European School of Molecular Medicine

IFOM

IEO European Institute of Oncology

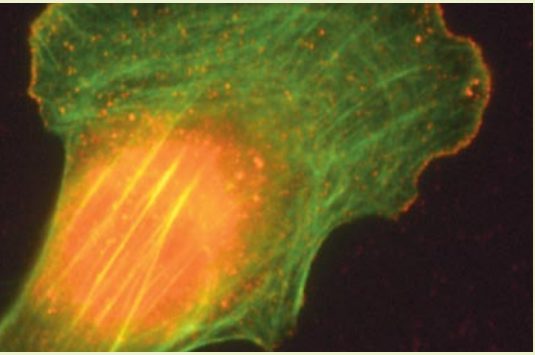
Università degli Studi di Milano

May 12-14, 2007

Milan, Italy

SEMM WORKSHOP ON

Cell Migration: from Molecules to Organisms and Diseases



Abstract book

SEMM WORKSHOP ON
CELL MIGRATION:

from Molecules to Organisms and Diseases

May 12/14, 2007
IFOM-IEO Campus
Milan, Italy

ORGANIZATION

SCIENTIFIC & ORGANIZING COMMITTEE

Marie-France Carlier
Dynamique du Cytosquelette, LEBS, CNRS - Gif-sur-Yvette, France
carlier@lebs.cnrs-gif.fr

Ugo Cavallaro
IFOM-IEO Campus - Milan, Italy
ugo.cavallaro@ifom-ieo-campus.it

Marina Mione
IFOM-IEO Campus - Milan, Italy
marina.mione@ifom-ieo-campus.it

Giorgio Scita
IFOM-IEO Campus - Milan, Italy
giorgio.scita@ifom-ieo-campus.it

EVENT COORDINATOR

Sabrina Frata
SEMM Foundation – Milan, Italy

TABLE OF CONTENTS

MAY 12, 2007

h. 12.00/2.00 pm - Registration and poster positioning

h. 1.00/2.00 pm - Light lunch at workshop venue

MAY 12, 2007 (h. 2.00/6.00 pm)

Session I: CYTOSKELETON - **Chair:** M.F. CARLIER

h. 2.00/2.40 pm
Control of filament barbed end growth in motile processes
Marie-France Carlier, Gif-sur-Yvette, France

h. 2.40/3.20 pm
Multi-Scale Approach to Adhesion Structures
Dorit Hanein, La Jolla, USA

h. 3.20/3.35 pm
Fascin, a novel target of beta-catenin-TCF signalling, increase migration, invasion and metastasis of colon cancer cells
Danijela Vignjevic, Paris, France

h. 3.35/4.15 pm
Regulation of the actin cytoskeleton and plasma membrane dynamics by MIM/ABBA family proteins
Pekka Lappalainen, Helsinki, Finland

h. 4.15/4.30 pm
uPAR-induced cell adhesion and migration: Vitronectin provides the key
Chris Madsen, Milan, Italy

h. 4.30/5.00 pm - Coffee break

h. 5.00/6.00 pm - **Poster session I**

MAY 12, 2007 (h. 6.00/7.35 pm)

Session II: SIGNALLING TO/FROM CYTOSKELETON - **Chair:** G. SCITA

h. 6.00/6.40 pm
EPH receptor/ephrin signaling: dynamic regulation of the actin cytoskeleton and cell motility
Catherine Nobes, Bristol, UK

h. 6.40/6.55 pm
Regulation of purified native WAVE complexes: tightly inhibited but susceptible to artifactual activation
Andres Lebensohn, Boston, United States

h. 6.55/7.35 pm
Connecting the membrane to the actin cytoskeleton by the BAR, EFC, and RCB domain proteins and WASP/WAVE proteins
Shiro Suetsugu, Tokyo, Japan

h. 7.35 pm - End of the session

MAY 13, 2007 (h. 9.00 am/2.00 pm)

Session III: SIGNALLING/ADHESION - **Chair:** U. CAVALLARO

h. 9.00/9.40 am
Stem cells and morphogenesis
Elaine Fuchs, New York, USA

h. 9.40/9.55 am
Expression of L1-CAM in human pancreatic carcinoma corresponds to increased endothelial migration in vitro and enhanced vascularization of tumor tissue in situ
Yasmin Issa, Heidelberg, Germany

h. 9.55/10.25 am - Coffee break

h. 10.25/11.05 am
Regulation of microtubules and cell polarity by Rho GTPases in migrating cells
Gregg Gundersen, New York, USA

h. 11.05/11.20 am
Rab5 regulates Rac internalization and trafficking promoting spatial restriction of signaling and cell migration
Andrea Palamidessi, Milan, Italy

h. 11.20 am/12.00 pm
Dynamic regulation of podosomes in primary human cells
Stefan Linder, München, Germany

h. 12.00/1.00 pm - Lunch

h. 1.00/3.00 pm - **Poster session II**

MAY 13, 2007 (h. 3.00/4.45 pm)

Session IV: IMAGING - **Chair:** K. HAHN

h. 3.00/3.40 pm
New live cell biosensor designs: windows on endogenous motility signaling
Klaus Hahn, Chapel Hill, USA

h. 3.40/3.55 pm
Molecular kinetics of AIP1, a cofilin-dependent actin barbed end interacting protein, in lamellipodia: Evidence for frequent filament severing
Naoki Watanabe, Kyoto, Japan

h. 3.55/4.35 pm
Chemotactic cell movement, a key mechanism during development
Cornelis J. Weijer, Dundee, UK

h. 4.35/5.00 pm - Coffee break

MAY 13, 2007

(h. 5.00/7.05 pm)

Session V: ANIMAL MODELS - **Chair:** M. MIONE

h. 5.00/5.40 pm
Insights into the regulation of epithelial border cell migration from genetics and live cell imaging
Denise Montell, Baltimore, USA

h. 5.40/5.55 pm
Regulation of actin-driven processes during Drosophila development
Sven Bogdan, Münster, Germany

h. 5.55/6.10 pm
Beta1 integrins and the migration of enteric neural crest cells in the gut
Marie Breau, Paris, France

h. 6.10/6.50 pm
Molecular and cellular mechanisms controlling directed germ-cell migration in zebrafish
Erez Raz, Göttingen, Germany

h. 6.50/7.05 pm
A combinatorial role for three matrix metalloproteinases in Xenopus embryonic macrophage migration
Grant N. Wheeler, Norwich, UK

h. 7.05 pm - End of session

h. 7.30 pm - Social Dinner

MAY 14, 2007 (h. 9.00/10.35 am)

Session VI: ORGANOGENESIS - **Chair:** R. ADAMS

h. 9.00/9.40 am
Molecular regulation of blood vessel morphogenesis
Ralf Adams, London, UK

h. 9.40/9.55 am
Polycystin-1 Induces Cell Migration by Regulating PI3kinase-dependent Cytoskeletal Rearrangements and GSK3beta -dependent cell-cell Mechanical Adhesion
Manila Boca, Milan, Italy

h. 9.55/10.35 am
Regulation of Cytoskeletal Dynamics During Neurite Initiation and Axon Navigation
Frank B. Gertler, Madison, USA

h. 10.35/11.05 am - Coffee break

MAY 14, 2007 (h. 11.05 am /1.40 pm)

Session VII: DISEASES - **Chair:** A. BEN-ZE'EV

h. 11.05/11.45 am
Cell motility at the invasive front of tumors: the role of novel Wnt/beta-catenin target genes
Avri Ben-Ze'ev, Rehovot, Israel

h. 11.45 am/12.25 pm
In vivo imaging of cancer invasion: from individual to collective cell migration
Peter Friedl, Würzburg, Germany

h. 12.25/12.40 pm
Identification of genes and pathways regulating tumor invasion and metastasis induced by e-cadherin loss in a conditional mouse model for ILC
Gilles Doumont, Amsterdam, The Netherlands

h. 12.40/1.40 pm - **CLOSING KEYNOTE**
Distinct mechanisms of tumor invasion and metastasis
Gerhard Christofori, Basel, Switzerland

h. 1.40 pm - End of the workshop and light lunch

Lectures

Marie-France Carlier
Gif-sur-Yvette, France

Dorit Hanein
La Jolla, USA

Pekka Lappalainen
Helsinki, Finland

Oral Presentations

Danijela Vignjevic
Paris, France

Chris Madsen
Milan, Italy

Session I

CONTROL OF FILAMENT BARBED END GROWTH IN MOTILE PROCESSES

MARIE-FRANCE CARLIER

Gif-sur-Yvette, France

Motile processes like cell protrusions are generated by site-directed barbed end growth of actin filaments. This process occurs in the context of treadmilling. Fluorescence speckle microscopy of actin and regulatory proteins in migrating cells show that morphologically and dynamically distinct networks of actin filaments initiated at the plasma membrane coexist in the cell and define cellular compartments. How are these different turnover rates coordinated to achieve directional movement in response to extracellular cues ? How can regulatory proteins recognize distinct actin networks ? What are the relationships between the force produced by filament barbed end growth that deforms the membrane and the molecular mechanism of the protein machineries that link barbed ends to the membrane ? A combination of in vitro polymerization assays and reconstituted motility assays provides some insight into these issues. Force is determined by the number of filaments. Velocity is determined by the rate of barbed end growth. Both are controlled by membrane-associated machineries like N-WASP-Arp2/3 and formins. These machineries operate with different molecular mechanisms and kinetic parameters, allowing them to display different velocities under the same steady state conditions. The branched filament array formed by N-WASP-Arp2/3 displays transient attachments to the membrane linked to Arp2/3-induced branching. These transient attachments control the segregation of N-WASP bound at the surface of giant liposomes in a motility assay, and determine the motility regime. In contrast, formins maintain permanent attachment to the filament barbed ends during processive growth, and truly behave as end-tracking stepping motors. The role of profilin and ATP hydrolysis in formin function has been debated. We show that profilin is the coupling device that links ATP hydrolysis on actin to the stepping movement of formin at barbed ends. Profilin caps barbed ends until ATP is hydrolyzed on the penultimate subunit. Evidence for synergy between different actin-based machineries like formin and Spire will also be briefly presented.

MULTI-SCALE APPROACH TO ADHESION STRUCTURES

DORIT HANEIN

Professor, Burnham Institute for Medical Research, Las Jolla, California, USA

My group studies molecular machines involved in the assembly and regulation of the actin cytoskeleton at the leading edge of motile cells. Structural characterization of these multimolecular protein complexes is likely to reveal potential mechanisms underlying cell growth, differentiation and migration as well as the role of these interactions in tumor invasion and metastasis. In particular, we study the role of Arp2/3 complex in actin filament network assembly, the role of myosin in cell migration, and the role of actin binding proteins in providing a scaffold for cell protrusions and adhesion. We combine various electron cryomicroscopy, image analysis and bioinformatics techniques to extract high-resolution structural information of these large dynamic assemblies in their fully hydrated state. Electron cryomicroscopy is the principal method for solving the structures of large complexes that remain beyond the reach of NMR and x-ray crystallography. The high-resolution electron microscopy field is in a state of rapid development both on the instrumentation and computational fronts, utilizing a variety of new technology breakthroughs and image analysis approaches for producing 3D reconstructions of key macromolecular complexes. Although high-resolution structural approaches provide critical information about individual molecules and complexes, a barrier to progress remains their structural and functional integration at the cellular level. Towards this end we are currently developing techniques and protocols that allows us to image whole cell, in their fully hydrate state, and to use bioinformatics tools, to correlate between the high-resolution structural information motives with the in situ characterization obtained from living cells.

FASCIN, A NOVEL TARGET OF BETA-CATENIN-TCF SIGNALLING, INCREASE MIGRATION, INVASION AND METASTASIS OF COLON CANCER CELLS

DANIJELA VIGNJEVIC[1], Marie Schoumacher[1], Nancy Gavert[2], Klaus-Peter Janssen[3], Gloria Jih2, Marick Laé4, Daniel Louvard1, Avri Ben-Ze'ev[2], Sylvie Robine[1]

1 UMR144/CNRS, Institut Curie, Paris 75005, France; 2 Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot 76100, Israel; 3 Department of Surgery, TUM, Munich, 81379, Germany; 4 Department of Pathology, Institut Curie, Paris, 75005, France;

Cancer cells become metastatic by acquiring a motile and invasive phenotype. This step requires remodeling of the actin cytoskeleton and the expression of exploratory, sensory organelles known as filopodia. Aberrant beta-catenin-TCF target gene activation plays a major role in colorectal cancer (CRC) development.
We identified fascin, a key component of filopodia, as a potential target of beta-catenin-TCF signaling in CRC cells. Fascin mRNA and protein expression were increased in primary cancers in a stage-dependent manner. Fascin was exclusively localized at the invasive front of tumors also displaying nuclear beta-catenin. Forced expression of fascin in CRC cells increased migration and invasion in cultures and caused cell dissemination and metastasis in vivo. Although expression of fascin in primary tumors correlated with the presence of metastases, fascin was not expressed in metastases.
Our studies show that fascin expression is tightly regulated during development of CRC metastases, as a novel target of beta-catenin-TCF signaling. We propose that transient up-regulation of fascin in colon cancer promotes the acquisition of migratory and invasive phenotypes that lead to metastasis. Further, expression of fascin is down-regulated once the tumor cells reach their metastatic destination where migration ceases and proliferation is enhanced.

REGULATION OF THE ACTIN CYTOSKELETON AND PLASMA MEMBRANE DYNAMICS BY MIM/ABBA FAMILY PROTEINS

PEKKA LAPPALAINEN, Pieta K. Mattila, Juha Saarikangas, Anette Pykäläinen, Ville O. Paavilainen, Helena Vihinen, Eija Jokitalo, Marjo Salminen

Institute of Biotechnology, University of Helsinki, P.O. Box 56, 0014 Helsinki, Finland

The actin cytoskeleton plays a fundamental role in various motile and morphogenetic processes involving membrane dynamics. Here, we show that actin-binding proteins MIM (missing-in-metastasis), ABBA (actin bundling protein with BAIAP2 homology), and IRSp53 directly bind PI(4,5)P2-rich membranes and deform them into tubular structures. This activity resides in the N-terminal IM-domain of these proteins that is structurally related to membrane tubulating BAR (Bin-Amphiphysin-Rvs) domains. We found that due to a difference in the geometry of the PI(4,5)P2 binding site, IM-domains induce a membrane curvature opposite that of BAR domains and deform membranes by binding to the interior of the tubule. This explains why IM-domain proteins induce plasma membrane protrusions rather than invaginations. Studies on ABBA revealed that this protein is strongly expressed in radial glia cells and that endogenous ABBA localizes to the actin rich end-feet in cultured radial glia like C6-R cells. Depletion of ABBA from C6-R cells results in defects in lamellipodial dynamics and impairs cell process outgrowth. Together, these data reveal that interplay between actin dynamics and a novel membrane deformation activity promotes cell motility and morphogenesis.

uPAR-INDUCED CELL ADHESION AND MIGRATION: VITRONECTIN PROVIDES THE KEY

[1] CHRIS D. MADSEN, [1]Annapaola Andolfo, [1]Gian Maria Sarra Ferraris, [1]Orla Cunningham and [1,2]Nicolai Sidenius

1 The FIRC Institute of Molecular Oncology (IFOM), Via Adamello 16, 20139 Milan; 2 Molecular Genetics Unit, DIBIT, Università Vita-Salute San Raffaele, Via Olgettina 58, 20132 Milan, Italy

Expression of the GPI-anchored membrane receptor uPAR induces profound changes in cell adhesion, migration and proliferation and its expression correlates with the malignant phenotype of cancers. To identify the key molecular interactions essential for uPAR function in these processes, we carried out a complete functional alanine scan of uPAR in HEK293 cells. Of the 255 mutant receptors characterized, 34 failed to induce changes in cell adhesion, cell morphology accompanied by actin-rearrangement and focal adhesion turn-over, and cell migration. Remarkably, the molecular defect of all of these mutants was a specific reduction in integrin-independent cell binding to vitronectin. A membrane tethered plasminogen activator inhibitor-1, which has the same binding site in Vn as uPAR, replicated uPAR-induced changes. A direct uPAR-Vn interaction is thus both required and sufficient to initiate downstream signalling leading to changes in cell morphology and migration. Together these data demonstrate a novel mechanism by which a cell adhesion molecule lacking inherent signalling capability evokes complex cellular responses, independently of lateral interactions with signalling receptors, by modulating the contact between the cell and the matrix. The importance of the uPAR/Vn-interaction was not cell-type specific as all mutants identified were subsequently confirmed in CHO cells.

Lectures

Catherine Nobes
Bristol, UK

Shiro Suetsugu
Tokyo, Japan

Oral Presentations

Andres Lebensohn
Boston, United States

Session II

EPH RECEPTOR/EPHRIN SIGNALLING: DYNAMIC REGULATION OF THE ACTIN CYTOSKELETON AND CELL MOTILITY

DR CATHERINE NOBES

Departments of Physiology and Biochemistry, University of Bristol, Bristol, BS8 1TD, UK.

Cell-to-cell contact between Eph receptor- and ephrin-expressing cells triggers bi-directional signalling that can regulate attractive or repulsive cell behaviours. These functions of Eph receptors and ephrins make them key players in controlling cell migrations and in establishing tissue patterning in embryos and adults. We aim to understand the molecular mechanisms underlying this complex dynamic relationship and how cell migration patterns are regulated by Eph/ephrin-contact. To do this we are focusing on how downstream signals, in both Eph receptor- and ephrin-expressing cells, impinge on the actin cytoskeleton and ultimately on the behaviours of receptor- and ligand-expressing cells after collision using a combination of cell biology, biochemical and live-cell-imaging approaches. Our time-lapse movies of Eph receptor- and ephrin-cell contacts and collisions demonstrate that this interaction is highly dynamic - a repeated 'love-hate' relationship with constant switching between membrane protrusion and membrane retraction behaviour.

REGULATION OF PURIFIED NATIVE WAVE COMPLEXES: TIGHTLY INHIBITED BUT SUSCEPTIBLE TO ARTIFACTUAL ACTIVATION

ANDRES M. LEBENSOHN and Marc W. Kirschner

Department of Systems Biology, Harvard Medical School, Boston, Massachusetts, USA

WAVE proteins, crucial regulators of Arp2/3 dependent actin assembly, exist in large complexes whose regulation has remained unclear due to conflicting biochemical studies. Previous work from our lab postulated that native WAVE1 complex was basally inactive but could be activated by Rac and/or Nck. Other researchers found that WAVE1 and WAVE2 complexes reconstituted in vitro, purified from cells over-expressing Flag-WAVE, and purified conventionally were basally active. Trying to understand these discrepancies, we found it is not the source or the WAVE variant, but the way in which the complexes are made and handled that affects their activity. We observed that native WAVE1 and WAVE2 complexes purified by conventional and immunoaffinity chromatography from a variety of tissues and cell lines are inactive. These complexes are tightly inhibited but contain activatable WAVE, because denaturing treatments such as heating or freeze-thawing without cryoprotectants result in actin assembly. Thus, previous reports of basally active WAVE complexes are most likely due to free or exposed WAVE from misassembled or unfolded complexes. Hitherto we were unable to activate our native complexes. These findings clarify what is known about WAVE complex activity, explain many of the discrepancies in the field, and highlight critical issues for future experiments.

