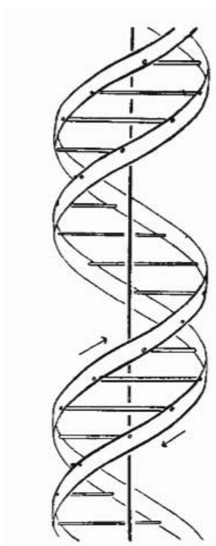
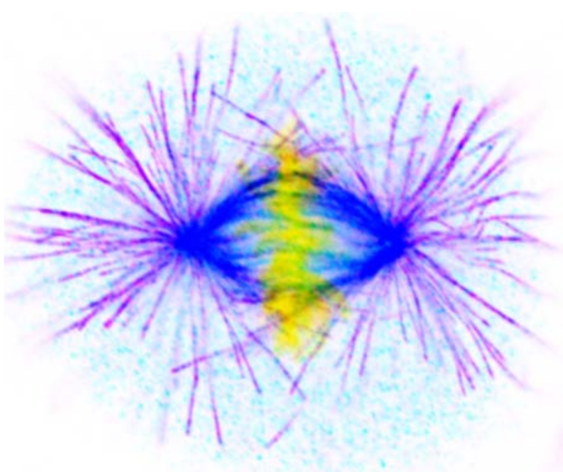
Cell, Molecular and Developmental Biology Research Retreat
June 11-13, Mont Gabriel, Ste-Adèle

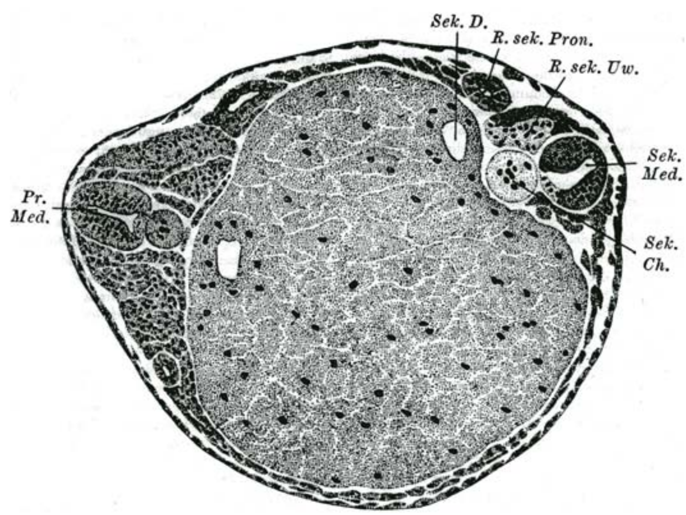
WEB SITE: biology.mcgill.ca/CIAN

Organisers:
Jackie Vogel (McGill), Damien D'Amours (IRIC), Alyson Fournier (McGill), Artur Kania (IRCM), Craig Mandato (McGill)

INVITED SPEAKERS:
Keith Burridge (UNC-Chapel Hill, USA)
Dan Kiehart (Duke University, USA)
Yves Barral (ETH-Zürich, Switzerland)







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Institut de recherches cliniques de Montréal
1967-2007

d b r i

Cell Imaging and Analysis Network
Developmental Biology Research Initiative (DBRI)
Department of Biology
McGill University
A CFI Innovation Fund Project

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ET EN CANCÉROLOGIE

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The Developmental Biology Research Initiative (DBRI), McGill University

Institut de recherches cliniques de Montréal (IRCM)

The Montreal Neurological Institute (MNI)

The Department of Anatomy and Cell Biology (ACB), McGill University

Faculty of Medicine, McGill University

Institut de recherche en immunologie et en cancérologie (IRIC)

Contents

Schedule for June 11-13, 2007

Monday, June 11th

4:00 pm – 6:00 pm	Welcome/Check-in
6:00 pm	Dinner

Keynote Lecture

7:00-7:45 pm Keynote Speaker 1: Keith Burridge
Rho GTPases and leukocyte transendothelial migration
(Sponsored by GE HealthCare, introduction A. Fournier)

Socials

8:00 pm Science Speed Dating

9:00 pm Mixer (cash bar)

Tuesday, June 12

7:00 – 8:00 am	breakfast
8:00 am	Welcome from the organizers

Session I: Science in Montreal (session sponsored by Waters)
(Session chair: J Vogel)

T1 8:05 Paul Lasko (DBRI/McGill)
Bicaudal-C and regulation of RNA stability in the Drosophila oocyte

T2 8:30 John Bergeron (ACB/McGill)
The Protein Microscope: An application of Cell Map Proteomics

T3 8:55 Stefano Stifani (MNI)
Generation of cell diversity in the mammalian nervous system

T4 9:20 Guy Sauvageau (IRIC)
Regulation of Hematopoietic Stem Cell Activity by Hox and PcG Genes

T5 9:45 Tarik Möröy (IRCM)
Research at the IRCM: Regulation of chromatin and alternative splicing during hematopoiesis

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Poster Session (all posters on display)

10:15 to 10:45 Coffee break and Poster Session I
(all posters on display)

Tuesday June 12, continued

Session II: Cell Biology and Human Disease
(Session chair: Ryan Petrie)

T6	10:45	Siegfried Hekimi (DBRI, McGill) ***A new way to look at the role of mitochrondria in the aging process***
T7	11:00	Edward Fon (MNI) ***A Regulated Interaction with the UIM-protein Eps15 Implicates Parkin in EGF Receptor Trafficking and PI3K-Akt Signaling***
T8	11:15	Gino Laberge (IRIC) ***Deciphering the molecular mechanisms leading to Nup98-HoxA9-dependent leukemia using Drosophila***
T9	11:30	Fiona Bedford (ACB, McGill) ***Regulation of chloride homeostasis- a means to control inhibitory neurotransmission***
T10	11:45	Slyvie Mader (IRIC, Biochemistry) ***Regulation of gene expression and cellular growth of breast tumor cells by estrogen receptors***
T11	12:00	Phillipe Roux (IRIC) ***The p90 ribosomal S6 kinase (RSK): Connecting Ras to transcription and translation***
T12	12:15	Nabil Seidah (IRCM) ***The Cellular Biology and Physiology of the Proprotein Convertases PC5 and PCSK9***
12:30		Lunch

(posters, all sponsor booths on display)

Scientific Workshops

Hands-on workshops by GE HealthCare, Quorum and Beckman will be held in the CIAN facility on June 14. Registration for all workshops is available at the meeting at the CIAN Welcome desk.

W1 2:00 – 2:45 GE HealthCare (Roberto Diez)

“DIGE: Spot-on for accuracy”

2D electrophoresis is a very powerful separation tool and is commonly used to isolate proteins of interest for proteomic studies. Some of the key problems facing researchers using this technique are sample preparation and gel-to-gel reproducibility. The application of DIGE (Difference Gel Electrophoresis) for performing 2D will be highlighted- a powerful technique that utilizes the multiplexing of samples on the same gel to incorporate an internal standard that virtually eliminates gel to gel variation. All the steps in the workflow will be discussed with suggestions on how best to maximize the quality of data generated: sample preparation, first dimension IEF, second dimension SDS PAGE, detection, image capture and analysis, and spot processing.

W2 2:45 – 3:15 Quorum (John Arbuckle)

“Grid Confocal Imaging using Structured Light Illumination”

The use of Structured light Illumination provides a means to obtain high quality confocal type images without capturing any out of focus information or haze. This technique can be added on to most microscopes and macroscopes and uses standard fluorescence illuminators.

coffee break: 3:15 – 3:30

W3 3:30 – 4:00 Beckman (Michela Juran)

“Miniaturization Solutions for Discovery Sciences”

As the scientific research community demands more quality results produced at a faster pace than ever before, there is a need to automate manual processes to obtain these results in a more productive and consistent fashion in order to allow research staff to shift their focus from manual work to interpreting the results. The solution to automating many repetitive tasks and processes, such as genomics preparation to drug discovery, is miniaturization. This presentation will discuss the means to increase the speed and capacity of research operations, produce higher quality data and reduce overall costs. Your scientific vision is within reach… let us show you how to achieve your goals sooner!

Tuesday June 12, continued

Poster Session (all posters on display)

4:00	Poster Session II	(Posters; presenters at odd#)
5:00	Poster Session III	(Posters; presenters at even#)
6:00	Buffet dinner in Grand Salon (lecture hall)	

Keynote Lecture

7:00 – 7:45 pm Keynote Speaker 2: Dan Kiehart
Biophysical and Genetic Analysis of Cell Sheet Morphogenesis: Dorsal Closure as a Model System
(Sponsored by DBRI, introduction by P. Lasko)

Session III: Cellular Morphology and Locomotion
(Session chair: Francisco Gomes)

T13 8:00 Nathalie Lamarche-Vane (ACB, McGill)
Trio mediates netrin-1-induced Rac1 activation and axon guidance

T14 8:15 Caroline Laplante (DBRI, McGill)
Differential expression of Echinoid drives epithelial morphogenesis in Drosophila

T15 8:30 Tim Kennedy (MNI)
Organization of Axo-Oligodendroglial Paranodal Junctions Requires DCC and Netrin-1

T16 8:45 Wolfgang Reintsch (ACB, McGill)
Inhibition of embryonic cell adhesion by the cadherin juxtamembrane domain is enhanced by Xp120/xARVCF

T17 9:00 Alyson Fournier (MNI)
CNS Regeneration: Molecular approaches to identify novel therapeutic strategies for nerve repair

T18 9:15 Tamara Western (DBRI, McGill)
Cell walls and cellular differentiation: Genetic dissection of the Arabidopsis mucilage secretory cells

T19 9:30 Simon Moore (MNI)
Inhibiting Rho Signaling Enhances Axon Chemo-attraction to Netrin-1

T20 9:45 David Hipfner (MNI)
Connecting growth and integrity of epithelial tissues in Drosophila

10:00 pm cash bar

Wednesday June 13

7:00 – 8:00 am		breakfast

Session IV: The Cytoskeletion and Interdisciplinary Approaches
(Session chair: Dalinda Liazoghli)

8:00 am		Invited Speaker: Yves Barral ***Asymmetric segregation of age during yeast budding*** (Sponsored by CIAN, introduction by J Vogel)
T21	8:45	Amy Maddox (IRIC) ***Roles for Anillin in Organizing Cortical Contractility***
T22	9:00	James Knockleby (DBRI, McGill) ***Aurora B/INCENP mediated sensing and repair of defective kinetochore-MT attachments requires Ame1***
T23	9:15	Dieter Reinhardt (ACB, McGill) ***Oligomerization of the C-terminus of fibrillin-1 provides the basis for microfibril assembly – Does the bead finally reveal its secret?***
T24	9:30	Matthais Trost (IRIC) ***System biology of an Organelle: Phosphoproteomic and proteomic analysis of Phagosomes***
T25	9:45	David Juncker (Dept Biomedical Engineering, McGill) ***Microfluidic systems for miniaturizing and parallelizing biological experimentation***
10:00		Coffee break (sponsor displays in breakout rooms)

Session V: Cell and Developmental Biology
(Session chair: Jimmy Ouellet)

T26	10:15	Francois Fagotto (DBRI, McGill) ***Revisiting the Wnt pathway: ask the natives to show us around***
T27	10:30	Dayana Krawchuk (IRCM) ***Myotopic organization of limb innervation by spinal motor neurons is defined by Netrin and Netrin receptor expression***
T28	11:00	Laura Nilson (DBRI, McGill) ***The hnRNP Squid is required cell-autonomously in the Drosophila germline to repress translation of unlocalized gurken mRNA in the oocyte***
T29	11:15	Marko Horb (IRCM) ***Differential ability of Ptf1a and Ptf1a-VP16 to convert stomach, duodenum and liver to pancreas***

Wednesday June 13, continued

T30 11:30 Michel Cayouette (IRCM)
Regulation of temporal competence in retinal progenitor cells

11:50 Future organization and closing remarks

Scientific Workshop

W4 12:00 – 12:50 Kelly Lundsten, (Molecular Probes)

"State of the Art Imaging: Generating the Perfect Image"

Fluorescence microscopy is a powerful, widely used application for the visualization of protein localization and cellular processes. This seminar will present an overview of the key elements and considerations of fluorescence and instrumentation that contribute to generating the perfect image. Emphasis will be placed on choosing the best reagents to capture the desired image.

Topics to be discussed include:
- Basic overview of organic-based fluorescent molecules
- Summary of techniques for secondary detection and amplification with the Alexa Fluor® dyes and QDot® nanocrystals
- Protein expression tags/GFP
- Reagents for subcellular localization in fixed and live cells
- Tools and techniques for improving and maintaining an ideal signal to noise ratio

Kelly will also lead a hands-on imaging workshop in the CIAN facility on June 14.
Registration for this workshop is available at the meeting at the CIAN Welcome desk.

1:00 Lunch

2:00 – 4:00 pm: sessions for departmental meetings, sponsor interactions

Abstracts (Talks)

T1 Bicaudal-C and regulation of RNA stability in the Drosophila oocyte
Paul Lasko, Department of Biology and DBRI, McGill University

Bicaudal-C (Bic-C) encodes an RNA binding protein required maternally for patterning the Drosophila embryo. We show that Bic-C is present in vivo in ribonucleoprotein complexes that contain Bic-C RNA, that Bic-C negatively regulates Bic-C mRNA stability, and that it associates with NOT3/5, a component of the CCR4 core mRNA deadenylase complex. Bic-C binds with high affinity to a specific segment of the Bic-C 5' untranslated region. Bic-C poly(A) tail length decreases upon Bic-C overexpression and isincreased in Bic-C mutant ovaries. Bic-C overexpression induces premature cytoplasmic streaming, a posterior-group phenotype, defects in Oskar and Kinesin heavy chain: bGal localization, and dorsal appendage defects. These phenotypes resemble those of orb mutants, are enhanced by mutations affecting orb and poly(A) polymerase, and are suppressed by mutations affecting CCR4 deadenylase. We conclude that Bic-C antagonizes orb by recruiting the CCR4 deadenylase complex to specific mRNAs.

T2 The Protein Microscope: An application of Cell Map Proteomics
John Bergeron Anatomy and Cell Biology, McGill University

The application of hierarchical clustering to a quantitative representation of comprehensive protein abundance in isolated organelles provides a ready method to sort contaminants from resident proteins and gain insight into organelle identity and function. Organelle based proteomics provides hundreds of proteins of unknown function. An application of the protein microscope is to assign locations and mechanistic functions to these proteins.

T3 Generation of cell diversity in the mammalian nervous system
Stefano Stifini, Montreal Neurological Institute

The regulation of cell diversity generation is critical to the correct development of the nervous system. Perturbations of the mechanisms that control nerve cell specification cause a number of developmental anomalies that result in neurological and cognitive conditions. This presentation will focus on the analysis of mechanisms important for a) the generation of immature post-mitotic progenies from pluripotent neural progenitor cells, and b) the post-mitotic development of specific neuronal phenotypes in the mammalian nervous system. It will also be discussed how these studies relate to other lines of investigation at the Montreal Neurological Institute.

T4 Regulation of Hematopoietic Stem Cell Activity by Hox and PcG Genes
Guy Sauvageau, Amélie Faubert, Mélanie Fréchette, Simon Girard and Jalila Chagraoui. Institute for Research in Immunology and Cancer, University of Montréal, Montréal, Québec, Canada, H3C 3J7

The ability to sustain rather than initiate self-renewal distinguishes long-term from short-term repopulating hematopoietic stem cell (LTR and STR-HSC). Using complementation and overexpression strategies, we show that *Bmi1* is dispensable for the program leading to symmetrical self-renewal divisions (i.e., expansion) of fetal HSC and confirm that this gene is essential for preserving the functional integrity of adult LTR-HSC. We document that *Hoxb4* induces self-renewal divisions in *Bmi1-/-* multipotent cells leading to their expansion, and subsequent rescue of their in vivo hematopoietic reconstitution activity. However, this activity was limited to 3-4 months. We also report that Hoxb4 and Bmi1 regulate the expression distinct genes in mouse HSCs (i.e. *Angptl3* - *Cbx7* and *Msi1,* respectively). Using a yeast two-hybrid approach, we also identified E4F1, an inhibitor of cell proliferation, as a novel partner of BMI1 in hematopoietic cells. Most importantly, our results show a strong genetic interaction between *Bmi1* and *E4F1* in the regulation of stem cell proliferation. Furthermore, we demonstrate that RNA interference mediated knock down of *E4F1* is sufficient to completely rescue the clonogenic and repopulating ability of *Bmi1-/-* hematopoietic cells. Together, these results support the emerging concept that the abilities of HSCs to engage versus sustain self-renewal divisions are differentially regulated.

T5 Research at the IRCM: Regulation of chromatin and alternative splicing during hematopoiesis

Tarik Möröy. The Institut de recherches cliniques de Montréal (IRCM)

The Institut de recherches cliniques de Montréal (IRCM) was founded in 1967 as the first independent biomedical research centre of its kind in Quebec. It is a non-profit organization with the aim to study the molecular mechanisms and the causes of disease, to develop new diagnostic procedures and discovering preventive and therapeutic approaches that help to enhance our quality of life.

The IRCM has been recognized as one of the top-performing research centres in the country, and presently houses over 30 specialized basic research laboratories working in immunology, cancer, neurobiology, cardiovascular diseases, bioethics and medicinal chemistry. At the same time the IRCM is a centre for translational research and runs four different clinical research programs in the area of hypertension, hyperlipidemia, diabetes and metabolic malfunction. But the IRCM also has a mission as a training centre and helps to educate the next generation of researchers. Over 120 young scientists at postgraduate and post-doctoral level work in the IRCM's different research laboratories. These young researchers will put the scientific and technical expertise they acquire at the IRCM to excellent use, contributing to the development of academic research, the success of pharmaceutical and biotechnology firms and the launch of biotech-companies. Thus, the investment of the IRCM in training young scientists generates a direct and tangible spin-off for a knowledge based economy. The IRCM is affiliated with the Université de Montréal and all IRCM laboratory directors hold appointments as professors at different university departments. At the same time our institute has a close association with McGill University and most laboratory directors are adjunct professor at the McGill faculty of medicine.

The research unit: Hematopoiesis and Cancer at the IRCM ("The Director's lab")

The research unit Hematopoiesis and Cancer is directed by the president and scientific director of the IRCM, Dr. Tarik Möröy. His unit follows the aim to better understand the molecular basis of the physiology, ontogeny and differentiation of all blood cells and to reveal the mechanisms leading to cancer in the hematopoietic system, i.e. to leukemia and lymphoma. The research programme focuses on the role of transcription factors and chromatin regulators such as the zinc finger proteins Gfi1 and Gfi1b and the POZ/BTB transcription factor Miz-1. As a general approach to understand their function, mutant alleles are generated by gene targeting in the mouse; an extremely powerful method that has permitted the group to gain deeper insight into a number of critical steps in stem cell self-renewal and hematopoietic differentiation but also in mechanisms underlying the development of lymphoma and leukemia.

T6 A new way to look at the role of mitochondria in the aging process

S. Hekimi, Department of Biology, McGill

The mitochondrial oxidative stress theory of aging suggests that the accumulation of damage due to the toxic properties of reactive oxygen species (ROS) is the cause of aging. The theory is based on evidence that indicates a strong correlation between chronological age and oxidative damage in individuals, and between oxidative potential and lifespan for species. Furthermore, mitochondria are the main source of ROS and the first targets of their toxicity, and the loss of mitochondrial function with age is well documented in many organisms. This last observation has even prompted the proposal that it is the loss of mitochondrial function with its possible impact on energy metabolism and the regulation of apoptosis that is the cause of the age-dependent deterioration of other cellular functions. We will present evidence from genetic studies in C. elegans and in mice that suggest that this theory needs revision.

T7 A Regulated Interaction with the UIM-protein Eps15 Implicates Parkin in EGF Receptor Trafficking and PI3K-Akt Signaling

Lara Fallon1, Catherine M.L. Bélanger1, Amadou T. Corera1, Maria Kontogiannea1, Elsa Regan-Klapisz2, France Moreau1, Jarno Voortman2, Michael Haber1, Paul M.P. van Bergen en Henegouwen2, **Edward A. Fon**1.
1Montreal Neurological Institute, McGill University, Montreal, Canada. 2Molecular Cell Biology, Institute of Biomembranes, Universiteit Utrecht, Utrecht, The Netherlands

Parkin encodes an E3 ubiquitin-ligase responsible for a common familial form of Parkinson's disease (PD). We have identified a regulated interaction between parkin and Eps15, an adaptor protein involved in EGF-receptor (EGFR) endocytosis and trafficking. The work implicates parkin for the first time in a ubiquitin-dependent trafficking pathway. Treatment of cells with EGF stimulates parkin binding to both Eps15 and the EGFR and promotes parkin-mediated ubiquitination of Eps15. Binding of the parkin ubiquitin-like (Ubl) domain to the Eps15 ubiquitin-interacting motifs (UIMs) is required for parkin-mediated Eps15 ubiquitination. Further, EGFR endocytosis and degradation are accelerated in parkin-deficient cells and EGFR signaling via the PI3K-Akt pathway is reduced in parkin knockout mouse brain. We propose that by ubiquitinating Eps15, parkin interferes with the ability of the Eps15 UIMs to bind ubiquitinated EGFR, thereby delaying EGFR internalization and degradation, and promoting PI3K-Akt signaling. Considering the role of Akt in neuronal survival, our results have broad new implications for understanding the pathogenesis of PD. Supported by the Canadian Institutes of Health Research and the Michael J. Fox Foundation.

T8 Deciphering the molecular mechanisms leading to Nup98-HoxA9-dependent leukemia using Drosophila

Gino Laberge[¶§], Amélie Casgrain[¶§], Guy Sauvageau[§] and Marc Therrien[¶].
[¶]Laboratory of Intracellular Signaling, [§]Laboratory of Molecular Genetics of Hematopoietic Stem Cells, Institute for Research in Immunology and Cancer, Université de Montréal, C.P. 6128 succursale Centre-Ville, Montréal, Québec H3C 3J7, Canada

About 75% of the genes involved in human diseases have a counterpart in Drosophila. This high degree of conservation combined to powerful genetic tools make the flies a suitable model to address specific questions related to human disorders. Here, we present evidence indicating that Drosophila is a relevant system to identify novel regulators and effectors of a leukemia-causing oncogene. Numerous chromosomal translocations are linked to leukemogenesis. These translocations often result in the production of a chimeric protein that participates in disease development/progression. One of these is the t(7;11)(p15;p15) translocation associated to acute myeloid leukemia (AML) and which fuses the N-terminal part of nucleoporin 98 (Nup98) to the C-terminal part of the transcription factor HoxA9. How Nup98-HoxA9 induces leukemia is currently unknown. As homologues of Nup98 and HoxA9 are present in flies, we reasoned that expression of Nup98-HoxA9 during development may interfere with basic cellular components analogously to the situation encountered in human hematopoietic cells. Expression of Nup98-HoxA9 during eye development produces a phenotype that is related to the one produced by overexpression of homothorax (hth), which encodes the fly homologue of Meis1, a HoxA9 co-factor. In addition, Nup98-HoxA9 and Hth collaborate when co-expressed in the eye, whereas a heterozygous hth loss-of-function allele dominantly suppresses the Nup98-HoxA9 phenotype. Together, these findings lend support to the specificity of the Nup98-HoxA9 phenotype and suggest that a screen for modifiers of Nup98-HoxA9 will identify key modulators of this potent oncogene. Interestingly, preliminary genetic interactions suggest a strong link between Nup98-HoxA9 and chromatin remodelling factors.

T9 Regulation of Chloride Homeostasis - a Means to Control Inhibitory Neurotransmission

*Beibei Zhao, †Adrian Wang, *Ayesha Murshid, †Derek Bowie, *John F. Presley and ***Fiona Kay Bedford**. From the *Department of Anatomy & Cell Biology and †Pharmacology & Therapeutics, McGill University, Montreal, Quebec, CANADA.

