

The 2012 METRAPOLIS



Membrane Trafficking and Polarity Interest Symposium

November 27th 2012

Albert Einstein College of Medicine

Seminars

8:45AM - 4:00PM

LeFrak Auditorium

Price Center

Poster Session

4:30PM - 6:00PM

Lubin Dining Hall

Mazer Building

Dear METRAPOLIS 2012 attendees,

We are happy that you will be joining us on November 27th 2012 at Albert Einstein College of Medicine.

As this is the birth year of our symposium, we hope that this event becomes a fulfilling experience for all of us. It is because of your own responsiveness and enthusiasm to share in this meeting that we are able to have a wonderful and strong group of scientists together under one roof in the Bronx. Over the course of this first METRAPOLIS gathering, we will have an opportunity to listen to nine excellent trafficking and polarity investigators, learn about new developments in the field from the work of students and postdoctoral fellows, and have an ample opportunity to discuss our research interests. We genuinely hope that on the day of the symposium, all the participants get a chance to discover something new and share ideas and perspectives of their own.

We look forward to seeing you.

Your METRAPOLIS 2012 Organizers,
Dawn Fernandez, Aleksandr Treyer, and Ryan Schreiner

The best way to have a good idea is to have a lot of ideas
–Linus Pauling

Links and Contact Information

Directions and Parking Information:

<http://www.einstein.yu.edu/visitors/>

Campus Map:

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Contact Information:

Aleksandr Treyer and Dawn Fernandez:

Telephone: 718-430-3509

Email: Metrapolis2012@gmail.com

We would like to thank our sponsors, the American Society for Cell Biology, and both The Sue Golding Graduate Division and The Cancer Center of Albert Einstein College of Medicine for their assistance in funding this symposium. We also thank Dr. Anne M \ddot{u} sch and Dr. Victoria Freedman for their mentorship and Carolyn Owens in the Graduate Division for her organizational assistance.

METRAPOLIS 2012

Membrane Trafficking and Polarity Interest Symposium

Tuesday, November 27 2012

Price Center LeFrak Auditorium

- 8:15am - 8:45am ***Check-In and Breakfast/Meet & Greet- Price Center Auditorium***
- 8:45am – 9:00am Welcome & Plan for the day- Dawn Fernandez and Aleksandr Treyer
Opening Remarks- Dr. Allen Spiegel, *The Marilyn and Stanley M. Katz Dean*
- 9:00am - 9:30am **Dr. Jeremy Nance**, Associate Professor in the Department of Cell Biology (The Skirball Institute) and Developmental Genetics at New York University- “Mechanisms of primordial gonad assembly during *C. elegans* embryogenesis”
- 9:45am - 10:15am **Dr. Sergei Sokol**, Professor in the Department of Developmental and Regenerative Biology at the Mount Sinai School of Medicine- “Cell polarity and signaling in vertebrate embryogenesis”
- 10:30am -11:00am **Dr. Marek Mlodzik**, Professor and Chair of the Department of Developmental and Regenerative Biology at the Mount Sinai School of Medicine- "Inter- and Intracellular Regulation of Wnt/Planar Cell Polarity Signaling"
- 11:00am -11:15am ***Coffee Break- Lower Level outside of Price Auditorium***
- 11:15am - 11:45am **Dr. Qais Al-Awqati**, Robert F. Loeb Professor of Medicine and Professor of Physiology and Cellular Biophysics at Columbia University-"Differentiation of Epithelial Cells"
- 12:00m - 12:30pm **Dr. Michael Caplan**, C. N. H. Long Professor and Chair of the Department of Cellular and Molecular Physiology at Yale University- “Playing in Traffic: New Pathways and Signals in Epithelial Polarity”
- 12:45pm -1:30pm ***Lunch- Lower Level outside of Price Auditorium***
- 1:30pm – 2:00pm **Dr. Enrique Rodriguez-Boulan**, Charles and Margaret Dyson Professor in Ophthalmology Research, and Professor of Cell and Developmental Biology at Weill Cornell Medical College- “Polarized trafficking in epithelial cells”
- 2:15pm - 2:45pm **Dr. Geri Kreitzer**, Associate Professor of Cell and Developmental Biology at Weill Cornell Medical College- “Kinesin family motors in polarization of epithelial cells”
- 3:00pm - 3:30pm **Dr. Gregg Gundersen**, Professor in the Departments of Anatomy and Cell Biology, and Pathology at Columbia University- “Organizing the cell from the inside-out: The role of nuclear positioning in cell polarity”
- 3:30pm - 4:00pm **Dr. Elaine Fuchs**, Investigator for the Howard Hughes Medical Institute, and Rebecca C. Lancefield Professor in the Laboratory of Mammalian Cell Biology and Development at the Rockefeller University- “Regulating Polarity in Epidermal Morphogenesis”
- 4:00pm - 4:15pm **Concluding Remarks**
- 4:30pm - 6:00pm ***Poster Session & Reception- Lubin Dining Hall – Mazer Building***