CONNECTING THE MEMBRANE TO THE ACTIN CYTOSKELETON BY THE BAR, EFC, AND RCB DOMAIN PROTEINS AND WASP/WAVE PROTEINS.

SHIRO SUETSUGU[1,2], Kazuya Tsujita[3], Atsushi Shimada[4], Kazutaka Murayama[5], Mikako Shirouzu[5], Shigeyuki Yokoyama[5], and Tadaomi Takenawa[3]

1 Institute of Molecular and Cellular Biosciences, University of Tokyo; 2 PRESTO, Japan Science and Technology Corporation (JST); 3 Kobe University School of Medicine; 4 RIKEN SPring-8 Center; 5 RIKEN Genome Science Center.

WASP/WAVE family proteins are important regulators of the Arp2/3 complex which causes a exponential growth of actin filaments. Each WASP/WAVE family protein forms stable protein heterocomplex through its N-terminal domain. The C-terminal domain activates the Arp2/3 complex. Recently, a large family of membrane-deforming proteins was found to interact with the proline-rich regions that locate in the middle of the WASP/WAVE family proteins. These proteins have the SH3 domains that interact with WASP/WAVE family proteins. The membrane-deforming ability of these proteins are provided by the N-terminal domains such as Bin-Amphiphisin-Rvs167 (BAR) domain, Extended-FCH (EFC) domain/FCH-BAR (F-BAR) domain, and Rac-binding (RCB)/IRSp53-MIM- homology (IM) domain.

We have analyzed the mechanism of membrane deformation caused by EFC and RCB domains. EFC domains cause invagination of the plasma membrane that appears to be consistent with the membrane tubulation in vitro. In contrast, RCB domains caused the membrane deformation in a inverted direction as the BAR domain. The active form of Rac enhances this deformation.

Time-lapse imaging indicated that the EFC domain-proteins and N-WASP are recruited simultaneously to clathrin-coated pit when clathrin disappears from the plasma membrane. Interestingly, majority of BAR and EFC/F-BAR containing proteins interacts with N-WASP and dynamin.

In contrast, proteins containing the RCB domain bind to WAVE and Ena/ VASP proteins. However, significance of membrane deformation by the RCB domain in lamellipodia and filopodia formation was not clarified yet. These protein networks suggest that BAR and EFC proteins regulate the actin polymerization by N-WASP for inward vesicle movement such as endocytosis, and that the RCB/IM proteins regulate the actin polymerization by WAVEs for outward directions such as lamellipodia and filopodia.

Department of Biochemistry, Institute of
Medical Science, University of Tokyo, Shirokanedai, Minato-ku, Tokyo,
108-8639 Japan.
tel+81-3-5449-5508
fax +81-3-5449-5417

Poster session I

A1	Ewa Aladowicz
A2	Andres M. Lebensohn
A3	Stefano Alemà
A4	Pontus Aspenström
A5	Danila Baldessari
A6	Costanza Ballarò
A7	Anna Baruzzi
A8	Melanie Barzik
A9	Barbara Belletti
A10	Giovanna Berruti
A11	Cristina Bianchi
A12	Katiuscia Bonezzi
A13	Giusi Caldieri
A14	Paola Chiarugi
A15	Paolo Cirri
A16	Maria C.Denis
A17	Andrea Disanza
A18	Elena Rainero
A19	Ramona Esposito
A20	Gabrielle Faure-Andre
A21	Sofia Francia
A22	Alexis Gautreau
A23	Silke Gerboth
A24	Chiara Giuliani
A25	Andrea Graziani
A26	Maud Hertzog

Last update: May 02, 2007

A1

RALP IS INVOLVED IN CELL ADHESION AND MIGRATION DURING MELANOMA PROGRESSION

EWA ALADOWICZ, Giuseppina Giardina, Cristina Spinelli Italy, Ernesta Fagiani, Luisa Lanfrancone

European Institute of Oncology, Milan, Italy

RaLP is a newly identified member of the Shc family of adaptor proteins that encodes a cytosolic protein highly expressed in aggressive and metastatic melanomas. When ectopically expressed in non-metastatic melanoma cells, it functions as a substrate of activated IGF-1 and EGF receptors, increasing Ras-MAPK signaling and cell migration; while its silencing in RaLP-positive melanoma cells abrogates migration in vitro, without affecting MAPK signaling, suggesting that RaLP activates both Ras-dependent and-independent migratory pathways in melanomas. Silencing of RaLP expression in melanoma cell lines by RNA interference inhibits tumorigenesis in vivo, thus suggesting that RaLP could be a critical determinant in the acquisition of the migratory phenotype by melanoma cells and a potential target for novel anti-melanoma therapeutic strategies.

To investigate the molecular basis of the decreased adhesive and migratory capacity mediated by RaLP silencing in melanoma cells, we analysed the pattern of expression of RaLP knocked down WM-266.4 cells compared to cells where an irrelevant gene (luciferase) was silenced. Gene expression profile was performed and attention focused on genes involved in regulating signalling (56), adhesion and motility (41). Protein levels of β3 integrin and L1-CAM were found down regulated by RaLP ablation in WM-266.4 cells, while ankyrin and caveolin expression was upregulated, suggesting a role of RaLP in mediating cell-cell and cell-matrix adhesion.

A2

REGULATION OF PURIFIED NATIVE WAVE COMPLEXES: TIGHTLY INHIBITED BUT SUSCEPTIBLE TO ARTIFACTUAL ACTIVATION

ANDRES LEBENSOHN

Harvard Medical School, Systems Biology, Boston, United States

For abstract please reach pag. 26

A3

EPS8 ASSOCIATES WITH P120 CATENIN TO REGULATE CADHERIN-DEPENDENT ADHESION AND CELL MOTILITY

C. Ballarò[1], A. Disanza[2], A.M. Salvatore[3], G. Scita[2] and S. ALEMÀ

1-Istituto di Biologia Cellulare, CNR, Monterotondo; 2-IFOM-IEO Campus, Milan; 3-Istituto di Neurobiologia e Medicina Molecolare, CNR, Rome, Italy.

The regulation of cadherin-mediated adhesion by growth factors resulting in plasticity of cell-cell contacts and cell migration has been extensively investigated, but whether and how cadherin function is directly affected, as well as the role of the underlying actin cytoskeleton, remain less well understood issues. Eps8, a substrate of receptor tyrosine kinases, in a complex with Abi1 and Sos-1 induces Rac activation leading to actin cytoskeleton remodelling. Recent studies have shown that Eps8 regulates the growth of actin filaments by capping their barbed ends, an activity essential for cell motility. Here, we report that Eps8 localizes to cell-cell junctions, potentially through its direct and constitutive association with the N-terminus of p120 catenin. Through the use of RNAi and the reconstitution with mutants selectively defective for Eps8 capping activity, the latter is shown to be required for normal cadherin-dependent adhesion, cortical actin organization, epithelial cell migration and HGF-driven cell scattering. This study provides novel functional information on Eps8 as a cell-cell adhesion regulator and increases our understanding of the roles of actin capping proteins in cell adhesion.

A4

CYTOSKELETAL DYNAMICS DOWNSTREAM OF THE ATYPICAL RHO GTPASE WRCH1 IS COORDINATED BY THE NON-RECEPTOR TYROSINE KINASES PYK2 AND SRC

PONTUS ASPENSTRÖM, Dirk Pacholsky, Åsa Fransson and Aino Ruusala

Ludwig Institute for cancer Research, Biomedical Center, Uppsala University, Box 595, SE-751 24 Uppsala, Sweden

Wrch1 belongs to Cdc42 subfamily of small RhoGTPases. The human Wrch1 protein shares 57% identity and 70% similarity with Cdc42. In contrast to Cdc42 it contains both N- and C- terminal extensions of 46 and 21 amino acids, respectively and the N-terminal extension contains several PXXP motifs. Wrch1 has a very rapid, intrinsic guanine nucleotide exchange activity. PAK1, Nck, Grb2 and PLC have been identified as proteins interacting with Wrch1. When expressed transiently in NIH3T3 cells Wrch1 reduced the stress fiber content about 90% and the cells had an up-rounded morphology. The expression of Wrch1 also induced extensive filopodia formation in these cells. We have made a yeast two hybrid screening using a constitutively active Wrch1 mutant as bait. Eight independent clones representing potential Wrch1 binding proteins were identified. The most prominent of these clones encoded the non-receptor tyrosine kinase Pyk2. We found that Pyk2 can function as an effector in regulating cell adhesion downstream of Wrch1. We also found that the activity of Src was needed for the formation of filopodia downstream of Wrch1. We propose a model in which Pyk2 and Src function to coordinate the Wrch1-dependent effects on cell adhesion and cytoskeletal dynamics.

A5

THE REELIN PATHWAY HAS ROLES IN NEURONAL MIGRATION AND AXON EXTENSION DURING ZEBRAFISH DEVELOPMENT

DANILA BALDESSARI[§], Arianna Costagli*, Stephen W. Wilson* and Marina Mione[§]

*§IFOM, FIRC Institute of Molecular Oncology, 20139 Milan, Italy; *Dept. Anatomy and Developmental Biology, University College London, London WC1E 6BT, UK*

The Reelin pathway plays a crucial role in neuronal migration during nervous system development. Throughout the forming central nervous system (CNS), newly born neurons migrate under the influence of chemo-attractive and chemo-repellent guidance cues to achieve correct position in the maturing CNS. An altered Reelin signalling pathway disrupts the layered cellular architecture of the cortex and causes diseases in humans (i.e. lissencephaly and cerebellar hypoplasia) and malformations in mouse (i.e. reeler, scrambler).

To shed light on Reelin pathway function in cell migration in the developing zebrafish nervous system, we analysed embryos with compromised Reelin activity following morpholino injection. We used a tg(islet1:GFP) transgenic line to analyse effects upon migration of the VII nerve nucleus and extension of the facial nerve axons. Abrogation of Reelin function impairs migration of the neuronal somata and disrupts axon formation and extension. Upon binding of the secreted glycoprotein Reelin to two lipoprotein receptors (VLDLR and ApoER2), the signal is conveyed through the cytoplasmic adaptor molecule Disabled 1 (Dab1). In zebrafish there are two Dab1 alternatively spliced isoforms, differing in the number of tyrosine residues phosphorylable upon Reelin-receptor binding. We focused on the distinct intracellular mechanisms of action of Dab1 isoforms in neuronal migration and axon extension.

A6

EPS8 ASSOCIATES WITH P120 CATENIN TO REGULATE CADHERIN-DEPENDENT CELL ADHESION AND MOTILITY

COSTANZA BALLARÒ [1], Andrea Disanza [2], Anna Maria Salvatore [3], Giorgio Scita [2] and Stefano Alemà [1]

(1) Istituto di Biologia Cellulare, CNR, Monterotondo; (2) IFOM-IEO Campus, Milan; (3) Istituto di Neurobiologia e Medicina Molecolare, CNR, Rome, Italy.

The regulation of cadherin-mediated adhesion by growth factors resulting in plasticity of cell-cell contacts and cell migration has been extensively investigated, but whether and how cadherin function is directly affected, as well as the role of the underlying actin cytoskeleton, remain less well understood issues. Eps8, a substrate of receptor tyrosine kinases, in a complex with Abi1 and Sos-1 induces Rac activation leading to actin cytoskeleton remodelling. Recent studies have shown that Eps8 regulates the growth of actin filaments by capping their barbed ends, an activity essential for cell motility. Here, we report that Eps8 localizes to cell-cell junctions, potentially through its direct association with the N-terminus of p120 catenin. Through the use of RNAi and the reconstitution with mutants selectively defective for Eps8 capping activity, the latter is shown to be required for normal cadherin-dependent adhesion, epithelial cell migration and HGF-driven cell scattering. This study provides novel functional information on Eps8 as a cell-cell adhesion regulator and increases our understanding of the roles of actin capping proteins in cell adhesion.

A7

A CROSS-TALK BETWEEN SRC FAMILY KINASES AND ABL REGULATES PHAGOCYTIC CELL MIGRATION

ANNA BARUZZI*, Giovanni Martinelli§ and (Giorgio Berton)*

**Department of Pathology, University of Verona; §Insitute of Hematology and Medical Oncology "Seragnoli", University of Bologna, Italy*

Src-family kinases (Fgr and Hck) regulate phagocytic cell migration. In COS cells expressing full length Fgr and Fgr with deletions of specific domains (N-terminus, SH1), induction of cell migration correlates with phosphorylation of a few substrates (FAK, cortactin, p190RhoGAP, and Vav2), formation of protein complexes between FAK, Fgr and p190RhoGAP and activation of Rac1 (Continolo et al. Exp Cell Res 302:253, 2005). SH2 or SH3 Fgr mutants failed to induce COS cell migration and this correlated with a decreased Rac1 activation. However, both mutants triggered tyrosine phosphorylation of several proteins, including Vav2, and induced formation of protein complexes between FAK, Fgr and p190RhoGAP. SH2 or SH3 Fgr mutants were unable to trigger phosphorylation of the Rac GEF Sos-1. Because tyrosine phosphorylation of Sos-1 by the tyrosine kinase Abl regulates Rac activation (Sini et al. Nat Cell Biol 3:268-74, 2004), we addressed whether a Src/Abl cross talk regulates phagocytic cell migration. In macrophages with the deficiency of Fgr and Hck tyrosine phosphorylation of Sos-1 and Abl and association of Sos-1/Abl was markedly reduced. Abl and Abl/Src inhibitors blocked macrophage and neutrophil migration. These findings identify a cross-talk between Src and Abl in regulating cell migration in phagocytic cells.

A8

ENA/VASP- AND MDIA2-INDUCED FILOPODIA ARE MECHANISTICALLY AND STRUCTURALLY DIFFERENT

MELANIE BARZIK and Frank B. Gertler

Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA, 02139, USA

Filopodia are involved in many cellular processes including cell migration, neuronal growth cone pathfinding and metastasis. Ena/VASP proteins play an important role in the formation of filopodia by protecting actin filament barbed ends from capping proteins and promoting filament bundling. Certain formin proteins nucleate and elongate unbranched actin filaments processively, thereby also initiating filopodia. We investigated the roles of VASP and the diaphanous-related formin mDia2 during filopodia formation in a cell line lacking these proteins (MVD7 cells). Both VASP and a constitutively active form of mDia2 (mDia2(M1041A)) stimulate filopodia formation independently of each other, a result that differs from previous findings in Dictyostelium. The rate of filopodia induction in MVD7 is significantly higher for mDia2(M1041A) than for VASP. When co-expressed, both proteins localize to the filopodial tips. Interestingly, mDia2(M1041A)- but not VASP-induced filopodia contain dynamic microtubules extending into the tip complex. Our study provides the first description of an engagement of dynamic microtubules with bundled actin filaments in filopodia of fibroblasts, and we suggest that this provides stability, persistency, and guidance for mDia2(M1041A)-stimulated filopodia. These results show that different types of molecules can support the formation of filopodia. Importantly, these filopodia have distinct molecular, morphological and mechanical characteristics.

A9

CELL MORPHOLOGY AND MOTILITY IN THREE-DIMENSIONAL CONTEXT: ROLES OF P27KIP1 AND STATHMIN

BARBARA BELLETTI[1], Stefania Berton[1], Katarina Wolf[2], Francesca Lovat[1], Ilenia Pellizzari[1], Sara D'Andrea[1], Alfonso Colombatti[1], Peter Friedl[2] and Gustavo Baldassarre[1]

1 Division of Experimental Oncology 2 CRO-IRCCS, Aviano; 2 Rudolf-Virchow Centre, DFG Centre for Experimental Biomedicine and Department of Dermatology, University of Wuerzburg, Wuerzburg, Germany

It is now increasingly recognized that both normal and transformed cells behave differently when included in a three-dimensional (3D) matrix, respect to their standard bi-dimensional (2D) growth in culture. We have recently demonstrated that p27kip1 inhibits the motility of normal and transformed fibroblasts through extracellular matrix (ECM) components due to its interaction with the microtubules (MT) destabilizing protein Stathmin. Here, using as a model system the Mouse Embryonic Fibroblasts (MEF) derived from mice WT, p27kip1 knock-out (KO) and Double KO (DKO) for both p27kip1 and Stathmin genes, we focus our work on cellular behavior in 3D-matrices. Several lines of evidence demonstrate that p27kip1 absence in mouse fibroblasts results in a more roundish morphology coupled with an amoeboid-like type of motility and an increased cell speed, when cell were included 3D-collagen gels. Cell shape and motility in 3D were specifically linked to p27kip1 activity on Stathmin, since they could both be rescued by changing stathmin levels in p27wt and ko cells. Moreover, p27kip1wt fibroblasts stably transfected with stathmin acquired a roundish shape coupled with increased migratory speed in 3D, thus resembling p27kip1ko cells. Conversely, MEFs double knockout for p27kip1 and stathmin reacquired the mesenchymal phenotype observed in p27wt MEFs.These alterations in cell morphology and motility were linked to a different stability in the MT network in cells expressing different amount of p27 and stathmin. Moreover, since ECM-dependent MTs stability is regulated also by RhoA via mDia, and MTs-dynamics regulates RhoA activity, we investigated the relationship between p27kip1, Stathmin and RhoA looking at RhoA activity in these cells. Our preliminary results show that Stathmin hyperactivity in p27kip1 null cells results in increased RhoA activity and this could explain their roundish morphology and the amoeboid motility we observed in these cells. Altogether these data demonstrate the existence of a complex interplay between p27kip1 Stathmin and RhoA, and point to this molecular interaction as an important determinant in the control of cellular shape and motility in 3D context.

A10

INSIGHTS INTO THE REGULATION OF APICAL ECTOPLASMIC SPECIALIZATION FROM THE GENERATION OF AN ANIMAL MODEL

GIOVANNA BERRUTI,* Evanthia Aivatiadou, * and Elisabetta Mattei, **

**Dipartimento di BIOLOGIA, Laboratorio di Biologia Cellulare e Molecolare della Riproduzione, Università di Milano, Italy, **Istituto di Neurobiologia e Medicina Molecolare, CNR, Roma, Italy*

The GTPase Rap1 serves as a critical point in various cellular processes including signal transduction, cell proliferation and differentiation; recently it is attracting much attention for its role in cell-cell adhesion. Ectoplasmic specialization (ES) is a testis-specific type of cell-cell adherens junction; specifically, the ES found between Sertoli cells is known as basal ES, while that found between Sertoli cells and spermatids is known as apical ES. During spermatogenesis, ES undergoes extensive restructuring to permit germ cells to move across the seminiferous epithelium and fully developed spermatids to be released at spermiation. We generated transgenic mice with a dominant negative mutant of Rap1 under the control of the spermatid-specific protamine I promoter so to achieve both tissue and temporal restriction in the transgene expression. Using this approach, we found that interfering with Rap1 in haploid cells results in an anomalous release of immature spermatids within the lumen of seminiferous tubuli and in low sperm counts; the loss of not differentiated cells correlated with impaired spermatid-Sertoli cell adhesion. Moreover, we found that germ cells express VE-cadherin with a timing coincident with the formation and function of apical ES. Our iRap1 mouse provides an in vivo model to study testis ES dynamic.