Fast synaptic inhibition in the brain is controlled by the GABAA receptor, which is as a heteromeric ligand-gated chloride channel and is dependent on the appropriate electrochemical gradient for chloride. Early in development, GABAAR responses are excitatory and transition to inhibition is linked to a shift in intracellular chloride concentrations from high to low. This is controlled primarily by the developmentally regulated expression of the K+/Cl- cotransporter, KCC2. In mature neurons, downregulation of KCC2 can also switch GABAAR responses from inhibitory back to excitatory. The consequences of this are a loss in control of neuronal activity, leading to a hyperexcitability state. This downregulation of KCC2 has been linked with the neuropathological conditions of epilepsy and chronic pain and appears to be controlled transcriptionally as well as post-translationally via regulated membrane trafficking. KCC2 is a 12 transmembrane K+/Cl- cotransporter belonging to the SLC12 transporter family. To begin to understand the mechanisms controlling KCC2 post-translational downregulation, we have examined the molecular mechanisms controlling the endocytosis of KCC2. We have identified a non-classical endocytic motif that is both necessary and sufficient to drive the constitutive clathrin-mediated endocytosis of KCC2. This KCC2 endocytic motif is localized in the carboxy terminus of KCC2 and is a clathrin-binding adaptor-protein 2 interacting motif. We have also found this motif is conserved between KCC transporters but not to the closely related NKCC transporters, suggesting it is likely a selective endocytic signal for KCC transporters. Future studies will address whether KCC2 regulated endocytosis utilizes this motif and characterize the mechanisms involved.

T10 Regulation of gene expression and cellular growth of breast tumor cells by estrogen receptors

Veronique Bourdeau, Julie Deschenes, David Laperriere, Malika Aid, Eric Duplan, **Sylvie Mader**
Institute for Research in Immunology and Cancer and Biochemistry Department, Universite de Montreal

Estrogens are steroid hormones that have pleiotropic physiological actions in numerous target tissues, but also play a role in breast tumorigenesis. Estrogens act through intracellular estrogen receptors (ERs), which are ligand-inducible transcription factors. ERs bind to specific estrogen response elements (EREs) which are present in large numbers throughout the human and mouse genomes. We have used chromatin immunoprecipitation experiments to verify that EREs present at large distances from the transcriptional start sites (TSS) of regulated genes can be functional binding sites, and chromatin conformation capture assays have indicated that these sites can form chromatin loops with the TSS of neighboring genes. EREs spread throughout large distances of flanking regulatory sequences can also cooperate with each other via chromatin loops.
Analysis of the conservation of EREs in the human and mouse genomes indicates narrow peaks centered around the TSS. However, EREs are enriched over much larger distances in the flanking sequences of primary target genes or in regions bound in ChIP-on-chip experiments. Surprisingly, EREs were found enriched only in up-regulated primary target genes, suggesting that mechanisms other and direct DNA binding are implicated in transcriptional repression. Expression of up-regulated primary estradiol target genes in MCF7 cells correlates positively with that of ERalpha in breast tumor cells, indicating that these genes constitute signature markers for the ER+ luminal type of breast tumors. Primary regulation of several proto-oncogenes by estradiol suggests that several of these genes may contribute to the proliferative effects of estrogens and represent novel targets for breast cancer treatment.

T11 The p90 ribosomal S6 kinase (RSK): Connecting Ras to transcription and translation

Philippe Roux, IRIC - Université de Montréal, P.O. box 6128, Station centre-ville, Montréal, Québec, Canada H3C 3J7

The p90 ribosomal S6 kinase (RSK) family comprises four serine kinases that are activated downstream of the Ras/extracellular signal-regulated kinase (ERK) signalling cascade. While the Ras/ERK pathway has been linked with a wide spectrum of biological effects, including cell survival, growth and proliferation, the contribution of the RSK protein kinases in these processes remain poorly understood. Some RSK isoforms have been shown to play important roles during embryonic development, while others were found to promote carcinogenesis. Activated RSK regulates several transcription factors that play roles in cell survival and proliferation, and recent data suggest that RSK is an important modulator of mRNA translation. How these kinases mediate these functions upon stimulation of the Ras/ERK signalling pathway will be addressed.

T12 The Cellular Biology and Physiology of the Proprotein Convertases PC5 and PCSK9

Nabil G. Seidah, Gaetan Mayer, Ahmed Zaid, Steve Poirier, Rachid Essalmani, Annik Prat
IRCM, Montreal, QC, Canada

The proprotein convertases (PCs) comprise 9 Serine proteinases that process a large cohort of secretory precursors. The Furin-like convertases (Furin, PC5, PACE4 and PC7) are activated in cis and then process their substrates in trans either in the TGN, cell surface or recycling endosomes. New data suggest that PC5 and PACE4 are activated at the cell surface and they are often implicated in the inactivation of various cell-surface precursor proteins bound to heparan sulfate proteoglycans. Little is yet known about the in vivo biological functions of the PCs during embryonic development and in the adult. Data will be presented showing the critical role played by PC5 during embryonic development and our attempt to rescue the lethal phenotype associated with the knockout of PC5 gene. This led us to define a novel function of PC5 in body axis regulation and the identification of the first in vivo substrate of this convertase. Both human and mouse studies on PCSK9 revealed that this last member of the PC-family is intimately involved in enhancing the degradation of the LDLR in endosomes and thus upregulates the levels of circulating LDL-cholesterol. This enzyme has been proven to be directly responsible for the development of dominant familial hypercholesterolemia in patients exhibiting single point mutations in the PCSK9 gene. On the other hand, mutations leading to loss of function of PCSK9 result in a hypocholesterolemia phenotype. We will present data towards defining the mechanism behind the function of PCSK9 and its role in the development of cardiovascular complications.

T13 Trio mediates netrin-1-induced Rac1 activation and axon guidance

Anne Briançon-Marjollet1, Atefeh Ghogha2, Homaira Nawabi3, Ibtissem Triki2, Camille Auziol1, Sylvie Fromont1, Hervé Enslen6, Karim Chebli4, Jean-François Cloutier7, Valérie Castellani3, Anne Debant*1,5 and **Nathalie Lamarche-Vane***2,5
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6Unité mixte de recherche Santé UMR-S536, INSERM, Institut du Fer à Moulin, 75005 Paris, France
7Montreal Neurological Institute, McGill University, Dept. of Neurology and Neurosurgery, Montreal, Quebec, Canada H3A 2B4

The chemotropic guidance cue netrin-1 promotes axon outgrowth through its receptor DCC (Deleted in Colorectal Cancer) via activation of Rac1. However, the guanine nucleotide exchange factor (GEF) linking netrin-1/DCC to Rac1 activation has not yet been identified. Here we show that Trio is this GEF. We found that Trio, Nck-1, PAK1, and DCC are present in the same signaling complex, and that netrin-1-induced Rac1 activation is impaired in the absence of Trio. Trio -/- cortical neurons fail to extend neurites in response to netrin-1, while they are able to respond to glutamate. Accordingly, netrin-1-induced commissural axon outgrowth is severely impaired in Trio -/- spinal cord explants and commissural axon projections are defective in Trio -/- embryos. In addition to defects in spinal cord development, the anterior commissure is absent in Trio-null embryos, and netrin-1/DCC-dependent axonal projections that form the internal capsule and the corpus callosum are also defective in Trio -/- embryos. Thus, Trio through its ability to activate Rac1 mediates netrin-1 signaling in axon growth and guidance.

T14 Differential expression of Echinoid drives epithelial morphogenesis in Drosophila.

Caroline Laplante and Laura Nilson DBRI, McGill Univ, Montreal, PQ, Canada.

Interaction between distinct cell types is thought to drive epithelial morphogenesis by locally altering the cytoskeleton. Our study of Echinoid (Ed), a putative cell adhesion molecule, has shown that differential expression of Ed between neighboring groups of cells is sufficient to trigger the assembly of a contractile actomyosin cable at their interface. In the ovary, we identified an endogenous Ed expression border between the two follicle cell types that form the epithelial tubes that secrete the dorsal appendages and showed that elimination of this border causes defective appendage formation. Additionally, we found an endogenous Ed expression border between the two cell types involved in embryonic dorsal closure, where the two lateral sheets of embryonic epidermis converge dorsally, covering the amnioserosa. Ed is expressed in the epidermis but absent from the amnioserosa creating an endogenous Ed expression border that corresponds to the location of a well-characterized actomyosin cable. Embryos mutant for ed lack the Ed expression border and fail to assemble the actomyosin cable resulting in aberrant dorsal closure. Similarly, ectopic expression of Ed in the amnioserosa thus eliminating the Ed expression border also impedes the assembly of the actomyosin cable. Additionally, we found that the expression of Ed during dorsal closure is independent of the JNK pathway suggesting that both the JNK pathway and the establishment of an Ed expression border are required for proper dorsal closure. This research proposes a mechanism by which the differential expression of a cell adhesion molecule locally modulates the cytoskeleton required for morphogenetic processes.

T15 Organization of Axo-Oligodendroglial Paranodal Junctions Requires DCC and Netrin-1

Andrew A. Jarjour, Sathyanath Rajasekharan, K. Adam Baker, Jeannie Mui, Jack P. Antel, and **Timothy E. Kennedy**

Paranodal axoglial junctions are essential for the segregation of myelinated axons into distinct domains and efficient conduction of action potentials. Here, we describe a role for netrin-1, a protein abundantly expressed in the mature CNS, in regulating paranodal adhesion between oligodendrocyte processes and axons. Netrin-1 and DCC are enriched at the paranode in CNS myelin both in vitro and in vivo. In myelinated cerebellar slice cultures derived from neonatal DCC-/- and netrin-1-/- mice, paranodes initially develop and mature normally but later become disorganized, as paranodal loops detach from the axonal surface and from each other, and transverse bands disappear. Furthermore, the domain organization of myelin is compromised in the absence of netrin-1 or DCC function: K+ channels inappropriately invade the paranodal region, and the normally restricted paranodal distribution of caspr expands longitudinally along the axon. Our findings identify an essential role for netrin-1 and DCC regulating the organization of axo-glial junctions.

T16 Inhibition of embryonic cell adhesion by the cadherin juxtamembrane domain is enhanced by Xp120/xARVCF

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Classical cadherins constitute an important family of cell-cell adhesion molecules. The cytoplasmic tail of these transmembrane glycoproteins is thought to regulate the strength and dynamics of adhesion, both as a central structural component and by forming a scaffold for signaling molecules. In Xenopus laevis, overexpression of the cytoplasmic tail efficiently disrupts cell adhesion, an activity that has been attributed to its membrane-distal beta-catenin binding domain. We show that overexpression of a membrane-proximal fragment, the juxtamembrane domain, also affects adhesion in the early embryo. While being a comparatively weak inhibitor when expressed alone, its activity becomes strongly enhanced by coexpression of p120catenin related proteins. Expression of a dominant negative form of rac produces a similar phenotype, while coexpression of constitutively active rac with the juxtamembrane domain and Xp120/xARVCF restores adhesion. This suggests that intracellular signaling is involved in the loss of adhesion phenotype. In addition, expression of the juxtamembrane domain or the complete cytoplasmic tail induces a rac-independent redistribution of cadherins from the plasma membrane to the cytoplasm and, using a heterologous system, we provide evidence for an activity of the juxtamembrane domain in clustering of cadherins. Our observations suggest that cadherin cytoplasmic domains may form protein complexes at the membrane that actively regulate the adhesion “status” of embryonic cells.

T17 CNS Regeneration: Molecular approaches to identify novel therapeutic strategies for nerve repair

Alyson Fournier, MNI

Trauma in the adult mammalian CNS results in devastating clinical consequences as a result of the failure of injured axons to spontaneously regenerate. Chondroitin sulfate proteoglycans (CSPGs) and the myelin-associated inhibitors (MAIs; myelin associated glycoprotein, Nogo-A, and oligodendrocyte-myelin glycoprotein) bind receptor molecules on injured axons initiating intracellular signaling cascades that block axonal regrowth. In part, CSPGs and MAIs inhibit axon regeneration by disrupting Rho-GTPase-dependent cytoskeletal dynamics. Blockade of RhoA and a downstream effector, Rho kinase (ROCK), promotes axon regeneration both in vitro and in vivo; however, the ability of RhoA and ROCK to affect multiple physiological processes in many cell types highlights the need to identify novel intracellular signaling substrates of neurite outgrowth inhibition to develop more specific and potent therapeutic avenues. In a screen to identify molecules that functionally interact with RhoA to mediate neurite outgrowth inhibition, we identified collapsin-response mediator protein 4b (CRMP4b) as a molecule that interacts with Rho GTPase in a Nogo-dependent manner. The CRMPs are a family of cytosolic phosphoproteins that regulate axon growth and pathfinding. We find that CRMP4b interacts with RhoA in a Nogo dependent manner, leading us to investigate the potential role of this complex in the inhibition of neurite outgrowth. We find that antagonism of CRMP4 or of the CRMP4b–RhoA interaction attenuates neurite outgrowth inhibition. This protein–protein interaction represents a novel, specific target for therapeutic intervention after CNS injury.

T18 Cell walls and cellular differentiation: Genetic dissection of the Arabidopsis mucilage secretory cells

Tamara L. Western, Biology Dept., McGill University

The mucilage secretory cells (MSCs) of the Arabidopsis seed coat undergo a complex differentiation process that includes the synthesis and apoplastic deposition of large quantities of pectinaceous mucilage. These mucilage pockets are formed on the apical surface of the cell below the primary cell wall and their formation is accompanied by intracellular rearrangement of the cytoplasm into a column in the centre of the cell. These developmentally regulated steps make the MSCs an excellent model system with which to dissect the processes not only of pectin synthesis and its regulation in cellular differentiation, but also polar secretion and plasma membrane-cell wall attachment. To understand these processes, we are taking a multi-prong approach to dissecting MSC differentiation. A number of genes required for MSC differentiation have been identified through mutant screens from wild type and sensitized backgrounds. One such mutant, known as "patchy", has patchy rather than complete mucilage release after seed hydration. Our results show that the patchy mutant is affected in AtBXL1, a gene that appears to be required for structural modification of the pectin network during MSC differentiation. Reverse genetic approaches are also underway, including datamining of publicly available datasets and microarray analysis of wild type seeds compared with a null MSC differentiation mutant. Isolation of knockout mutants and detailed expression analyses of candidate genes is underway, with a focus on cell wall synthesis genes, putative plasma-membrane cell wall attachment genes, and genes that may be involved in targeted secretion.

T19 Inhibiting Rho Signaling Enhances Axon Chemoattraction to Netrin-1

Simon W. Moore, James Correia, Karen Lai Wing Sun and Timothy E Kennedy

Extracellular guidance cues direct axon extension by regulating growth cone cytoskeletal organization and adhesive contacts. The Rho GTPases Rac and Cdc42 play well-recognized roles in axon extension and chemoattraction, while Rho is primarily thought to mediate growth cone repulsion or collapse. Here we investigate the hypothesis that Rho signaling might influence chemoattraction. Embryonic rat spinal commissural neurons express the three Rho family members (Rho A, B & C), as well as, their downstream targets PRK2, ROCKI, ROCKII and diaphanous homolog 1. We demonstrate that netrin-1, through its receptor DCC, inhibits RhoA in embryonic spinal commissural neurons. Given the key role of Rho in regulating actin dynamics, we anticipated that inhibiting Rho would disrupt the capacity of axons to turn in response to netrin-1. Surprisingly, when Rho signaling was inhibited commissural axon chemoattraction was not only intact, but enhanced, with axons turning over a greater distance toward a source of netrin-1. This increased responsiveness of commissural axons to netrin-1 was accompanied by increased levels of plasma membrane DCC and enhanced DCC mediated adhesion to substrate bound netrin-1. Our findings are consistent with described roles for RhoA regulating receptor trafficking and adhesive remodeling in non-neuronal cells, and support the conclusion that netrin-1 inhibition of RhoA promotes axonal chemoattraction by increasing plasma membrane DCC in growth cones.

T20 Connecting growth and integrity of epthelial tissues in Drosophila

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Uncontrolled cell proliferation and loss of polarized cell architecture are two of the hallmarks of epthelial cancers. Classical studies of the behavior of transformed cells in culture, including such phenomena as anchorage-independent growth and contact inhibition, lead to the notion that cell architecture and proliferation control are intimately connected at the level of signaling. The same relationship holds true in vivo, as best demonstrated by studies of a number of genes involved in epithelial tissue growth in Drosophila melanogaster. However, the underlying signaling mechanisms in tissues remain largely unknown. We have been characterizing a Sterile20-family kinase called Slik that plays a dual role in growth control and epithelial architecture during Drosophila development. Both gain- and loss-of-function genetic analyses indicate that Slik promotes tissue growth by accelerating cell proliferation rates. Slik activity is also important for proper maintenance of epithelial cell polarity, and in its absence epithelial tissue integrity is compromised. Our results suggest that Slik has separable functions in promoting growth and epithelial cell polarity through distance effector pathways, and may thus contribute to the coordination of these processes in vivo.

T21 Roles for Anillin in Organizing Cortical Contractility

Amy Maddox, IRIC Montreal QC Canada

Cytokinesis is the dramatic cell shape change when one cell is pinched into two, so that each contains one daughter nucleus of segregated chromatin. A contractile band rich in actin filaments and myosin motor protein assembles at the cell equator in anaphase, and its constriction drives this cell shape change. Anillin is conserved cytoskeletal protein that enriches specifically in the contractile ring and is required for cytokinesis in many systems. Anillin can be thought of as a cytoskeletal crosslinker: it can bundle actin filaments, and bind active myosin and septin filaments via distinct domains. I am studying anillin using the *C. elegans* early embryo due to its amenability to quantitative live assays and thorough depletion of essential proteins. I have found that anillin organizes actomyosin contractility during polarity establishment in the zygote. During cytokinesis, anillin is required for timely shape changes and for symmetry breaking in the contractile ring. Older embryos depleted of anillin undergo occasional cytokinesis failures, indicating that anillin contributes to the robustness of cytokinesis. I am now expressing truncations to examine how anillin's predicted actin-, myosin and septin-interaction domains contribute to its multiple roles in organizing contractility. I am also investigating the role of anillin in polar body extrusion, a specialized cytokinesis event that fails often when anillin is depleted. Together, this work is implicating anillin in facilitating the cell shape changes that occur during cell division.

T22 Aurora B/INCENP mediated sensing and repair of defective kinetochore-MT attachments requires Ame1

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High fidelity chromosome segregation in all cells requires the formation of bi-oriented attachments between spindle microtubules (MT) and kinetochores. The conserved Aurora B kinase (Ipl1) and its INCENP (Sli15) adaptor play a crucial role in repairing defective attachments, and in checkpoint mechanisms that detect defective attachments. Indicative of its crucial function in genome stability, Aurora B mis-regulation is found in many cancer cell types. Ame1 is an essential component of the COMA sub-complex (Ctf19, Okp1, Mcm21, Ame1) of the kinetochore of budding yeast. Using two conditional mutants of the COMA, ame1-4 and okp1-5, we examine the role of Ame1 in the formation and repair of kinetochore-MT attachments. ame1-4 cells have a compromised COMA but do not affect the stability of DNA binding and MT binding kinetochore complexes. However, ame1-4 cells exhibit defective sister chromatid attachments that are not repaired, and are unable to maintain a checkpoint arrest. We find that disruption of the COMA results in a failure to maintain the localization of Sli15 to the kinetochore. In turn, Sli15 functions in checkpoint maintenance and passenger protein migration to the spindle are abrogated. Finally, over-expression of OKP1 in ame1-4 cells restores localization of ame1-4p and re-establishes checkpoint maintenance, indicating that Ame1 is necessary and sufficient for Sli15 localization and function. We propose that Ame1 provides an essential, regulatable and potentially functionally conserved scaffold for Sli15 and maintains genomic stability by promoting the formation of proper kinetochore-microtubule attachments through stabilization of the interaction between Sli15/Ipl1 and the kinetochore.

T23 Oligomerization of the C-terminus of fibrillin-1 provides the basis for microfibril assembly – Does the bead finally reveal its secret?

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Fibrillin-1 provides the backbone of microfibrils, which are essential components of the vascular extracellular matrix. Mutations in human fibrillin-1 cause a number of connective tissue disorders including Marfan syndrome characterized by aortic aneurysms as well as skeletal and ocular pathologies. Microfibrils assemble close to the cell surface and an N- to C-terminal linear interaction of fibrillin-1 mediated by domains at the ends of the molecules is believed to represent one of the initial assembly steps. Microfibrils extracted from tissues show a typical "bead-on-the-string" structure with a bead diameter of ~22 nm and a periodicity of 50-60 nm.

To study the initial mechanisms of microfibril assembly, we constructed a number of C-terminal recombinant fibrillin-1 deletion fragments. Analyzing these constructs revealed distinct disulfide-bonded oligomers, and the oligomerization process is dependent on the presence of the last three EGF modules in fibrillin-1. The unique C-terminal domain does not influence this oligomerization. The N- to C-terminal self interaction is strongly dependent on the oligomerization status of the C-terminus, whereby the largest oligomers showed the strongest interaction. Surprisingly, a monomeric C-terminus does not at all interact with the N-terminus. Electron microscopy after rotary shadowing shows for the oligomer a structure with a dense core and “arms” stretching out. The inner diameter (27.1 nm) corresponds to the diameter of the beads in microfibrils, whereas the outer diameter (99.6 nm) corresponds to double the interbead distance. Data obtained by dynamic light scattering revealed that the largest oligomeric fraction is composed of 10-12 monomers. Taken together, we show that fibrillin-1 self assembly is dependent on the presence of C-terminal oligomers. Our studies indicate that the bead of the microfibrils is generated primarily by the C-terminus.

T24 Systembiology of an Organelle: Phosphoproteomic and proteomic analysis of Phagosomes

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Specialized cells of the immune system such as macrophages can internalize particles such as microbes and apoptotic cells into membrane-bound organelles, called phagosomes. In these cells, phagosomes fuse with lysosomes forming phagolysosomes in a process called maturation, leading to the killing and degradation of the pathogens. The molecular mechanisms involved in phagolysosome biogenesis and the signaling events involved in the maturation of this compartment in response to pathogen infection and the strong effect of Interferon-γ on the ability to kill pathogens are still poorly understood. In the present investigation, we used a sensitive MS-based proteomics approach to profile changes in protein composition and abundance of this organelle and to characterize its phosphoproteome in control and interferon-g activated macrophages.

First, we analyzed the expression changes of phagosomal proteins through Interferon-γ activation. In 102 LC-MS runs we identified 2,881 proteins, providing the by far most thorough analysis of this important organelle.

By combining the highly efficient TiO2-phosphopeptide enrichment with the high mass accuracy of the Orbitrap mass spectrometer we were able to identify 2,770 different phosphorylation sites on 1,064 phagosomal proteins by LC-MS and 2D-LC-MS. Although several other large-scale phosphoproteome analyses have been performed recently, 66% of these sites are unknown so far, proving the strength of sub-cellular fractionation. The identified proteins consist of 82 kinases of which some can be assembled into complete signaling transduction chains. Also identified were phosphorylations on 48 different receptors, including all known receptors involved in phagocytosis, and many GTPases.

We characterized changes in several functional groups of proteins that provide additional insights into the mechanism of Interferon-γ activation on the level of the phagosome, as well as the immunological mechanism of phagosome maturation and its possible subversion by intracellular pathogens.

T25 Microfluidic systems for miniaturizing and parallelizing biological experimentation

David Juncker

Miniaturization, parallelization, and integration are the concepts that drove the advances of microelectronics and of computer science, and which have lead to the "digital revolution". Miniaturization and parallelization have started to bear on the life sciences with the emergence of large-scale cDNA microarrays and high throughput sequencers.

