotype.

In the near future, the *Mos*TIC technique will be used to tag other AChR subunits. We are currently performing a *Mos1* mutagenesis to isolate additional *Mos1* insertions into AChR subunit genes. Progress from this screen will be presented.

BACK

# Progress of the Localization of Expression Mapping Project

**Jane Shingles**[1], John Reece-Hoyes[1], Denis Dupuy[2], Marc Vidal[2] and Ian Hope[1]
[1]Institute of Comparative and Integrative Biology, University of Leeds, Leeds, LS2 9JT, United Kingdom; [2]Centre for Cancer Systems Biology and Department of Cancer Biology, Dana Farber Cancer Institute and Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115, USA

The Promoterome library containing 6500 *Caenorhabditis elegans* promoter fragments has previously been generated and used to construct promoter ::GFP fusions to allow determination of gene expression patterns in *C. elegans*. Optimization of the *C. elegans* micro-particle bombardment transformation technique, to give a medium through-put system, allowed the generation of expression patterns for over 300 *C. elegans* promoter::GFP fusions. Promoter fusion constructs of transcription factors genes and genes with homology to human genes with no assigned function were selected for the initial analysis and the results are shown.

Initial analysis of the results highlights several significant differences between the two sets of genes. Promoters from *C. elegans* homologues of human genes with no known function were far more likely to yield either no GFP expression (35% vs. 6%) or ubiquitous GFP expression (23% vs. 8%) under the standard laboratory conditions utilized. In contrast, promoters from *C. elegans* transcription factor genes were far more likely to drive neuronal expression (62% vs.16%).

Full results are presented on the Hope Laboratory Expression Pattern database – URL:http://bgypc059.leeds.ac.uk /~web

BACK

# CGC and WormBase

**Mary Ann Tuli**[1], Jonathan Hodgkin[2], Theresa Stiernagle[3], Robert Herman[3], Richard Durbin[1]
[1]WormBase, Sanger Institute, Cambridge, UK; [2]University of Oxford, Oxford, OX1 3QU, UK; [3]University of Minnesota, Minneapolis, MN 55455, USA

WormBase is an international consortium of biologists and computer scientists from Caltech (USA), Cold Spring Harbor Laboratory (USA), Wellcome Trust Sanger Institute (UK) and the Genome Sequencing Center at Washington University (USA).

WormBase is dedicated to providing the research community with accurate, current, accessible information concerning the genetics, genomics and biology of *C.elega*ns, and some related nematodes. WormBase can be freely accessed at www.wormbase.org, and is also available for download at ftp://ftp.wormbase.org/pub/wormbase. A new data release is produced every three weeks.

The UK WormBase group works closely with the Caenorhabditis Genetics Center (CGC) to curate genetic nomenclature and maintain the *C.elegans* genetic map. Detailed guidelines are accessible via http://www.cbs.umn.edu/CGC/Nomenclature/nomedguid.htm
All CDSs, transcripts and pseudogenes are assigned an identifier of the form WBGene00000001. This identifier is unique and remains stable when the gene object is updated. Release WS150 (November 2005) contains 44644 WBGeneIDs of which 25043 are from *C.elegans*, and 19587 from *C.briggsae*. The number of CDS objects, which have been assigned a CGC approved gene name is 6638.

In release WS140 (March 2005) the Variation class was introduced as a more efficient way to handle most of what was in the Allele and Locus class. The Variation class incorporates the following: Alleles, SNPs (both confirmed and predicted), RFLPs and transposon insertions. Curation of allele data by WormBase biologists is an ongoing project and in response to the WormBase 2005 Users survey (http://www.wormbase.org/announcements/newsletters /pdf/2006-01.pdf) we will curate these more intensely. Allele information is either captured from scientific journals or submitted directly be researchers using the WormBase submission forms at http://wormbase.org/db/curate.base.

WormBase is supported by a grant from the National Human Genome Research Institute at the US National Institute of Health #P41 HG02223 and the British Medical Research Council. CGC is supported by NIH NCRR.

BACK

# Molecular Characterization of MOS Transposon-Tag Strains Produced by the NemaGENETAG Project

**Vallin E.,** Granger L., Martin E. & Segalat L.
CGMC, CNRS-UMR 5534, Université Lyon 1, France

A collection of *Caenorhabditis elegans* insertion mutants is being created as part of the NemaGENETAG project. The aim is to produce characterized transposon-tagged mutants for the European and international scientific community. We use the *Drosophila* mariner element Mos-1, which has been shown to be active in *C.elegans*.