A11

ENDOGENOUS EXPRESSION OF ARG ISOFORMS THAT DIFFER AT N- AND C-TERMINI, SUBCELLULAR LOCALIZATION AND CYTOSKELETON INTERACTIONS OF THE TRANSFECTED ISOFORMS

*BIANCHI CRISTINA, *Torsello Barbara, *Angeloni Valentina, *Brambilla Paola, *Invernizzi Lara, *Bombelli Silvia, *Corizzato Matteo, ^Sarto Cecilia, *Perego Roberto A.

**Dept Experimental Medicine, Milano - Bicocca University, via Cadore 48, 20052 Monza; ^Dept. Laboratory Medicine, Desio Hospital - Italy.*

Arg (or Abl2) proteins belong to the Abelson family of non receptor tyrosine kinases. In the C-terminal domain Arg has two actin- and one microtubule binding sequences and through cytoskeletal interactions is required for neurulation, adhesion-dependent neurite branching and fibroblastic/epithelial cell adhesion and migration. We have previously shown that: a) the exons 1A, 1B and 2 are alternatively spliced, producing 1AL, 1AS, 1BL and 1BS N-termini; b) the alternative loss of part of the first C-terminal F-actin binding domain produces CTL and CTS C-termini. Combining the different splicing events, eight putative Arg isoforms may be predicted. All eight transcripts are expressed and different protein spots are detected in Caki-1 cell line by 2-DE western blot. The Arg isoform cellular localization and interaction with cytoskeletric proteins has been analyzed. The cDNA isoforms cloned in pFLAG-CMV2 and transfected in COS-7 and SH-SY-5Y cells evidenced, by immunofluorescence, a cytosolic distribution of all isoforms with a granular signal only for some. In transfected COS-7 cells the Arg isoforms co-localize with F-actin in a sub-membranous cell region. Confocal microscopy analysis and co-immunoprecipitation assays are ongoing to elucidate these results. Putative different interactions of Arg isoforms with microtubules will be also studied.

A12

A POSSIBLE MECHANISM OF ANTIMOTILITY ACTIVITY BY TAXANES: MODULATION OF TUBULIN ACETYLATION

BONEZZI KATIUSCIA[1], North Brian [3], Manzotti Carla [2], Riva Antonella [2], Verdin Eric [3], Giavazzi Raffaella [1], Taraboletti Giulia[1]

1Department of Oncology, Mario Negri Institute for Pharmacological Research, Bergamo, Italy; 2INDENA Spa, Milan; Italy; 3University of California, San Francisco CA

The microtubule stabilizing taxanes show potent anti-motility activity. We previously reported that the anti-motility ability of taxanes is unrelated to their cytotoxicity. The aim of this study was to investigate the induction of tubulin acetylation as a possible mechanism of the anti-motility activity of taxanes. Paclitaxel, at concentrations that affect cell motility but not proliferation (10-8-10-9 M, for 4h), induced tubulin acetylation in endothelial cells (HUVEC). IDN 5390, a seco-derivative that has the same antimotility potency of paclitaxel but lower cytotoxicity (Clin Cancer Res 8: 1182, 2002), was as active as paclitaxel in inducing tubulin acetylation. In addition both taxanes inhibited cell motility and induced tubulin acetylation at similar concentrations in 1A9 ovarian carcinoma cells and in its paclitaxel-resistant variant 1A9/PTX22. Inhibitors of histone deacetylases reproduced the effects of taxanes on motility and tubulin acetylation. Moreover ongoing experiments using siRNA confirmed the involment of tubulin acetylation in cell motility. Conversely tubulin deacetylation, by overexpressing histone deacetylases, increased cell motility and decreased sensitivity to the antimotility effects of taxanes. These results show that paclitaxel and IDN 5390 promote tubulin acetylation suggesting this as a possible mechanism of the antimotility, but not antiproliferative, activity of taxanes.

A13

INVADOPODIA AS SPECIALIZED PLASMA MEMBRANE DOMAINS

GIUSI CALDIERI, Giada Giacchetti, Massimiliano Baldassarre and Roberto Buccione

Tumour Cell Invasion Laboratory, Department of Cell Biology and Oncology, Consorzio Mario Negri Sud, S. Maria Imbaro (Chieti), Italy.

Invasive tumoral cells in vitro extend proteolytically active protrusions, termed invadopodia, into the surrounding extracellular matrix (ECM). They are enriched in integrins, signaling machinery, proteases and actin-associated proteins. We found that PtdIns4,5P2, typically formed in cholesterol-rich raft-like plasma membrane (PM) domains, is enriched at invadopodia. Also, Brefeldin A and the microtubule disruptor Nocodazole, that inhibit anterograde and polarized protein trafficking respectively, blocked ECM degradation at invadopodia. This supports our hypothesis that invadopodia might be specialized PM domains where polarized transport occurs. To test if invadopodia are raft-like domains, we depleted cholesterol from the PM. We observed invadopodia disassembly and block of ECM degradation. The same was obtained upon transfection with a caveolin mutant known to block cholesterol delivery to the PM. Next, we analyzed the floatation profile of some typical invadopodia components by equilibrium ultracentrifugation from cells with or without active invadopodia. We observed a shift of these proteins towards lighter fractions (i.e. lipid raft fractions) in actively degrading cells. We suggest that invadopodia are specialized lipid raft-like PM domains acting as a platform for signaling and specialized transport. Studies are in progress to analyze polarized trafficking at invadopodia and the role of caveolin. Supported by AIRC.

A14

THE ROLE OF EPHA2 TYROSINE PHOSPHORYLATION IN TUMOUR CELL MOTILITY AND INVASION

Taddei Maria Letizia*, Parri Matteo*, Francesca Bianchini**, Ramponi Giampietro*, Lido Calorini** and CHIARUGI PAOLA*

**Department of Biochemical Sciences, University of Florence, Italy; **Department of Experimental Pathology and Oncology, University of Florence, Italy.*

Several studies have correlated the upregulation of Eph tyrosine kinases and ephrin ligands in several tumours with their ability to regulate cell migration, likely contributing to tumour progression and metastasis. The EphA2 receptor is highly expressed in the more aggressive stages of breast and prostate cancers, and in advanced melanomas. Our interest is focused on the role of EphA2 tyrosine phosphorylation in the motility and invasion of prostatic carcinoma and melanoma cells and we generated several EphA2 mutants within the juxtamembrane, the kinase or the C-ter domains, together with the kinase dead and a dominant negative mutant, deleted of the entire cytoplasmic region. We first show that in prostatic carcinoma cells overexpression of active EphA2 causes a ligand-independent EphA2 phosphorylation leading to inhibition of Rho-mediated cell migration and invasion. Conversely inhibition of EphA2 signaling by overexpression of kinase deficient mutants results in the reversion of these effects, confirming a key role of tyrosine phosphorylation of EphA2 in prostatic carcinoma cell motility and in the organization of actin cytoskeleton. Ongoing studies indicate that in melanoma cells the overexpression of active EphA2, but not of its kinase deficient mutants, significantly increases cell motility and invasion, thus suggesting an histotype-related effect.

A15

PROLIFERATION VS MIGRATION IN PDGF SIGNALING: THE KEY ROLE OF ENDOCYTOSIS

CIRRI P, Buricchi F, De Donatis A, Camici G, Vinci M.C, Ramponi G.

It is a common knowledge that Platelet-Derived Growth Factor (PDGF) is a critical regulator of mesenchymal cell migration and proliferation. Nevertheless, these two phenotypic response are mutually exclusive. In order to solve this apparent contradiction we have studied the behaviour of NIH3T3 fibroblasts in response to increasing concentrations of PDGF-BB. We have found that there is a strong cell proliferation induction only with PDGF-BB concentration above 5 ng/ml while cell migration response arise starting from 1 ng/ml but is negligible at higher PDGF-BB concentration. Our data indicate that cells display a differential activation of the main signaling pathways in response to stimulation with increasing concentration of PDGF-BB. At low PDGF-BB concentrations there is the maximal activation of signaling pathways that are linked to cytoskeleton rearrangement (Rac1 and FAK) whereas at high PDGF-BB concentrations there is the activation of pathways more strictly linked to mitogenesis induction. Our results suggest a possible mechanism by which cells switch from a migrating to a proliferating phenotype, sensing the increasing gradient of PDGF-BB. In addition we propose that cell decision to proliferate or to migrate relays on different endocytotic routes of PDGF-R in response to various PDGF-BB concentrations.

A16

THE ROLE OF TNFR2 IN THE EFFICIENT REMYELINATION OF THE CENTRAL NERVOUS SYSTEM

MARIA C. DENIS, Eumorphia Remboutsika, George Kollias

B.S.R.C. "Al. Fleming", Institute of Immunology, Institute of molecular biology, Vari, Greece

Experimental Autoimmune Encephalomyelitis (EAE), the animal model of multiple sclerosis, develops through the immunologic attack of the myelin sheath (demyelination) of the central nervous system (CNS). The adult nervous system maintains the ability to self-repair (remyelination) by recruiting neuronal stem cells and precursors (NPCs). Tumor Necrosis Factor (TNF) is a multipotent cytokine with a vital role in CNS pathogenesis. It signals through TNFR1, which mediates apoptosis and tissue damage; and TNFR2 implicated in immunoregulation and reparative processes. Our goal is to elucidate the specific role of TNFR2 in remyelination. TNFR2-/- animals exhibit exacerbated EAE clinical profile with sustained motor deficits. Histological examination of inflamed CNS revealed in wild type animals characteristic foci of astrocytes encircling inflammatory and NPCs. TNFR2-/- animals have reduced numbers of such foci while exhibiting dispersed inflammatory infiltrates. We hypothesized that lack of organized cellular microarchitecture may be responsible for the sustained disease observed in the absence of TNFR2. We have examined the cellular constituents of the foci and have observed that TNFR2 promotes the efficient migration and proliferation of astrocytes. We are currently dissecting genetically and molecularly the role TNFR2 in the migration of these cells and consequently their role in the recovery of the disease.

A17

REGULATION OF CELL SHAPE BY CDC42 IS MEDIATED BY THE SYNERGIC ACTIN-BUNDLING ACTIVITY OF THE EPS8-IRSP53 COMPLEX.

ANDREA DISANZA[1, 2], Sara Mantoani[1, 2], Maud Hertzog[1,2], Silke Gerboth[1, 2], Emanuela Frittoli[1, 2], Anika Steffen[1,2, 3], Kerstin Berhoerster[4], Hans-Juergen Kreienkamp[4], Francesca Milanesi[1, 2], Pier Paolo Di Fiore[1, 2,5], Andrea Ciliberto[1, 2], Theresia E. B. Stradal[3] & Giorgio Scita[1, 2,5]

1Istituto FIRC di Oncologia Molecolare (IFOM), Via Adamello 16, 20139, Milan, Italy; 2 Department of Experimental Oncology, Istituto Europeo di Oncologia (IEO), Via Ripamonti 435, 20141, Milan, Italy; 3 Signalling and Motility Group, German Research Centre for Biotechnology (GBF), Mascheroder Weg 1, D-38124 Braunschweig, Germany; 4 Institute for Human Genetics, University Hospital Eppendorf, Hamburg Martinistrasse 52, D-20246 Hamburg, Germany; 5 Dipartimento di Medicina, Chirurgia ed Odonoiatria, Università degli Studi di Milano, 20122 Milan, Italy.

Actin-crosslinking proteins organize actin into highly dynamic and architecturally diverse subcellular scaffolds that orchestrate a variety of mechanical processes, including lamellipodial and filopodial protrusions in motile cells. How signalling pathways control and coordinate the activity of these crosslinkers is poorly defined. IRSp53, a multi-domain protein that can associate with the Rho-GTPases Rac and Cdc42, participates in these processes mainly through its amino-terminal IMD (IRSp53 and MIM domain). The isolated IMD has actin-bundling activity in vitro and is sufficient to induce filopodia in vivo. However, the manner of regulation of this activity in the full-length protein remains largely unknown. Eps8 is involved in actin dynamics through its actin barbed-ends capping activity and its ability to modulate Rac activity. Moreover, Eps8 binds to IRSp53. Here, we describe a novel actin crosslinking activity of Eps8. Additionally, Eps8 activates and synergizes with IRSp53 in mediating actin bundling in vitro, enhancing IRSp53-dependent membrane extensions in vivo. Cdc42 binds to and controls the cellular distribution of the IRSp53-Eps8 complex, supporting the existence of a Cdc42-IRSp53-Eps8 signalling pathway. Consistently, Cdc42-induced filopodia are inhibited following individual removal of either IRSp53 or Eps8. Collectively, these results support a model whereby the synergic bundling activity of the IRSp53-Eps8 complex, regulated by Cdc42, contributes to the generation of actin bundles, thus promoting filopodial protrusions.

A18

DIACYLGLYCEROL KINASE ALPHA PHOSPHORILATION BY SRC ON Y335 IS REQUIRED FOR ACTIVATION, MEMBRANE RECRUITMENT AND HGF INDUCED CELL MOTILITY

Baldanzi G.[1], RAINERO E.[1], Chianale F.[1], Cutrupi S.[2], Gnocchi VF.[1], Porporato P.[1], Traini S.[1], Brignoli G.[1], Gaggianesi M.[1], Pighini A.[1], Parolini O.[3], Graziani A.[1].

1 Department of Medical Sciences, University Amedeo Avogadro of Piemonte Orientale, Novara 28100, Italy; 2 University of Torino, c/o Bioindustry Park of Canavese, Colleretto Giacosa (TO), Italy; 3 Centro Di Ricerca E.Menni, Fondazione Poliambulanza-Istituto Ospedaliero, Brescia 25124 Italy

Diacylglycerol kinases (Dgk), which phosphorylate diacylglycerol to generate phosphatidic acid, act as either positive or negative key regulators of cell signaling. We have previously shown that Src mediates growth factors-induced activation of Dgkα, whose activity is required for cell motility, proliferation and angiogenesis. Inhere we demonstrate that both Hepatocytes Growth Factor (HGF) stimulation and v-Src transformation induce tyrosine phosphorylation of Dgkα on Y335, through a mechanism requiring the proline-rich C-terminal sequence. Moreover we show that both proline-rich sequence and phosphorylation of Y335 of Dgkα mediate i) its enzymatic activation, ii) its ability to interact respectively with SH3 and SH2 domains of Src, iii) its recruitment to the plasma membrane. In addition we show that phosphorylation of Dgkα on Y335 is required for HGF-induced motility, while its constitutive recruitment at the membrane by myristylation is sufficient to trigger spontaneous motility in absence of HGF. Providing the first evidence that tyrosine phosphorylation of Dgkα is required for growth-factors-induced activation and membrane recruitment, these findings underscore its relevance as a rheostat, whose activation is a threshold to elicit growth factors-induced migratory signaling.

A19

IDENTIFICATION OF GENES CONTROLLED BY THE FOXE1 TRANSCRIPTION FACTOR

ESPOSITO RAMONA[1], Esposito Rosaria[2], De Luca Pasquale[3], Sepe Leandra[4], Paolella Giovanni[5], Garbi Corrado[6] and Di Lauro Roberto[7]

1DBPCM, University "Federico II", Naples, Italy. 2: DBPCM, University "Federico II", Naples, Italy 3: Biogem s.c.ar.l., Ariano Irpino (AV), Italy. 4:DBBM, University "Federico II", Naples, Italy. 5: DBBM, University "Federico II", Naples, Italy. 6: DBPCM, University "Federico II", Naples, Italy. 7: DBPCM, University "Federico II", Naples, Italy and Biogem s.c.ar.l., Ariano Irpino (AV), Italy

Foxe1 is a transcription factor that plays an important role in the migration of the thyroid bud. Both in mice and humans, mutations in the foxe1 gene result in aberrant development. To identify genes controlled by foxe1, undifferentiated rat thyroid epithelial cell line FRT, which does not express any endogenous foxe1 mRNA, have been stably transfected with a foxe1 expression vector. The positive clone for high expression of foxe1 protein, FRT-Foxe1.1, has been selected for further studies. Confirming the involvement of foxe1 in cell migration, FRT-Foxe1.1 clone shows:
? increased number of lamellipodia in comparison with wt cells;
? increased capacity of cell migration detected by Boyden Chamber Assay and Time Lapse Microscopy.
To identify genes controlled by foxe1, we compared the transcriptome of FRT-Foxe1.1 with that of FRT wt cells, using Affymetrix® Gene Chips. We found 45 differentially expressed genes with a fold change higher that 3. They were analyzed using Gene Ontology and compared to the FRT wt gene expression profile, demonstrating a great enrichment in genes involved in the locomotory behaviour. In order to establish the role of foxe1 downstream target genes in the migratory process we are planning iRNA experiments in FRT-Foxe1.1.

A20

ANTIGEN-DEPENDENT REGULATION OF DENDRITIC CELL MIGRATION

FAURE-ANDRE GABRIELLE[1], Vargas Pablo[1], Piel Matthieu[2], Diaz Jheimmy[1], Hugues Stéphanie[1], Lennon-Dumenil Ana-Maria[1]

1 U653 INSERM, Institut Curie, Paris, France; 2 UMR144, Institut Curie, Paris, France

Dendritic cells (DC) capture antigens in peripheral tissues and process them in peptides that are presented to T cells. Concomitantly, DC migrate to lymph nodes to encounter T cells. We have shown that one of the major components of the antigen-processing pathway, the Invariant Chain (Ii), associates to the actin-based motor protein Myosin II, thereby regulating antigen presentation. As Myosin II is a key regulator of cell motility, we investigated whether this association also controls DC migration. We found that the migratory capacity of murine DCs relies on Myosin II activity as well as on the expression of Ii, both in vivo and in vitro. DC migration is strongly increased in cells lacking Ii whereas it is reduced when DC accumulate Ii. Three-dimensional time-lapse analysis in microchannels showed that Ii expression affects their intrinsic migratory capacity in correlation with an abnormal formation of podosomes, a process known to depend on Myosin II. We propose that Myosin II directs vesicle transport either toward the antigen-processing pathway, or toward the migratory apparatus to form podosomes. Therefore, Myosin II and Ii allow DC to regulate in a coordinated fashion two essential events required for T cell activation: antigen processing and cell migration.