We have developed and are further developing a toolbox of microfluidic and soft lithography technologies for (i) miniaturizing and parallelizing biological experiments, and (ii) for tailoring of the cellular microenvironment. I will discuss microfluidic capillary systems which we have used for ultra-miniaturized protein analysis, and which can be cloned into large arrays for large scale proteomics. We are also developing a versatile microfluidic technology using electrostatic valves that will be useful for complex fluid manipulation for cell culture and screening on a chip. Finally, we are developing the microfluidic probe which can be used to "write" and erase" patterns and objects on surfaces with a microfluidic jet. The versatility of the probe is illustrated with a series of processing examples, including protein microarrays, arrays of surface gradients, and the selective staining and contact-free removal of single living cells. We will use the probe for changing the microenvironment of cells by creating for example surface gradients and diffusible cues, and study the cellular behavior in response to a combination of different stimuli. Large scale proteomics and cell biological experiments in well defined microenvironments will drive systematic and quantitative biological studies, and provide a rich source of data for bioinformatics and systems biology.

T26 Revisiting the Wnt pathway: ask the natives to show us around

Francois Fagotto, Brian Siu, Renu Heir, Biology, McGill

The Wnt pathway has been extensively studied (3800 publications (>400 review) with Wnt, Axin, APC or Dishevelled in title). Many proteins (> 20) have been identified that are involved directly or indirectly in the pathway, and dozens of potential interactions between these proteins have been reported. Despite this wealth of information, the actual mechanism of signal transduction remains unclear, and there are probably about as many models for regulation of the pathway as molecules. All models agree on the central role of the scaffold protein Axin, which recruits most components in a large complex. The composition, localization, activity and modulation of this complex have not been established.

We are trying to answer to this crucial question by studying the endogenous components in a simple cellulal model, using biochemical and cell biological techniques. While we are still far from our final goal, i.e. a precise desciption of the pathway including qualitative,quantitative and kinetic data on the interactions and localization of the native complexes, our data have already yielded many surprises, and have infirmed several assumptions on which the current models where based.

T27 (and P81) Myotopic organization of limb innervation by spinal motor neurons is defined by Netrin and Netrin receptor expression

Dayana Krawchuk, Frederic Charron and Artur Kania

A simple myotopic topography is evident in the organization of the lateral motor column (LMC) spinal motor neurons and their axonal projections to limb muscles. Motor neurons located in the lateral LMC innervate dorsal limb muscles, whereas medial LMC motor neurons innervate ventral limb muscles. LMC motor axon trajectories are controlled by LIM homeodomain transcription factors expressed in the soma (Lim1 and Isl1) and limb (Lmx1b), in part by controlling expression of Eph tyrosine kinase receptors and their Ephrin ligands. However, genetic evidence suggests the existence of additional effector molecules controlling LMC axon guidance in the limb.

To uncover new LMC axon guidance determinants, we completed a screen for molecules whose limb expression is restricted dorsally or ventrally at the time of axon trajectory choice. We found that Netrin1, a gene encoding a diffusible molecule required for attractive or repulsive guidance of many classes of axons, is expressed specifically in the dorsal limb mesenchyme and is under the control of Lmx1b. Our analysis of Netrin receptor mRNA distribution demonstrates that receptors associated with attraction towards Netrin are expressed by lateral LMC motor neurons innervating the dorsal limb, and receptors associated with repulsion from Netrin are expressed by medial LMC motor neurons projecting ventrally. This restricted Netrin receptor expression leads to our hypothesis that Netrin1 is a bifunctional motor axon guidance cue in the limb that controls LMC myotopy. We are testing this hypothesis through gain and loss of function of Netrin pathway components in both the mouse and the chick.

T28 The hnRNP Squid is required cell-autonomously in the Drosophila germline to repress translation of unlocalized gurken mRNA in the oocyte.

Lucia Caceres and **Laura Nilson**, Department of Biology, McGill University

The Drosophila dorsal-ventral axis is established during oogenesis, when gurken mRNA and protein become strictly localized to the anterior-dorsal region of the oocyte. The Gurken protein is homologous to vertebrate transforming growth factor alpha and establishes asymmetry by activating the Drosophila epidermal growth factor receptor in the overlying somatic epithelium. We demonstrated previously that this gurken mRNA is provided to the oocyte by the adjacent germline cells, the nurse cells, that are associated with each oocyte. Our current work focuses on how factors in the nurse cells might govern gurken localization within the oocyte. One candidate is Squid, a heterogeneous nuclear ribonucleoprotein; in squid mutant ovaries, gurken mRNA is detected throughout the anterior circumference of the oocyte. To ask whether Squid is required in the nurse cells for proper regulation of gurken within the oocyte, we generated mosaic egg chambers in which only a subset of the nurse cells can produce Squid. In these mosaics, gurken transcripts are properly localized, indicating that Squid function is not required in individual nurse cells to localize gurken mRNA within the oocyte. In contrast, Squid is required cell-autonomously in the nurse cells to maintain unlocalized gurken in a translationally repressed state within the oocyte, suggesting that Squid provides gurken mRNAs with a nuclear history that regulates their translation within the oocyte cytoplasm. Our results uncouple the localization and translation functions of Squid and provide a link between nuclear history and translational repression in Drosophila.

T29 Differential ability of Ptf1a and Ptf1a-VP16 to convert stomach, duodenum and liver to pancreas

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Determining the functional attributes of pancreatic transcription factors is essential to understand how the pancreas is specified distinct from other endodermal organs, such as liver, stomach and duodenum, and to direct the differentiation of other cell types into pancreas. Previously, we demonstrated that Pdx1-VP16 was sufficient to convert liver to pancreas. In this paper we characterize the functional ability of another pancreatic transcription factor, Ptf1a, in promoting ectopic pancreatic fates at early stages throughout the endoderm and later in during organogenesis. Using the transthyretin promoter to drive expression in the early liver region/bud of transgenic Xenopus tadpoles, we find that Ptf1a-VP16 is able to convert liver to pancreas. Overexpression of the unmodified Ptf1a on the other hand, has no effect in liver, but is able to convert stomach and duodenum to pancreas. When overexpressed at earlier embryonic stages throughout the endoderm, Ptf1a activity is similarly limited, whereas Ptf1a-VP16 has increased activity. Interestingly, in all instances we find that Ptf1a-VP16 is only capable of promoting acinar cell fates, whereas Ptf1a promotes both acinar and endocrine fates. Lastly, we demonstrate that, similar to mouse and zebrafish, Xenopus Ptf1a is essential for the initial specification of both endocrine and exocrine cells during normal pancreas development.

T30 Regulation of temporal competence in retinal progenitor cells

Jimmy Elliot, Vasanth Ramamurthy, Christine Jolicoeur, **Michel Cayouette**
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In the developing retina, the earliest retinal progenitor cells (RPCs) are multipotent and at least some RPCs are competent to generate all the different retinal cell types but, as development proceeds, they loose the competence to gernerate early-born cell types, and acquire the competence to generate late-born cell types. Although it is clear that the various retinal cell types are generated in a strict, but overlapping order of cell genesis, the molecular machinery regulating how RPCs change over time to generate the right cell types at the right time remains unknown. Here, we test the hypothesis that the zinc finger transcription factor Ikaros might be controlling RPC temporal competence. Consistent with this possibility, we found that Ikaros is expressed in all early embryonic RPCs, whereas at mid-stages of retinogenesis only a sub-population of of RPCs express Ikaros, and none express it in the late postnatal retina. Using retroviral lineage analysis, we showed that misexpression of Ikaros in RPCs at late stages of retinogenesis, when RPCs no longer express Ikaros, forced the generation of early-born neurons at the expense of late-born cell types. In contrast, analysis of retinas of Ikaros null and Ikaros dominant negative mice revealed that early-born cell types are reduced. Together these results suggest a model in which Ikaros expression is both necessary and sufficient to confer early temporal competence to RPCs

Abstracts (Highly Recommended Posters)

P-HR1 Neurotrophins-Induced Cleavage of p75NTR is Dependent on Trk Activation and Potentiates Trk Signaling in PC12 Cells and in Primary Neurons.

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p75NTR potentiates survival and axonal growth of sensory neurons, possibly by enhancing Trk receptor signaling. A number of studies suggest that the p75NTR-TrkA functional interaction occurs at the cell surface where p75NTR sensitizes Trk receptors to low concentrations of neurotrophins. Intriguingly, p75NTR is a substrate for alpha-secretase, which leads to extracellular domain shedding, and for g-secretase, which releases the intracellular domain (ICD) from its transmembrane tether. The mechanism(s) that regulate generation of the ICD and the nature of its biological activity remain uncertain but recent studies suggest that p75NTR cleavage and signaling is induced by ligands that bind p75NTR (Kenchappa RS et al. 2006) or bind a p75NTR co-receptor (Domeniconi M et al. Neuron 2005).
In this study, we have examined the role of neurotrophins (NTs) in mediating p75NTR cleavage events. Initially using PC12 cells, we found that p75NTR undergoes robust cleavage in response to NGF treatment, demonstrated that NGF-induced p75NTR cleavage is dependent upon a and g- secretase activity, and required activation of TrkA and MEK. The p75NTR ICD generated via TrkA actvation accumulates in the cytosol but not to the nucleus. An analogous cascade has been identified in primary rat cerebellar granule cells, where BDNF-induced activation of TrkB induces a and g-secretase-dependent cleavage of the p75NTR. Finally, we found that a and g- -secretase inhibitors reduced phosphorylation of both Akt and Erk in response to Trk activation, indicating that p75NTR cleavage enhances Trk signaling.
Taken together, these data suggest that cleavage of p75NTR may play an important role in pro-survival neurotrophin signaling.

P-HR2 Cell lineage analysis in vertebrate retina.

Francisco L.A.F. Gomes, Michel Cayouette. IRCM – Institut de recherches clinique de Montreal

The generation of cell diversity in the central nervous system is though to involve both cell-intrinsic mechanisms and negative feedback environmental signals. The vertebrate retina contains seven major cell types, which are generated from a pool of multipotent retinal progenitor cells (RPCs) in a strict, but overlapping, chronological order of cell birth. Both cell intrinsic and extrinsic signals act to regulate RPC fate decisions during development. However, recent results indicate that intrinsic signals may be more important than currently believed in determining retinal cell fate. In addition, increasing evidence suggests that by controlling the orientation of cell division, progenitor cells can distribute cell-fate determinant to one or both daughter cells, thereby producing symmetric or asymmetric outcomes. To test the relative importance of cell-intrinsic mechanisms and extracellular signals in cell fate choice, we have cultures RPCs at clonal density and reconstructed their entire lineage using long-term time-lapse video microscopy. We identified the different retinal cell types by morphology and the expression of specific markers. Additionally, to determine whether the orientation of cell division influences cell-fate choice, we have analyzed the movies and the immunocytochemistry data and determined the fate of daughter cells generated from differently oriented divisions that developed from a a single RPC. By studying these lineage trees we should be able to identify if a particular combination of cell types arise by stereotyped patterns of cell division or, alternatively, if the clones are randomly generated.

P-HR3 Defining the role of the brat family in C. elegans embryonic polarity

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Our laboratory is interested in understanding the molecular mechanisms controlling cell polarity and asymmetric cell division using C. elegans embryo as a model. The evolutionary conserved PAR proteins (PAR-1 to -6 and PKC-3) are essential for zygote polarization and for its asymmetric division, however, the molecular mechanisms involving them remain elusive. Recently, several genes have been identified as new modulators of PAR-dependent cell polarity, for their capacity to suppress par-2(it5ts) lethality. Orthologs of two of these genes, nos-3 and fbf-1/2, function as translational repressors, together with the tumor suppressor Brat, to regulate Drosophila embryonic polarity. There are 5 predicted Brat orthologs in C. elegans (CeBrats), suggesting that they might also be involved in C. elegans embryonic polarity. Indeed, we have shown that 3 of the 5 CeBrats, (ncl-1, nhl-1 and nhl-2), are able to suppress par-2(it5ts) lethality. This result suggests that they could function in C. elegans PAR-dependent polarity through translational repression. In addition, we have shown that ncl-1, nhl-1 and nhl-2 suppress most of the early polarity phenotypes associated with par-2 depletion. Besides, the three brats mutants able to suppress par-2 lethality display distinct polarity phenotypes. These data suggest that ncl-1, nhl-1 and nhl-2 could function through at least partially independent pathways.
We are currently establishing whether ncl-1, nhl-1 and nhl-2 function, together or independently, within a complex with nos-3 and fbf-1/2 to inhibit translation and we are searching the mRNA targets of this complex, using a combination of biochemical, bio-informatic and genetic approaches.

P-HR4 Elaboration of new tools to study CNS and PNS myelination

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Myelination is a highly regulated developmental process whereby oligodendrocytes in the CNS and Schwann cells in the PNS adhere, produce and extend membranous processes to ensheath and wrap axons. Fast electrochemical transmission in the nervous system is critically dependent on the ensheathment of myelin around axons, and its destruction results in severe motor and sensory deficits, as seen in patients with multiple sclerosis. Although the myelinating cells of the CNS and PNS have been long identified, the processes by which they myelinate axons, as well as the mechanisms by which myelin degenerates, remain unknown. To better understand how myelinating cells ensheath their targets, we are developing novel systems to study this process in vitro. Using textured fibres (carbon/glass), we have observed that oligodendrocytes contact and ensheath these artificial fibers in the absence of axons, suggesting that they can be promiscuous in target selection. A second model system we are developing is to culture DRG neurons along with myelinating cells on a micropatterned surface. In this way, we can manipulate select neurites and myelinating cells in order to study the effect of laser ablation on myelin. These systems represent a novel strategy by which we can study the early events in myelination, as well as the effects of various therapeutic agents on the myelinating properties of oligodendrocytes.

P-HR5 Investigating non-canonical functions of γ-tubulin using genome-scale structure function analysis

Thao Nguyen, Lara Cuschieri, Megan Zadworny, Guillaume Lesage and Jackie Vogel

γ-Tubulin is a conserved component of microtubule organizing centers (MTOCs) that functions in the dominant pathway of microtubule nucleation in vivo. Recent studies suggest that γ-tubulin may have additional functions in dictating the organization and function of microtubules. For example, γ -tubulin in budding yeast (Tub4) appears to function in spindle positioning, as a mutation in the carboxyl terminal DYSL domain of Tub4 abrogates the Kar9-dependant pathway for spindle positioning. Tyr445 of this domain is phosphorylated in vivo, and a phospho-mimetic mutation (Tyr445Asp) results in defects in chromosome segregation. We hypothesized that non-canonical functions of Tub4 might be coordinated through differential phosphorylation of Tyr445, and that phospho-mimetic and phospho-inhibiting mutations at Tyr445 would yield sepration of function alleles. As such, these mutations would be expected to have distinctive genetic interaction profiles relative to each other, and to a generalized loss of function mutant. To investigate this hypothesis, we conducted genome-scale structure-function analysis using high-throughput Synthetic Genetic Array (SGA) analysis of synthetic genetic interactions between a given tub4 mutant and ~4600 deletion mutants. Our strategy relies on the integration of interaction data derived from multiple SGA analyses. Here, we present the first SGA analysis of the phospho-inhibiting allele tub4-Y445F. Data clustering using hierarchical algorithm was performed on gene interaction matrix to identify major clusters/pathways that Tub4 might involve and to make predictions in potential functions of uncharacterized hits. The results revealed previous characterized as well as novel pathways including spindle positioning, actin organization, checkpoints and, interestingly, DNA damage repair.

P-HR6 Notch signaling is required in differentiated neurons to maintain developmental quiescence in C. elegans

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The Notch signaling pathway is highly conserved among higher metazoans and is used repeatedly throughout development to specify distinct cell fates among populations of equipotent cells. Mounting evidence suggests that Notch signaling may also be critical in neuronal function in post-mitotic, differentiated neurons. Here we demonstrate a novel role for the canonical Notch signaling pathway in differentiated neurons in maintaining a specialized post-embryonic developmental stage in the nematode C. elegans called dauer. Our data suggest that cell signaling downstream of the developmental decision to enter dauer leads to the activation of Notch–responding genes in post-mitotic neurons to properly maintain the "diapause-like" dauer stage. Consistent with this, we provide evidence that GLP-1, one of the two C. elegans Notch receptors, is indeed expressed in differentiated neurons. Furthermore, activation of the Notch pathway requires a minimal cluster of Forkhead binding sites sufficient for the cell-type/stagespecific expression of the Notch ligand LAG-2, yet does not require the FOXO homologue DAF- 16, which is required for dauer formation. We also found that lag-2 repression during reproductive development is dependent on the C. elegans FOXC orthologue UNC-130. We propose that signaling associated with the onset of dauer ultimately initiates a Notch signaling cascade in specific, differentiated neurons and thereby affects global changes throughout the animal, that are critical to maintain developmental quiescence.

P-HR7 Imaging sensitized emission FRET in live Hela cells reveals amplification and redistribution of Cdc42 activity by the netrin-1 receptor DCC

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The receptor deleted in colorectal cancer (DCC) directs migration of growth cones towards the source of its ligand netrin-1 in part through the activation of the Rho family GTPases Rac1 and Cdc42. To visualize the distribution of active Rac1 or Cdc42 downstream of DCC two forms of Forster resonance energy transfer (FRET) based biosensors were evaluated for correct intracellular distribution and ability to report the activation state of the GTPases. The intramolecular biosensors Raichu-Cdc42 and –Rac1 consist of YFP, GTPase, Pak1 CRIB, and CFP expressed as a single molecule. The Raichu constructs displayed a dynamic range of FRET detecting changes in Rac1/Cdc42 activities in Hela cells, but both were strictly targeted to the plasma membrane. The intermolecular biosensors CFP-Rac1 or CFP-Cdc42 co-expressed with YFP-Pak1 CRIB (ICY-Rac1 or –Cdc42 respectively) reported a dynamic range of GTPase activation in addition to intact subcellular targeting and were selected to monitor Rac1/Cdc42 signaling downstream of DCC. In live Hela cells ICY-Cdc42 revealed that DCC colocalized with the most active Cdc42 and induced a gradient of Cdc42 activity, which peaked at the cell edge and declined toward the cell centre. Live cell imaging showed DCC on the cell surface undergoing Cdc42-dependent trafficking from the periphery towards the centre of the cell. Together these data indicate that DCC expression can amplify and reorganize Cdc42 signaling within a cell and identifies Cdc42 as a mediator of DCC intracellular trafficking.

P-HR8 Differential activation of RhoA, Rac1 and Cdc42 by the axon guidance cue netrin-1 and Unc5a.

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Netrin-1 is a bifunctional chemotropic cue that attracts or repels different axons and guides their migration. Two distinct families of netrin-1 receptors have been identified in vertebrates: the Deleted in Colorectal Cancer (DCC) and the Unc5 receptors (Unc5a, b, c and d) mediating the attractive and repulsive responses respectively. The intracellular molecular mechanisms underlying axonal migration are still unclear but it is known that this process requires remodeling of the actin cytoskeleton. There is now compelling evidence that Rho GTPases, in particular RhoA, Rac1 and Cdc42, are important signaling elements within the neuronal growth cone and we believe that they link netrin-1-mediated Unc5a signaling to cytoskeletal remodeling and repulsion. We found that overexpression of Unc5a or a mutant lacking the cytoplasmic tail in N1E-115 neuroblastoma cells expressing endogenous netrin-1, stimulated the formation of neurites, a process that is often associated with Rac1/Cdc42 activation and RhoA downregulation. This effect is netrin-1 dependent since it is inhibited by the addition of antibody blocking the function of netrin-1. Rho GTPase activation assays in COS-7 cells expressing Unc5a and stimulated with netrin-1 also showed a transient 1.5-and 2-fold- increase in the levels of activated Rac1 and Cdc42, respectively and interestingly, a nine-fold increase in the level of activated RhoA after 2 minutes of stimulation. In future experiments, we will monitor Rac1, Cdc42 and RhoA activities in N1E-115 cells by Fluorescence Resonance Energy Transfer (FRET), to delineate how Rho GTPases mediate the response of neurites to Unc5a/netrin-1 in a spatially and timely fashion.

Abstracts (Posters)

P1 An atypical di-leucine endocytic motif mediating the constitutive internalization of the K+/Cl- cotransporter, KCC2.

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Inhibitory neurotransmission is critical for the proper function of the central nervous system (CNS). The neuron-specific K+/Cl- cotransporter, KCC2, plays an essential role in driving the developmental transformation of GABAergic and glycinergic transmissions from excitatory to inhibitory and maintaining the strength of inhibition in the adult CNS. While it has been shown that the overall expression level of KCC2, and hence KCC2 transport activity, can be regulated by neuronal activity and neurotrophin signalings, the post-translational mechanisms such as membrane trafficking that mediate KCC2 are yet poorly understood. In this study, we have been engaged in understanding the molecular mechanisms regulating the constitutive endocytosis of KCC2 and mapping the endocytic motif(s) involved. By utilizing a fluorescence-based endocytosis assay, we show that KCC2 undergoes clathrin-mediated constitutive endocytosis in heterologous HEK293 cells and that endocytosed KCC2 is sorted to the recycling pathway. Using a series of KCC2 chimeras where we fused different regions of KCC2 to endocytosis deficient reporter proteins, we identified an endocytic di-leucine motif, LL657,658, located in the KCC2 carboxyl terminus. We demonstrate this motif plays an essential role in mediating the constitutive endocytosis of KCC2. Furthermore, this di-leucine motif is also responsible for binding the adapter protein 2 (AP-2), which is known to recruit cargo proteins to the clathrin-mediated endocytosis pathway. Altogether our studies suggest the endocytosis of KCC2 may be a means by which its transport activity can be regulated. Thus, KCC2 endocytosis could be a potential mechanism by which KCC2-coupled synaptic inhibition and neuronal plasticity are regulated.

P2 The role of CdGAP in cell migration, adhesion, and invasion using a mouse breast cancer metastasis model system

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RhoA, Rac1, and Cdc42, the best-characterized members of the Rho family of small GTPases, are critical regulators of many cellular activities. Rho GTPases act as molecular switches and exist in either an inactive GDP-bound or an active GTP-bound conformation. Guanine nucleotide exchange factors (GEFs) catalyze the exchange of GDP for GTP, leading to activation in response to various stimuli. GTPase-activating proteins (GAPs) increase the intrinsic GTPase activity, resulting in inactivation of the protein. A significant number of RhoGAPs have been shown to present altered expression patterns in a variety of human cancers and cell lines. CdGAP (Cdc42 GTPase-activating protein) is a serine- and proline-rich RhoGAP protein showing GAP activity against both Cdc42 and Rac1 but not RhoA. We have previously demonstrated that CdGAP is phosphorylated downstream of the MEK-ERK pathway in response to serum and that CdGAP is a serum-inducible gene. CdGAP is also required for normal cell spreading, polarized lamellipodia formation, and cell migration. More recently, we have observed a high increase in the expression levels of CdGAP proteins and mRNAs in explant cultures made from mammary tumors arising from NMuMG immortalized mouse mammary cells expressing the oncogenic ErbB-2/Neu-NT receptor. These cells induce rapidly growing mammary tumors, which aggressively metastasize to the lung compared to control cells. Using an siRNA approach to downregulate the expression of CdGAP, we will investigate whether CdGAP regulates cell migration, adhesion, and invasion using this mouse breast cancer metastasis model system.