The J. Ewbank laboratory (Marseille) generates and provides us with Mos-positive strains, which we molecular characterize. The localization of the insertion is determined by inverse PCR and sequencing. The sequence is compared with the *C. elegans* genome sequence to determine the insertion sites using the blast program. At the beginning of 2006, we have characterized approximately 2000 strains. Strains are currently analyzed sequentially with enzymes Hae III and Mbo I. Approximately 70% of the strains received from Marseille give a workable PCR band in these conditions. The percentage of PCR bands that give an unambiguous genome localization is approximately 66%. These strains are frozen in triplicate in both -80°C freezer and liquid nitrogen.

In case of request or for quality control, strains are thawed and PCR-tested with a Mos primer and an insertion-specific primer to check the presence on the insertion. On 30 strains tested in recovery tests, 28 were positive. These results prove that the insertions are stable and can be easily recovered from frozen samples.

The aim of the project is to generate and provide strains for the worm community. The list of the insertions can be found on a database available on the web.

BACK

# Curating Wormbase Gene Structures

**Gary Williams[1],** Paul Davis[1], Anthony Rogers[1], Philip Ozersky[2], John Spieth[2], Tamberlyn Bieri[2], Darin Blasiar[2]
[1]WormBase, Sanger Institute, Cambridge, UK; [2]The Genome Sequencing Center Washington University School of Medicine, St. Louis, MO, USA

WormBase is an international consortium of biologists and computer scientists from Caltech (USA), Cold Spring Harbor Laboratory (USA), Wellcome Trust Sanger Institute (UK) and the Genome Sequencing Center at Washington University (USA).

WormBase is dedicated to providing the research community with accurate, current, accessible information concerning the genetics, genomics and biology of *C. elega*ns, and some related nematodes. WormBase can be freely accessed at www.wormbase.org, and is also available for download at ftp://ftp.wormbase.org/pub/wormbase A new data release is produced every three weeks.

Recently some new datasets have been added to the database to aid curation.The protein products from automated gene prediction in *C. remanei* have been mapped to the *C. elegans* genome; InterPro motifs are no longer taken from SwissProt annotations, but are directly predicted from the *C. elegans* proteins; contigs of ESTs from other nematode species produced by the NEMBASE and Nematode.net projects have been mapped to the genome and a new set of TEC-REDs have been mapped to the genome resulting in 3,325 new trans-spliced sites which mark the 5' ends of genes or isoforms. Work is underway to classify and curate transposons and we intend to add Mass-Spec data from several sources to improve coding sequence validation.

Over the last year the curated coding sequences have increased from 19,854 to 20,060 and alternately spliced isoforms have increased from 2,772 to 2,883. The number of genes where every base of every exon is confirmed by EST evidence has increased from 6,427 to 6,584.

In the *C. elegans* proteins there have been 630 modified entries, 440 deleted entries, 709 new entries and 64 reappeared entries.

WormBase is supported by a grant from the National Human Genome Research Institute at the US National Institute of Health #P41 HG02223 and the British Medical Research Council.

BACK

# Multi-Parameter Axial Profiling of Transgenic *C. elegans* Expressing Fluorescent Proteins from Various Cell-Specific, Tissue Specific and Developmentally Regulated Promoters

Bo Wang[1], Julia Thompson[1], Yanping Zhang[2], Michael Herman[2], Mariya Lomakina[1], Bruce Holcombe[1], **Rock Pulak**[1]
[1]Union Biometrica, Holliston, MA; [2]Division of Biology, Kansas State University, Manhattan, Kansas

The COPAS Biosort instrument automates the analysis, sorting, and dispensing of all stages of *C. elegans*, measuring the animal's size and the intensity of expressed fluorescent markers. Once analyzed, animals can be selected according to user defined criteria, and then dispensed into multi-well plates for high throughput screening or collected in bulk for further analysis. With this technology, time required for large scale screening for certain changes in the optical properties of the animals, such as changes in the levels of expression of a fluorescent protein, can be dramatically reduced and human error minimized. Recent enhancements to an add-on module, called the Profiler II, have been tested for its ability to collect positional information of fluorescent expression. The instrument can simultaneously collect fluorescence information in three separate regions of the spectrum. Here we show that the instrument can analyze multi-colored transgenic animals and can be used to compare the amounts and relative positions of expression of two or three different colors of fluorescence. Furthermore, this technology can be used to screen for independent changes in the intensity or position of each reporter protein. We have tested various transgenic animals expressing green, yellow and/or red fluorescing proteins from a collection of promoters that include *myo-2*, *str-1*, *egl-17*, *mab-5*, and various others, separately and in certain combinations. We present some proof of principle examples of how these could be used in genetic screens.

BACK