A21

DIAL 911 FOR TELOMERE ELONGATION

SOFIA FRANCIA[1], Robert S. Weiss[2], M. Prakash Hande[3], Raimundo Freire[4], Fabrizio d'Adda di Fagagna[1]

1 IFOM Via Adamello, 16 ; 20139 Milano; 2 Department of Biomedical Sciences, Cornell University; 3, National University of Singapore, Singapore; 4 Hospital Universitario de Canarias, Tenerife, Spain.

Telomeres cap the ends of chromosomes and are maintained via a specialized mechanism involving the telomerase reverse transcriptase enzyme. Here we report that the mammalian Rad9/Rad1/Hus1 cell cycle checkpoint complex (911) is a novel constituent of the mammalian telomere and is required for telomere homeostasis. Significantly, Hus1-deficient primary mouse embryonic fibroblasts, as well as thymocytes from conditional Hus1 knockout mice, demonstrated severe telomeric shortening, as well as chromosomal fusions. 911 was found in association with telomerase enzyme activity and it was required for efficient telomerase activity in vitro. Taken together, these findings identify an unanticipated function for 911 at telomeres in mammals and provide a mechanistic link between the activity of DNA-damage checkpoint proteins and the telomere maintenance machinery.

A22

ASSEMBLY OF A FUNCTIONAL WAVE COMPLEX REQUIRES THE DISSOCIATION OF PRECURSOR TRIMERIC BRK1

Emmanuel Derivery[1], Jenny Fink[2], Davy Martin[3], Anne Houdusse[3], Daniel Louvard[1], Matthieu Piel[2], Theresia E. Stradal[4], and ALEXIS GAUTREAU[1].

[1]"Morphogenesis and cell signaling", [2]"Biology of Cell Cycle and Cell Motility", [3]"Structural Motility" laboratories, UMR144 CNRS/Institut Curie, 26 rue d'Ulm, 75248 Paris Cedex 05, France; [4]HZI-Helmholz Center for Infection Research, Mascheroder Weg 1, D-38124 Braunschweig, Germany.

The Wave Complex activates the Arp2/3 complex at the leading edge of migrating cells. It is a multi-protein complex, composed of five subunits. The small subunit Brick1 (Brk1, 75 amino-acids, also known as Hspc300) is the most conserved subunit. RNAi mediated knock-down of Brk1 destabilizes the Wave Complex, and abolishes lamellipodia and ruffle formation. Moreover, we found that Brk1 exists in two pools with two structural conformations : an homotrimer when free or a single molecule within the Wave Complex. Importantly, this novel homotrimeric complex of Brk1 has been evolutionary conserved in plants and animals. An assay in which tagged trimers were purified and reintroduced into human cells showed that exogenous trimeric Brk1 is dissociated into Wave Complex subunits. Since converted Brk1 associates specifically with neo-synthesized subunits and that trimeric Brk1 is sufficient to reconstitute the Wave Complex in RNAi treated cells, trimeric Brk1 represents a precursor pool required to assemble the Wave Complex. Furthermore, a mutant impaired in trimer dissociation is unable to assemble into a Wave Complex. This study is the first one on the in vivo assembly of the Wave Complex. It suggests that Brk1 is a keystone in this assembly process.

A23

INVESTIGATION OF MOLECULAR MECHANISMS CONTROLLING SOS1 RAC GEF ACTIVITY

SILKE GERBOTH and Giorgio Scita

IFOM, The FIRC Institute for Molecular Oncology, 20139 Milano, Italy

Sos1 is a dual nucleotide exchange factor towards Ras and Rac. Sos1 DHPH domain displays GEF activity towards Rac, but is autoinhibited under resting conditions. The engagement of Sos1 into a complex with Eps8 and Abi1 or Tyrosine phosphorylation of Sos1 by Abl can activate its Rac GEF activity. To investigate how Tyrosine phosphorylation regulates Sos1 Rac GEF activity, we mapped the phosphorylated Tyrosine. A c-terminal Tyrosine is the major phosphorylation site of Abl kinase. Mutation of this Tyrosine to Phenylalanin prevents activation of Sos1 Rac GEF activity by Abl, but not its Ras GEF activity. Interestingly, we found that the region encompassing Sos1 pY undergoes intramolecular interactions with the DHPH domain. The affinity of this interaction is altered upon Tyrosine phosphorylation. Thus, we propose that Tyrosine phosphorylation leads to intramolecular changes, which may account for the opening of the DHPH domain and consequently activation of Rac GEF. Finally, we observed that tyrosine phosphorylation promoted by entry of Sos1 within an Sos1/Eps8/Abi1/Abl complex further increases its Rac GEF activity, providing evidence that multiple signaling mechanisms converge in fine tuning Sos-1 functions. The significance of Sos1 tyrosine phosphorylation in response to physiological and transforming stimuli will be addressed.

A24

TOCA-1 HOMOLOGS IN CAENORHABDITIS ELEGANS

GIULIANI, C., Troglio, F., Malabarba, M.G., Cassata, G. and Scita G.

IFOM, FIRC Institute of Molecular Oncology Foundation, Milan, Italy.

Toca-1 (Transducer of Cdc42-dependent actin assembly) is a recently identified member of F-BAR (Bin-Amphiphysin-Rvs)-protein family. F-BAR domain-containing proteins are a class of cytosolic molecules with membrane-bending properties, which participate in the regulation of actin dynamics and membrane trafficking. This is achieved at least in part by association of Toca-1, via its SH3 domain, with the actin nucleator promoting factor N-WASP, and the endocytic protein Dynamin. In addition to Toca-1, two other highly conserved proteins, FNBP1 and Cip-4 exist in mammals, forming a distinct subgroup within this protein family. In C. elegans two homologs of mammalian F-BAR proteins exist: TOCA-1 and TOCA-2, whose functional roles have never been defined. Conversely, the known mammalian interactors of Toca-1 have been characterized in the nematode: wsp-1/N-WASP mutant and its related family member wve-1/WAVE knock-down show reduced brood size and embryonic lethality. We generated mutant alleles for both toca-1 and toca-2 and analyzed their phenotypes. toca-1(tm2056);toca-2(ng11) double mutant shows reduced brood size and embryonic lethality, albeit at a low penetrance. These phenotypes are somewhat similar to the ones obtained by RNA-interference with wsp-1 and wve-1. However, genetic analysis demonstrated that toca-1 and toca-2 interact with wve-1, but not with wsp-1, suggesting that toca-1, toca-2 and wve-1 function together. Detailed phenotypic, biochemical and immunolocalization analyses are currently ongoing to validate and extend this latter interaction and gain clues as to the function of toca-1 and toca-2 in the nematode.

A25

DIACYLGLYCEROL KINASE-ALPHA MEDIATES HGF-INDUCED CELL SCATTERING, BY REGULATNG RAC MEMBRANE TARGETING AND ACTIVATION.

Chianale F, Reinero E., Baldanzi GL., Porporato P. Traini S, Cutrupi S, GRAZIANI A.

Dip. Scienze Mediche, Università del Piemonte Orientale Amedeo Avogadro, Novara, Italia

Diacylglycerol kinase (Dgk) enzymes phosphorylate diacylglycerol (DG) to phosphatidic acid (PA), regulating reciprocally DG-mediated and PA-mediated signalling pathways. We previously showed that HGF and VEGF activate Dgk-alpha in endothelial cells through a Src-mediated mechanism, that activation of Dgk-alpha is required for chemotactic and angiogenic signaling in vitro (Cutrupi et al. EMBO J 2000; Baldanzi et al. Oncogene 2004), and that Dgk-alpha activation by Alk receptor tyrosine kinase and npm-Alk oncogene is required for their proliferative activity (Bachiocchi et al. Blood, 2005). However neither the mechanism of Src-mediated activation of Dgk-alpha has been elucidated, neither its role in growth factor-induced signalling. We showed that HGF and v-Src activate Dgk-alpha by phosphorylating it on Tyr335, providing a docking site for SrcSH2 interaction, which in epithelial cells is responsible for HGF-induced recruitment of Dgk-alpha to the membrane. By inhibition of Dgk-alpha either pharmacologically or by expression of a dominant negative mutant or siRNA-mediated downregulation, we show that Dgk-alpha is required for HGF- and v-Src-induced cells scattering and motility, without affecting HGF-induced downregulation of cadherin intercellular adhesions. Inhibition of Dgk-alpha severely impairs membrane ruffle formation upon 15 minutes of HGF stimulation, by inhibiting activation of Rac and its targeting at new adhesion sites.

A26

STRUCTURAL CHARACTERIZATION OF EPS8 PROTEINS

M. HERTZOG[1], F. Milanesi, A[1]. Disanza, S. Pasqualato[1], Guittet E[2], Van-Heijenoort C[2], Hanein D, G. Scita[1];

1: IFOM Foundation, Milan, Italy; 2: ICSN, Gif sur Yvette, France; Burnham Institute, La Jolla, USA.

Eps8, is the founding member of a recently identified family of actin binding proteins involved in signaling to actin remodelling. Eps8 posses two distinct activities on actin dynamics: firstly, it acts as a cross-linking protein that organizes actin into highly dynamic and architecturally diverse subcellular scaffolds, thus orchestrating a variety of fundamental mechanical functions in motile cells. Additionally, Eps8 acts as a barbed ends capping proteins, blocking actin filaments elongation and thus regulating actin-based motility. The holo Eps8 full-length protein is autoinhibited as a capper, whereas it displays constitutively bundling activity. The structural and molecular mechanisms underlining these activities are unknown. Here, we show that the isolated C-terminal capping region of Eps8 (residues 648-821) contains a putative SAM-PNT domain, whose structure has been solved and shown to be composed of 5 amphipatic helices (H1 to H5). NMR analysis revealed that a complete change of folding occurs upon actin binding, suggesting that extensive surfaces if interaction. Consistently, Eps8 648-821 forms a high affinity (50nM) and stable complex with monomeric actin. Chemical cross-linking and sedimentation assays revealed the existence of two high affinities binding surface (Kd=50 nM and 2 μM, respectively) corresponding to the H1-H2 pair and H5 helices. Single point mutations disrupting the hydrophobic faces of these amphipatic helices significantly reduced capping activity, suggesting that multiple surfaces of interaction are required for barbed end capping. A model of how Eps8 caps actin filament will be discussed.

Lectures

Elaine Fuchs
New York, USA

Gregg Gundersen
New York, USA

Stefan Linder
München, Germany

Oral Presentations

Yasmin Issa
Heidelberg, Germany

Andrea Palamidessi
Milan, Italy

Session III

STEM CELLS AND MORPHOGENESIS

ELAINE FUCHS

Laboratory of Mammalian Cell Biology and Development, The Rockefeller University, 1230 York Avenue, Box 300, New York, NY, 10021

Stem cells (SCs) can self-renew and to differentiate along multiple lineages to generate different tissues. In the embryo, multipotent SCs respond to various cues to undergo morphogenesis and produce these tissues. For tissues to function, cells must orient themselves and interact with their neighbors. The skin is an excellent model to explore how an undifferentiated SC generate 3 functional tissues: epidermis, sebaceous gland and hair follicles. Embryonic skin begins as a single layer of multipotent SCs, which adhere to each other and to an underlying basement membrane rich in extracellular matrix. Both cell-cell and cell-substratum interactions are essential for the epidermis to form. As development proceeds, these SCs respond to key signals, including Wnts, Bmps, Shh, Notch and Fgfs, to either stratify to produce epidermis or grow downward to generate hair follicles. Basal cells remaining in contact with the basement membrane maintain proliferative potential, while suprabasal cells that are removed from their substratum differentiate. We've begun to dissect how extrinsic signaling to SCs sets off a cascade of changes in transcription that govern cell fate decisions, cell-cell and cell substratum remodeling, stratification and tissue morphogenesis. When this process is defective, cells fail to integrate morphogenetic signals and polarize the cytoskeleton, resulting in tissue dysfunction.

EXPRESSION OF L1-CAM IN HUMAN PANCREATIC CARCINOMA CORRESPONDS TO INCREASED ENDOTHELIAL MIGRATION IN VITRO AND ENHANCED VASCULARIZATION OF TUMOR TISSUE IN SITU

YASMIN ISSA-[1], Daniel Nummer-[2], Hubertus Schmitz-Winnenthal-[3], Dalibor Antolovich-[3], Jürgen Weitz-[3], Markus W. Büchler-[3], Philipp Beckhove-[2] and Peter Altevogt-[1]

1_Department of Cellular Immunology, German Cancer Research Center, Heidelberg, Germany; 2_T Cell Tumor Immunity Group, German Cancer Research Center, Heidelberg, Germany; 3_Department of Visceral Surgery, University Hospital of Heidelberg, Germany

INTRODUCTION

L1-CAM is expressed on different human tumor-entities and is correlated to poor survival-rates. It promotes tumor-growth and mediates adhesion, migration and invasion processes.

We therefore analyzed the endothelial cell (EC) expression of L1-CAM in situ and its role in migration, comparing EC from human pancreatic non-malignant- and carcinoma-tissue, which shows high metastasis-rates and bad prognoses.

METHODS

We used immunohistochemical-stainings for detection and automated quantification of L1-CAM and endothelial CD31. Endothelial L1-CAM, CD24 and Neuropilin-1 (NRP-1) expression was analyzed using Flow-cytometry. Angiogenic- and transmigration-capacity of isolated tumor- and control-EC (HUVEC) was analyzed using matrigel tube-formation-assay and transmigration-assay.

RESULTS

Compared to non-malignant pancreas, carcinoma tissue showed higher vascularization (2,4±0,6% vs. 0,7±0,2% CD31+area/mm2 tissue), enhanced L1-CAM expression, mainly coexpressed on EC (n=10). Isolated tumor-EC, not control-EC, showed increased L1 expression after TNFα stimulation (MFI 435 vs.14).

Angiogenic capacity of tumor-EC was specifically decreased compared to control-EC after mAB inhibition of L1 or respective ligands (CD24 and NRP-1) in tube-formation-assays (L1 71,7%±6% vs. 15%±8%, CD24 90,9%±2,2% vs. 36,5%±13%, NRP-1 46,7%±16,6% vs. 18,8%±5,9%,). In addition, migration rates of tumor-EC could be blocked by mAB (L1 17%±7%, CD24 51%±13%, NRP-1 44,8%±10,6%,) suggesting a role for L1-CAM in tumor selective angiogenic migration of endothelial cells.

REGULATION OF MICROTUBULES AND CELL POLARITY BY RHO GTPASES IN MIGRATING CELLS.

GREGG GUNDERSEN, Edgar Gomes, Jan Schmoranzer, Francesca Bartolini, Christina Eng and Ying Wen.

Department of Pathology & Cell Biology, Columbia University, New York, NY.

In migrating cells, microtubule (MT) arrays are polarized by at least two mechanisms: the selective stabilization of MTs in the leading edge and the reorientation of the centrosome to a position between the leading edge and the nucleus. These two rearrangements are independently regulated by small GTPases: Rho regulates selective stabilization while Cdc42 regulates centrosome reorientation. Our previous studies have identified downstream factors in each of these pathways: the formin, mDia1, GSK3b, nPKC and the MT TIP proteins EB1 and APC function downstream of Rho in the MT stabilization pathway (Palazzo et al., Nat Cell Biol (2001); Science (2004); Wen et al., Nature Cell Biol (2004); Eng et al., MBC (2006)) and Par6, aPKC, MRCK, and the MT TIP proteins dynein and dynactin function downstream of Cdc42 in the centrosome reorientation pathway (Palazzo et al., Curr Biol 2001; Gomes et al., Cell (2005)). In our study of centrosome reorientation we found a novel actin and myosin-dependent movement of the nucleus away from the leading edge that was responsible for centrosome reorientation. We have explored the basis for this actin-dependent movement for the nucleus and have used dominant negative approaches and siRNA knock down to identify nesprin-2G as the outer nuclear membrane protein that attaches the nucleus to the actin cytoskeleton and SUN2 protein as an inner nuclear membrane protein that anchors nesprin-2G. Both nuclear proteins accumulate in membrane "lines" that colocalize with actin filaments on the nucleus, suggesting that the anchoring of actin filaments to the nucleus involves a complex spanning the inner and outer nuclear membranes. In wounded monolayer experiments, cells depleted of nesprin-2G exhibit an unusual phenotype: instead of migrating directional into the wound, they migrate in random directions, including back into the monolayer. These results suggest that correct nuclear positioning is important for cells to respond in a polarized fashion to a migratory stimulus.

RAB5 REGULATES RAC INTERNALIZATION AND TRAFFICKING PROMOTING SPATIAL RESTRICTION OF SIGNALING AND CELL MIGRATION

ANDREA PALAMIDESSI[1,2]; Emanuela Frittoli [1,2]; Massimiliano Garre' [1,2]; Mario Faretta [1,2]; Giorgio Scita [1,2]; Pier Paolo Di Fiore [1,2]

1 IFOM, Ist. Firc Oncologia Molecolare (Milan); 2 Dept. Experimental Oncology, Ist. Europeo Oncologia, IEO (Milan)

Membrane trafficking ensures localized intracellular responses by stimulating spatial restriction of signaling, thus controlling actin dynamics. Rab5 and Rac are essential mediators of endocytosis and actin remodeling, respectively. Recently, Rab5 was shown to act as key switch for the formation of specialized actin-based protrusions, circular ruffles, which are sites of internalization and associated to cell migration. Here, we show that Rab5 and Rac are functionally linked in a spatial fashion. Rab5 activates Rac in response to HGF-stimulation by controlling its intracellular trafficking to endosomes where Tiam-1, a Rac-specific GEF, is also recruited. Activated Rac is detected in Rab5-dependent, EEA1- and transferrin-positive endosomes. This ensures localized activation of Rac required for dorsal ruffles formation. Consistently, impairing endocytosis prevented Rab5 and HGF-mediated Rac relocalization to endosome and its activation. Whereas blocking the re-delivery of endosomal-enriched active Rac back to plasma membrane abrogated circular ruffling. Importantly, Rab5 control of Rac trafficking is critical to promote cell migration. Thus, Rab5 stimulates spatial restriction of Rac signaling, by promoting its internalization, intracellular trafficking and re-delivering to specialized plasma membrane sites essential for cell locomotion.

DYNAMIC REGULATION OF PODOSOME IN PRIMARY HUMAN CELLS

STEFAN LINDER

Institut für Prophylaxe und Epidemiologie der Kreislaufkrankheiten; Ludwig-MaximiliansUniversität; Pettenkofer-str. 9, 80336 München, Germany; Email: stefan.linder@med.uni-muenchen.de

Podosome–type adhesions establish close contact to the substratum but are also able to degrade components of the extracellular matrix. This ability is thought to contribute to cellular invasiveness in both physiological and pathological situations. Cell types showing podosome formation include monocytic, endothelial or smooth muscle cells, whereas the related invadopodia have been mostly observed in carcinoma cells. The talk will provide an introduction to podosomes and invadopodia and will then focus on the dynamic regulation of podosomes in primary human cells. In particular, the regulatory relationship between podosomes and the microtubule cytoskeleton as well as the motor proteins that connect both structures will be addressed.