P3 CdGAP interacts with the SH3D domain of the scaffolding endocytic protein intersectin through a novel and atypical motif

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The small guanine nucleotide binding proteins of the Rho family of proteins (Rho GTPases) act as molecular switches by cycling between an inactive GDP-bound state and an active GTP-bound state. They are inactivated by GTPase-activating proteins (GAPs), which bind the GTPases in their GTP-bound state in order to stimulate their intrinsic GTP-hydrolysis activity. CdGAP is one of the 70 RhoGAPs expressed in mammals and is specific for Cdc42 and Rac1. Our laboratory has previously showed that the endocytic protein intersectin interacts with CdGAP through a subset of its SH3 domains to inhibit CdGAP activity. Interestingly, the binding region on CdGAP contains no consensus PxxP binding site for SH3 domains. Our goal is to identify the motif required to mediate this interaction and to determine the structure of this complex. Deletion mutant analysis revealed that an 80a.a. domain conserved among three other RhoGAPs is necessary for the interaction. One of these RhoGAPs, ARHGAP30, also binds through the same domain to intersectin SH3D. Substitution of three conserved basic residues to alanines in this domain of CdGAP reduced its affinity for intersectin. Therefore, our current data suggest that the SH3D domain of intersectin binds to an atypical motif. To get a better understanding of the function of CdGAP, we are also generating a knockout mouse for the CdGAP gene using a LacZ-neomycin cassette. The combination of these genetic, cell biology and biochemistry experiments should allow us to get a better understanding of the function of CdGAP and intersectin.

P4 The Effects of Manipulating Tensegrity and the Contractile Array

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The overall stability and framework of any cell depends upon the tensional prestress brought about by its cytoskeletal components. Therefore, changes to this tensional homeostasis within the cell should have consequences upon the biochemical signaling pathways that affect the dynamics of the cytoskeleton. Using the Xenopus laevis oocyte model system, this study looks at the effects of manipulating global tension on wound-induced actomyosin contractile rings (Mandato and Bement, 2003). Decreasing cytoskeletal tension coincides with biochemical signals that give rise to a contractile array that is markedly reduced. Our results indicate that there is indeed a reduction and that it is dose-dependent, with a drastic change in the contractile ring morphology in the presence of a hypertonic environment. This attenuation in the tensegrity of the cell also prevents the contractile array from ever closing. It is well known that the Rho GTPases, Cdc42 and RhoA, specifically play a role in the regulation of contractile ring assembly, mediating its organization and function in distinct ways (Benink and Bement, 2005). Upon decreasing cellular tension RhoA expression appears to be decreased at the contractile array, while Cdc42 appears to be upregulated, suggesting crosstalk between the two GTPases. Furthermore, a constitutively-active RhoA mutant rescues contractile array formation and closure, and is resistant to decreases in tension brought on by a hypertonic environment. These results suggest for the first time, that biochemical signals such as the Rho GTPases can sense and mediate tensional changes in the cell.

P5 Growth-arrest-specific Protein 2 Stops Cell Division Possibly by Disrupting Microtubules Dynamics

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Growth-arrest-specific 2 (gas2) gene was initially identified at high level of expression in murine fibroblasts under growth arrest conditions and followed by down-regulation upon serum stimulation to re-entry into the cell cycle (Schneider et al., 1988). It has been shown that gas2 gene product—Gas2 protein may interact with both F-actin and microtubules (Brancolini et al., 1992 and Sun et al., 2000). In our study, full-length Gas2 protein was microinjected into Xenopus laevis embryos, and this permanently stops subsequent cell division. Cryo-confocal microscopy analysis also suggested that Gas2 overlaps microtubules. A cytokinesis analog contractile array assay (Mandato and Bement, 2001) showed that GFP-Gas2 co-localizes with double F-actin rings during the cell wound healing, which is a typical phenotype when the Xenopus laevis oocytes are treated with microtubule stabilization drug Taxol. Furthermore, preliminary data suggested that Gas2 may play a role in microtubule dynamic instability; therefore, we hypothesize Gas2 arrests cell division by disrupting cell cycle metaphase checkpoint.

P6 Investigation of the role of the small Ras-family GTPase Rab35

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Rabs belong to the largest family of monomeric small GTPase proteins. There are more than 60 members identified in mammals. They have distinct subcellular localization and often are involved in inter-organelle transport. Rab35, one of these members, is poorly characterized so far and its localization and role are unknown. In this study, a fluorescent-tagged Rab35 was analyzed to characterize its localization and better understand its role. It is found mainly at the plasma membrane, and also is present in small unidentified structures in the cytosol. This Rab35 do not colocalize to any classical endocytic markers like clathrin or caveolin but do colocalize with some GPI anchored proteins. Using pulse chase experiment, a fluorescent folate which binds to its GPI-anchored receptor was found to colocalize with over-expressed Rab35 construct after short period of time. GPI proteins are believed to be endocytosed in a clathrin- and caveolin-independent fashion into endocytic compartments termed GEEC (GPI-AP enriched early endosome compartment). We also found that over-expression of activated form of Rab35 on Baby Hamster Kidney cells; highly affect the morphology of these cells. Indeed we found that Rab35 colocalize with actin to some extent, and that motility of Rab35 positive vesicles depends on actin but not on microtubules. The process and mechanisms of endocytosis via GEEC and cargo sorting are poorly understood. It is not clear at which step Rab35 is involved, but investigating the role of this protein and finding its effectors may help to unravel the mechanisms involved in that transport.

P7 Dose-Regulated Clathrin-Dependent and Independent Endocytosis of Nerve Growth Factor Receptor in PC12 Cells

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We find that ligand-induced TrkA/NGF endocytosis is clathrin-mediated at low extracellular concentrations of NGF (1 ng/ml) with targeting to a transferrin/EEA1 containing conventional early endosomal compartment in PC12 cells. This endocytosis is inhibited by overexpression of truncations of the clathrin adaptor protein beta-arrestin 1. Moreover, by co-IP we find a ligand-induced interaction between TrkA and beta-arrestin 1, and ligand-induced recruitment of beta-arrestin 1 to clathrin pits. At higher extracellular doses (50 ng/ml) we find TrkA/NGF uptake is independent of clathrin, dynamin, caveolin and beta-arrestin 1, but requires actin, Cdc42 and sphingolipids. At 50 ng/ml, TrkA/NGF is targeted to endosomes lacking EEA1, but enriched in fluid phase markers and GPI-anchored proteins similarly to previously described GPI-AP enriched early endosomes (GEECs). The mode of endocytosis is correlated with ultimate fate of the proteins, with actin-mediated endocytosis leading to degradation, and clathrin-mediated endocytosis leading to long-term intracellular retention of intact NGF. We propose that the route of NGF/TrkA endocytosis (clathrin-dependent vs. independent) determines fate of ligand with clathrin-mediated endocytosis required for long-term cellular retention in signaling endosomes and Cdc42/actin/mediated endocytosis leading to efficient degradation of ligand. Beta-arrestin 1 may be the adaptor protein for phosphorylation/ligand-dependent clathrin-mediated uptake.

P8 Expression of fibrillin-3 in early human development

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Fibrillins are the major ~350 kDa components of extracellular microfibrils found in many tissues including blood vessels, bone and the eye. Fibrillin-1 and -2 are well characterized and mutations in these proteins have been shown to cause a number of fibrillinopathies including Marfan syndrome and congenital contractural arachnodactyly. Fibrillin-3 was very recently discovered and is thus much less well characterized. It has been suggested as a candidate for the recessive form of Weill-Marchesani syndrome. The goal of this project is to characterize the expression and the function of fibrillin 3.

The C-terminal half (rFBN3-C, ~190 kDa) of fibrillin-3 was recombinantly expressed in human embryonic kidney cells. Expression in eukaryotic cells is important since the protein is stabilized by numerous intramolecular disulfide bonds requiring the folding apparatus in the endoplasmic reticulum of eukaryotic cells. The protein was purified to homogeneity by immobilized metal ion affinity chromatography. A polyclonal antiserum against rFBN3-C was raised in rabbit and any cross-reactivity against other fibrillins and homologous fibulins was eliminated by pre-clearing the anti-serum with cross-reactive fibrillins and fibulins coupled to sepharose. The resulting high quality monospecific antiserum was used to characterize fibrillin-3 expression patterns in early human development (6-12 weeks of development). Fibrillin-3 was found to be expressed in perichondrium, perineurium, in developing bronchi, glomeruli, pancreas and gut, but most interestingly, in developing epithelium. The overview of the developmental fibrillin-3 expression will be presented. This work establishes fibrillin-3 as an important connective tissue protein in early human development.

P9 Identification of New Zealand Chironomids: does morphology match molecules?

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The aquatic non-biting midges (Diptera: Chironomidae) are globally distributed and one of the most abundant group of insects found in freshwater ecosystems. They play an important role in aquatic food chains as a major food source for both vertebrate and invertebrate species. Chironomids are also important bio-monitoring tools for a range of studies; from environmental quality to eco toxicity assays. Unfortunately, their accurate identification is often complicated by morphological conservatism and the existence of cryptic species, which can make ecological studies of these species problematic. However, advances in molecular techniques such as the banding pattern of larval polytene chromosomes (Martin, 1979) or the use of mitochondrial sequences for Cytochrome b or Cytochrome c oxidase subunit I locus (COI) have the potential to distinguish such cryptic species. To test the utility of molecular techniques, I used New Zealand Chironomus spp., and compared traditional morphological identification with polytene chromosome characterization and DNA sequencing of the mitochondrial enzyme COI. Based on morphological evaluation, 5 species of Chironomus were identified. DNA sequences and polytene chromosome characterization, matched morphological identifications and were successful in discriminating even closely related Chironomus species (COI divergences >5% in all cases). Due to the challenges of traditional morphological taxonomy within this genus genetic "bar-coding" techniques using the CO1 gene would appear to be the most promising method to easily distinguish these species. On going work involving additional species and sampling locations is currently underway.

P10 The Developmental Basis of Evolutionary Changes in Vestigal Disc Morphology in Ants

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Wing polyphenism in ants evolved once, giving rise to colonies with a winged reproductive caste and a wingless sterile caste. Even though the wingless caste does not produce any wings, it possesses vestigial wing imaginal discs. There is a remarkable diversity in the shape and form of these vestigial wing imaginal discs in different ant species. Here we analyze vestigial wing disc morphology in over 60 ant species. We constructed a molecular phylogeny using wingless, LWR, COI and Cytb in order to analyze the rates of evolution and elucidate the ancestral state of imaginal disc morphology. Our phylogenetic comparative analyses to date reveal that disc morphology is conserved within genera, but is variable across genera with the sporadic appearance of convergent disc morphology in different subfamilies of ants.

P11 WITHDRAWN

P12 Identification of the restorer of fertility genes for cytoplasmic male sterility in Brassica napus.

Ryan Nantel-Smith and Dr. Gregory Brown

Cytoplasmic Male Sterility (CMS) is a maternally inherited defect in pollen production specified by novel open reading frames in mitochondrial DNA. CMS can be suppressed by nuclear restorer of fertility (Rf) genes. Two forms of CMS, nap and pol, are native to the canola species Brassica napus and have corresponding restorers of fertility Rfn and Rfp, which have been shown to be allelic. Most restorer genes found to date encode proteins with multiple tandem copies of a degenerate, 35 amino acid repeat sequence, the pentatricopeptide repeat (PPR). We expect Rfn and Rfp genes to encode PPR proteins.

A genomic library possessing Rfp was initially used to identify the genomic region spanning the restorer. Additionally, a 170 Kb BAC of the region specifying the Rfn gene has been identified and preliminarily sequenced. 6 putative PPR containing genes have been found in the sequenced region thus far. The Rfn and Rfp allelic forms of these PPR have been amplified and will be sequenced to identify differences between the two genotypes. Candidate genes will be introduced into the appropriate B. napus CMS line to assess their biological function as restorer genes by their capacity to rescue the CMS phenotype.

Analysis of the allelic Rfn and Rfp may provide insight into the molecular features of restorer genes which are necessary for their specificity. Furthermore, we hope to gain insight into the molecular events underlying the evolution of restorer genes in the nucleus in response to the appearance of CMS genes in mitochondrial DNA.

P13 Mclk1 heterozygosity affects Ras-induced tumor formation in mice

D.O. Cliche and S. Hekimi

Heterozygous Mclk1 mice have extended lifespan and altered mitochondrial functions. Since cancer is highly correlated to age, we hypothesized that Mclk1+/- mice have cancer fighting properties. In C.elegans, clk-1 was found to decrease a ras pathway. Tg.AC mice carry the v-ha-ras oncogene the expression of which is inducible by skin wounding. Upon induction, skin papillomas form. This study focuses on the occurrence of papillomas in Mclk1+/- mice and tumor development. In a BALB/CxFVB/N background, Mclk1+/- tumor growth is compromised. Mclk1+/- mice have decreased tumor size and decreased growth rates but the tumor incidence is the same as control mice. In contrast, C57BL/6xFVB/N Mclk1+/- mice have increased tumor incidence, increased tumor multiplicity and increased tumor volume and growth rates. These differences may be due to the use of different genetic backgrounds or the fact that the animals were not at the same age at the time of induction. In the BALB/CxFVB/N experiment, the mice had 21 weeks, compared to 11 weeks in the C57BL/6xFVB/N experiment. In this cancer model, tumor formation is remarkably age-dependent. The tumor multiplicity increases linearly with age. Therefore, in addition to the background, the results may have been affected by the age of the animals. Consequently, C57BL/6xFVB/N mice will be aged to 40 weeks to see if older Mclk1+/- animals have increased tumorigenesis compared to wild type animals. This could nicely illustrate the notion of trade-offs in Mclk1+/- mice that are thought to be unavoidable in long-lived mutants.

P14 Long-Lived Mclk1+/- Mice are not Resistant to Experimentally-Induced Atherosclerosis

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Mice that are heterozygous for Mclk1, a mitochondrial enzyme required for the biosynthesis of ubiquinone, live longer than wild type mice. The prolonged survival implies a decreased mortality from lethal pathologies. Atherosclerosis, one of the main age-dependent pathologies in humans, develops in mice that lack Apolipoprotein E (ApoE) or the Low Density Lipoprotein Receptor (LDLr). Using these models, we sought to determine if Mclk1 heterozygosity protects against this disorder which does not normally affect mice. ApoE mice were sacrificed at 10 and 18 months of age. LDLr mice were fed a high-fat, high-cholesterol diet and sacrificed at 6 and 10 months of age. Mclk1 heterozygosity did not clearly protect against atherosclerosis in any condition. ApoE-/-;Mclk1+/- females sacrificed at 10 months of age actually had more severe atherosclerosis than their controls. Plasma ubiquinone levels, which can modulate atherosclerosis severity, were not affected. Plasma cholesterol and lipid peroxidation were not altered, although plasma triglycerides were decreased in ApoE-/-;Mclk1+/- males sacrificed at 10 months of age. In several experiments, Mclk1+/- mice had less white adipose tissue, but this was independent of any effect on atherosclerosis. Furthermore, Mclk1 heterozygosity did not extend lifespan of mice in the ApoE background. In conclusion, Mclk1+/- mice do not appear to be protected against experimentally-induced atherosclerosis and the longevity promoting effect was not observed in the ApoE model of chronic disease. Extended lifespan in mice therefore does not necessarily confer protection against diseases, such as atherosclerosis, that are age-related in humans but not in mice.

P15 Bicaudal-C regulates nos expression during oogenesis

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Bicaudal-C (BIC-C), a KH domain RNA binding protein, functions during oogenesis to establish anterior to posterior polarity in the Drosophila oocyte. Homozygous Bic-C mutant females arrest oogenesis at stage 10. Heterozygous Bic-C mutant females produce embryos with a range of patterning defects, including bicaudal embryos. We mapped the BIC-C binding site to an evolutionarily conserved region of about 20 nucleotides in the nos 3' UTR. This is within a large regulatory module for the nos mRNA that was identified genetically and that contains multiple elements for mediating its localization and translational control. nos translational control is crucial during ovarian and early embryonic development and it is tightly regulated both spatially and temporally. Immunostaining of Bic-C homozygous mutant ovaries reveals higher levels of NOS protein, particularly in the oocyte. Furthermore, embryos produced by Bic-C/+ heterozygote mothers exhibit anterior ectopic expression of the NOS protein. This suggests that NOS de-repression may underlie the observed bicaudal phenotype. Together, these data imply that BIC-C is a key regulator of NOS expression in vivo.

P16 Regulation of Vasa expression by the SPRY-domain and SOCS-box containing protein Gustavus?

Jan-Michael Kugler and Paul Lasko, Department of Biology, McGill University

Gustavus (Gus) binds Vasa through its SPRY-domain and ElonginBC through its SOCS-box, suggesting that Gus may act as substrate recognition subunit of a ubiquitin E3-ligase that regulates Vasa expression.
PiggyBac gus[f07073] disrupts gus expression. Vasa levels are significantly reduced in gus[f07073] ovary extracts. gus[f07073] embryos rarely hatch and often collapse, but can partly be rescued by maternal expression of transgenic Venus::Gus. Venus::Gus is enriched perinuclear in ovarian germ-line cells and transiently accumulates posteriorly in mid-oogenesis oocytes. Posterior, but not perinuclear accumulation requires oskar and vasa.
In apparent contradiction to reduced Vasa levels in gus[f07073] ovaries, GFP::Vasa deleted or substituted for the entire Gus binding site is expressed at high levels, localizes similar to non-mutant Vasa and rescues vasa phenotypes. Surprisingly, however, we were hitherto unable to isolate well expressing transgenes containing similar substitutions within the Gus binding site. We speculate that multiple factors might compete for the Gus binding site to fine-tune Vasa expression.
Our candidate is F-box protein CG4643, which contains a SPRY-domain strikingly similar to Gus. We detect CG4643 transcripts in ovaries and transgenic CG4643::HA is enriched perinuclearly in nurse cells and in the oocyte posterior. Currently, we are phenotypically characterizing PiggyBac CG4643[f06595] and testing for Vasa-CG4643::HA interaction.

P17 Translational regulation of Drosophila germ plasm component germ cell-less.

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During Drosophila oogenesis and embryogenesis, gene products are sequestered in discrete regions to ultimately achieve the characteristic asymmetry of the early embryo. germ cell-less (gcl) RNA, like other germ plasm components, is present throughout the oocyte during oogenesis, then accumulates in the germ plasm, at the posterior pole of the embryo, after fertilization. Although gcl RNA is detected outside of the germ plasm during early embryogenesis, Gcl protein is detected only in the germline. In addition, previous studies have shown that somatic expression of Gcl is developmentally detrimental.
Using transgenic constructs, we find that gcl RNA localization and translation are controlled by its 3'UTR. Our results indicate that gcl translation is repressed outside of the germ plasm and that mediators of translational control can be competed by both endogenous and transgenic gcl. We are currently investigating candidate mediators of translational control of gcl during oogenesis and embryogenesis. A promising candidate is Bruno, which represses the translation of other germline transcripts. Loss of function and overexpression of Bruno cause reciprocal effects on Gcl expression during oogenesis. The gcl 3'UTR contains one Bruno Response Element (BRE), a U-rich sequence found in other Bruno targets that has been shown to interact directly with Bruno. Bruno can bind to the gcl 3'UTR in vitro, however, this binding is at least partially independent of the BRE. Ongoing investigation addresses the developmental consequences of disrupting the Bruno-gcl interaction. This work demonstrates the conservation and divergence of mechanisms of translational control in early Drosophila development.
Please thank Jocelyn for all of her good work in organizing this event (perhaps buy her a beer or a glass of wine!)

P18 Dauer-dependent regulation of germline quiescence in C. elegans

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Under suboptimal growing conditions, Caenorhabditis elegans can enter a developmentally arrested stage known as dauer that is specialized for dispersal and survival. Entry into this stage is controlled by three independent signaling pathways that affect morphological, physiological and behavioral changes. One such change includes the establishment of cell cycle quiescence within the dauer germ line.

Mutations in aak-2(AMPK) or par-4(LKB1) were found to cause germline hyperplasia during dauer. We conducted a genetic screen to isolate mutations that phenocopy the aak-2 and par-4 phenotypes in the germ line. Three independent recessive mutants (rr93, rr94 and rr95) with dauer-dependent germline hyperplasia were found to complement both aak-2 and par-4 mutants. rr93 mutant dauers possess three-fold more germ cells than wild type dauers, while rr94 and rr95 both exhibit similarly increased germ cell counts.

Interestingly, rr94 mutants display dauer-dependent sterility, a phenotype not shared with rr93 and rr95. The gonads of rr94 adults that have recovered from the dauer stage are disorganized and contain an excessive number of germ cells. This phenotype is independent of the time spent in dauer before recovery. In addition, both rr94 and rr95 mutants exhibit a reduced dauer lifespan, although the lethality is less penetrant in rr95 dauers. The germ line hyperplasia in dauer observed in rr94 mutants occurs independently of whether dauer formation is induced by reduced insulin-like or TGF-β signaling, suggesting that these signals converge on this genetic pathway. Cloning and molecular characterization of rr94, rr93 and rr95 are underway.

P19 Identification of lethal regulators of cell division and cell growth using a high throughput strategy in C. elegans

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The intestinal cells of C. elegans undergo three types of developmentally-regulated cell cycles: mitosis during embryogenesis, then karyokinesis (nuclear division) and endocycles postembryonically. Endocycles are very specialized in that successive S-phases proceed without intervening mitosis, enabling these cells to grow without undergoing cell division.

To uncover the regulatory mechanisms that allow these cells to bypass the mitotic checkpoint, we designed an RNAi-based genetic screen to identify genes that affect endocycle timing or execution in the intestine. To circumvent any potential RNAi-associated lethality, rde-1 (argonaute-like) mutant animals, which cannot elicit the RNAi response, were rescued specifically in the intestine with rde-1+ expressed under the control of gut-specific promoters from mid-embryogenesis to adulthood, restoring the RNAi pathway exclusively in the intestine. Feeding these animals with the RNAi library will allow us to identify genes that act in the intestine to regulate the developmental switch from mitotic cell cycles to the endocycle, without adverse effects on other tissues or during early embryogenesis.

We demonstrated the effectiveness of our approach by phenocopying the intestinal hyperplasia of cki-1(p27/KIP1) mutants and by inducing intestinal cell cycle defects when targeting essential licensing replication factors like cdt-1 or orc-2. Thus our strategy provides a novel and efficient method to identify tissue-specific functions of essential regulatory genes that would have been impossible to detect by classical genetic approaches.

P20 Functional analysis of a novel RING domain protein (RNF-1) during development of C. elegans

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Cell cycle progression is regulated by several classes of cyclin-dependent kinases (CDK), whose activities are in turn constrained by CDK inhibitors (CKIs). In C. elegans, cki-1 and cki-2 encode p21/27 family CKIs. Unlike cki-1, little is known about the function of cki-2. To understand its roles, we identified CKI-2 interacting partners PCN-1, SMO-1 (SUMO orthologue), and RNF-1 (a RING domain protein).
Using RNF-1 anti-serum, we observed that RNF-1 is present at the nuclear periphery. We are currently determining the biological importance of this localization. Previous work revealed that misexpression of CKI-2 causes 20%-30% embryonic arrest. However, this arrest was suppressed by 60% when RNF-1 was co-expressed with CKI-2, suggesting that RNF-1 antagonizes CKI-2. Western blotting with CKI-2 anti-serum revealed that co-expression of RNF-1 and CKI-2 accelerate the degradation of CKI-2. Since most RING domain proteins are ubiquitin ligases, we tested whether RNF-1 affected CKI-2 stability through ubiquitination-dependent proteolysis. We found that removal of other critical components of the ubiquitination or proteasome machinery greatly reduced the modification and the degradation of CKI-2, while these results were not observed when the critical components required for SUMOylation were removed, suggesting that RNF-1 is unlikely to be involved in SUMOylation. Our previous work also revealed that depletion of CKI-2 may cause DNA-damage sensitivity. rnf-1 (RNAi), on the other hand, shows some degree of DNA-damage resistance, which is consistent with the idea that RNF-1 genetically antagonizes CKI-2. We are currently determining how CKI-2 and its partners function in response to DNA damage.

P21 PTEN, LKB1 and AMPK block divisions in the germline stem cell population during dauer development in C. elegans

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When C. elegans larvae encounter adverse environmental conditions, they can arrest reproductive development and execute an alternative diapause-like "dauer" stage. This decision is genetically controlled by the activity of three parallel signalling pathways: insulin-like, TGF-ß, and cGMP signalling. We found that all three signals rely on the activities of PTEN and LKB1 to appropriately arrest germ cell divisions during dauer development. In humans, mutations in genes involved in TGF-β signal transduction, in PTEN or in LKB1 give rise to three similar syndromes that share a cancer predisposition. We therefore hypothesized that mutants that would form abnormal dauers exhibiting germline hyperplasia, and phenocopy PTEN or LKB1 mutant dauers, could result from mutations in their downstream effectors, or regulators.
From a genetic screen based on this idea, we characterized two mutant (aak-2 and aakb-2) in which dauer larvae show pronounced germline hyperplasia. These genes encode C. elegans AMP-activated kinase alpha-2 (aak-2) catalytic and beta-2 (aakb-2) regulatory subunit homologs. Our work suggests that aak-2 functions together with aak-1 (AMP-activated kinase alpha-1 catalytic subunit), both of which require aakb-2 to progressively block germline proliferation during dauer development, linking metabolic status, through insulin-like signaling, to proliferation of the germline stem cell population.

P22 Pellino, a novel component of dorsoventral patterning?

Amir H. Haghayeghi, Amila Sarac, Frieder Schöck

Our research is focused on a zygotic lethal mutation that we named “7T2”. This mutation affects embryonic development of Drosophila. 7T2 has been mapped to the pellino gene. We have used the UAS/GAL4 system to rescue the 7T2 phenotype by ubiquitously expressing Pellino, confirming the mapping results. The scaffold protein Pellino was initially found in a yeast two hybrid screen as a binding partner of Pelle, a protein which plays a crucial role in dorsoventral patterning. Additionally, recent studies revealed a function of the human pellino homolog in the Toll and JNK pathways suggesting that Pellino in Drosophila might have a similar role.
Using germline clones we found that 7T2 mutant embryos exhibit three phenotypes: germband retraction failure (U-shaped phenotype), head defects and twisted phenotype. Immunostaining of mutant embryos show alterations in ventral expression of Twist that is consistent with a perturbation of dorsoventral patterning.
We will report our progress in investigating the function of Pellino in the Toll and JNK pathways in Drosophila.

P23 Zasp is a cytoskeletal adaptor regulating the attachment of integrins to actin filaments

Klodiana Jani and Frieder Schöck

The integrin family of heterodimeric transmembrane receptors mediates cell-matrix adhesion via intracellular adaptor proteins that connect integrins to the actin cytoskeleton. Using an RNAi screen in Drosophila tissue culture cells we identified a gene we named zasp, which encodes a member of the Alp/Enigma family of PDZ-LIM domain proteins. Here we show that Zasp localizes to focal adhesions and loss of Zasp prevents integrin-dependent cell spreading by disrupting focal adhesions. In tissues, focal adhesions can mature into muscle attachment sites. We demonstrate that Zasp colocalizes with βPS integrin in muscle attachment sites and with α-actinin in muscle Z-lines. Fly larvae lacking Zasp do not form Z-lines and fail to localize α-actinin to the Z-line. In addition, zasp mutants exhibit muscle attachment defects characterized by a detachment of actin fibers. Finally, Zasp strongly interacts genetically with integrins. Our data demonstrate that Zasp is a novel cytoskeletal adaptor regulating the attachment of integrins to actin filaments.

P24 Withdrawn

P25 γ-tubulin is required for functional Kar9-Bim1 complexes during early spindle placement in budding yeast

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Proper spindle placement prior to anaphase is essential for high fidelity chromosome segregation. Much of what is known regarding spindle placement comes from studies on the budding yeast, Saccharomyces cerevisiae, which undergoes polarized growth and an asymmetric cell division. In yeast, the microtubule +end interacting proteins, Kar9 and Bim1, promote interactions between the +ends of astral microtubules and cortical actin to facilitate spindle placement. Paradoxically, Bim1 and Kar9 also associate with spindle pole bodies (SPBs), the fungal centrosome, suggesting that SPBs facilitate their function during spindle placement. Consistent with this, we previously demonstrated that a mutation within a SPB component, γ-tubulin (tub4-Δdsyl), perturbs early spindle placement by disrupting the SPB localization of Kar9 and Bim1. In tub4-Δdsyl cells, Kar9 complexes accumulate on microtubule +ends with reduced levels of Bim1. These complexes suppress microtubule dynamics and promote prolonged tethering of microtubule +ends to the cortex. Previous studies have shown that differential phosphorylation of Kar9 at the SPB by the Cdk1 orthologue, Cdc28, is important for proper spindle placement. We observe that Bim1 is also a phospho-protein and that this phosphorylation is perturbed by the tub4-Δdsyl mutation. Moreover, cells lacking the Cdc28 mitotic cyclins, Clb3 and Clb4 exhibit suppressed microtubule dynamics similar to tub4-Δdsyl cells. Finally, we report that γ-tubulin itself co-immunoprecipitates with Cdc28. We propose that γ-tubulin facilitates the formation and Cdc28 dependent regulation of Kar9-Bim1 complexes at SPBs. This event is essential for the ability of Kar9-Bim1 complexes to mediate interactions between microtubule +ends and the cortex during spindle placement.

P26 PATCHY encodes a putative beta-xylosidase/arabinofuranosidase required for pectin sidechain modification in Arabidopsis mucilage secretory cells (MSC)

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In Arabidopsis, the epidermal cells of the outer ovule integument differentiate through a complex process into specialized cells that produce mucilage between the primary cell wall and plasma membrane. Upon imbibition, the mucilage expands rapidly, breaking through the primary cell wall and enveloping the seed. A mutation in PATCHY (PTY) causes a peculiar phenotype where mutants have patchy and delayed mucilage release compared with wild type seeds. These mutants appear to undergo normal mucilage production and mucilage secretory cell development. Cloning of PTY revealed a T-DNA insertion in AtBXL1, a gene encoding a beta-xylosidase/arabinofuranosidase. Molecular complementation, and two independent Salk T-DNA knockout lines in the same locus producing a similar 'patchy' release phenotype, confirms the gene is involved in mucilage release. Expression analysis with qualitative RT-PCR and a promoter GUS fusion showed expression of PTY in all tissues tested, including 7 and 10 day old seeds, consistent with its proposed role in mucilage release and cell wall modification. Preliminary chemical analysis suggests that pty mutants may have an increase in (1-5)-linked alpha-D-arabinan in their seed coat mucilage when compared to wildtype. Genetic analysis suggests PTY may act independently of known MSC regulators.

P27 The seed coat of Arabidopsis thaliana as a model system to study plant plasma membrane to cell wall adhesion.

McFarlane, H.E., Kwok, M., and Western, T.L.

Traditionally, plant cells are thought of as static and constrained because of their rigid cell walls. However, new evidence suggests a model in which plasma membrane and cell wall interactions are dynamic. As a result, there is considerable interest in determining the key proteins that act to link the plasma membrane and the cell wall. While there are many hypotheses as to which gene families are involved in adhesion, few proteins have been directly implicated in this process. The goal of my work is to use the seed coat of Arabidopsis thaliana as a model system to study plasma membrane to cell wall adhesion. Twenty-two candidate genes were identified as potential adhesion proteins using publicly available gene expression profiles and data from seed-specific microarrays that were recently performed in this lab. Homozygous mutants were isolated and confirmed for all of these genes. In order to phenotype mutant plants for defects in plasma membrane to cell wall adhesion, this adhesion was first characterized in the wild type seed coat. Mutant plants were screened for defects in plasma membrane to cell wall adhesion and changes in seed coat morphology that may result form defects in adhesion. Preliminary results indicate that some arabinogalactan proteins may be involved in this process. Currently, further phenotypic analysis is being preformed to confirm these potential plasma membrane to cell wall adhesion mutants and double mutants are being produced and analyzed.

P28 A Reverse Genetics Approach to Understanding Mucilage Synthesis in the Aarabidopsis Seed Coat

James Schafhauser, Helene Virasith, Felicia Kim, and Tamara Western

In Arabidopsis, differentiation of the epidermal cells of the seed coat after fertilization leads to the production of a pectinaceous mucilage, which is synthesized in the Golgi apparatus and secreted between the plasma membrane and the primary cell wall. Very little is known about which genes are involved in the synthesis of pectins. We are using two reverse genetics approaches to identify genes that may be involved in the synthesis of mucilage in the Arabidopsis seed coat. The first is datamining the expression patterns of predicted cell wall genes. Putative knockout mutants for genes which showed high expression levels in seed and silique tissues were selected. None have shown reduced amounts of seed coat mucilage, suggesting genetic redundancy. Double mutants are being constructed and analyzed to further investigate these genes. The second approach is a microarray experiment performed between wild type seed and those of a known mucilage secretory cell developmental mutant. Expression results from the microarray data are being confirmed through RT-PCR and knockout mutants are being isolated. Through the examination of these genes we hope to refine the proposed genetic pathway for the regulation of mucilage production and secretion during seed coat epidermal cell differentiation.

P29 Mapping and characterization of a new meiosis specific mutant

Sara Labella and Monique Zetka
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Meiosis takes place in diploid germ cells that have to undergo two rounds of cell division in order to produce haploid gametes. In the first meiotic division homologous chromosomes are separated from each other, a process that depends on a physical connection (chiasma) between the homologs. In order to establish chiasmata, homologous chromosomes have to align, synapse and recombine earlier in prophase. Any defect in one of these processes causes aneuploidy and thus embryonic lethality.

Caenorhabditis elegans is a powerful model to study meiosis because it allows the identification of meiosis specific mutants by simply screening for worms with Him (High Incidence of Males) and Emb (Embryonic lethality) phenotypes that occur as a result of chromosome missegregation. We have isolated one such mutant, vv44, which shows a new phenotype in recombination: using RAD-51 antibody staining as read-out for the formation of double strand breaks (DSBs), we noticed RAD-51 foci at much higher levels than in wild-type, suggesting a significant increase of recombination initiation events. In addition, RAD-51 foci also persist longer, indicating possibly additional defects in the repair of DSBs. Accordingly, we observe elevated levels of apoptotic corpses, as these unrepaired chromosomes activate the pachytene checkpoint.

We mapped vv44 to chromosome I, within an interval of about 0.65cM containing 64 genes. We are currently in the process of identifying the mutation by means of sequencing and RNA interference experiments.

P30 Suppressor Analysis of meiotic chromosome pairing and recombination defects in C. elegans

Ka-Lun Law and Monique Zetka, Department of Biology, McGill University

Proper chromosome segregation at meiosis I depends on initial alignment of homologous chromosomes, establishment of the synaptonemal complex, and formation of chiasmata between homologues. Previous studies have demonstrated that HIM-3, a component of the meiotic chromosome core, is required for these processes. In him-3(vv6) mutant germ lines, wild-type levels of HIM-3 are loaded onto the chromosome core, but severe defects in homologue alignment, extensive non-homologous synapsis and defects in chiasmata formation are observed. To identify proteins that interact with HIM-3 and other proteins that function in the HIM-3 pathway, an EMS-based suppressor screen has been performed using the vv6 allele to isolate candidates that suppress the embryonic lethality characteristic of vv6.

In the strongest suppressor strain vv6; vv39, the initial homologue alignment defects of vv6 are not rescued, however the non-homologous synapsis defects are suppressed. This is supported by the observation that recombination is initiated and resolved normally in the suppressor mutants leading to a partial restoration of chiasmata formation. I hypothesize that in the suppressor mutants, homologues fail to align initially during early prophase I, but they undergo homologous synapsis, which is sufficient to increase the frequency of crossing over. I am currently further characterizing and mapping this suppressor.

P31 GFP Based Screen For Meiotic Mutants

Tom Nolis, Zetka Lab

Meiosis causes the reduction of diploid chromosomes into haploid gametes playing a significant role in all sexually reproducing organisms. The progression of meiosis can be broken down into meiosis I and meiosis II. In meiosis I, homologous chromosomal segregation depends on the formation of a chiasmata – a secure connection that arises between homologs – its formation is absolutely critical for chromosomal segregation. In order to establish a chiasmata, three major landmarks must occur between homologous chromosomes: recognition and alignment, synapsis, and recombination. A defect in any one of these key events can cause chromosomal non-disjunction or cell aneuploidy.

The nematode Caenorhabditis elegans has many attributes that make it a well-suited model for studying various aspects of meiosis. The germ-line nuclei in the gonad mature in a spatiotemporal manner and the nuclei can easily be distinguished during different stages of meiotic prophase by using DAPI staining. Should a mutation impair homologous chromosomal segregation of the X chromosome the progeny of the animal will bear a visibly higher percentage of males, presenting a ‘High Incidence of Males’, or a ‘Him’, phenotype.

The goal of this study is to screen, identify, and isolate more Him mutations by building on the screen “Green Eggs and Him”. The screen makes use of the yIs34 injection that has a GFP tag driven by a male-specific-promoter. The injection is tuned to have male embryos shine bright green in utero of the mother – the GFP is only expressed in embryos with homozygous Him mutations. Therefore, we can isolate any hermaphrodite that has a homozygous Him phenotype by selecting only those worms with one or more GFP positive egg(s) in utero.

To date seventeen Him mutants have been isolated. DAPI staining has allowed us to categorize the mutants into recognition and alignment, synapsis, and recombination defective mutants. With the use of SNIP-SNP mapping we are continuing to narrow towards the exact genetic location of each mutant allele.

P32 Cloning and characterization of the C. elegans meiotic mutant vv33

Yvonne Quan and Monique Zetka

Caenorhabditis elegans is a powerful tool in genetics and developmental biology, partly for its small, sequenced genome and because it is easily manipulated in laboratory conditions. C. elegans is useful for studying meiotic processes due to the spatiotemporal organization of germ line nuclei. During meiosis, fidelity of homologous chromosome segregation is dependent upon early prophase processes that stably pair homologous chromosomes and join them through genetic exchange. Meiotic mutants are characterized by a high embryonic lethality (Emb) and a high incidence of males (Him) phenotypes due to autosomal and X-chromosome missegregation. Our laboratory is conducting large-scale screening for new meiotic mutants based on the Him phenotype.

My objectives are to map and to characterize a newly isolated mutant, vv33. My mapping data presently indicate that vv33 lies in a 4.05 m.u. interval on the left arm of chromosome I. Further mapping will be performed to refine the interval. Once candidate genes are identified, the RNAi phenotype of these genes will be investigated. Candidates that mimic the vv33 phenotype will be sequenced in vv33 mutants to identify the mutation. DAPI stainings of mutant gonads reveal the presence of unusually large and disorganized nuclei, suggesting a defect in cell-cycle progression. Preliminary data also suggest that vv33 mutants are defective in homologue alignment and recombination. Antibody stainings specific for monitoring synapsis and recombination will be performed to further examine meiotic defects in vv33. Cloning and characterization of vv33 will provide insight into its function during meiosis and germ line development.

P33 Analysis of yeast PP1/Glc7 functional networks: Investigation of the role of Glc7 in Kar9 and dynein-dependent spindle positioning

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The essential and highly conserved budding yeast PP1-type phosphatase, Glc7, plays a critical role in several essential processes such as glucose metabolism, cell polarity, spindle positioning and chromosome segregation. To gain a greater understanding of PP1 functional networks, we created a novel catalytic mutant (glc7-E101Q) and investigated genetic interactions at the genome-level by Synthetic Genetic Array (SGA). Characterization of the glc7-E101Q mutant revealed growth and glycogen accumulation defects, similar to other glc7 mutants. SGA analysis revealed a broad network of 147 synthetic sick/lethal (SSL) that cluster in the processes of sister chromatid cohesion, Kar3-, Kar9- or dynein-dependent nuclear migration, S-phase checkpoint and Cla4-mediated cell polarity. SSL interactions identified within the Kar9 and dynein pathways (which regulate early and late spindle positioning, respectively) suggest Glc7 may regulate both of these pathways. Our finding builds on a previous study that suggested Glc7 and its subunit, Bud14, may promote dynein activation at the bud cortex. We are currently investigating the role of Glc7/Bud14 and Kel1 (a cortical protein required for Bud14 localization) in these pathways. Single kel1-delta and bud14-delta mutants showed no obvious defects in spindle positioning. Loss of Kel1 but not Bud14, however, exacerbated the spindle positioning defects of kar9-delta cells. In contrast, loss of either Bud14 or Kel1 had negligible impact spindle positioning defects of dyn1-delta cells. Collectively, these observations suggest a role for Kel1 in the regulation of the dynein pathway independent of Bud14/Glc7.

P34 Retinal Degeneration in Dystroglycanopathies: Analysis of Cell Death in Developing Dystroglycan Deficient Fly and Mouse Retina

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Dystroglycanopathies are a group of congenital muscular dystrophies resulting from deficiencies in the extracellular matrix receptor dystroglycan (Dg). Dystroglycanopathies are associated with mental retardation and eye pathologies. Dg is evolutionarily conserved and we show that Dg is required during retinal development in both invertebrates and mammals. Mosaic D. melanogaster retinas deficient in Dg, are structurally disrupted and do not function properly. This can be rescued by re-expressing Dg specifically in photoreceptors. We are exploring when cell death initiates and whether it is caspase mediated. Terminal dUTP nick-end labeling (Tunel) assay coupled to confocal imaging are used to determine levels of cell death during retinal development. Expression of the viral caspase inhitior p35 is used to test whether the cell death is caspase mediated. To date, we show that in third instar larvae cell death levels are significantly greater in Dg deficient mosaics but this cell death is not in photoreceptors. It also appears that the cell death is not caspase mediated. We continue to examine the levels of cell death during pupal stages of Dg deficient retina to determine whether Dg plays a role in caspase independent cell death.

P35 Molecular mechanism of SRY action during testicular differentiation in the mouse.

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SRY is the master gene on the Y-chromosome known to trigger testis determination in mammals. Despite its identification 15 years ago, the molecular mechanism of its action remains poorly understood. We tested the effect of a dominant negative SRY fusion protein, TAT-HMG, on testicular differentiation in culture. HMG is the DNA binding motif of SRY whereas the TAT sequence of HIV makes the fusion protein penetrable into cells. Gonadal primordia with adjacent mesonephroi were isolated from CD1 mouse fetuses at embryonic day 11.5. One of each pair was cultured in a control medium whereas the other was incubated in the presence of the TAT-HMG protein for up to 3 days. The treatment with the TAT-MHG protein resulted in (1) upregulation of Sry and downregulation of Sox9, 1 day after culture, (2) delay in SRY downregulation and MIS expression during culture, (3) massive cell death in the mesonephros, and (4) disruption of testis cord formation. We speculate that the competition between the endogenous SRY and the TAT-HMG protein may lead to downregulation of Sox9, an immediate Sry downstream gene, and the upregulation of Sry by a feed back mechanism. The temporary downregulation of Sox9 and delay in MIS expression may be associated with the disruption of testis cord formation. Alternatively, the massive loss of mesenchymal cells may prevent the migration of cells from the mesonephros, which is essential for testis cord formation.

P36 The role of Numb during asymmetric cell division

Amel Kechad and Michel Cayouette. IRCM

Cell diversity can arise from exposure to different environmental signals or through asymmetric cell divisions. The latter process generated daughter cells that acquire different fates by segregating cell fate determinants into only one of the two daughter cells during mitosis. Asymmetric cell division occurs in a variety of organisms, including rats, mice, chicken, fish and frogs, in various tissues such as the hindbrain, the cortex, and the retina, but the mechanisms regulating asymmetric cell divisions remain poorly understood, especially invertebrates.

In the developing rat retina, the cell fate determinant Numb, a natural inhibitor of Notch signaling, is asymmetrically localized at the apical pole of the retinal progenitor cells (RPCs) where it is thought to segregate asymmetrically to one daughter cell to influence its fate.

Previous studies have shown correlation between asymmetric inheritance of Numb and asymmetry in cell fate choice, but it remains unclear whether the asymmetric segregation of Numb directly influences cell-fate choice in the retina. To address this question, we used retroviral vectors to infect individual RPCs in the NumbloxP/loxP mouse at different time points in retinal development. The resulting knockout clones obtained from individual RPCs were then analyzed by couting the number and the type of cells present in each clone. Preliminary results indicate that inactivating Numb early in retinal development significantly reduces clone size, whereas inactivating Numb in older RPCs does not affect clone size but drastically changes cell fate decisions. These results suggest a two different roles for Numb during retinal development: promoting RPC proliferation at early stages of development, but promoting neuronal differentiation at later stages.

P37 Role of Numb in Photoreceptor Development

Vasanth Ramamurthy, Michel Cayouette

Retinitis Pigmentosa (RP) is the most common inherited cause of blindness. RP is generally characterized by photoreceptors (PR) degeneration. Currently there are no cures for RP. Recently, we have obtained preliminary evidence that the protein Numb, which is known to regulate endocytosis of different receptors such as Notch, is expressed in the developing OS of photoreceptors, and is strongly expressed in the OS and outer limiting membrane in the mature retina. Here, we propose to investigate the role of Numb in the differentiation of photoreceptors. Specifically, we will test the hypothesis that Numb plays a part in the establishment and/or homeostasis of photoreceptor OS. To study the function of Numb in photoreceptors, we propose to inactivate both Numb and its homologue Numblike (Nbl) in the mouse. Numb and Nbl knockout mice are embryonically lethal, and to overcome this we will use a Cre-loxP system to generate a conditional Numb/Nbl knockout mouse. Previous reports have implicated numb as a critical player in cell fate decision in retinal progenitor cells. In order to bypass this crucial role of Numb, we will inactivate Numb (and Nbl) in post mitotic undifferentiated photoreceptors by using a Cre recombinase regulated by the Opsin promoter. Outer segments are critical in photoreceptor function and mutations in genes that affect the development and integrity of OS often lead to photoreceptor degeneration. The is study should help us better understand the role of numb in photoreceptor OS formation and could potentially lead to the identification of new mutations affected in RP and other photoreceptor degenerative diseases.

P38 Building up muscle: a strong role for the atypical Rac activator Dock180

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Dock180 is the prototype of a new atypical family of RhoGTPase activators. Genetic studies in Drosophila and C. elegans demonstrated that Dock180 orthologues not only play a role in the activation of the GTPase Rac in various types of cell migration, but also in the phagocytosis of apoptotic cells and the fusion of myoblasts. On the other hand, the function of Dock180 in mammals still remains unknown and is studied here for the first time.

Expression profile studies demonstrate that in the developing mouse embryo, Dock180 is first expressed in the somites, consistent with a role in muscle development. Characterization of a knock-out mouse for the Dock180 gene indicates that this mutation is lethal at birth. Histological analyses show the muscular tissue as being dramatically reduced and disorganized in KO embryos, notably at the levels of the intercostals, limbs and diaphragm. An in vitro model of primary myoblast differentiation was established and used to demonstrate that the fusion of myoblasts into mature fibers is defective in a Dock180-null background.

During muscle differentiation, transcription factors that register the acquisition of muscle identity have been extensively studied. However, molecular mechanisms needed for myoblasts to differentiate into mature fibers still remain obscure. Our studies have identified an essential and conserved function of Dock180 at the fusion step of muscle differentiation.

P39 Unraveling the Dock180/Elmo molecular complex and its implication in Rac activation

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In C. elegans, Ced-12 (Elmo) and Ced-5 (Dock180) are genetically linked and act as regulators of Ced-10 (Rac)-mediated cell migration and phagocytosis during development. While it is known that Dock180 and Elmo interact physically, the domain requirements for this coupling remain elusive. In addition, the role of Elmo in this complex is controversial. Here, we sought to (i) determine the molecular mechanism by which DOCK180 and Elmo interact and (ii) test the importance of Elmo-binding to Dock180 in Rac-GEF activity as well as in cell spreading and migration.

To decipher the mechanism of interaction between Dock180 and Elmo, we used pull-down and co-IP methodologies and found that the atypical PH domain of Elmo is mainly responsible for binding Dock180. We crystallized and solved the structure of the atypical PH domain of Elmo. While the core of the PH domain is highly similar to known PH domains, we found a unique and long hydrophobic a-helix at the beginning of the domain. Mutagenesis and biochemical analysis revealed that this hydrophobic portion of the PH domain is responsible for binding to a novel surface on Dock180. Using novel Elmo mutants, we found that the formation of a Dock180-Elmo complex is essential for proper cytoskeletal rearrangement and cell migration. Further studies are ongoing to elucidate if the Elmo-Dock180 interaction contributes to the GEF activity of this complex.