Lectures

Klaus Hahn
Chapel Hill, USA

Cornelis J. Weijer
Dundee, UK

Oral Presentations

Naoki Watanabe
Kyoto, Japan

Session IV

NEW LIVE CELL BIOSENSOR DESIGNS: WINDOWS ON ENDOGENOUS MOTILITY SIGNALING

KLAUS HAHN

UNC Department of Pharmacology, Chapel Hill, NC, USA

Over the past ten years there has been a revolution in our ability to visualize signaling dynamics in living cells. This has shown that the spatio-temporal dynamics of signaling is a critical determinant of cell behavior. In many cases localized generation of particular protein states (i.e. phosphorylation, particular conformations etc), rather than simple protein translocation, determine the outcome of receptor stimulation. In this talk I will describe several new tools to study protein conformational changes in living cells, and demonstrate their utility in studies of Rho family GTPase signaling for motility and macropinocytosis. The talk will cover methods to study endogenous, untagged proteins using novel dyes, simultaneous imaging of coordinated signaling activities, and biosensors for previously inaccessible targets made via phage display screening of biosensor libraries.

MOLECULAR KINETICS OF AIP1, A COFILIN-DEPENDENT ACTIN BARBED END INTERACTING PROTEIN, IN LAMELLIPODIA: EVIDENCE FOR FREQUENT FILAMENT SEVERING

Takahiro Tsuji, Chiharu Higashida, NAOKI WATANABE

Department of Pharmacology, Kyoto University Faculty of Medicine, Kyoto, Japan

Actin undergoes rapid polymerization-depolymerization cycles in lamellipodia. Our recent work (J. Cell Biol. 175: 947-955, 2006) revealed the astoundingly fast (0.58 per sec) dissociation rate of Capping protein from the actin network. Notably, this fast Capping protein dissociation occurs in a manner dependent on cofilin-mediated actin disassembly. These results suggested that filament severing and end-to-end annealing take place fairly frequently during the reorganization of dendritic nucleation actin arrays in lamellipodia. To test this frequent filament severing-annealing hypothesis, we extended our single-molecule speckle analysis to AIP1. AIP1 enhances the actin severing activity of cofilin and interacts with the actin barbed end only in the presence of cofilin. Therefore, AIP1 is thought to bind the new barbed end formed by cofilin-catalyzed filament severing. In agreement with this idea, our single-molecule speckle analysis now shows that the association of AIP1 is rapidly inhibited by an actin depolymerization inhibitor, jasplakinolide, which also interferes with cofilin binding to the actin network. These data provide in vivo evidence for the dependence of AIP1-actin interaction on cofilin-mediated filament severing. We are currently analyzing the single-molecule kinetics of AIP1 in live XTC fibroblasts in order to elucidate the frequency and the role of AIP1-associated filament severing in lamellipodia.

CHEMOTACTIC CELL MOVEMENT A KEY MECHANISM DURING DEVELOPMENT

CORNELIS J WEIJER

Division of Cell and Developmental Biology, Wellcome Trust Biocentre, College of Life Sciences, University of Dundee, Dundee DD1 5EH, UK

Cell movement is a key mechanism during embryonic development of most organisms, and has to be precisely regulated in space and time through extensive cell-cell signalling. We investigate how cells signal each other and these signals are detected and translated in directed coordinated movement. We study these questions in two different experimental systems, in the social amoebae Dictyostelium discoideum, a simple genetically tractable micro-organism, showing a relatively simple starvation induced multicellular development, and during gastrulation in the chick embryo, a model system for early amniote development.

Dictyostelium cells aggregate by chemotaxis in response to propagating waves of cyclic-AMP, which are periodically initiated by aggregation centres and travel trhough the population of cells. All stages of Dictyostelium morphogenesis are critically dependent on the dynamical interactions between cAMP wave propagation and chemotactic cell movement. We investigate how the cells signal each other and how they detect gradients of cAMP and translate this information into polarised activation of the actin-myosin cytoskeleton to result in force generation and directed movement up a cAMP gradient. We are particularly interested in the process of chemotactic cell sorting, in which differentiating prespore and prestalk cells sort out in the mound to form an axial pattern of cell types in the slug.

To investigate whether chemotaxis also plays a major role during the early development of higher organisms, we have analysed the migration of mesoderm cells, during gastrulation in the chick embryo. We have found that the movement of mesoderm is controlled by a combined action of chemo-attractants and repellents. We have identified some of theses attractants and repellents as belonging to the families of Fibroblast (FGF) and Vascular Endothelial Growth Factors (VEGF). We are now investigating how these factors are detected to result in directed cell movement and how signalling and movement are integrated to result in gastrulation.

Lectures

Denise Montell
Baltimore, USA

Erez Raz
Göttingen, Germany

Oral Presentations

Sven Bogdan
Münster, Germany

Marie Breau
Paris, France

Grant N. Wheeler
Norwich, UK

Session V

INSIGHTS INTO THE REGULATION OF EPITHELIAL BORDER CELL MIGRATION FROM GENETICS AND LIVE CELL IMAGING

DENISE MONTELL

Department of Biological Chemistry, Center for Cell Dynamics, Johns Hopkins School of Medicine, Baltimore, MD, USA

My laboratory studies the molecular mechanisms that control cell migration in development and disease. We have developed the border cells in the Drosophila ovary as a model system to study the developmental regulation of cell migration and its coordination with other developmental events. We have described multiple extracellular signals that are required for the correct cells to move in the proper direction, at the appropriate developmental stage. These signals include a steroid hormone (ecdysone), growth factors that activate receptor tyrosine kinases, and a cytokine that stimulates the JAK/STAT pathway. Each signal emanates from a distinct anatomical source, ensuring proper coordination of border cell migration with other cell types and developmental events. We have recently shown that spatial localization of Notch activity can be achieved by localized Kuzbanian gene expression, a previously unrecognized mechanism by which Notch activity can be localized during development. Ongoing screens continue to identify new genes, including Par-1, that control additional aspects of border cell migration. We have also shown that a number of these genes contribute to ovarian cancer. Recently we have defined conditions that allow us to observe border cell migration directly, in living organ culture, using time-lapse movies. This allows us to combine powerful genetic manipulations with live-imaging to decipher and observe the mechanisms by which cell migration is regulated in vivo.

REGULATION OF ACTIN-DRIVEN PROCESSES DURING DROSOPHILA DEVELOPMENT

R. Fricke, R. Stephan, C. Gohl, A. Mertens, C. Klämbt and S. BOGDAN

Institute of Neurobiology, University of Münster, Germany

Developmental processes involving movement and cell shape changes are based on a highly dynamic reorganization of the actin cytoskeleton. Actin polymerization is controlled by members of WASP protein family, WASP, N-WASP and WAVE that activate the Arp2/3 complex. Biochemical work of a number of laboratories showed that a protein complex comprising Sra-1, Kette and Abi regulates WAVE function. We have recently shown that this complex also plays a central role in activating WASP mediated processes during Drosophila development. To understand how WAVE and WASP are differentially activated we have screened for additional regulatory factors. Here, we present a biochemical and functional analysis of a novel Abi interacting protein, the Cip4/Toca1 protein, a highly conserved member of the PCH-protein family. Our genetic data reveal a so far unknown function of Cip4 for the correct establishment of cell polarity during wing development, a process that requires the coupling of actin dynamics and membrane trafficking. Genetic data suggest that Cip4 could possibly modulate not only WASP but also WAVE activity. Moreover, Cip4 can form protein complexes with both WASP and WAVE and is able to recruit WASP and WAVE in vivo to membrane tubules induced by Cip4 overexpression. Thus, Cip4 may define a new key component in directing cytoskeletal dynamics during membrane trafficking.

BETA1 INTEGRINS AND THE MIGRATION OF ENTERIC NEURAL CREST CELLS IN THE GUT

BREAU MARIE[1], Pietri Thomas[1,§], Eder Olivier[1], Blanche Martine[1], Brakebusch Cord[2], Fässler Reinhardt[2], Thiery Jean Paul[1] and Dufour Sylvie[1]

1 UMR144, CNRS - Institut Curie, 26, rue d'Ulm, 75248 Paris cedex 05, France; 2 Max Planck Institute of Biochemistry, Department of Molecular Medicine, Martinsried, Germany; § Present address : Institute of Neuroscience, University of Oregon, Eugene, OR 97403-1254

Enteric nervous system (ENS) development is a fascinating process whereby vagal neural crest cells (NCCs) colonise the whole intestine by migrating through its mesenchyme. During this colonisation, vagal NCCs actively proliferate, then differentiate into neurons and glial cells and aggregate to form the ganglia of the future ENS. We are studying the roles played by beta 1 integrins in this tissular invasion process. We use the Ht-PA-Cre mouse line, which targets the disruption of the beta1 integrin gene in the NCCs and their derivatives, when crossed with beta1 floxed mice. Beta1 integrin-null vagal NCCs fail to completely colonise the gut, leading to an absence of ganglia in the most distal part of the intestine, which resembles the human Hirschsprung's disease. In addition, mutant NCCs form abnormal aggregates in the gut wall, leading to a severe alteration of the ganglia network organisation. Beta1-null NCCs survive, proliferate, and differentiate normally within the gut. However, a variety of organotypic cultures revealed that beta1-null NCCs show impaired adhesion on extracellular matrix, enhanced intercellular adhesion properties, and a migration defect. In order to better characterise this defect, we are now analysing the dynamic behaviour of these cells during the gut colonisation.

MOLECULAR AND CELLULAR MECHANISMS CONTROLLING DIRECTED GERM-CELL MIGRATION IN ZEBRAFISH

EREZ RAZ

Germ Cell Development, Max-Planck-Institute for Biophysical Chemistry; Goettingen, Germany

The migration of Primordial Germ Cells (PGCs) in zebrafish relies on directional cues provided by the chemokine SDF1a and its receptor CXCR4b. These cells serves as an excellent model system for understanding long-range cell migration in development and disease. Using time-lapse microscopy we have been able to monitor the behavior of PGCs from early stages of development to later stages when they have reached their target. We show that following their specification the cells undergo a series of morphological alterations that precede the acquisition of motility and responsiveness to attractive cues. The cells then migrate towards their target while correcting their path following phases of loss of morphological cell polarity. In the following stages the cells gather at specific locations and move as cell clusters towards their final target. In all of these stages, zebrafish PGCs individually respond to alterations in the shape of the sdf-1a expression domain by directed migration towards their target, the somatic part of the gonad.
To determine the molecular basis for PGC polarization and migration we have followed the distribution of cytoskeletal elements in the migrating cells. Interestingly, unlike findings in some other cell types, this analysis did not support the idea that actin polymerization propels cellular protrusions. Rather, we could demonstrate that zebrafish primordial germ cells generate bleb-like protrusions that are powered by cytoplasmic flow. Protrusions are formed at sites of higher levels of free calcium where activation of myosin contraction occurs. Separation of the acto-myosin cortex from the plasma membrane at these sites is followed by a flow of cytoplasm into the forming bleb. We propose that polarized activation of the receptor CXCR4 leads to a rise in free calcium that in turn activates myosin contraction in the part of the cell responding to higher levels of the ligand SDF-1. The biased formation of new protrusions in a particular region of the cell in response to SDF-1 defines the leading edge and the direction of cell migration.

A COMBINATORIAL ROLE FOR THREE MATRIX METALLOPROTEINASES IN XENOPUS EMBRYONIC MACROPHAGE MIGRATION

Matthew L. Tomlinson, Carla Garcia-Morales, Muhammed Abu-Elmagd and GRANT N. WHEELER

School of Biological Sciences, University of East Anglia, Norwich, UK

We have developed an in vivo model to look at the role of Matrix metalloproteinases (MMPs) in macrophage cell migration in the Xenopus embryo. Xenopus embryonic macrophages act as the main mechanism of host defence, before lymphoid cells have developed. During development the embryonic macrophages differentiate in the ventral blood island at neurula stages and then migrate over the whole embryo. MMPs are a family of zinc dependant endoproteases with multiple roles in extracellular matrix remodelling and the activation of signalling peptides. MMPs are known to be expressed by macrophages but it is unclear what their precise role is in cell migration. We have shown by wholemount in situ hybridisation that XMMP?s 7, 9 and 18 are expressed in Xenopus macrophages. Using morpholino antisense oligonucleotides to knockdown the endogenous protein we have shown that all three MMPs are necessary for macrophage migration in the embryo. In addition, double and triple knockdowns of these MMPs using combinations of the morpholinos show that the MMPs work synergistically to enable macrophage migration. The work presented here represents the first triple knockdown and functional investigation into three macrophage MMPs in-vivo and demonstrates the importance of MMPs in mediating cell migration in the whole organism.

Poster session II

B1	Chiharu Higashida
B2	Natsuko Imaizumi
B3	Thomas Kledal
B4	Christophe Le Clainche
B5	Daria Leali
B6	Sandrine Medves
B7	Francesca Milanesi
B8	Subhanjan Mondal
B9	Jlenia Monfregola
B10	Francesca Orso
B11	Barbara Ortensi
B12	Matteo Parri
B13	Maria Carla Parrini
B14	Elaine Pinheiro
B15	Elisa Ridolfi
B16	Krista Rombouts
B17	Mette Rosenkilde
B18	Mika Sakurai-Yageta
B19	Cristina Santoriello
B20	Julia Sero
B21	Ziv Shulman
B22	Harvey Smith
B23	Maria Letizia Taddei
B24	Katsiaryna Tarbashevich
B25	Iva Tolic-Norrelykke
B26	Giorgia Volpi
B27	Adriana Albini

Last update: May 2, 2007

B1

RAPID ACTIVATION OF MDIA1 TO RESTORE CELLULAR ACTIN POLYMERS REGULATED BY G-ACTIN.

CHIHARU HIGASHIDA, Shingo Kobayashi, James Monypenny, Shuh Narumiya, and Naoki Watanabe

Department of Pharmacology, Kyoto University Faculty of Medicine, Yoshida Konoe-cho, Sakyo-ku, Kyoto 606-8501, Japan

mDia1 belongs to the formin family proteins that share highly conserved formin homology 1 (FH1) and formin homology 2 (FH2) domains. Many actin-based cellular structures such as yeast actin cables, cytokinetic contractile ring and actin bundles in mammalian cells are dependent on formins. Recent studies characterized that FH2 or FH1-FH2 domains nucleate actin filaments and they remain continually associated to the growing barbed-end of filaments. However, the precise mechanisms that regulate formin-mediated actin filament formation, both temporally and spatially within the cell, are still unknown. Here our single-molecule imaging reveals that cells possess an acute actin polymer restoration mechanism involving mDia1. Increase in the G-actin pool induced by actin monomer sequestering drugs rapidly activated mDia1. Expressing nonpolymerizable actins sufficiently induced frequent activation of mDia1. Rho activity was required for activation of mDia1, but the FH2 region alone can be activated by latrunculin B. These results suggest that accumulation of G-actin works as a cue to activate mDia1 to execute rapid assembly of actin filaments.

B2

MECHANISMS OF ANGIOGENESIS IMPAIRMENT BY RADIATION THERAPY.

N. IMAIZUMI, Y. Monnier, C. Rüegg.

1, Centre Pluridisciplinaire d'Oncologie (CePO), and Swiss Institute for Experimental Cancer Research (ISREC), NCCR Molecular Oncology, Epalinges, Switzerland

Clinical evidence indicates that tumors recurring within previously irradiated fields are highly invasive and metastatic, suggesting a role of the tumor stroma in this effect. Angiogenesis plays a critical role in tumor progression. Ionizing radiation is known to induce apoptosis of angiogenic endothelial cells, while the effect on quiescent endothelial cells and de novo angiogenesis is not well characterized. We observed that irradiation of normal tissue prevents tumor- and growth factor-induced angiogenesis and decided to investigate the putative mechanisms involved. We found that irradiation did not kill quiescent vessels within irradiated skin. Also, we observed that impaired angiogenesis was not due to decreased recruitment of inflammatory cells within the irradiated field. We used mouse aortic ring assay to test the sprouting capacity of endothelial cells in vitro, and found that irradiation of the aorta completely suppressed sprouting angiogenesis. Using HUVEC cells, we could show that irradiation of quiescent confluent endothelial cells did not induce cell death but suppressed subsequent migration in a scratch wound assay. We are now assessing the effect of radiation in migration and proliferation ability at the molecular level. Inhibition of endothelial cell migration and proliferation by radiation may explain reduced angiogenesis in tumors growing in previously irradiated fields.

B3

CELL MIGRATION ON NANOSTRUCTURED 2D MICROENVIRONMENTS

Gertrud M. Hjortø, Thomas H.R. Carlsen, Morten Hansen, Chris D. Madsen*, Niels B. Larsen, Nicolai Sidenius*, Henrik Flyvbjerg, THOMAS N. KLEDAL

*Biosystems Department, Risoe National Laboratory, The Danish Technical University, Frederiksborgvej 399, DK-4000, Roskilde, Denmark. (Correspondence to Thomas.kledal@risoe.dk); *IFOM Foundation, Via Adamello 16, 20139 Milan, Italy*

Investigations of cell migration in tissue like microenvironments have been hampered by the difficulties in establishing a well defined environment. Additionally, current experimental setup only allows the investigator to extract data from one or very few cells, even though there may be hundreds to thousands of cells interacting, adhering, braking down ECM or moving in the experiment. By tracking cell movements on nano/micro structured polymer surfaces using time lapse experiments followed by mathematical modelling, we can overcome these limitations, and obtain data from all the individual cells in the experiments. We are structuring 2D microenvironments by micro contact printing and In-mould patterning. We will present the two technologies and we will present data and models from our studies of cell migration on nano structured microenvironments. We are currently conducting studies how cells migrate w/wo expression of endogenous and viral chemokine receptors, and studies of how migration of cells that express the uPA receptor, depends on the presence of vitronectin. Thus, by constructing specific, well-defined microenvironments, and model cell behavior in these environments, we can assay the significance of specific components of the microenvironment. This way we can quantify the importance of e.g. cell adhesion, proteolysis and chemotaxis for the invasion process or for e.g. viral dissemination.

B4

TALIN REVEALS THE BARBED END CAPPING ACTIVITY OF VINCULIN

CHRISTOPHE LE CLAINCHE and Marie-France Carlier.

CNRS, Laboratoire d'Enzymologie et Biochimie Structurales, Gif-sur-Yvette, France.