We identified novel binding sites between Elmo and Dock180 and demonstrated that the formation of this complex is key for Rac-mediated cell migration. Novel strategies targeting this pathway may provide a means to block the spread of metastatic cancers.

P40 Dorsal-Ventral Pancreas Development in Xenopus Laevis

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Pancreatic cells are derived from separate dorsal and ventral progenitor populations that are induced by different signaling factors. Since they are regulated differently understanding how they are specified will help elucidate the molecular signals that underlie pancreatic cell fate specification. The initiation of pancreas development involves interactions between endodermal progenitors and mesoderm. It has been well established that different tissue interactions specify development of dorsal versus ventral pancreas. However, less is known about the molecular signals that act downstream of these initial inductive interactions, prior to fusion of the pancreatic buds, and their subsequent differentiation and morphogenesis. Understanding how committed pancreatic progenitors proliferate and differentiate into acinar, ductal and endocrine lineages will provide insight into the molecular mechanisms that specify one cell type versus another. Previously, we showed that, in Xenopus, prior to pancreatic cell differentiation, exocrine progenitor cells first arise and are specified in the ventral pancreas, while endocrine progenitor cells originate from the dorsal pancreas. Therefore, to identify differentially expressed genes involved in specifying endocrine versus exocrine cells, we have compared the gene expression profiles of dorsal and ventral pancreatic buds isolated prior to their fusion. As a result of this screen we have identified numerous dorsal and ventral specific genes. In this poster, we will summarize the current functional data we have obtained with several of these genes.

P41 Specification of neuronal position within the spinal cord

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The somata and axons of motor neurons of the lateral motor column (LMC) of the vertebrate spinal cord are organized myotopically: medially positioned LMC (LMCm) motor neurons innervate ventral limb muscles, while laterally positioned LMC (LMCl) motor neurons innervate dorsal limb muscles. LMCm and lateral LMCl neurons are distinguished by the expression of the LIM homeodomain proteins Isl1 and Lim1, respectively. Previous studies show that Isl1 and Lim1 can control the medio-lateral positioning of LMC cell bodies, suggesting that Isl1 and Lim1 regulate the expression of the still undetermined effectors of cell migration required for the correct settling of LMC neurons.

Our preliminary experiments have demonstrated that in mouse and chick spinal cord, Reelin expression domain is adjacent to LMC neurons and the gene encoding the intracellular adaptor protein Dab1, implicated in relaying Reelin signaling, is highly expressed in LMCl neurons. Using gain and loss of function experiments, we are currently testing the involvement of Reelin signaling in the positioning of LMC motor neuron cell bodies.

P42 Regulation of apoptosis by hox genes during limb development: identification of hox target genes

Damien GREGOIRE and Marie KMITA

Hox genes, that encode for transcription factors, are central players in the establishment of body architecture during embryogenesis. In addition to their patterning function, evidences have been obtained that suggest an important role of Hox genes in the control of cell proliferation and apoptosis. We have obtained evidences that loss of HoxA-HoxD gene clusters in limb buds induces impaired outgrowth of these organs, due to drastic apoptosis at early stages of limb development. Our working model is that Hox genes maintain cell survival in developing limbs through the regulation of genes that control apoptosis. The aim of my research project is to identify these genes and characterize their functional interplay with Hox genes.

For this purpose, we looked for genes whose expression is deregulated in limb buds lacking HoxA-HoxD gene clusters. mRNA from wild type and HoxA/HoxD mutant limb buds have been isolated at E10, soon after limb bud emergence (E9.5) but just before morphological defects in mutants are detectable. Comparative expression profiling was performed using Affymetrix microarrays, at the ‘Genome Quebec’ genomic core facility.

Our microarray screen highlighted around 150 genes as being misexpressed in HoxA/D mutants, including the few already known Hox target genes. Results of the screen and validation will be presented.

Our approach allowed us to identify putative Hox downstream genes during limb development. Focusing on the most compelling candidates, we will now characterize how their regulation by Hox genes is implicated in the control of cell survival during limb development.

P43 The protein convertase PC5/6 is involved in the anterior-posterior axis patterning during mouse development.

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A large number of secreted proteins are produced as precursors that are cleaved after basic sites to generate mature forms. This cleavage is achieved by subtilisin/kexin like proteases known as the proprotein convertases (PCs). So far, nine PCs are known, seven of which cleave at basic residues: PC1/3, PC2, PC4, PC5/6, PC7, PACE4, Furin, and two others cleave at non-basic sites: SKI-1/S1P and PCSK9. In vitro studies suggest that PC5 is involved in post-translational endoproteolytic processing of numerous pro-proteins, such as renin, Alzheimer's beta-secretase BACE1, transforming growth factor-beta, vascular enthothelial growth factor C, HIV gp160, integrin-alpha subunit and mullerian-inhibiting substance. To provide insight into the physiological role of PC5 in vivo, we have generated a knockout (KO) mouse of the PC5 gene. KO embryos fail to implant and die between E4.5 and E6.5. In situ hybridization revealed that around the implantation time PC5 is strongly expressed in the extraembryonic tissue but not detectable in the embryo proper. In order to overcome the early embryonic lethality, we have developed a conditional knockout, in which the exon 1 and the proximal promoter were targeted. Using a Meox-cre knockin mice the PC5 gene was inactivated in the embryo but not in the extraembryonic tissue. The PC5 deficient embryos get implanted but die after birth and present a smaller size and a shortened or absent tail with an increased number of thoracic and lumbar vertebrae. Furthermore, the kidneys do not develop. Since these phenotypes are similar to those observed in growth / differentiation factor-11 (Gdf11) KO embryos, we have assessed in vitro and ex vivo the processing of Gdf11 by PCs. The results indicate that the active form of Gdf11 is produced only by PC5 and PACE4. In order to rule out the possibility of in vivo maturation of Gdf11 by PACE4, we are analyzing the tissue distribution of Gdf11, PC5 and PACE4 using in situ hybridization.

P44 The proprotein convertase PC5A is activated via a cAMP/PKA-dependent pathway: A novel regulated mechanism for the processing of extracellular proteins.

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Regulation of the expression of proprotein convertases (PCs) has been demonstrated on several occasions. For example ACTH treatment of Y1 adrenocortical cells triggers an increase in the mRNA level of the convertase PC5. To define the physiological significance of this regulation, we have analyzed: (1) the localization of the zymogen pro-PC5 and its mature form PC5 under steady state conditions and following PKA stimulation by ACTH or 8-Br-cAMP, (2) the impact of ACTH or 8-Br-cAMP on the autocatalytic secondary cleavage of the prodomain of PC5, and (3) the activity of PC5 on its substrates following such stimulation. Microscopy and biochemical experiments revealed that endogenous PC5 is complexed to its prodomain at the plasma membrane of Y1 cells. This labeling is lost upon treatment with heparin and is increased by overexpressing members of the Syndecan family, suggesting attachment of secreted pro-PC5A to heparan sulfate proteoglycans (HSPGs). Following incubation of Y1 cells with ACTH or 8-Br-cAMP, the cell surface labeling of the prodomain of PC5 is greatly diminished, while the signal for mature PC5 is increased. Moreover, after PKA stimulation, the protease activity of PC5 is increased, as evidenced by the cleavage of PC5's substrates Lefty, ADAMTS-4, endothelial lipase and PCSK9. Our data suggest a novel mechanism for PC5A activation at the cell surface, through a PKA-dependent removal of its prodomain. A similar mechanism may also apply to the convertase PACE4, thereby extending our knowledge of the molecular details of the zymogen activation and functions of these HSPG-bound convertases.

P45 The proprotein convertase PCSK9 induces the degradation of LDLR and its closest family members VLDLR and ApoER2

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The proprotein convertase PCSK9 gene is the third locus implicated in familial hypercholesteromia, emphasizing its role in cardiovascular diseases. Loss of function mutations and gene disruption of PCSK9 resulted in a higher clearance of plasma low density lipoprotein cholesterol (LDL-C), likely due to a lower degradation of the liver LDL receptor (LDLR). In this study, we show that two of the closest family members to LDLR represent new PCSK9 targets. These include the very-low-density-lipoprotein receptor (VLDLR) and apolipoprotein E receptor 2 (ApoER2) implicated in neuronal development and lipid metabolism. Our results show that wild type PCSK9 and more so its natural gain of function mutant D374Y can efficiently degrade the LDLR, VLDLR and ApoER2 either following co-expression or re-internalization of secreted human PCSK9. We next designed membrane-bound PCSK9-chimeras, which demonstrated that as compared to the secreted wild type enzyme, the enhanced intracellular targeting of PCSK9 to late endosomes/lysosomes resulted in a much more efficient degradation of the three receptors. In vivo validation of the enlarged functional activity profile of PCSK9 was achieved by demonstrating that PCSK9 modulates VLDLR levels in skeletal muscles in both Pcsk9 knockout mice and in liver-specific transgenic mice. This provided evidence for the endocrine-like function of secreted hepatic PCSK9, which enhances the degradation of the VLDLR in skeletal muscle. Thus, the effect of PCSK9 on these three receptors suggests that this convertase plays a major role in cholesterol and lipid homeostasis, and through the enhanced degradation of the VLDLR it could also reduce the development of adiposity.

P46 WITHDRAWN

P47 A Functional Recycling Endosome is Necessary for Cell Migration in vivo

Stefanie Wculek and Gregory Emery, Institute for Research in Immunology and Cancer (IRIC)

The recycling endosome (RE) has emerged as a key regulator of different polarized processes like epithelial maintenance, cell-boundary rearrangement and asymmetric cell division. It has also been proposed to be involved in cell migration, however without direct evidences.

During Drosophila melanogaster oogenesis, a cluster of polar follicle cells, called border cells, perform an invasive migration through surrounding nurse cells to reach the oocyte. This event resembles metastasis formation since it requires epithelial-mesenchymal transition (EMT), reorientation and arrest. Furthermore, it is easily accessible for protein expression and mutant analysis, making it an ideal in vivo system to test the role of the RE in cell migration.

We observe that overexpression in border cells of a dominant negative form of Rab11, the small GTPase regulating vesicular trafficking through the RE, blocks migration or leads to severe migration defects in about 80% of examined egg chambers, suggesting that the RE is implicated in both EMT and cell migration.

Different proteins involved in cell migration, like matrix proteins of the Integrin family or the cell-cell adhesion protein E-Cadherin have been shown to traffic through the RE. Furthermore, receptor tyrosine kinases involved in guiding border cells during their migration - the EGF receptor and the sole Drosophila homolog of the PDGF and VEGF receptors (PVR) - are regulated by endocytosis. We suspect that one or several of these proteins might be mislocalized or misregulated by the expression of dominant negative Rab11. Therefore we are currently examining their spartial distribution and activation.

P48 Analyse des mécanismes de régulation de l'expression des gènes par le récepteur des oestrogènes alpha dans les cellules de carcinome mammaires (MCF7) par une approche bioinformatique.

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La régulation de l'expression des gènes chez les eucaryotes fait intervenir des activateurs ou répresseurs transcriptionnels liant des séquences d'ADN spécifiques. Les techniques à haut débit (ChIP-on-chip, puces d'ADN) permettent de révéler de nouveaux mécanismes de régulation de l'expression génique par recherche de sites enrichis dans les régions liées par un facteur de transcription (FT) donné et/ou dans les séquences flanquantes des gènes régulés. L'analyse des résultats de ChIP-on-chip permet d'identifier des régions où un FT d'intérêt est lié à l'ADN de façon directe ou indirecte (tethering). D'un autre côté, l'analyse des régions flanquantes de gènes régulés de façon primaire par ce FT permet d'identifier les sites médiant une régulation par liaison directe, tethering ou par interference avec d'autres voies de signalisation (effets non-génomiques).
Nous avons analysé les mécanismes de régulation de l'expression des gènes par le récepteur des oestrogènes alpha dans les cellules de carcinome mammaires (MCF7) par recherche de sites dans des régions liées par ce récepteur en ChIP-on-chip et dans les séquences flanquantes de gènes cibles primaires identifiés par puces d'ADN. Nous avons utilisé une banque de matrices de sites de FT (TRANSFAC) et testé l'influence du choix des ensembles de référence (background) sur l'enrichissement observé de ces sites afin de déterminer l'effet des biais de composition (%GC, séquences répétées ou de faible complexité). Deux tests statistiques (z-score et p-value) ont été appliqués afin de supporter la signification biologique des sites prédits et d'éliminer les faux positifs. L'analyse comparative de ces deux jeux de données est présentée.

P49 Studying transcriptional regulation by estrogens through functional genomics

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Estrogen receptors (ERs) mediate the pleiotropic physiological actions of estrogens but also contribute to breast tumorigenesis. ERs are ligand-dependent transcription factors modulating expression of primary target genes through direct DNA binding, interaction with other transcription factors (tethering), or with signaling proteins (non-genomic mechanisms). Altered expression of primary targets can influence expression of secondary targets, resulting in broader regulation of gene expression.
To identify estrogen target genes in breast cancer cells, we performed microarray analysis (Affymetrix HG-U133 2.0 Plus chips representing over 47, 000 human transcripts) using MCF7 cells treated for 24 hours with estradiol (E2) in the absence or presence of the translation inhibitor cyclohexamide. This approach identified fore than 500 up-regulated and 200 down-regulated primary target genes.
To analyze the mechanisms of gene regulation by estrogens, we monitored the distribution of putative estrogen response elements (EREs) at up to 25kb from transcriptional start sites using position weight matrices. EREs were enriched near up-regulated, but under-represented near down-regulated primary genes. However, siRNA depletion of ERalpha antagonized the effects of E2 on both up- and down-regulated primary target genes. These results suggest that ERalpha acts on up- and down-regulated genes through different mechanisms, possible acting on down-regulated targets through transcription factrs whose binding sites were enriched in promoter sequences. Secondary target genes were enriched in binding sites for several other transcription factors, including E2F family members, whose expression is itself regulated by estradiol, consistent with a role of these factors in regulating estradiol-induced cellular growth.

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P50 Raloxifene and ICI182,780 increase estrogen receptor-alpha association with a nuclear compartment via overlapping sets of hydrophobic amino acids in activation function 2 helix 12.

Khalid Hilmi, David Cotnoir-White, Mathieu Lupien and Sylvie Mader

The basis for the differential repressive effects of antiestrogens on transactivation by estrogen receptor-alpha (ERalpha) remains incompletely understood. Here, we show that the full antiestrogen ICI182,780 and, to a lesser extent, the selective ER modulator raloxifene (Ral), induce accumulation of exogenous ERalpha in a poorly soluble fraction in transiently transfected HepG2 or stably transfected MDA-MB231 cells and of endogenous receptor in MCF7 cells. ERalpha remained nuclear in HepG2 cells treated with either compound. Replacement of selected hydrophobic residues of ERalpha ligand-binding domain helix 12 (H12) enhanced receptor solubility in the presence of ICI182,780 or Ral. These mutations also increased transcriptional activity with Ral or ICI182,780 on reporter genes or on the endogenous estrogen target gene TFF1 in a manner requiring the integrity of the N-terminal AF-1 domain. The antiestrogen-specific effects of single mutations suggest that they affect receptor function by mechanisms other than a simple decrease in hydrophobicity of H12, possibly due to relief from local steric hindrance between these residues and the antiestrogen side chains. Fluorescence anisotropy experiments indicated an enhanced regional stabilization of mutant ligand-binding domains in the presence of antiestrogens. H12 mutations also prevent the increase in bioluminescence resonance energy transfer between ERalpha monomers induced by Ral or ICI182,780 and increase intranuclear mobility in correlation with transcriptional activity in the presence of these antiestrogens. Our data indicate that ICI182,780 and Ral locally alter the ERalpha ligand binding structure via specific hydrophobic residues of H12 and decrease its transcriptional activity through tighter association with an insoluble nuclear structure.

P51 WITHDRAWN

P52 Anti-proliferative effects of retinoic acid on SkBr-3 breast cancer cells

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Retinoic acid (RA) is a potent regulator pf cell proliferation, differentiation and death. Ectopic administration of RA strongly induces growth inhibition, differentiation or apoptosis of malignant cells. Clinical trials using RA for treatment of numerous types of cancer are ongoing. However, side effects and resistance to treatment make it necessary to elucidate the underlying mechanisms of RA action on tumorigenic cells in order to improve therapeutic approaches. Even though RA is already used in the treatment of a number of cancers, little is known about target genes mediating its actions. We hypothesize that some primary RA target genes contribute to the anti-proliferative effects of RA, and that those genes could function as therapeutic targets in cancer treatment, increasing efficiency of treatment and/or minimizing side effects.

From a panel of breast cancer cell lines, we identified the ER-negative, ErbB2-overexpressing cell line SkBr-3 as the strongest responder to RA. Further analysis of the growth arrest in SkBr-3 revealed an increase in G0/G1 phase of the cell cycle. The nature of this arrest is being analyzed using markers for different G0/G1 arrest phenotypes. We are using microarray analysis combined with bioinformatics approaches to identify RA regulated genes in those cells. Target gene regulation will be confirmed and the role in proliferation inhibition of the most interesting RA target genes will be assessed using knockdown and over-expression studies. We seek to identify by this approach potential candidates to be used as targets for novel cancer treatments, minimizing resistance and toxicity.

P53 MC-View : An online tool for RNA motif visualization

Louis-Philippe Lavoie, Emmanuelle Permal, Romain Rivière, Véronique Lisi, Caroline Louis-Jeune and François Major (all from IRIC)

RNA structural motifs are the building blocks of RNA molecules. A large number has been found and characterized so far and their study becomes more refined each year. Different methods of discovery and analysis have been proposed, but one common thread is that the study of RNA motifs is key to understanding the elusive nature and mechanisms of RNA as a whole. While fast, fully automated tools begin to appear, visualization is still one of the simplest and yet most effective recourse to gain deeper insight into the structures. To this end, a tool to rapidly produce complete renderings of studied structures should be in the arsenal of every structural biologist.
MC-View is an online tool that allows for searching and visualization of structural motifs in submitted RNA structures. It uses predefined structural motifs such as GNRA tetraloop or sarcin-ricin motif and searches for them in a PDB file. Using a flexible graph annotation to describe nucleotides and their interactions, the input molecule is searched for all instances of the selected motifs. The result is a fully annotated secondary structure diagram and a PyMOL script overlaying the initial three-dimensional structure with the studied motifs. Putative protein-RNA hydrogen bonds (h-bonds) are also identified.

P54 Homology modeling with RNA molecules: an example on viral hairpins

Emmanuelle Permal and Francois Major - IRIC

There are few examples of homology modeling with RNA structures, despite its widespread use for proteins. However, the few approaches for RNA homology modeling use the same technique, which is to replace nucleotides within the coordinates of the native structure. As an alternative, we propose a new way for RNA homology modeling with structural fragments combined with a modeling system: MC-Sym.
We propose a multi-step example of RNA homology modeling based on fragment modeling and protein homology modeling processes. Each fragment can be either extracted from an existing structure or built with our modeling system MC-Sym. We choose top test our approach on a well-known and conserved viral motif: the Stem-loop D that is essential to translation in Coxsackie viruses. Homology models produced with our methods showed a strong similarity (RMSD criterion from 2Å to 3.5 Å) with the model 1 from PDB file of the stem-loop D from Coxsackie virus B3 used as template.

P55 Skp2 phosphorylation controlled by CDK1/2 and Cdc14B plays an important role in regulating G1-phase length

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The SCFSkp2 ubiquitin ligase controls the stability of key cell cycle regulators, notably the cyclin dependent kinase inhibitor p27. The activity of SCFSkp2 is believed to be regulated by the APCCdh1, another important cell cycle ubiquitin ligase, which targets the F-box protein Skp2 for degradation during mitosis exit and the G1 phase. However, it is well documented that Skp2 expression occurs during G1 phase progression, a period of high APCCdh1 activity. How and why Skp2 accumulates in G1 is not known. Moreover, Skp2 has oncogenic properties and is often overexpressed in human tumors, underscoring the importance of understanding the mechanisms regulating Skp2 expression. Here we show that Skp2 is phosphorylated on Ser64/Ser72 in vivo. Phosphorylation plays an important role in controlling Skp2 expression. Specifically, Skp2 phosphorylation protects it from APCCdh1-mediated degradation by interfering with its association to Cdh1. Phosphorylation of Ser64 of Skp2 is catalyzed by CDK2/CDK1, and is reversed by the proline-directed phosphatase Cdc14B. Ser64 phosphorylation by CDK2 during G1 phase progression was found to be essential for optimal Skp2 expression. On the other hand, dephosphorylation of Skp2 by Cdc14B at the M/G1 transition promotes its degradation by the APCCdh1. Importantly, depletion of Cdc14B accelerates cell cycle progression from mitosis to S phase in a Skp2-dependent manner, demonstrating epistatic relationship of Cdc14B and Skp2 in the regulation of G1 length. Thus, reversible phosphorylation plays a key role in the timing of Skp2 expression in the cell cycle.

P56 Phosphorylation of the activation loop of ERK3/ERK4 is necessary for MK5 activation

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The ERK3 and ERK4 protein kinases are 50% identical to ERK1/2 in the kinase domain. Thus, they belong to the mitogen-activated protein (MAP) kinase family, but several features distinguish ERK3/4 from other family members. (i) They have a long C-terminal extention. (ii) They present a SEG sequence in the activation loop instead of the classical TXY phosphorylation motif found in classical MAP kinases. The impact of these features on ERK3 or ERK4 function and activity remains unknown. ERK3/4 were shown to interact with the MK family member MK5, a recently derscribed regulator of cellular senescence. Unfortunately, kinase-substrate relationships between ERK3/4 and MK5 remain unclear. Here we have investigated the impact and regulation of MK5 and ERK3/4. As shown by immunoprecipitation and in vitro kinase assay, ERK3/4 directly phosphorylate MK5. Moreover, phosphorylation of ERK3/4 on the activation loop is mandatory for interaction with MK5, for its activation and for the correct subcellular localisation of ERK3/4 and MK5. Because activation loop phosphorylation of ERK3/4 is not mediated by MK5, we have developed a mass spectrometry strategy to identify the MK5 phosphorylation sites on ERK3. These data clearly indicate that phosphorylation of ERK3/4 catalytic loop on Ser189/186 appears to be involved not only in the catalytic activation of the enzyme but also in the interaction process with MK5. Finally, ERK3/4 appears to be key regulators of MK5 which could be of crucial importance for understanding it apparent function in senescence and tumor suppression.

P57 Nuclear localization of MEK1 transforms intestinal epithelial cells

Duhamel S., Voisin L. and Meloche S.

The role of ERK1/2 MAP kinase signaling in colorectal cancer initiation and progression is unclear. In a large tissue microarray (TMA) analysis, we have found that 44% of colorectal cancers display expression of phosphorylated MEK1/2, compared to 10% of normal tissues. The spatial localization of ERK1/2 signalling plays a critical role in the control of cell proliferation. Interestingly, we observed that 81% of colorectal cancers display aberrant p-MEK1/2 staining in the nucleus, as compared to 8% of normal colon tissue.
To mimic the mislocalization of MEK1/2 observed in colon cancers and test its impact on tumorigenesis, we introduce mutations in the NES of MEK1 cDNA to prevent nuclear export. The MEK constructs were expressed in intestinal epithelial cells by retroviral gene transfer and the subcellular localization, activity and cellular impact of MEK1 mutants was assayed. Nuclear localization of MEK1 drastically transformed the cells. They showed morphological transformation, increase in cell proliferation rate, resistance to anoikis and loose their anchorage-dependance. Importantly, the cells formed agressive tumours in athymic mice. In conclusion, we demonstrate for the first time that localization of MEK1 in the nucleus is sufficient to transformed epithelial cells and to induce the formation of tumours in mice. These results further illustrate the importance of MEK1/2 in tumorigenesis and the interest of testing MEK inhibitors in clinical trials.

P58 Identification of new phosphorylation sites on the MAP kinase ERK1.

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The extracellular signal-regulated kinase (ERK1/2) cascade is a central pathway that transmits signals from many extracellular stimuli to regulate cellular processes such as proliferation, differentiation and senescence. Signaling via the ERK1/2 cascade is mediated by sequential phosphorylation and activation of a cascade of kinases: Raf – MEK1/2 – ERK1/2. Activation of ERK1/2 occurs through phosphorylation of threonine and tyrosine residues at the sequence T-E-Y by the upstream kinases MEK1/2. The two activating phosphorylation sites (T and Y) were identified in 1991. Further phosphoamino acid analysis of ERK1 have showed that ERK1 is also phosphorylated on serine and other threonine residues in serum starved cells and following mitogenic stimulation. Surprinsingly none of these sites were identified despite the great interest in the MAPK pathway. In order to identify ERK1/2 phosphorylation sites, we expressed a GST tagged ERK1 in 293 cells (either exponentially growing; serum-starved or stimulated with serum) and purified the protein by GST pull-down. After SDS PAGE electrophoresis, the corresponding bands were excised, digested in gel and subjected to MS/MS analysis. These analyses led to the identification of eight potential novel phosphorylation sites. Five of them are serine and three are threonine. Most of them are conserved from C.elegans to human and in ERK2, except for one serine conserved only in ERK1 and another serine which is present exclusively in mammalian ERK1 and ERK2.

Further studies will characterize the role of these phosphorylation sites by testing the effect of their mutation on the function of ERK1.

P59 Regulation of ERK4 and ERK3 activation loop

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MAP kinases are activated by dual phosphorylation of the conserved TXY motif present in their activation loop. ERK4 and ERK3 are atypical MAP kinases that possess the SEG sequence instead of the TXY motif. Consequently, these kinases contain only one putative phosphorylation site, S186 and S189 respectively, potentially regulating kinase activity. The phosphorylation status of this serine residue has not been characterized mainly because stimuli that activate ERK4 and ERK3 or upstream kinases have not been identified. Recently, MK5 was identified has a direct substrate of ERK4 and ERK3. It was also shown that once activated, MK5 is subsequently able to phosphorylate ERK4 resulting in a hyperphosphorylated form of the protein. Here we have investigated the phosphorylation of ERK3 S189 and ERK4 S186 in vivo and the total phosphorylation of ERK4 in the presence of MK5. Our results indicate that S189/S186 are constitutively phosphorylated in response to classical MAP kinase stimuli such has serum and sorbitol. Interestingly, phosphorylation of ERK4 on S186 increases in the presence of MK5. This increase is independent of MK5 activity, demonstrating for the first time the existence of an ERK4 S186 kinase. We also show that phosphorylation of ERK4 S186 is required for stable interaction with MK5. Finally, we show that phosphorylation of ERK4 by MK5 is exerted on serine residues other than S186. Together, these results demonstrate that ERK4 S186 phosphorylation is regulated in vivo by MK5 association and that ERK4 and MK5 are mutual, but sequential, kinases.

P60 The atypical MAP kinase Erk3 is a novel substrate for CDK1/Cyclin B in mitosis

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Erk3 is a MAP kinase homolog whose function is currently unknown. Previous work of the laboratory has shown that Erk3 is an unstable protein in proliferating cells regulated by the ubiquin-proteasome system. Moreover, expression of stable mutants of Erk3 inhibits cell cycle progression and blocks entry into S phase. To better understand the mechanisms regulating Erk3 abundance and activity throughout the cell cycle, we have studied its level and stability in different phases of the cell cycle in HeLa cells. We observed that Erk3 is hyperphosphorylated and that its half-life considerably increases during mitosis. Preliminary results from in vivo and in vitro experiments suggest that several sites are phosphorylated at the C-terminus of the protein. In addition, inhibition of CDK activity lead to partial suppression of the electrophoretic mobility changes and overexpression of cyclin B1 in vivo reproduces the shift observed in mitosis. Altogether, these data suggests that CDK1/Cyclin B1 phosphorylates Erk3 in mitosis. Using mass spectrometry analysis of immunoprecipitated GFP-Erk3-Flag, we are currently identifying the sites that are phosphorylated in this phase of the cell cycle as well as the role of stabilizing Erk3.
Results obtained to date suggest that Erk3 is hyperphosphorylated and stabilized specifically during mitosis. This study will help us to better understand the involvment of Erk3 as a cell cycle regulator and which mecanisms regulate its abundance in proliferating cells.

P61 A Genome-Wide High Content Screen to Identify Modulators of RAS/MAPK Signalling

Dariel Ashton-Beaucage, Anne-Sophie Guenier and Marc Therrien; IRIC

The RAS/MAPK pathway participates in a wide array of cellular processes including proliferation, differentiation and survival. Also, disruption of normal MAPK signalling is often a key process in oncogenesis. The principal components of the pathway, RAS, RAF, MEK and MAPK, form an evolutionarily conserved signalling unit called the MAPK module. This module has been extensively studied in a variety of model organisms. However, the specific means by which the pathway is regulated are still poorly understood.
In order to identify new pathway components, we have developed a high-content screening method in S2 cells to measure pathway activity. Immunofluorescence, microscopy and image analysis techniques were automated to allow for standardised treatment of a large quantity of samples. We are currently using the Open Biosystem whole-genome dsRNA set (~16000 individual dsRNAs) to systematically test the effect of the depletion of each of the genes of the drosophila genome on pathway activity. The results from this screen should provide us with a better picture of the components involved in the intracellular mechanisms of MAPK signalling.

P62 Characterization of the tyrosine phosphorylation and sub-cellular localization of CNK, a scaffolding protein of the Ras/MAPK pathway

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The Ras/MAPK pathway is a major route that regulates basic cellular events including cell proliferation/differentiation. Connector enhancer of KSR (CNK) is a pivotal regulator of the Ras/MAPK pathway by its ability to assemble a RAF-activating complex. Besides its relevance with respect to Ras/MAPK signaling, mounting evidence suggest that CNK is also involved in other pathways. Recently, we found that Drosophila CNK associates with the tyrosine kinase Src42A in and RTk-dependent manner and that this interaction is part of a mechanism regulating RAF activation. Although the binding of Src42A to CNK is sufficient to drive Ras/MAPK signaling, Src42A also appears to phosphorylate CNK on multiple tyrosines. To ascertain the significance of those events, first we sought to identify the relevant residues by testing a series of CNK mutants for their ability to become phosphorylated in and RTK/Src42a-dependent manner. This approach identified several tyrosines and two proline-rich regions. Interestingly, rescue experiments using cell-based assays showed that phospho-site mutants are not incapacitated in their ability to contribute to Ras/MAPK signaling. Those residues may thus participate in other events. Genetic approached are currently being pursued to test this possibility. In parallel, we seek to define the subcellular localization of CNK. Using S2 cells, our preliminary findings revealed a punctuated cytoplasmic distribution. However co-localization studies with numerous markers failed to uncover CNK localization and thus will require additional work. Together, these new findings form the basis to explore the regulation of CNK as well as its potential role in Ras/MAPK-independent signaling events.

P63 A genetic screen for modifiers of pdz-gef

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The Ras/MAPK pathway is a key conduit regulating cell proliferation and differentiation. To identify new partners of this pathway, we have conducted a genetic screen for modifiers of a rough eye phenotype caused by the eye-specific expression of a dominant negative form of Connector eNhancer of KSR (CNK); a scaffolding protein of the pathway. In addition to isolating mutations in bona fide components of the Ras/MAPK pathway, the screen led us to identify the small GTPase Rap1 and its upstream activator PDZ-GEF as dominant enhancers, thus suggesting that their activity is required in concert to Ras/MAPK signaling during eye development. It also opened the possibility that CNK regulates Rap1 signaling and may even link the Ras and Rap1 pathways. While a connection between Ras and Rap1 signaling has been suggested, the molecular events, if any, are currently unknown. Moreover, among the multiple GEFs for Rap1 that have been characterized to date, PDZ-GEF remains the only one for which the mode of activation has not been elucidated. To investigate how Ras and Rap1 signaling may be connected and possibly how PDZ-GEF is itself regulated, we set out a new genetic screen for modifiers of a pdz-gef transallelic combination. This configuration produces a mild rough eye phenotype caused by a loss of cells as well as a wing bristle phenotype whereby the anterior wing margin bristles are mis-positioned with respect to one another. The phenotypes and initial phases of the screen will be presented.

P64 Enrichment of ubiquitinated proteins and a novel proteomic approach for the identification of modification sites.

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Conjugation of ubiquitin molecules on target proteins is one of the major regulating systems in cell. This process involves wide range of events such as proteasomal degradation. Deregulation of the later can cause many diseases like cancer, inflammatory disorders, viral infections and neurodegenerative diseases. So far, few large-scale studies of protein ubiquitylation have been reported due to the difficulty of isolating these low abundance and rapidly degraded proteins. To identify ubiquitylated proteins, a differential proteomic strategy combining affinity purification and the deubiquitylating enzyme USP2 is proposed. Ubiquitin-conjugated proteins from a cell extract are enriched using GST-Rad23 ubiquitin binding protein. The sample of purified Ub-proteins is split into a control and an USP2-treated samples. The control and the USP2-treated protein extracts are then digested with trypsin and analyzed by nano-LC-MS-MS on a high resolution Orbitrap mass spectrometer. Peptide ions conjugated with ubiquitin bare a tractable side chain di-glycine adduct following tryptic digestion. Peptide maps representing the coordinates (m/z, time, charge and abundance) are obtained for each nanoLC-MS/MS analysis using in-house peptide detection software. Comprehensive data mining and clustering analyses on peptide maps reveals two distinct ion populations representing peptides containing or not the di-glycine tag. Targeted nanoLC-MS-MS analyses using inclusion lists are performed to identify potential ubiquitylated peptides. By limiting the MS-MS acquisition to only these distinct ion populations, more efficient identification of modified peptides can be obtained. Preliminary results using this approach have allowed the identification of more than 60 putative ubiquitylated proteins and 2 ubiquitination sites.

P65 Phosphorylation of PCNA and its importance during DNA replication and DNA repair

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Faithful replication of the genetic material and DNA repair are highly controlled cellular mechanisms that are essential for genomic stability and cell survival. Impaired DNA can cause mutations or chromosomal rearrangements that potentially lead to carcinogenesis or cell death. Proliferating Cell Nuclear Antigen (PCNA) is a homo-trimeric ring shaped protein that encircles DNA and acts as a processivity factor for replicative DNA polymerases, a necessary requirement for DNA replication. Post translational modifications such as sumoylation and ubiquitination have been shown to regulate PCNA function in both DNA replication and repair processes, respectively. However, doubts persist as for the role of phosphorylation in regulating PCNA function. By using mass-spectrometry, we identified seven phosphorylation sites in yeast PCNA some of which were also identified in human PCNA. In yeast, PCNA phosphorylation appears to be highly modulated throughout the cell cycle. In fact, two of the seven phosphorylation sites showed a 3 to 10 fold increase in the phosphorylation level at the G1/S-phase transition. In addition, three of the identified PCNA phosphopeptides comprise a Cdc7-Dbf4 consensus sequence, a kinase that is essential for initiation of DNA replication in eukaryotes. Preliminary results indicate that both yeast and human PCNA are substrates for Cdc7-Dbf4 in vitro and that mutation of some of these sites to alanine resulted in temperature sensitive (Ts) mutants, thus affecting DNA replication. These results clearly indicate that PCNA phosphorylation is tightly linked to DNA replication in yeast.

P66 Phosphoproteome profiling approaches to monitor global cell signaling changes in macrophages

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The cascade of signalling pathways leading to host immune response in macrophage cells is primarily guided through phosphorylation and dephosphorylation events. In addition, studies have shown that secretion of interferon-γ□□ by T-cells activates the JAK/STAT signalling pathway in macrophages and induces phosphorylation of many additional substrates. Hence, phosphoproteome analysis in macrophages is essential for a better understanding of the immune response. In the present study, we conducted kinetic profiles (0, 5, 10, 30, 60 & 180min) of differential phosphoproteome following interferon-γ administration to mouse J774 macrophages. Phosphopeptides from tryptic digests of cytosolic proteins were isolated using TiO_2 affinity media and analyzed by liquid chromatography-mass spectrometry (LC-MS). Amongst the different trends observed, a higher proportion of phosphopeptides showed a sudden increase in phosphorylation 5min after interferon-γ induction. The comparison of the macrophage phosphoproteome with and without interferon-γ using in-house bioinformatic tools indicated that 23% and 18% ion clusters showed more than 3-fold over- and under-expression, respectively. A total of 1607 phosphopeptides were identified including 1314 novel phosphorylation sites. The kinetic profiles of phosphopeptide abundances lead to the identification of early signalling and regulatory events including the enhanced phosphorylation of members of ROS complex (p40 & p67 phox) and vesicle trafficking (VAMP-4 & α-taxilin). Furthermore, significant increase of putative phosphopeptide identification was obtained using 2D-LC-MS and alkaline phosphatase treatment. Among new potential phosphoproteins showing statistically meaningful change in abundance were CAD protein, cytosolic Phospholipase-A2, p47 phox and AP-3 complex. Overall, analytical tools enabled the identification of important phosphorylated candidates upon interferon-γ induction of macrophages which leads to a better understanding of the signalling pathways of the immune response.

P67 FACT is essential for DNA replication

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In eukaryotic cells, the replication and transcription of DNA packaged into chromatin is a challenge in terms of accessibility and maintenance of chromatin organization. These processes require both to overcome the nucleosome barrier and the restoration of epigenetic information encoded in chromatin structure. The FACT complex (Facilitates Chromatin Transcription) is a heterodimer of Spt16 and Pob3 that alters nucleosomal structure by removal and reassembly of histone H2A-H2B dimers, thus stimulating RNA polymerase II activity. On the other hand, several links also exist between FACT and genomic DNA replication during S-phase. Indeed, FACT binds directly to Pol1 (DNA polymerase α) and interacts genetically with several replication factors. FACT also interacts with Replication Protein A (RPA) and the MCM complex, a putative helicase that likely unwinds DNA ahead of DNA polymerase. Our hypothesis is that FACT is recruited to replication origins, where it acts as an essential component of the replication apparatus that evicts parental histones located ahead of the fork and transfers the histones to the neo-synthesized strands. Our results argue that DNA replication completion in yeast is not prevented by either thiolutin (an inhibitor of the three RNA polymerases) nor cycloheximide (a protein synthesis inhibitor). Thus, the transcription and replication functions of FACT can be separated. Moreover, we found that inactivation of temperature-sensitive Spt16 or Pob3 blocks DNA synthesis in early S-phase. These results indicate that FACT plays an essential role in DNA synthesis that is independent of its role in transcription.

P68 Role of histone H3 lysine 56 acetylation in the maintenance of chromosomal integrity during replication

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Lysine 56 acetylation was recently described as a modification found in newly synthesized histone H3 molecules deposited behind replication forks in Saccharomyces cerevisiae. This genome-wide mark is cell cycle regulated, being present mostly in S phase histones and removed during G2. Cells that are unable to either acetylate or deacetylate this lysine residue are sensitive to DNA damage occurring during DNA replication. Moreover, cells lacking the putative H3 lysine 56 deacetylases display a temperature sensitive (Ts) phenotype, which could be linked to problems associated with rapid replication. In order to understand the role of this modification, suppressors of the temperature sensitive phenotype were identified. Interestingly, some of these suppressors were found to be involved in the formation of boundaries between euchromatin and heterochromatin. We hypothesized that that these sites were frequently damaged during replication and thus that removal of the boundaries could alleviate the Ts phenotype of deacetylase mutant cells. Consistent with this hypothesis, we found that the Ts suppressors did not reduce the level of H3 lysine 56 acetylation or the sensitivity of mutant cells to genotoxic agents that randomly damage genomic DNA. Preliminary ChIP on Chip experiments against DNA damage-induced phosphorylated H2A suggest that active-silent chromatin boundaries are indeed the sites of frequent DNA damage in wild-type and deacetylase mutant cells. Intriguingly, the overall quantity of DNA damage is not influenced by the suppressors, suggesting that spontaneous DNA damage at specific loci is problematic for deacetylase mutant cells. We anticipate that this work will help us understand how cells cope with DNA damage associated with replication of euchromatic-heterochromatic boundaries.

P69 NRAGE/Maged1, an apoptotic receptor adaptor protein, facilitates p75NTR-dependent and independent apoptosis in vivo.

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NRAGE/MAGED1 is an intracellular adaptor protein that binds the p75 neurotrophin receptor (p75NTR) and may facilitate p75NTR-induced apoptosis. To examine the physiological role of MAGED1, we generated Maged1 null mice. These animals show reduced developmental cell death in sympathetic and dorsal root ganglia and display defects in hair follicle catagen. Sympathetic neurons derived from Maged1 null mice show sharply reduced BDNF-mediated apoptosis and cortical neurons from Maged1 null mice are defective in p75NTR-dependent apoptosis. Intriguingly, defects in developmental cell death of motoneurons were observed in Maged1 null but not in p75NTR null mice. These data demonstrate that Maged1 is a physiological adaptor protein that facilitates p75NTR-dependent apoptosis and in addition, reveal that Maged1 participates in p75NTR-independent apoptotic pathways.

P70 ProNGF activates TrkA signaling in PC12 cells in a p75NTR dependent manner

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The uncleaved, pro-form of nerve growth factor (proNGF) has recently been proposed to be a specific ligand for the p75 neurotrophin receptor (p75NTR), a receptor that plays key roles in development and in neuronal injury. The processing, receptor binding properties, and cellular responses to ProNGF remain controversial and it is still unknown how ProNGF functions in the developing and adult nervous system under physiological conditions. ProNGF has been shown to be cleaved in vitro by furin to produce mature NGF so to clearly elucidate the effects of ProNGF we have produced and validated a series of ProNGF constructs that have been mutated in the furin cleavage site that have allowed us to perform a variety of functional studies in PC12 cells, a model of developing sympathetic neurons. Recombinant ProNGF is stable, is not cleaved extracellularly in PC12 cells, and binds to p75NTR at the cell surface. ProNGF exposure leads to phosphorylation of TrkA, activation of sustained ERK and AKT signaling and results in neurite outgrowth in PC12 cells, similar to its mature counterpart NGF. However, unlike mature NGF, our data indicates that proNGF initially binds p75NTR and that receptor internalization is a prerequisite for subsequent TrkA activation which in turn governs ERK and AKT activation and neurite outgrowth. Therefore, trafficking of proneurotrophins and neurotrophin receptors appears to regulate the cellular response to proneurotrophins.

P71 LGI1 is a novel antagonist of myelin-based growth inhibitors

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The failure of damaged adult central nervous system neurons to regenerate can have devastating consequences, often resulting in permanent loss of sensory and motor function. Growth inhibitors present in myelin have been identified as one of the major factors preventing CNS axonal regeneration. Current evidence points to a multicomponent receptor complex comprised of p75NTR, NgR and Lingo-1 that responds to these myelin-associated inhibitors and transduces the inhibitory signal to the neuron. We have identified a protein, termed LGI1 that interacts with components of the myelin-associated inhibitor receptor complex and functions to antagonize the growth inhibitory effects of myelin. We will present data concerning our identification of LGI1 as a myelin-based growth inhibitor antagonist, and discuss avenues we are exploring to determine how LGI1 mediates this effect.

P72 The Role of GDNF Family Members in Axon Guidance in the Accessory Olfactory System

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The establishment of proper connectivity in the nervous system is essential for its function. In sensory systems, neurons in the periphery must project axons into exact locations in the CNS so that sensory stimuli can be translated into neural information. The olfactory systems play a critical role in the survival and mating behavior of most terrestrial vertebrates. While the main olfactory system is involved in the detection of odorants, the accessory olfactory system (AOS) detects pheromones and dictates innate actions. In both systems, axons of chemosensory neurons form stereotypic connections with higher-order neurons in the CNS. The formation of these connections is essential for olfactory function and relies on axon guidance molecules to direct pathfinding axons to their correct targets.
The mechanisms involved in dictating the formation of precise connections between sensory neurons in the vomeronasal organ (VNO) and their target field, the accessory olfactory bulb, are not yet fully understood. The GDNF family of ligands has recently been shown to act as chemoattractants for neurons in the rostral migratory stream and as attractants for sympathetic neuron axons. My project is aimed at determining whether GDNF family ligands function as attractants for VNO neurons during development of the AOS. Expression patterns of the GDNF family ligands and their receptors in the AOS have been assessed using immunohistochemistry. VNO explant assays have also been used to test the responsiveness of VNO axons to sources of GDNF family ligands as well as their ability to promote neurite outgrowth from the VNO.

P73 Micropatterning hippocampal neurons in culture.

Wiam Belkaid, Dalinda Liazoghli, Peter Thostrup, David Juncker and David R.Colman

Neurons have a highly complex and polarized morphology that contributes to their function. During the development of the nervous system, formation of specific connections between nerve cells depends on the stability of growing axons to reach appropriate target cells and form synapses. To elucidate principles of neuronal signaling and network behavior, creation of neuronal network in which connectivity and pathways can be experimentally controlled are of high interests. In culture, hippocampal neurons form numerous synapses by developing axonal and dendritic extensions. In the present study we used a microcontact printing technique to control and study neurite outgrowth of hippocampal neurons in vitro. Polylysine micropatterning on glass substrate was optimized by contact printing using Polydimethylsiloxane (PDMS) stamps. Neurons were seeded on poly-D-lysine micropatterned coverslips. Our results show that hippocampal neurons followed the microcontact printed pattern of poly-D-lysine. Micropatterning is a promising technique that will eventually permit the precise study of different neurobiological mechanisms on a cellular-interaction level such as cell-cell communication and cell signaling.

P74 Parkin-Mediated Monoubiquitination of the PDZ Protein PICK1 Regulates Acid-Sensing Ion Channel Activity

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Mutations in the parkin gene result in an autosomal recessive juvenile-onset form of Parkinson's disease. As an E3 ubiquitin-ligase, parkin promotes the attachment of ubiquitin onto specific substrate proteins. Defects in the ubiquitination of parkin substrates are therefore believed to lead to neurodegeneration in Parkinson's disease. Here, we identify the PDZ protein PICK1 as a novel parkin substrate. We find that parkin binds PICK1 via a PDZ-mediated interaction, which predominantly promotes PICK1 monoubiquitination rather than polyubiquitination. Consistent with monoubiquitination and recent work implicating parkin in proteasome-independent pathways, parkin does not promote PICK1 degradation. However, parkin regulates the effects of PICK1 on one of its other PDZ partners, the acid-sensing ion channel (ASIC). Over-expression of wild-type, but not PDZ binding- or E3 ubiquitin-ligase-defective parkin abolishes the previously described, protein kinase C-induced, PICK1-dependent potentiation of ASIC2a currents in non-neuronal cells. Conversely, the loss of parkin in hippocampal neurons from parkin knockout mice unmasks prominent potentiation of native ASIC currents, which is normally suppressed by endogenous parkin in wild-type neurons. Given that ASIC channels contribute to excitotoxicity, our work provides a mechanism explaining how defects in parkin-mediated PICK1 monoubiquitination could enhance ASIC activity and thereby promote neurodegeneration in Parkinson's disease.