In order for cells to migrate, focal adhesions (FA) assemble at the front of crawling cells and disassemble at the trailing edge. Focal adhesions are associated with actin-myosin stress fibers that provide traction on the substrate on which cells move. The traction force depends on the regulation of acto-myosin contractility and actin assembly by FA proteins associated with stress fibers. Talin, vinculin and paxillin are the first proteins found in nascent FAs. Vinculin is a 116 kDa protein of unknown function that contains a N-terminal globular head (Vh) and a C-terminal elongated tail (Vt) linked by a central poly-proline rich flexible region. An intra-molecular interaction Vh-Vt masks the actin binding domain in the tail. The binding of several ligands including talin, ?-actinin and the Shigella protein IpaA to the head domain of the auto-inhibited vinculin disrupts the intra-molecular interaction and exposes the cryptic actin binding domain. Here we show that vinculin activated by talin regulates actin assembly by capping the filament barbed ends specifically. This activity is mediated by the C-terminal domain of vinculin. We are mapping the capping domain precisely and studying the regulation of this activity by other ligands of vinculin.

B5

OSTEOPONTIN OVEREXPRESSION INHIBITS IN VITRO RE-ENDOTHELIALIZATION VIA INTEGRIN ENGAGEMENT

DARIA LEALI, Emanuela Moroni, Federico Bussolino*, and Marco Presta.

*Unit of General Pathology, Department of Biomedical Sciences and Biotechnology, School of Medicine, University of Brescia, Brescia, Italy; *Institute for Cancer Research and Treatment, Department of Oncological Sciences, University of Torino, Turin, Italy.*

The extracellular matrix protein osteopontin (OPN) plays a non-redundant role in atherosclerosis and restenosis. Here we investigated the impact of OPN upregulation in an in vitro model of re-endothelialization. Murine aortic endothelial (MAE) cells interact via alphaV-integrins with the integrin-binding Arg-Gly-Asp OPN sequence and adhere to immobilized OPN. On this basis, MAE cells were stably transfected with a wild-type OPN cDNA (OPN-MAE cells), with an OPN mutant lacking the Arg-Gly-Asp sequence (dRGD-OPN-MAE cells), or with vector alone (mock-MAE cells). When compared to mock-MAE and dRGD-OPN-MAE cells, OPN-MAE cells showed a reduced capacity to repair a wounded monolayer. Accordingly, OPN-MAE cells at the edge of the wound were unable to form membrane ruffles, to reorganize their cytoskeleton, and to activate key regulators of cell migration cycle as FAK, Rac1, RhoA and ERK. Also, parental MAE cells showed reduced re-endothelialization after wounding when seeded on immobilized OPN and exhibited increased adhesiveness to OPN-enriched extracellular matrix. In conclusion, OPN upregulation impairs re-endothelialization by inhibiting cell migration via alphaV-integrin engagement by the ECM-immobilized protein. This may contribute to the adverse effects exerted by OPN in restenosis and atherosclerosis.

B6

EFFECTS OF TES, A ZYXIN-BINDING PARTNER, ON THE ORGANISATION AND DYNAMICS OF THE ACTIN CYTOSKELETON IN LIVING CELLS.

SANDRINE MEDVES[1], Mikalai Yatskou[1], Marie Catillon[1], Delphine Lentz[1], Marleen Van Troys[2], Christophe Ampe[2] and Evelyne Friederich[1].

1 Laboratory of Molecular Biology, Genomics and Modelling, CRP-Santé, 84, Val Fleuri, L-1526, Luxembourg; 2 Department of Biochemistry, Faculty of Medicine and Health Sciences, Ghent University, and Medical Protein Research, Flanders Interuniversity Institute for Biotechnology (VIB09), Belgium

Tes is a tumour suppressor protein localised in focal adhesions, stress fibers and areas of cell-cell contacts. The protein is composed of an N-terminal part that contains a cystein-rich sequence and a PET domain. This domain targets the protein to actin stress fibers. The C-terminal part contains LIM domains that target the protein in focal adhesions where it co-localises with zyxin, another LIM containing protein implicated in the actin cytoskeleton regulation.
We investigated the effects of the N-terminal domain of testin on the actin cytoskeleton. Transfected fibroblast-like Vero cells expressing this domain exhibited thick, aligned stress fibers that were linked to large focal adhesions and that spanned the whole cell body. Confocal microscopy-based FRAP analysis of GFP-actin dynamics in living cells producing Tes Nt, revealed that this domain has an effect on actin dynamics. In vitro co-sedimentation of Tes Nt with F-actin supported direct binding of this domain to actin filaments.
All together, our results show that Tes contains a novel, N-terminal actin-binding domain through which this protein affects actin turnover in structures like focal adhesions and stress fibers. We propose that Tes might contribute to zyxin-mediated regulation of actin dynamics.

B7

STRUCTURAL CHARACTERIZATION OF EPS8 PROTEINS

F. MILANESI[1], M. Hertzog[1], S. Pasqualato[1], A. Disanza[1], E.Guittet[2], C. Van Heijenoort[2], D. Hanein[3], G. Scita[1]

1 IFOM Foundation Milan Italy; 2 ICSN Gif sur Yvette France; 3 Burnham Institute La Jolla USA

Eps8, is the founding member of a recently identified family of actin binding proteins involved in signaling to actin remodelling. Eps8 posses two distinct activities on actin dynamics: firstly, it acts as a cross-linking protein that organizes actin into highly dynamic and architecturally diverse subcellular scaffolds, thus orchestrating a variety of fundamental mechanical functions in motile cells. Additionally, Eps8 acts as a barbed ends capping proteins, blocking actin filaments elongation and thus regulating actin-based motility. The holo Eps8 full-length protein is autoinhibited as a capper, whereas it displays constitutively bundling activity. The structural and molecular mechanisms underlining these activities are unknown. Here, we show that the isolated C-terminal capping region of Eps8 (residues 648-821) contains a putative SAM-PNT domain, whose structure has been solved and shown to be composed of 5 amphipatic helices (H1 to H5). NMR analysis revealed that a complete change of folding occurs upon actin binding, suggesting that extensive surfaces if interaction. Consistently, Eps8 648-821 forms a high affinity (50nM) and stable complex with monomeric actin. Chemical cross-linking and sedimentation assays revealed the existence of two high affinities binding surface (kd=50 nM and 2 μM, respectively) corresponding to the H1-H2 pair and H5 helices. Single point mutations disrupting the hydrophobic faces of these amphipatic helices significantly reduced capping activity, suggesting that multiple surfaces of interaction are required for barbed end capping. A model of how Eps8 caps actin filament will be discussed.

B8

RASGEF Q, A DICTYOSTELIUM ACTIN BINDING EXCHANGE FACTOR FOR RASB, AFFECTS MYOSIN II FUNCTIONS

SUBHANJAN MONDAL, Deenadayalan Bakthavatsalam, Berthold Gaßen, Francisco Rivero, Angelika A. Noegel

Centre for Biochemistry, Institute of Biochemistry I, Medical Faculty and Centre for Molecular Medicine Cologne, University of Cologne, D-50931 Cologne, Germany

The social amoeba Dictyostelium discoideum has a surprisingly large repertoire of GTPases of the Ras subfamily that regulate a diverse range of cellular processes. Compared to our understanding of downstream effects of Ras, our understandings of mechanisms that activate Ras GTPases are relatively poor. We report on a Ras guanine nucleotide exchange factor (RasGEF), RasGEF Q in Dictyostelium. RasGEF Q is a 143 kDa protein containing a RasGEF and RasGEF amino terminal domain separated by a DEP domain. RasGEF Q binds to F-actin and also interacts with myosin II and myosin II heavy chain kinase A (MHCK A) in an F-actin dependent manner. We also show that RasGEF Q is the predominant exchange factor for RasB, and that RasB is activated upon starvation when RasGEF Q expression is at its peak. Overexpression of the GEF domain of RasGEF constitutively activates RasB and leads to a cytokinesis defects in suspension resembling the phenotype of cells expressing a constitutively active RasB and of myosin null mutants. Furthermore, RasGEF Q- mutants show myosin II overassembly, are defective in suppression of lateral pseudopods during chemotactic migration. Together our results suggest that cAMP induces RasGEF Q and thereby activates RasB, which could regulate processes requiring myosin II. The characteristics of RasGEF Q- mutants also suggest that it has roles during later stages of development in cell sorting and developmental patterning and in phototaxis.

B9

ORF19, A NEW WH2- CONTAINING ACTIN BINDING PROTEIN

JLENIA MONFREGOLA[(1)], Nicola Silva[(1)], Stefania Crispi[(1)], Enni Bertling[(2)], Pekka Lappalainen[(2)] and Matilde Valeria Ursini[(1)].

(1) Institute of Genetics and Biophysics "Adriano Buzzati Traverso?, P. Castellino 111, Naples, Italy.; (2) Institute of Biotechnology, P. O. Box 56,University of Helsinki, Helsinki 00014, Finland

Orf19 is a murine gene ubiquitously expressed that encodes for a protein of 475 amino acids with unknown function. To unravel the biological function of the ORF19 protein we performed a bioinformatic prediction of functional domains and we found that the C-terminal portion contains a 13-residues proline stretch followed by a WH2 domain and an acidic region (CA). All these sequence features are commonly found in regulators of the actin cytoskeleton. We observed that the portion from residues 304-475 shows a high similarity with the WASP-region that promotes actin filament nucleation through the Arp2/3 complex activation. Therefore, we demonstrated that the full-lenght ORF19 fused to GFP co-localizes with G-actin in U2OS cells. In vitro binding assay of actin monomer showed that the ORF19 C-terminal portion (aa 304-475) binds the ATP-G-actin with high affinity. Moreover, we produced several constructs in which the myc tag is fused to the ORF19 C-terminal (aa 304-475) and the ORF19 C-terminal lacking of the CA region (aa 304-464), which we will over-express in U2OS cells to assay their ability to disrupt cellular actin organization by ARP2/3 activation. Collectively, those preliminary results strongly suggest that ORF19 is a new actin binding protein .

B10

THE AP-2ALPHA TRANSCRIPTION FACTOR REGULATES TUMOUR CELL MIGRATION

FRANCESCA ORSO, Elisa Penna, Alessandra Solero, Piero Sismondi, Michele De Bortoli, Daniela Taverna

Department of Oncological Sciences, Univ. of Turin c/o IRCC- Candiolo

AP-2 transcription factors are a family of developmentally-regulated DNA binding protein. They are encoded by five different genes (alpha, beta, gamma, delta and epsilon) but they share a common structure. AP-2 play relevant roles in growth, differentiation and adhesion via the regulation of specific genes. Many evidences suggest that AP-2 act as tumour suppressors in particular in melanomas and mammary carcinomas. Here we are investigating the roles of AP-2 proteins in cancer formation and progression. We knocked down AP-2 alpha expression in tumour cells using RNAi and we obtained significant reduction of migration, chemotherapy-induced apoptosis and enhanced tumour growth in vivo. The migration defects are due, at least in part, to secreted factors. Microarray analysis of the siRNA-expressing cells revealed that many genes involved in adhesion, migration and invasion are regulated by AP-2alpha (MMPs, chemokines, neuropilin-like proteins). We are currently analyzing the biological properties of some of these genes. Preliminary data suggest that ESDN (endothelial and smooth-muscle neuropilin-like protein) could be a good candidate for the migration defects. In parallel we are analyzing the roles of AP-2 gamma in tumour cells by performing knock out experiments. Our goal is to dissect the differential functions of the two AP-2 isoforms.

B11

RAI (SHC C) IS REQUIRED FOR NEURAL STEM CELL MIGRATION AND BRAIN TUMOR DEVELOPMENT.

BARBARA ORTENSI[1], Daniela Osti[1], Paolo Gaetani[2], Maria Capra[3], Micaela Quarto[3], Paolo Nuciforo[3], Serena Pellegatta[4], Gaetano Finocchiaro[4] and Giuliana Pelicci[1].

1 Department of Experimental Oncology, European Institute of Oncology, via Ripamonti 435, 20141 Milano, Italy; 2 Department of Neurosurgery, Istituto Clinico Humanitas, via Manzoni 56, 20089 Rozzano (MI), Italy; 3 IFOM Fondazione Istituto FIRC di Oncologia Molecolare, Milan, Italy; 4 Unit of Experimental Neuro-Oncology, Istituto Neurologico Besta, via Celoria 11, 20133 Milan, Italy

Rai (Shc C) belongs to the family of Shc adaptor proteins and is expressed in the brain, exclusively in neural stem cells (NSC) and in neurons. In a microarray screening of human tumoral tissues arising from a wide range of organs we found that the only kind of cancer expressing Rai is the glioblastoma multiforme (GBM), the most aggressive and infiltrating of primary brain tumors. To study the role of Rai in gliomagenesis, we isolated cancer stem cells (CSC) from GBM specimens and analysed Rai expression in different conditions. We produced lentiviral vectors to silence Rai expression by means of RNA interference and infected the CSCs obtained from several patients subjected to surgery. We demonstrate by in vitro experiments that Rai is involved in the migration of cancer progenitors and this could be important for the invasiveness of the tumor in the surrounding normal brain. In order to test this hypothesis we are performing in vivo experiments, inoculating the CSCs (interfered or not for Rai) in the brain of nude mice. These results can provide important information about molecular mechanisms of tumor development and identify Rai as a new potential diagnostic/prognostic marker and, possibly, as a target of therapeutic intervention.

B12

EPHRIN-A1 ACTIVATES A FAK/SRC MEDIATED MOTILITY RESPONSE LEADING TO ACTIVATION OF ACTINO/MYOSIN CONTRACTILITY

MATTEO PARRI, Francesca Buricchi, Elisa Giannoni, Giovanni Raugei, Giampietro Ramponi, and Paola Chiarugi

Dipartimento di Scienze Biochimiche, Università degli Studi di Firenze Italy

Eph receptors and ephrin ligands are widely expressed in epithelial cells and mediate cell repulsive motility through heterotypic cell-cell interactions. Several Ephs, including EphA2, are highly overexpressed in many tumours correlating with poor prognosis and high vascularity in cancer tissues. We report herein that in prostatic carcinoma cells (PC3) ephrinA1 elicits a repulsive response carried out through actino/myosin contractility activation, finally leading to retraction of cell body. This events occur through the assembly of an EphA2-associated complex involving the two kinases Src and Focal Adhesion Kinase (FAK). EphrinA1-mediated repulsion leads to the specific phosphorylation of Tyr576/577 of FAK, enhancing FAK kinase activity. Finally ephrinA1 induced repulsive and motility responses are achieved through a Rho-mediated phosphorylation of Myosin Light Chain II, in which FAK and Src activation are again required steps. Hence, Src and FAK are upstream regulators of the overall response induced by ephrinA1/EphA2, instructing cells to retract the cell body and to repulse from the original site. This may account for a Rho-driven round like cell shape and amoeboid motility shift, likely affecting invasiveness of ephrin-sensitive carcinomas.

B13

NOVEL INSIGHTS ON THE ROLE OF PAK1 KINASE IN CELL MOTILITY BY FUNCTIONAL FRET MICROSCOPY

MARIA CARLA PARRINI, Jacques Camonis, Michiyuki Matsuda* and Jean de Gunzburg

*Institut Curie, Inserm U830, Paris, France, *Kyoto University, Kyoto, Japan*

Pak1 kinase, a down-stream effector of Cdc42 and Rac1 GTPases of the Rho family, regulates cell motility by controlling actin cytoskeleton and adhesions. We developed a novel Pak1 biosensor based on the FRET technology, named Pakabi (Pak activation biosensor), which allowed us to follow Pak1 conformational changes and activation in living cells. We found that translocation to the plasma membrane, a well-established mechanism to induce Pak1 activity, leads to transition from a closed to a semi-open conformation accompanied by partial autophosphorylation events. Binding to GTPases is required to reach the fully open conformation with the crucial threonine 423 phosphorylated in the catalytic site. We visualized the spatio-temporal dynamics of Pak1 activation at the membrane of protrusions during cell spreading and we established the correlation between Pak1 activity and protrusion formation, as well as between Pak1 and Cdc42 activities. We also demonstrated that Pak1 kinase activity is necessary for cellular protruding activity, as when it is blocked, cell spreading is inhibited. The emerging function of Pak1 in cancer calls for a better understanding of this protein. Our approach allows for the first time the investigation of the complex dynamics in space and time of Pak1 regulation during live protruding activity.

B14

ANALYSIS OF THE RA AND PH DOMAIN CONTAINING MRL PROTEINS IN NERVOUS SYSTEM DEVELOPMENT

ELAINE M. PINHEIRO[1], Matthias Krause[2], Mine Kimura[1] and Frank Gertler[1]

1 Department of Biology and Center for Cancer Research, Massachusetts, Institute of Technology, Cambridge, MA; 2 Randall Division of Cell & Molecular Biophysics, King's College London, London, UK

The MRL (MIG-10/RIAM/LPD) protein family are known regulators of lamellipodial dynamics, adhesion, and cell motility. The vertebrate members, Lamellipodin (LPD) and RIAM, and the C. elegans ortholog, MIG-10 share a conserved domain structure: a Ras-association domain (RA), a pleckstrin homology domain (PH) and various proline rich motifs such as SH3, profilin and EVH1 binding sites. The PH and RA binding domain is believed to link signaling between phosphoinositides and the Ras superfamily to actin dynamics via Ena/VASP proteins. Such signaling events could affect cell motility in response to environmental cues in such processes as axon guidance and growth cone translocation. In addition, RIAM and LPD, localize to the tips of lamellipodia and filopodia, structures important for axonal migration. They also interact with the Ena/VASP protein family, which have known functions in the nervous system. Mutants of mig-10 exhibit axon guidance defects that overlap with those of unc-34 mutants, the C. elegans Ena/VASP ortholog. In addition, MIG-10 has been shown to act downstream of the Slit and Netrin pathways.

We are interested in the role of the vertebrate family members in the developing nervous system. LPD and RIAM are highly expressed in the brain including the cortex. Using primary cortical cultures, we are examining the role of RIAM and LPD in vivo. Interestingly, these molecules show different levels of expression in the cortex throughout embryonic development. Overexpression of either molecule gives a distinct phenotype, suggesting that RIAM and LPD may have different roles. In addition, mice carrying a conditional allele of Lpd or Riam are being analyzed. Preliminary results will be presented.

B15

HGF ACTIVATES ENDOCYTOSIS OF MET AND E-CADHERINS: ROLE OF C-SRC IN SIGNALLING OF LOW AND HIGHLY INVASIVE BREAST CARCINOMAS

E. RIDOLFI, E. Matteucci and M.A. Desiderio

Institute of General Pathology, University of Milan, Italy

Hepatocyte growth factor (HGF) determines epithelial-mesenchymal transition of carcinoma cells, that implies cell dissociation and cytoskeleton rearrangement. HGF exerts the biological functions through signal cascade-activation at Met receptor docking site, which includes c-Src transducer. c-Src contributes to growth/survival of breast cancer cells, but also to motility, invasiveness and thus metastatic potential. We have studied the consequences of HGF-induced c-Src activation in breast carcinoma cells. In MCF-7 cells (low invasive) HGF induced phospho-c-Src mostly overlapped with E-cadherin/Met that have been internalised, as resulted by confocal analysis. At 30 min, HGF caused Met and E-cadherin tyrosine phosphorylation and Erk 1/2 activation, evaluated by co-immunoprecipitation and Western Blot experiments, respectively. In MDA-MB 231 cells (highly invasive), lacking E-cadherins, HGF determined Met trafficking to perinuclear compartment while phospho-c-Src enhancement at cell membrane triggered negative signals to block transcription. Our data raise provocative ideas about the trafficking and signalling mechanisms of Met/c-Src.

B16

THE ROLE OF MYRISTOYLATED ALANINE-RICH PROTEIN KINASE C SUBSTRATE (MARCKS) IN THE REGULATION OF CYTOPLASMIC AND NUCLEAR ACTIN DYNAMICS IN HUMAN COLON CANCER CELLS

KRISTA ROMBOUTS, Benedetta Lottini, Tommaso Mello, Alessandra Caligiuri, Francesco Liotta, Vinicio Carloni, Fabio Marra and Massimo Pinzani.

Dipartimento di Medicina Interna, and Centro di Ricerca, Trasferimento e Alta Formazione DENOTHE, Università degli Studi di Firenze, Florence, Italy.

It is increasingly clear that actin plays a key role not only in the cytoplasm but also, and possibly even more relevant, in the regulation of nuclear events such as chromatin remodelling and transcription. The proteins regulating these actin functions are not yet defined. MARCKS, a filamentous actin binding protein was determined as a new nuclear matrix protein co-localizing with nuclear actin. During mitosis MARCKS showed to behave like Aurora B kinase, a chromosome passenger protein, and a current target for anticancer therapy. As previously shown for Aurora B kinase, RNAi against MARCKS resulted in the presence of multinucleated cells i.e. a common feature of tumorigenesis. MARCKS protein expression was determined in different human colorectal carcinoma cell lines and studied in detail in Clone A cells, a highly invasive and metastatic cell line with high expression of MARCKS. By employing RNAi for MARCKS cell migration and cell cycle were significantly reduced concomitantly with morphological changes and an important reduction in Aurora B kinase protein expression. The present project is directed at assessing the functional role of MARCKS, actin and Aurora B kinase and their partners to explore the possibility of a therapeutic approach.

B17

STRUCTURAL AND FUNCTIONAL DELINEATION OF ORPHAN 7TM RECEPTORS EXPRESSED ON LEUKOCYTES

METTE M. ROSENKILDE*, Tau Benned-Jensen*, Claus B. andersen** and Elisabeth Ralfkjaer**.

**Lab. for Molecular Pharmacology, Department of Neuroscience and Pharmacology, The Panum Institute, Copenhagen University; **Dep. of Clinical Pathology, Copenhagen University Hospital Denmark.*

Seven transmembrane (7TM) receptors, also known as G-protein coupled receptors, constitute the largest superfamily of proteins in the human genome. Within the 7TM receptors, the rhodopsin-like - family A - constitute the largest group. Chemokine receptors control leukocyte movements and constitute a large group of rhodopsin-like 7TM receptors. Presently, 22 different chemokine rceeptors and >50 different chemokine ligands acting at these receptors have been identified. The chemokine receptors have been a major research area within my group for the last many years. Orphan 7TM receptors, with similar expression pattern - and low structural homology - to chemokine receptors have been included in our research lately. Presently, we are interested in the two leukocyte expressed orphan 7TM receptors, EBI2 and GPR18, that are both regulated by Epstein-Barr herpesvirus cell-entry. We are interested in the signalling pathways initiated by these two receptors, and the structural basis for the activation pattern. We are also interested in the expression pattern of both receptors, and have described the differential expression on the different subsets of leukocytes within the peripheral blood mononuclear cells (PBMCs). Given the signalling pathways initiated and the expression pattern, it is tempting to believe that both receptors may play important roles for cell migration, as observed for the chemokine receptors.

B18

ANALYSIS OF THE ROLE OF EXOCYST-IQGAP1 COMPLEX DURING CANCER CELL INVASION

MIKA SAKURAI-YAGETA [1, 2], Philippe Chavrier [1, 2]

1 Institut Curie, Centre de Recherche, Paris, F-75248 France; 2 CNRS, UMR144, Paris, F-75248 France.

Mammalian exocyst complex is involved in the targeting and tethering of secretory vesicles to dynamic regions of the plasma membrane and has recently been shown to play a role during cell migration. Using yeast two-hybrid (in collaboration with Hybrigenics, Paris, France), we identified IQGAP1, an effector of Cdc42/Rac1, as a binding partner of one of the exocyst subunit, Sec3. IQGAP1 has been shown to bind to CLIP-170, APC and actin and regulate actin cytoskeleton and microtubules dynamics during cell migration. In vitro, IQGAP1 was found to associate with the amino-terminal coiled-coil domain of Sec3 and Sec8 through its C-terminal coiled-coil domain. In vivo, we observed that the association of IQGAP1 with Sec3/Sec8 is dependent on the GTP-bound form of Cdc42 and RhoA. Sec3/Sec8 associates strongly with GTP-bound Cdc42/RhoA, suggesting that these GTPases allow exocyst binding with IQGAP1. In highly invasive MDA-MB-231 breast cancer cells, endogenous Sec8 and IQGAP1 formed a complex and colocalized at membrane protrusions called invadopodia where degradation of extracellular matrix occurs during cell invasion. siRNA-based knock-down of either the exocyst complex (Sec8, Sec6 or Sec10) or IQGAP1 inhibited matrix degradation in these cells. Expression of a constitutively active mutant form of IQGAP1, which does not interact with Cdc42/Rac1, stimulated matrix degradation. Deletion of the C-terminal Sec3/Sec8 binding domain abolished the capacity of this mutant to promote matrix degradation. All together, our results demonstrate that the association of IQGAP1 with the exocyst complex plays a role in the formation and function of invadopodia during cell invasion.

B19

TRANSGENIC ZEBRAFISH TO STUDY SUBCELLULAR ORGANELLE DYNAMICS

SANTORIELLO CRISTINA, Sigismund Sara, Polo Simona, Scita Giorgio and Mione Mione.

IFOM- Firc Institute of Molecular Oncology, Via Adamello, 16, 20139 Milan, ITALY

Cell migration is controlled by internal and external signals, resulting in highly dynamic and localized remodeling of the cytoskeleton, cell-cell interactions and cell-substrate interactions. At the moment a powerful approach to understand the relation between signals and cellular effectors during migration is provided by the use of imaging, which provides visible readouts in real time of these events. This technique is even more informative if applied to an entire living organism, where cellular diversity and organ formation provide a ground to observe diverse cell migration activities. In order to study and observe cytoskeleton dynamics during cell migration, we decided to generate several zebrafish transgenic lines, where intracellular fluorescent markers label organelles and cytoskeleton components. These zebrafish transgenic lines express, under an ubiquitous promoter, the following markers as EGFP or RFP fusion protein: kinase dead dcklk (microtubule binding protein), N-WASP (actin polymerization), Abi1 (actin capping protein), MENA (actin binding protein), clathrin (endocytosis) and dynamin2 (endocytosis). We have studied the dynamic localization of these fluorescent proteins in different cell types, during development and organogenesis, using fast image acquisition at the confocal microscope. We will propose and discuss the use of these lines in mutational analysis, drug screening and gene functional assays.

B20

PAXILLIN COORDINATES PHYSICAL AND CHEMICAL STIMULI TO DIRECT SPATIAL LAMELLIPODIA FORMATION

JULIA E. SERO*, Donald E. Ingber**

**Harvard Medical School, Biological and Biomedical Sciences/Vascular Biology Program Children's Hospital, Boston, MA, USA; ** M.D., Ph.D, Judah Folkman Professor of Vascular Biology, Harvard Medical School/ Vascular Biology Program, Children's Hospital, Boston, MA, USA*

Cell morphology and behavior are influenced not only by chemical factors but also by physical signals transmitted from the extracellular matrix (ECM) to the cytoskeleton through focal adhesions. Cellular geometry (cell shape and orientation) can guide the formation of motile processes in response to soluble growth factors. We have used microcontact printing to confine cells to adhesive islands of particular shapes and sizes. Cells plated on cell-sized square islands, for example, will spread and take on a square shape. Upon addition of the soluble growth factor PDGF, square-shaped fibroblasts initially form lamellipodia from all directions, but after 10 minutes extend new processes predominantly from corners. Previous work has shown that this effect depends on the spatio-temporal activation of Rho family small GTPases. Cells plated on square adhesive islands orient their actin cytoskeletons along the diagonal lines of tension and form large focal adhesions in corner regions. These focal adhesion complexes may act as platforms that coordinate signaling in subcellular microcompartments, such as regions of high tension, as cells migrate in response to chemical and physical cues. We have found that the focal adhesion scaffold protein paxillin is necessary for cells to maintain the corner lamellipodia localization. Paxillin -/- mouse embryonic fibroblasts, as well as human dermal fibroblasts in which paxillin is depleted by RNAi, show migration defects as well as impaired responses to PDGF. In the absence of paxillin, cells show little bias for lamellipodia formation even after 30 minutes. Here we explore the molecular mechanisms by which paxillin controls the regulation of ECM-guided lamellipodia formation in response to stimulation with a soluble motogen.

B21

HIGH AFFINITY LFA-1 IN LYMPHOCYTES REARRANGE TRANSIENT ICAM-1 CLUSTERS AT APICAL, JUNCTIONAL AND SUBLUMENAL ENDOTHELIAL COMPARTMENTS DURING TRANSENDOTHELIAL MIGRATION

ZIV SHULMAN[1] Vera Shinder[2], Sara Feigelson[1], and Ronen Alon[1]*

1 Department of Immunology, The Weizmann Institute of Science, Rehovot, Israel; 2 Department of Chemical Research Support, The Weizmann Institute of Science, Rehovot, Israel

LFA-1, chemokines and shear stress signals play critical roles in transendothelial migration (TEM) of T-cells. Lymphocytes must rapidly locomote from their site of arrest to paracellular endothelial junctions, where TEM takes place. We studied in-vitro the role of high affinity (HA) LFA-1 subsets in primary T-cell TEM across TNFalpha-activated HUVEC under physiological conditions of shear flow. In T-cells locomoting on endothelial surfaces presenting the prototypic chemokine CXCL12, HA-LFA-1 subsets were exclusively localized at numerous focal points where they rearranged endothelial ICAM-1 in 1 micron clusters. These ICAM-1 specific focal points were not formed by artificially activated T-cells. Strikingly, locking LFA-1 in HA conformation did not interfere with T-cell locomotion or TEM, while LFA-1 blockage perturbed TEM, reduced ICAM-1 clusters, and slowed down locomotion. HA-LFA-1 and ICAM-1 also colocalized in rings that surround T-cells during active TEM. ICAM-1 clusters were also reversibly formed during locomotion of the extravasating lymphocytes underneath the endothelial monolayer. Shear stress signals, although mandatory for lymphocyte TEM, did not further trigger HA-LFA-1 or ICAM-1 co-clustering. Collectively, our results suggest that T-cell locomotion and TEM require active clustering of endothelial ICAM-1 by chemokine triggered high affinity LFA-1 at both apical, junctional and subluminal endothelial compartments.

B22

UPAR ACTIVATES RAC VIA THE P130CAS-CRK-DOCK180 PATHWAY

HARVEY W. SMITH*,Professor Christopher J. Marshall**

**Institute of Cancer Research, London, UK; **Insitute of Cancer Research, London, UK*

The urokinase receptor (uPAR) is crucial for the migration and invasion of some cancer cell lines, and its expression is associated with poor prognosis in many cancers. uPAR also activates Rac by a potentially uPA-independent mechanism. Being GPI-anchored, uPAR uses transmembrane partners in signal transduction. A large body of evidence suggests these partners are integrins. This project aims to characterise the pathway linking uPAR, through its integrin partners, to Rac activation. Rac is activated by guanine nucleotide exchange factors (GEFs), which stimulate the exchange of GDP for GTP. Using RNA interference strategies, I have shown that the Rac GEF DOCK180 and its upstream adaptor proteins p130Cas and Crk are essential for normal morphology and motility in cancer cell lines expressing uPAR. In BE colon carcinoma cells, which require uPAR to activate Rac, and in HEK 293T cells overexpressing uPAR, DOCK180 and its upstream adaptors are required for Rac activity. uPAR also drives tyrosine phosphorylation of p130Cas and formation of p130Cas-Crk complexes in these systems. These data show that signalling through p130Cas and Crk to DOCK180 is a major mechanism of Rac activation by uPAR and suggests that this pathway is important in the Rac-driven motility of cancer cells.

B23

INHIBITION OF INTEGRIN-MEDIATED CELL ADHESION BY EPHRINA1 IS REDOX DEPENDENT

Francesca Buricchi, MARIA LETIZIA TADDEI, Elisa Giannoni, Giovanna Grimaldi, Matteo Parri, Giovanni Raugei*, Giampietro Ramponi* and Paola Chiarugi*

*Department of Biochemical Sciences, University of Florence, V.le Morgagni 50, 50134 Florence, Italy; *Center for Research, Transfer and High Education "Study at molecular and clinical level of chronic, inflammatory, degenerative and neoplastic disorders for the development of novel therapies"*

Interactions linking the Eph receptor tyrosine kinase and ephrin ligands transduce short-range repulsive signals regulating several motile biological processes including axon pathfinding, angiogenesis and tumour growth. These ephrin-induced effects are believed to be mediated by alterations in actin dynamics and cytoskeleton reorganization. The members of the small Rho GTPase family elicit various effects on actin structures and are probably involved in Eph receptor-induced actin modulation. In particular, a number of ephrin ligands lead to a decrease in integrin-mediated cell adhesion and spreading. Here we show that in prostatic carcinoma cells the ability of ephrinA1 to inhibit cell adhesion and spreading is strictly dependent on the decrease in the activity of the small GTPase Rac1. Given the recognized role of Rac-driven redox signalling for integrin function, reported to play en essential role in focal adhesion formation and in the overall organization of actin cytoskeleton, we investigated the possible involvement of oxidants in ephrinA1 signalling. We now provide evidence that Reactive Oxygen Species are an integration point of the ephrinA1/integrin interplay. Indeed H2O2 treatment or constitutive active Rac1 mutant overexpression revert the ephrinA1 induced inhibitory effect on cell migration and adhesion. We identify a redox circuitry in which the ephrinA1-mediated inhibition of Rac1 leads to a negative regulation of integrin redox signalling affecting the activity of the tyrosine phosphatase LMW-PTP. The enzyme in turn actively dephosphorylates its substrate p190RhoGAP, finally leading to RhoA activation. Taken together, our data propose a redox-based Rac-dependent upregulation of Rho activity, concurring to the inhibitory effect elicited by ephrinA1 on integrin-mediated adhesion strength. Furthermore, we speculate that through this redox-based mechanism ephrinA1 may control the migratory and invasive ability of epithelial cancer cells inducing an amoeboid motility, specifically correlated with increased Rho and decreased Rac activities.

B24

CHARACTERIZATION OF XGRIP2 - A NOVEL XENOPUS LAEVIS GERM PLASM AND PRIMORDIAL GERM CELLS SPECIFIC MRNA

KATSIARYNA TARBASHEVICH, Katja Koebernick and Tomas Pieler

GZMB, dept. Developmental Biohemistry, Göttingen, 37077, Germany

In amphibians, polarization of oocytes determines the future embryonic body plan. There are two major pathways of mRNA localization to the vegetal pole of Xenopus oocytes. The transcripts localized by the early pathway are believed to be involved in Xenopus germ line formation. Here we report on the identification and functional characterization of the new vegetally localized Xenopus mRNA xGRIP2. During oogenesis, xGRIP2 mRNA is localized via the early pathway. A single 210 nt cis-acting element, necessary and sufficient for xGRIP2 mRNA localization was mapped within its 3?UTR. UV-crosslinking experiments with this fragment display a protein binding pattern similar to another germ line specific mRNA XDead end. xGRIP2 is specifically expressed in germ plasm and PGCs throughout Xenopus embryogenesis. Loss-of-function experiments performed by injecting antisense morpholino oligonucleotides or mRNAs encoding putative dominant-negative protein fragments of xGRIP2 resulted in reduction of PGC numbers in xGRIP2-depleted embryos at tailbud stages. Misexpression of a putative xGRIP2 dominant negative protein encoding PDZ domains 2 and 3 also resulted in reduced PGC survival and interfered with the proper positioning of PGCs along the A/P axis of an embryo. Our results thus suggest that xGRIP2 is required for normal PGC development and migration in Xenopus.

B25

MICROTUBULE-BASED MECHANICAL SIGNALING ORGANIZES THE CELLULAR INTERIOR

Sven K. Vogel[1], Isabel Raabe[1], Leonardo Sacconi[2], Francesco S. Pavone[2], IVA TOLIC-NORRELYKKE[1]

1: Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany; 2: LENS, University of Florence, Italy

Microtubules (MTs) play a central role in the spatio-temporal organization of the cell. Cells of the fission yeast Schizosaccharomyces pombe have a centrally placed nucleus and divide by fission at the cell center. We have investigated the role of MTs in nuclear positioning, and the role of the nucleus in division plane positioning, by displacing the nucleus away from the cell center using optical tweezers. A nucleus displaced during interphase returned to the cell center by MT pushing against the cell tips. Nuclear displacement during late G2 phase or early prophase resulted in asymmetric cell division. These data imply that interphase MTs position the nucleus, which in turn positions the cell division plane. Furthermore, we show that the mitotic spindle is aligned with the cell axis already at the onset of mitosis, by growing along the axis of the adjacent interphase MT. This result suggests a new role for interphase microtubules: in addition to determining the nuclear and hence the division plane position, they also determine the initial alignment of the mitotic spindle.