P75 Long-Term Potentiation in Isolated Dendritic Spines

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In brain, N-methyl-D-aspartate (NMDA) receptor (NMDAR) activation can induce long-lasting changes in synaptic alpah-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptor (AMPAR) levels. These changes are believed to mediate the expression of several forms of synaptic plasticity, including long-term potentiation (LTP). Such plasticity is generally believed to reflect the regulated trafficking of AMPARs within dendritic spines. However, recent work suggests that the movement of molecules and organelles between the spine and the adjacent dendritic shaft can critically influence synaptic plasticity. To determine whether such movement is strictly required for plasticity, we have developed a novel system to examine AMPAR trafficking in brain synaptosomes, consisting of isolated and apposed pre- and postsynaptic elements. We report here that synaptosomes can undergo LTP-like plasticity in response to stimuli that mimic synaptic NMDAR activation. Indeed, KCl-evoked release of endogenous glutamate from presynaptic terminals, in the presence of the NMDAR co-agonist glycine, leads to a long-lasting increase in surface AMPAR levels, as measured by [3H]-AMPA binding; the increase is prevented by an NMDAR antagonist 2-amino-5-phosphonopentanoic acid (AP5). Importantly, we observe an increase in the levels of GluR1 and GluR2 AMPAR subunits in the postsynaptic density (PSD) fraction, without changes in total AMPAR levels, consistent with the trafficking of AMPARs from internal synaptosomal compartments into synaptic sites. This plasticity is reversible, as the application of AMPA after LTP depotentiates synaptosomes. Together, the results indicate that the minimal machinery required for LTP is present and functions locally within isolated dendritic spines.

P76 Role of NgR shedding in myelin inhibition

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The adult mammalian central nervous system is characterized by poor regeneration. This has been attributed to both the glial scar and myelin-associated inhibitors (MAIs). The Nogo-66 receptor (NgR) is expressed in neurons and can be activated by MAIs, which mediate growth inhibition. Cell surface expression of NgR is regulated by membrane type matrix metalloproteinase (MT-MMP)-dependent cleavage. NgR cleavage generates a dominant negative NgR fragment that could decrease the sensitivity of neurons to myelin. We have examined cell surface NgR cleavage in primary neurons and in transfected neuroblastoma and 293T cells. We find that NgR shedding is a property of multiple neuronal cell types including neuroblastoma cells, dorsal root ganglion (DRG) neurons and cerebellar neurons. Expression profiling indicates that neurons express multiple MT-MMPs. Further, we find that NgR shedding is regulated by overexpression of specific MT-MMPs. We also observe that other components of the NgR signalling complex, including Lingo-1, can be cleaved by specific MT-MMPs. We are currently examining the effects of MT-MMP overexpression on DRG neurite outgrowth in the context of CNS inhibitory substrates. We have generated cleavage resistant versions of

NgR to examine the relative contribution of NgR shedding to myelin-dependant inhibition in neurite outgrowth and growth cone collapse assays.

P77 The Role of CRMP4 in Nerve Regeneration

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The inability of CNS neurons to regenerate and reform functional connections following spinal cord injury has devastating clinical consequences. The failure of CNS neurons to spontaneously regenerate following injury can be partially attributed to the expression of neurite outgrowth inhibitory myelin associated inhibitors (MAIs). MAIs signal through a tripartite receptor complex to activate the cytosolic protein RhoA and influence cytoskeletal dynamics. RhoA antagonists promote neuronal survival and regeneration in animal models of nerve injury. However, RhoA's potential as a therapeutic target may be limited by its widespread roles in multiples cellular processes and cell types. In an attempt to discover more specific therapeutic targets to promote nerve regeneration, our lab identified the cytosolic phosphoprotein CRMP4b (Collapsin Response Mediator Protein 4b) as a protein that functionally interacts with RhoA to mediate neurite outgrowth inhibition. siRNA-mediated knockdown of CRMP4 and blockade of the RhoA-CRMP4b interaction with a competitive peptide (C4RIP) attenuates myelin-dependent neurite outgrowth inhibition. Analysis of the proximal tip of extending axons (growth cones) by time lapse video microscopy reveals that C4RIP regulates filopodial dynamics indicating that C4RIP modulates the actin cytoskeleton. We are currently investigating the in vivo roles of CRMP4 in regeneration in an optic nerve injury model by developing readily deliverable C4RIP and a CRMP4 knockout mouse. Elucidating the role of CRMP4 in nerve regeneration may provide insight into the molecular mechanisms following nervous system injury.

P78 Nogo signaling in the immune system

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Multiple sclerosis (MS) is an inflammatory disease characterised by myelin destruction and axonal transection in the central nervous system (CNS). Successful therapeutic intervention may require a multi-faceted strategy that is immuno-modulatory and promotes both remyelination and regeneration of damaged axons. In a mouse model of MS, experimental autoimmune encephalitis (EAE), recent studies have demonstrated that clinical outcome is improved by neutralization of a myelin-based inhibitor of axonal outgrowth, Nogo. Surprisingly, the improvement in these mice is associated with a modified immune cell response rather than improvements in axonal regeneration. To investigate the role of Nogo signaling in the immune system, we completed an expression profile of Nogo and its receptor, NgR1, in immune cells and performed associated functional studies. We find that immune cells upregulate expression of Nogo and NgR1 upon activation. The expression of NgR1 by immune cells suggests that they may be responsive to Nogo in their environment. Incubation of immune cells with NgR1 agonists fails to alter the proliferation and cytokine profiles of the immune cells. However, immune cells expressing NgR1 display reduced adhesion to myelin substrates. We propose that Nogo/NgR1 signaling may alter the adhesion properties of immune cells. This modulation could influence the ability of immune cells to cross the blood brain barrier and/or to migrate through the CNS. Nogo and NgR1 are therefore potential therapeutic targets for both regeneration and immuno-modulation based strategies to repair and prevent the CNS damage in MS.

P79 Investigating the role of LIM and TES Kinases in regulating actin cytoskeleton dynamics during myelin-associated inhibition of axonal growth.

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Physical trauma to the mammalian Central Nervous System results in irreversible functional loss. Spontaneous recovery of neuronal connectivity fails to occur, in part due to the inhibitory effects of Myelin-Associated Inhibitors (MAIs) on axon growth. MAIs, such as Nogo, signal via Rho-GTPase and Rho Kinase (ROCK) to regulate actin cytoskeleton dynamics. We have recently shown that Nogo-66-mediated ROCK activation signals a transient increase in the phosphorylated (inactive) form of the actin depolymerizing factor cofilin, followed by a persistent de-phosphorylation phase. This regulation of cofilin activity is mediated through ROCK-dependent phosphorylation of LIM Kinase (LIMK), followed by activation of Slingshot Phosphatase (SSH). Overexpression of Dominant-Negative LIMK in Dorsal Root Ganglion (DRG) neurons prevents the early increase in phospho-cofilin and significantly reduces neurite outgrowth inhibition on myelin substrate. Interestingly, pharmacological inhibition of Nogo-66-mediated ROCK signaling causes a nearly three-fold increase in phospho-cofilin during the late signaling phase. This suggests the possibility of a ROCK-independent Nogo-66 signaling pathway regulating cofilin activity. In this regard, we are developing reagents to investigate a possible role for Testis-Specific Protein Kinase (TESK), a serine/threonine kinase which phosphorylates cofilin, in regulating the neuronal actin cytoskeleton. TESK is expressed in several neuron types, including Purkinje, retinal ganglion, hippocampal and DRG neurons. Overexpression in cerebellar and DRG neurons causes a significant increase in phospho-cofilin levels and an inhibition of neurite outgrowth, while overexpression of Dominant-Negative TESK increases neurite outgrowth. TESK is targeted by numerous serine/threonine kinases, possibly as a key component of signaling pathways regulating actin cytoskeleton dynamics in neurons.

P80 Cool-1/b-Pix, a Cdc42 and Rac specific GEF, is required for DCC dependent axon chemoattraction to netrin-1.

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Axonal chemoattraction depends on directed reorganization of the growth cone cytoskeleton, regulated in part through the activation of the RhoGTPases Cdc42 and Rac. Binding of the chemoattractant guidance cue netrin-1 to its receptor DCC causes phosphorylation and activation of the serine threonine kinase Pak-1 (p21-activated kinase), a key effector of Cdc42 and Rac (Shekarabi & Kennedy 2002; Shekarabi et al. 2005). Although the signalling mechanisms required for DCC regulation of growth cone morphology are beginning to be elucidated, the molecular events leading to activation of Cdc42 and Rac downstream of DCC are not clear. Here we provide evidence that a protein complex including the Cdc42 and Rac specific GEF Cool-1/b-Pix and Git-2/PKL is constitutively associated with DCC in commissural neurons. We show that Cool-1/b-Pix dimerization and association with Pak-1 is required for netrin-1 mediated filopodia formation and growth cone expansion. Furthermore, Cool-1/b-Pix function is required for axon extension toward the floor plate at the midline of the embryonic spinal cord. Our findings provide evidence that Cool-1/b-Pix, a GEF specific for Rac and Cdc42, is required for DCC dependent regulation of growth cone morphology and axonal actin dynamics during commissural axon chemoattraction to netrin-1.

T27 (and P81) Myotopic organization of limb innervation by spinal motor neurons is defined by Netrin and Netrin receptor expression

Dayana Krawchuk, Frederic Charron and Artur Kania

(abstract provided on page 18)

P82 Variations of the chromatin at the beta-globin locus during development

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Chromatin marks have been identified and are presumed to be important for the proper expression of genes. The correlation between these marks (such as histone covalent modifications) and the status of gene expression is not indicative of their influence on gene regulation. We made use of the highly characterized human beta-globin locus to investigate this matter. The expression of the erythroid-specific Beta-globin genes is regulated during development. Using a transgenic model expressing normaly human globin genes (ln2 mice), we characterized variations of the chromatin at the human beta-globin locus during development. We analysed the patterns of histone covalent modifications, recruitment of transcription factors (TFs), co-factors and basal transcription machinery in primitive (e10.5) and definitive (e12.5) erythroid cells. We observed that in both cases, the chromatin organization and the recruitment of TFs correlate with the tissu-specific gene expression. We next used another transgenic model expressing abnormally the globin genes (delta2B) and found that when compared to ln2, the locus chromatin in delta2B (e12.5) is characterized by abnormal histone acetylation as well as impared recruitment of TFs and RNAP II. Surprisingly, in e10.5 EryC, we observed that the low level expression of human gamma globin genes is characterized by minor variations in the locus chromatin and decreased recruitment of GATA-1, NF-E2 as well as RNAP II. Finally, since during development we observed no variations in TF recruitment at the LCR no matter if the chromatin is restrictive or not, our results suggest that HS2 is important for chromatin organization and gene activation in EryC.

P83 Cellular adhesion of normal and cancerous colon cells - effects of surface topography

Cécile M. Perrault, Allison Andrews, Roger Tran-Son-Tay

Normal cellular adhesion is a key factor to maintain healthy human biological functions. Adhesion processes are influenced by many factors such as detachment forces and extra cellular topography, but the mechanisms involved are not well understood because of a lack of well-defined, simple, experimental systems for long-term cell adhesion. The literature also suggests that in the context of cancerous organs, an altered cellular adhesion can promote the spread of metastases. Therefore, in order to better characterize and gain new insights into the effects of some key mechanical factors, we have developed several simple, easy to use, techniques for evaluating the roles of both normal and shear forces, and surface roughness in cell adhesion, with a focus on cancer cells.

The results show as expected that, as the flow rate or the normal force is increased, more cells are detached from the surface substrate. However, it is found that, for a smooth surface, 20\% of the healthy cells will detach at the onset of an applied normal force, and that the rate of detachment is negligible after that. In contrast, cancer cells continuously detach as the normal force is increased. Another difference seen between healthy and cancer cells is in the behavior of doublet cells. Healthy doublets have a much stronger adhesion than single cells. However, this difference is not seen with cancer cells. Finally, it is observed that surface roughness produces different effects on the adhesion of healthy and cancerous colon cells. More specifically, the use of microchannel patterns on a substrate shows that cancer cells do not attach well on a flat surface, and that their adhesion is much weaker than for healthy cells. A small variation in the width or spacing of the patterns will make cancer cells more adherent and behave more like healthy cells. Changes in the feature depth yield a more gradual effect on the adhesion behavior of cancer cells, with an apparently maximum effect for a depth of about 2mm. On the other hand, changes in the topographical features do not affect very much the strength of adhesion of healthy cells.

In conclusion, the developed techniques for cultured cells offer new tools for studying long-term cell adhesion. They provide a mean for investigating the effects of both shear and normal forces, as well as surface roughness. Our results clearly indicate that the topography of the extra cellular matrix will play a major role on the adhesion of the cells, and have a more critical impact on the adhesion of cancer cells than on healthy cells.

P84 Gold surface functionalization for specific immobilization of human Olfactory Receptors carried by liposomes

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Olfactory receptors (OR) belong to the G-protein coupled receptors superfamily with a putative 7-transmembrane domains topology. The first common step in mammalian odorant detection is the odorant specific binding to an OR, expressed on the ciliae of olfactory sensory neurons, embedded in the olfactory mucosa located in the nasal cavity. The activated OR interacts with the Gαolf subunit of a heterotrimeric G-protein which mediates the signalling cascade transmission to the olfactory bulb, where olfactory signals are processed before reaching cortical structures. Given the prominent properties of animal olfaction (specificity, sensitivity, low thresholds for odorant detection), it is tempting to harness olfactory receptors to some device to yield an olfactory biosensor.
In the present contribution we confirm these expectations by showing the first experimental evidence of the sensitive action of OR immobilized onto inorganic substrates.
Three strategies for immobilization of human OR carried by liposomes are presented. Liposomes bearing human OR1740 were immobilized onto gold substrates through the use of self-assembled monolayers containing biotinyl groups. Biotinyl groups are subsequently used to attach neutravidin, then biotinylated monoclonal antibody directed against the receptor to allow its specific grafting. Surface Plasmon Resonance technique is implemented for real-time monitoring of step-by-step surface functionalization and for testing the functional response of immobilized OR. We show that OR1740 is functional when immobilized via a tag attached to its C-terminus, but not via its N-terminus. Present results confirm the possibility to develop a new generation of bioelectronic devices for the assessment of odorants, in particular for disease diagnostics.

P85 Microfluidic probes: microfabrication and use for cell biology

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Cellular experiments are often done using Petri dishes or glass slides with little control over the local cellular microenvironment. There is mounting evidence that the interaction between the cell and its environment is essential in defining the properties and response of the cell. Using microtechnologies it is now becoming possible to control and tailor the cellular microenvironment at cellular and sub-cellular scales.

Here we discuss the microfluidic probe and its application to tailor the microenvironment of cells. The probe combines the concepts of microfluidics and scanning probes and it can be used to “write” on immersed substrates in non-contact mode by flushing across a confined microjet. The probe consists of a silicon microfluidic head clamped with a small rod (similar to a pen) that can be moved across the surface. The programmed displacement of the probe across a substrate allows “drawing” arbitrary patterns of biomolecules on the surface. Cells can subsequently be cultured on these patterns, or alternatively, cells can be directly processed with the probe by flushing the microjet across them.

We are presently developing microfabrication processes using polymers and glass for fabricating transparent, low cost probes compatible with phase contrast imaging. We will use the probes to pattern gradients of proteins such as Netrin which is a well known guidance cue in neuronal development. We propose to create gradients with different slopes (linear vs exponential) and to observe the effect of the slope on axonal guidance, and then to challenge the cells with diffusible cues.

P86 Intravital Blood Flow Imaging : User Interface to Analyze Data Video Images

Dominic Filion. IRCM

Video acquisition of blood flow in arteriole and venule using brightfield transmission imaging are presented. Image processing algorithm and user interface programming using Matlab are detailed. Image processing includes video stabilization by cross-correlation of non-moving regions following another cross-correlation between subsequent frames to determine blood flow profile. Video acquisitions of rolling leukocyte using the fluorescent dye Rhodamine 6G are also presented. Those videos show leukocyte rolling along blood vessels. Image processing and algorithm for automated determination of quantitative criteria regarding the rolling are described in details.

P87 Adhesion strength regulates the integrin-actin linkage during cell migration

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Cell migration is regulated in part by the connection between the substratum and the actin cytoskeleton. However, the very large number of proteins involved in this linkage and their complex network of interactions makes it difficult to assess their role in cell migration. Here we apply a novel image analysis tool, spatio-temporal image correlation spectroscopy (STICS), to quantify the directed movements of adhesion-related proteins and actin in protrusions of migrating cells. The STICS technique reveals hidden protein dynamics even in situations where protein densities are either very low or very high, and it also works in the presence of large, static molecular complexes. We construct detailed cellular velocity maps for actin and the adhesion-related proteins alpha-actinin, alpha5-integrin, talin, paxillin, vinculin and focal adhesion kinase. From a comparison of velocities of the adhesion-related molecules to actin we characterize the efficiency of the linkage between integrin and actin in different cell types, identify potential mechanisms that regulate efficiency of the linkage, and identify two likely points of slippage or disconnect in this linkage, one at the integrin level and the other at the alpha-actinin level. Our data suggest that the efficiency of the linkage increases as actin and adhesions become more organized.

P88 Mechanical properties of microtubule and composite microtubule-actin networks

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We study in vitro models of the cytoskeleton composed of reconstituted microtubules, actin and composite networks made of both. We measure the elastic and viscous moduli of such networks using passive microrheology: Micrometer-sized polystyrene beads are embedded in the networks, their motion tracked to subpixel, <10 nm accuracy, and the ensemble average of their motion used to report on the properties of the network. The autocorrelation reports on the beads' local environment, whereas the cross-correlated motion between pairs of beads reports on the bulk material. We find a nontrivial frequency dependence of the network's viscoelastic properties. The viscoelastic moduli measured (~1Pa) are smaller than those reported for in vivo measurements – cells – by three of magnitude. The addition of motor proteins such as myosins and kinesin, dynein or Eg5 should "pre-stress" our in vitro networks and give insights into the essential components of cell mechanics.

P89 Multiplex fluorescent 1D immunoblot as a tool to visualize reversibly oxidized intracellular proteins

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During oxidative stress, intracellular proteins are reversibly or irreversibly oxidized. Recently, reversible protein oxidation has attracted attention as a possible redox signaling mechanism. Protein s-glutathionylation (i.e. formation of mixed disulfide between glutathione and protein thiol group) is one such reversible oxidative post-translational modification. We studied protein s-glutathionylation in respiratory epithelial cell lines under conditions of mild (hydrogen peroxide, H2O2) and strong (diamide) oxidative stress. Intracellular proteins were resolved on 4-20% gradient gel and electrotransferred onto a low-fluorescent PVDF membrane (Hybond LPF, GE Healthcare). The membrane was simultaneously incubated with two primary antibodies, to detect glutathionylated proteins and GRB2 (as a gel loading control) followed by Cy5 secondary antibody (GE Healthcare, detection of glutathionylated proteins) and Cy3 secondary antibody (GE Healthcare, GRB2 detection). Fluorescence was documented on a Typhoon Trio+ fluorescent scanner (2D/DIGE facility).
Protein s-glutathionylation was more prominent in diamide-stimulated samples, than in H2O2-stimulated samples. Surprisingly, we observed that GRB2 was reversibly oxidized with diamide, but not by H2O2. Specifically, the GRB2 band was shifted upward (indicating an increased molecular weight) in the diamide lane and this shift disappeared when the sample was chemically reduced, suggesting that diamide stimulation caused a reversible binding of a low-molecular weight substance to the protein. The overlay Cy5/Cy3 image demonstrated that the GRB2 band indeed appeared amongst the oxidized proteins.
Conclusion: In the present study, we demonstrate the usefulness of multiplex fluorescent 1D immunoblot to detect post-translational protein modifications on the level of individual proteins.

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P90 14-3-3 Proteins and Growth Cone Dynamics

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The distal tip of a developing axon, termed the growth cone, is a highly regulated dynamic structure, critical to establishing the proper speed and direction of neuronal outgrowth. This structure must integrate responses to numerous cues through precise spatial regulation of the dynamic actin and microtubule cytoskeleton. An initial proteomics screen of growth cones found members of the 14-3-3 adaptor protein family present. These proteins regulate a wide variety of Serine/Threonine phosphorylated targets in diverse cell mechanisms such as cell-cycle control, apoptosis, sub-cellular localization and cytoskeletal reorganization. The presence of these proteins in growth cones and the implication of their modulating the activity of Serine/Threonine Kinases and Phosphatases known to be involved in the regulation of actin dynamics, led us to hypothesize that 14-3-3 proteins could be integration points in growth cones allowing multiple ligand effects to be properly coordinated at the level of cytoskeleton dynamics. We have characterized the expression of multiple isoforms within growth cones of different cell types and developmental time points. Also, we present preliminary evidence from a number of experiments using viral mediated overexpression of the various isoforms and the 14-3-3 binding inhibitor R18 peptide to look for changes in basal neurite outgrowth as well as changes in response to a myelin substrate. Live-imaging has been used to examine the effects of the R18 peptide on the dynamic structures of the growth cone. In addititon, we present preliminary evidence of the regulation of 14-3-3 binding in response to ligands known to act as guidance cues.

P91 N-acetyl β-D-glucosaminidase, a cuticle enzyme secreted by axenic Steinernema carpocapsae suppresses the immune system of the Greater wax moth, Galleria mellonella

Jason F. Lapointe, Walter Tita, Gary B. Dunphy, and Craig A. Mandato

The nematode-bacterium complex, Steinernema carpocapsae-Xenorhabdus nematophila, is a virulent insect pathogenic system for larvae of the pest insect, the Greater wax moth, Galleria mellonella. Upon reaching the lepidopteran's hemocoel, there is a period during which the nematode must avoid initiating the insect's non-self humoral and cellular systems to ensure subsequent bacterial release. Immunosuppressive and evasive activities have been associated with steinernematid cuticle. However axenic S. carpocapsae are known to release trypsin-like and chymotrypsin-like enzymes, which partially limit hemocyte responses to foreign antigens implying other factors may be involved. Herein an APIZYM enzyme analysis of axenic exudate revealed a negative correlation with N-acetyl β-D-glucosaminidase (NAG) activity and hemocyte adhesion to slides. Confirmation of NAG as an immunosuppressant was based on blocking the inhibition of hemocyte attachment to glass slides by the exudate and commercial NAG sources using the NAG inhibitors α-D-mannose and p-nitrophenyl-α-D-mannopyranoside. Glucose and trehalose, at physiological levels in G. mellonella, did not impair NAG activity of either the exudate or commercial source; precluding these plasma sugars limiting immunosuppression by the nematode. The immunosuppressive activities of NAG suggests it is a virulence factor of the entomopathogenic nematode, Steinernema carpocapsae.

P92 The Expression of human Interleukin-7 in stably transfected Spodoptera frugiperda (Sf9) cells

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Development of insect cells capable of expressing recombinant proteins in a stable continuous manner is an attractive alternative to the BEVS processes for recombinant protein production. The ability to grow insect cells to high cell densities in either fed batch or perfusion cultures can now be further exploited to design processes which aim to maximize the production of recombinant proteins using the stable cell lines.

There are earlier reports of the stable expression of recombinant proteins in Drosophila as well as in Trichoplusia insect cell cell lines. In this work we have generated a new Sf-9 insect cell line expressing human Interleukin-7. Human Interleukin-7 is a 25 kDa glycoprotein secreted by bone marrow, thymus liver cells and stromal cells. It is used for the development of T- and B-cells also it has an important role in survival and differentiation of antigen presenting cells and lymphoid dendritic cells in thymus. The toxicity of IL-7 at therapeutic doses is limited. Human IL-7 has promising potential therapeutic uses in cancer therapy, hematopoietic antigen-specific responses (HSCT) and HIV infection also it has not shown serious side effect.

The cloning of a commercially available h-IL-7 gene into pIE1/153A (V4) Triple expression vector system for stable transfection of sf9 insect cells is described. After selection of the positive clones with puromycin, and screening using Western blot analysis. The most productive clone, Sf9hIL-7A1 was selected for further study. The growth of Sf9hIL-7A1 cells and production performance of the clone in small-scale shaker flask as well as in laboratory scale bioreactor is described. The performance of the clone with respect to growth and production is compared in a conventional stirred tank bioreactor and a disposable Wave bioreactor system.