B26

ARRESTING CELL MOVEMENT FROM THE INSIDE - A NOVEL PARADIGM OF METASTASIS FORMATION

G. VOLPI[1] , N. Bertani[1], P. Malatesta[1,2], G. Scita[3], R. Perris[1,4]

1 Department of Genetics, Microbiology and Anthropology, University of Parma, Italy; 2 Department of Oncology, Biology and Genetics, University of Genoa, Italy; 3 Institute FIRC for Molecular Oncology, IFOM, Milan, Italy; 4 National Cancer Institute, Aviano, CRO-IRCCS, Italy

Cell movement is known to be initiated by intricate molecular interplays between the outside and inside of the cell and to be propagated and guided through analogous interactions governed by environmental signals. Conversely, it is less clear how tumour cell migration may be stopped in presumptive migration-permissive host environments. Through a genetic screen we have identified two candidate genes, XTP1 and SDP35, that may act as putative internal controllers of milieu-independent cell motility arrest. Both genes are de novo expressed in primitive tumour lesions and strongly upregulated in meta-?static? formations where cell migration may have ceased. They encode proteins with non-conventional RhoGAP and DEP (Dishevelled-Egl10-Pleckstrin) homology domains through which they shuttle between nucleus and cytoplasm and differentially modulate the Rac1 and cdc42 control of cytoskeletal dynamics associated with shape changes, spreading and cell-substrate interactions conducive to motility interruption. Their putative motility-arresting role was confirmed by the impaired convergent extension movements observed during Xenopus gastrulation following spatiotemporally regulated ectopic expression of the genes. Retroviral transduction of the proteins in syngenic metastatic and non-metastatic murine tumour models are currently being exploited to clarify the molecular basis for their influence on a novel tumour spreading paradigm entailing a precise start/dissemination-stop/metastasis formation relationship.

B27

SIGNALING PATHWAYS OF ENDOTHELIAL CELL MIGRATION AND ANGIOGENESIS INHIBITED BY CHEMOPREVENTIVE MOLECULES

A. ALBINI*, N. Ferrari°, F. Tosetti°, G. Fassina °, G. Lorusso*^, N.Vannini °^, DM Noonan^

**IRCCS Multimedica, Milan, Italy; ° Laboratory of Molecular Biology National Cancer Research Institute, Genova; ^University of the Insubria, Varese, Italy*

Angiogenesis is necessary for solid tumor growth and dissemination, a promising target not only in cancer therapy but also in prevention. We have shown that various molecules, such as flavonoids, antioxidants and retinoids, act in the tumor micro-environment (1) inhibiting the recruitment and/or activation of endothelial cells and phagocytes of innate immunity. N-acetyl-cysteine, the green tea flavonoid epigallocatechin-3-gallate (EGCG), the calchone xanthoumol from bier hop, the rotenoid deguelin, the synthetic retinoid 4-hydroxy-fenretinide (4HPR) all prevent migration of endothelial cells, angiogenesis in the Matrigel sponge assay in vivo and inhibit the growth of the highly angiogenic Kaposi's sarcoma tumor cells (KS-Imm) in nude mice. Taken together, these data indicate that migration in vitro and in vivo of endothelial cells and angiogenesis are a common and key target of most chemopreventive molecules. These compounds most likely suppress the angiogenic switch in pre-malignant tumors, a concept we termed "Angioprevention". 4HPR induced members of the TGFß-ligand superfamily, which, at least in part, explains its anti-angiogenic activity. In contrast, functional genomics analyses of gene expression regulation by anti-angiogenic anti-oxidant chemoprevention compounds in primary human umbilical endothelial cells (HUVEC) in culture through Affymetrix GeneChip arrays identified overlapping sets of angiogenesis and inflammation specific genes down-regulated. NAC and the flavonoids all suppressed the IkB/NF-kB signalling pathway even in the presence of NF-kB stimulation by TNF, and showed reduced expression of many NF-kB target genes. A selective apoptotic effect on transformed cells, but not on endothelial cells, of the anti-oxidants may be related to the reduced expression of the NF-kB dependent survival factors Bcl2 and Birc5/survivin, that are selectively over-expressed in transformed cells, by these factors. Inflammation is increasingly recognized as an angiogenic staimulus in cancer, the repression of the NF-kB pathway suggests anti-inflammatory effects for the anti-oxidant compounds that may also have an indirect role in angiogenesis inhibition. For example, the green tea flavonoid EGCG inhibits inflammation-associated angiogenesis by targeting inflammatory cells, in particular neutrophils, and has proven to be chemopreventive in a pilot clincial trial for prostate cancer.

Lectures

Ralf Adams
London, UK

Frank B. Gertler
Madison, USA

Oral Presentations

Manila Boca
Milan, Italy

Session VI

POLYCYSTIN-1 INDUCES CELL MIGRATION BY REGULATING PI3KINASE-DEPENDENT CYTOSKELETAL REARRANGEMENTS AND GSK3BETA-DEPENDENT CELL-CELL MECHANICAL ADHESION

MANILA BOCA [1], Lisa D'Amato [1], Gregory G. Germino [2] and Alessandra Boletta [1].

1 Molecular Basis of PKD, Dulbecco Telethon Institute (DTI) and Dibit San Raffaele; 2 Johns Hopkins University School of Medicine, Baltimore , MD, USA

ADPKD is a common inherited disorder characterized by bilateral renal cyst formation. Mutations of PKD1 are responsible for ~85% of all cases. Polycystin-1 (PC-1), its gene product, is a large (520kDa) non-tyrosine kinase receptor involved in cell-cell/matrix interactions and in primary cilia function. We have shown that PC-1 expression in renal epithelial cells induces spontaneus tubologenesis in 3D collagen gels. We now find that PC-1 expressing cells have a scattered phenotype with profound cytoskeletal re-arrangements, suggestive of an acquired migratory phenotype. Time-lapse videomicroscopy of wound healing assays show that control cells move forward as a compact unit, while PC-1 expressing cells start pulling away from the edge as individual cells with a polarized migratory phenotype and the rate of migration is considerably higher. All these effects require PI3Kinase activity. Furthermore, in wound healing experiments PC-1 expressing cells tend to re-absorbe adherens and tight junctions quicker as compared to controls. We have found that PC-1 is able to control the turnover of cytoskeletal-associated beta-catenin and consequently adherens junctions trough the activation of Glycogen Synthase Kinase 3b directly at sites of cell-cell junctions. We propose that PC-1 induces cell migration through regulation of the actin cytoskeleton and the turnover of adherens junctions.

REGULATION OF CYTOSKELETAL SYNAMICS DURING NEURITE INITIATION AND AXON NAVIGATION

GERTLER Frank

Massachusetts Institute of technology, Cambridge, MA, USA

Extension of neurites from a spherical cell body is essential for the formation of a functional nervous system; however, the mechanisms underlying neuritogenesis are poorly understood. Ena/VASP proteins regulate actin dynamics, modulate elaboration of cellular protrusions, and function in axon navigation downstream of several guidance pathways. Surprisingly, analysis of mice lacking all three Ena/VASP proteins revealed an unexpected requirement for Ena/VASP in cortical axon fiber tract formation. When cultured, Ena/VASP-null cortical neurons failed to elaborate neurites, a step that precedes axon formation and neuronal polarization. This phenotype results from the inability of Ena/VASP-null neurons to form bundled actin filaments that comprise the core of filopodia. Neuritogenesis in Ena/VASP-null neurons can be rescued by several different approaches that induce filopodia including expression of the processive actin nucleating protein mDia2, revealing an unappreciated cellular function for filopodia. Furthermore, neurite initiation requires microtubule extension into filopodia, suggesting that interactions between actin filament bundles and dynamic microtubules within filopodia are crucial for neuritogenesis.

Lectures

Avri Ben-Ze'ev
Rehovot, Israel

Peter Friedl
Würzburg, Germany

Closing Keynote

Gerhard Christofori
Basel, Switzerland

Oral Presentations

Gilles Doumont
Amsterdam, The Netherlands

Session VII

CELL MOTILITY AT THE INVASIVE FRONT OF TUMORS: THE ROLE OF NOVEL WNT/β-CATENIN TARGET GENES

AVRI BEN-ZE'EV

Department of Molecular Cell Biology, Weizmann Institute of Science

Aberrant β-catenin signaling is prevalent in most colorectal cancer patients. β-catenin, an important cell-cell adhesion component binding cadherin receptors to the cytoskeleton, is also a key co-transcriptional activator of target genes (in complex with LEF/TCF factors) in the nucleus. Hyperactive β-catenin induces genes regulating the cell cycle and the invasion and metastasis of cancer cells. We investigated the coordination between cell-cell adhesion and signaling by the E-cadherin/β-catenin molecular complex in colon cancer development. In human colon cancer cells, we found that sparse cell cultures mimic cells at the invasive front of tumors displaying low levels of E-cadherin, but highly active nuclear β-catenin that transactivates Slug, a negative transcriptional regulator of E-cadherin. Slug is also induced during epithelial to mesenchymal transformation (EMT). In sparse cells we found high levels of ErbB1/2 and activated ERK. In contrast, dense cultures had distinct membranal E-cadherin and β-catenin, only limited β-catenin signaling, no Slug, and therefore high E-cadherin levels, but low levels of ErbB1/2 and MAPK signaling. This cell density-regulated phenotypic conversion is reminiscent of the plasticity in β-catenin and E-cadherin in the invasive versus the differentiated areas of colorectal carcinoma tissue. We identified two members of the L1 immunoglobulin-like cell adhesion receptors (normally expressed in nerve cells where they regulate neuronal guidance, fasciculation and motility), as novel target genes of β-catenin. We found these gene products exclusively localized at the invasive front of colon carcinoma tissue. Forced expression of L1 and Nr-CAM in fibroblasts induced motility, transformation, and conferred tumorigenesis in mice and, in colon cancer cells, caused liver metastasis. Suppression of L1 and of Nr-CAM in cancer cells reduced their motility and invasion into the ECM. The extracellular domain of Nr-CAM was sufficient for conferring growth in low serum by constitutive activation of MAPK/ERK and AKT, and stable expression of the shed ectodomain transformed NIH3T3 cells that also formed tumors in mice. We suggest that colon cancer cells exploit opportunistically these "neuronal" cell adhesion molecules, whose transcription is aberrantly activated by β-catenin - to promote metastasis. Since the extracellular domains of L1 and Nr-CAM are often shed by metalloproteinases, they could serve as diagnostic markers and anticancer therapy targets. We identified L1 and the metalloproteinase ADAM10 (a novel β-catenin target gene) at the invasive front of human colorectal cancer tissue.

Relevant References:
Gavert, N., Conacci-Sorrell, M., Gast, D., Schneider, A., Altevogt, P., Brabletz, T., and Ben-Ze'ev, A. L1, a novel target of β-catenin signaling, transforms cells and is expressed at the invasive front of colon cancers. J. Cell Biol. 168, 633-642 (2005).
Conacci-Sorrell, M., Kaplan, A., Raveh, S., Gavert, N., Sakurai, T., and Ben-Ze'ev, A. The shed ectodomain of Nr-CAM stimulates cell proliferation and motility, and confers cell transformation. Cancer Res. 65, 11605-11612 (2005).
Conacci-Sorell, M., Simcha, I., Ben-Yedidia, T., Blechman, J., Savagner, P., and Ben-Ze'ev, A. Autoregulation of E-cadherin expression by cadherin-cadherin interactions: the roles of β-catenin signaling, Slug and MAPK. J. Cell Biol. 163, 847-857 (2003).
Conacci-Sorrell, M., Ben-Yedidia, T., Shtutman, M., Feinstein, E., Einat, P., and Ben-Ze'ev, A. Nr-CAM is a target gene of the β-catenin/LEF-1 pathway in melanoma and colon cancer and its expression confers enhanced motility and tumorigenesis. Genes Dev. 16, 2058-2072 (2002).

IN VIVO IMAGING OF CANCER INVASION: FROM INDIVIDUAL TO COLLECTIVE CELL MIGRATION

PETER FRIEDL

Rudolf Virchow Center for Experimental Biomedicine and Dept. of Dermatology, University of Wuerzburg, Josef-Schneider-Str. 2, 97080 Wuerzburg, Germany

Cancer cell dissemination and metastasis in vivo result from a diverse set of migration strategies including individual cells and multicellular strands and clusters, referred to as collective invasion. Using 3D collagen lattices and in vivo intravital microscopy of cancer cell invasion, we have resonstructed at high resolution the subcellular location of pericellular proteolysis during the migration process, the resulting ECM remodeling, and invasion mechanism. The findings show how cell invasion and proteolytic ECM remodeling form a functional unit to generate collective cell invasion aling realigned tissue structures and further reveal novel compensation strategies that rescue single-cell migration after protease inhibitor-based treatment.

IDENTIFICATION OF GENES AND PATHWAYS REGULATING TUMOR INVASION AND METASTASIS INDUCED BY E-CADHERIN LOSS IN A CONDITIONAL MOUSE MODEL FOR ILC

DOUMONT GILLES, Derksen Patrick, De Bruin Michiel, Jonkers Jos

The Netherlands Cancer Institute (NKI) - Division of Molecular Biology, Amsterdam, The Netherlands

Invasive lobular breast carcinoma (ILC) is the second most common human breast malignancy. ILC is characterized by loss of E-cadherin expression, a cell adhesion molecule involved in maintaining epithelial integrity.
Our group has developed a highly relevant mouse ILC model based on epithelium-specific deletion of E-cadherin and p53. We intend to perform different functional genetic screens to identify genes and pathways that function downstream of E-cadherin in mammary tumorigenesis. We will perform these screens in clonal E-cadherin proficient and deficient tumor cell lines that we have derived from mammary tumors arising in our conditional mouse models. Specifically, we will screen for changes in their in vitro anoikis response (detachment-induced apoptosis), since all E-cadherin proficient cell lines are sensitive to anoikis whereas E-cadherin deficient cell lines are anoikis resistant.
Multiple independent clones are now available and tested for in vitro anoikis response and spontaneous survival on ultralow cluster plates. We are currently using these cell lines for the screens.
These studies will shed light on the genes and pathways that mediate the tumorigenic effects of E-cadherin loss in ILC, and may contribute to the identification of novel drug targets and the development of improved therapies for treatment of metastatic breast cancer.

DISTINCT MECHANISMS OF TUMOR INVASION AND METASTASIS

Andreas Wicki, François Lehembre, Mahmut Yilmaz, Stefan Grotegut, Angleika Kren, Anna Fantozzi, Lucie Kopfstein, and GERHARD CHRISTOFORI

Institute of Biochemistry and Genetics, Department of Clinical-Biological Sciences, Center of Biomedicine, University of Basel

Tumor cell invasion into the surrounding tissue can exhibit a phenotype of either single cell or collective cell migration. Single cell migration is mainly dependent on signaling pathways within migrating cells themselves and is usually accompanied by an epithelial-mesenchymal transition (EMT), which involves several genetic and epigenetic alterations including the loss of E-cadherin and the gain of N-cadherin expression (the cadherin switch). Gene expression profiling experiments together with biochemical analysis reveal that the distinct stages of EMT are tightly regulated by epistatic cascades of transcriptional control circuits involving activation and repression of a large number of genes that modulate the migratory and invasive behavior of tumor cells.

In contrast, collective cell migration requires the maintenance of cell-cell adhesion and a certain multicellular organization of the tumor tissue. Recently, we have shown that the expression of podoplanin, a small mucin-like protein, is upregulated in a number of human carcinomas, in particular squamous cell carcinomas. We have investigated podoplanin function in cultured human breast cancer cells, in a mouse model of pancreatic β cell carcinogenesis, and in human cancer biopsies. Podoplanin induces tumor cell spreading, migration and invasion in vitro and in vivo by a novel molecular pathway that does not involve the loss of E-cadherin function or EMT. Furthermore, it induces actin cytoskeleton rearrangement and the formation of filopodia by modulating the activities of Rho-family GTPases, which ultimately leads to collective cell invasion, a phenotype often observed in human squamous cell carcinomas. Finally, we propose a third type of metastatic tumor cell dissemination which involves an upregulated expression of lymphangiogenic factors, such as vascular endothelial growth factors C and D, and the subsequent induction of lymphangiogenesis. Increased lymphatic vessel density within and around expanding tumors leads to lymph node metastasis, most likely by a passive washing out and trapping of tumor cell clusters in regional lymph nodes.

In conclusion, we propose the existence of at least three distinct mechanisms of metastatic tumor cell invasion and dissemination: single cell invasion involving EMT, collective cell migration in the absence of EMT, and the lymphogenic dissemination to local lymph nodes.

Ralf H. Adams
Vascular Development Laboratory,
Cancer Research UK London Research Institute,
London, UK
ralf.adams@cancer.org.uk

Avri Ben-Ze'ev
The Lunenfeld-Kunin Professor of Cell Biology and Genetics,
Department of Molecular Cell Biology
The Weizmann Institute of Science
Rehovot, ISRAEL
avri.ben-zeev@weizmann.ac.il
http://www.weizmann.ac.il/mcb/avri/

Marie-France Carlier
Dynamique du Cytosquelette, LEBS, CNRS
Gif-sur-Yvette, France
carlier@lebs.cnrs-gif.fr

Gerhard Christofori
Institute of Biochemistry and Genetics
Department of Clinical-Biological Sciences (DKBW)
University of Basel
Basel, Switzerland
gerhard.christofori@unibas.ch
http://www.unibas.ch/dbmw/biochemie

Peter Friedl
Molecular Cell Dynamics Laboratory
Rudolf Virchow Center for Experimental Biomedicine and
Department of Dermatology, University of Würzburg
Würzburg, Germany
peter.fr@mail.uni-wuerzburg.de

Elaine Fuchs
Rebecca C. Lancefield Professor
Investigator
Howard Hughes Medical Institute, Laboratory of Mammalian Cell Biology and Development, The Rockefeller University
New York, USA
fuchs@mail.rockefeller.edu

Frank Gertler
Massachusetts Institute of Technology,
Cambridge, MA, USA
fgertler@mit.edu

Gregg G. Gundersen
Department of Anatomy & Cell Biology, Columbia University
New York, USA
Ggg1@columbia.edu

Klaus Hahn
UNC Department of Pharmacology, University of Virginia
Chapel Hill, NC, USA
khahn@med.unc.edu

Dorit Hanein
Burnham Institute for Medical Research
La Jolla, USA
fuchs@mail.rockefeller.edu

Pekka Lappalainen
Institute of Biotechnology
University of Helsinki
Helsinki, Finland
pekka.lappalainen@helsinki.fi

Stefan Linder
Institut für Kreislaufkrankheiten
München, Germany
stefan.linder@med.uni-muenchen.de

Denise Montell
Department of Biological Chemistry, Center for Cell Dynamics
Johns Hopkins School of Medicine
Baltimore, USA
dmontell@jhmi.edu

Catherine Nobes
Depts. of Physiology and Biochemistry, School of Medical Sciences, University of Bristol
Bristol, UK
catherine.nobes@bristol.ac.uk

Erez Raz
Germ Cell Development
Max-Planck-Institute for Biophysical Chemistry
Goettingen, Germany
eraz@gwdg.de

Shiro Suetsugu
Frontier Research Program for Young Investigators, Institute of
Molecular and Cellular Biosciences, the University of Tokyo.
Tokyo, Japan
http://www.iam.u-tokyo.ac.jp/suetsugu/

Cornelis J Weijer FRSE
Wellcome Trust Biocentre, University of Dundee
Dundee, UK
c.j.weijer@dundee.ac.uk

Last update: May 2, 2007