Poster Session- Lubin Dining Hall 4:30- 6:00PM (Set Up 4:00-4:30)

<u>Presenter</u>	<u>Poster Title</u>
Bipul R Acharya	KIF17 activity is regulated by its C-terminal tail domain and by EB1
Diane Santana Alves	AS160: a new Na,K-ATPase partner that regulates the trafficking of the sodium pump in response to energy depletion and renal ischemia.
Frank Batista	Complex N-glycans are Essential for Spermatogenesis
Francisco Lazaro-Diequez	Protein sorting in the direct and indirect apical targeting pathways: simultaneous sorting of apical and basolateral cargo in the transcytotic pathway
Ellen Ezratty	An Emerging Role for Arl/Arf Family GTPases in Regulation of Cilia Function during Notch Signaling and Epidermal Differentiation
Jeremy Fagan	Characterization of CG10732, a Novel Drosophila Rho Kinase Substrate Candidate and its Potential Role in Planar Cell Polarity Signaling
Dawn Fernandez	Identification of novel Par1b substrates that regulate cell-extracellular matrix signaling
Allison Gilder	Role of Calcineurin in Polycystin protein trafficking to the primary cilium in LLCPK cells
Keiji Itoh	GEF-H1 regulates myosin function and morphogenetic movements during neural tube closure
Kyeongmi Kim	Rab11 Regulates Planar Polarity and Migratory Behavior of Multiciliated Cells in Xenopus Embryonic Epidermis
Diana Klompstra	Identifying mechanisms of contact-mediated cell polarization
Xin Li	The adhesion GPCR Gpr125 modulates Dishevelled distribution and planar cell polarity signaling
Adrian Santos-Ledo	Identification of Rho Kinase Substrates and their Role During Zebrafish Morphogenesis
Sandra Veronica Lopez	Blood Brain Barrier model to test permeability of nanoparticles designed to target brain tumors
Sam Maung	Identifying Frl as a putative Rho Kinase substrate and down stream effector protein of the non-canonical Wnt PCP pathway in <i>Drosophila</i>
Jeannette Nachbar	The Role of KifC3 in Mitosis
Rashmi Nanjundappa	Role of Klarsicht, a KASH domain protein, in collective migration of the Drosophila embryonic salivary gland
Olga Ossipova	Neural crest specification by non-canonical Wnt signaling and the polarity protein PAR-1
Kimberly Reidy	Podocyte Deletion of LKB1 Results in Increased Severity of Diabetic Nephropathy in Mice
Pramodh Seneviratne	Modulation of ERR α Nuclear Localization by KIF17
William Smith	Regulation of vesicle transport to the apical membrane by kinesins in epithelial cells
Sozanne Solmaz	A flexible mid-plane ring in the nuclear pore complex
Sunandini Sridhar	The lipid kinase PI4KIII β regulates lysosomal efflux
Emily Stoops	Biosynthetic sorting of the sodium pump: Visualization of the segregation of newly synthesized epithelial Na,K-ATPase from apically directed proteins
Randy Stout	Organization of the gap junction Nexus: Looking beyond intercellular communication to roles in tissue organization and function
Aleksandr Treyer	Deciphering protein sorting itineraries at the trans-Golgi network of polarized epithelial cells
Ann Wehman	The lipid flippase TAT-5 inhibits the budding of extracellular vesicles from the surface of <i>C. elegans</i> embryos
Hua-Xing Wei	Mammalian GDP-Fucose Transporters Required for Notch Signaling
Yuliya Zilberman	A novel role of Rho GTPase PAC-1 in <i>C. elegans</i> epithelial adherens junctions
Hong Zhu	Mutation of SIMPLE in Charcot-Marie-Tooth 1C Alters Production of Exosomes

Presenters by Laboratory and Affiliation

<u>Presenter</u>	<u>Laboratory</u>	<u>Institution</u>
Bipul R Acharya	Geri Kreitzer	Weill Cornell Medical College
Diane Santana Alves	Michael Caplan	Yale University
Frank Batista	Pamela Stanley	Albert Einstein College of Medicine
Francisco Lazaro-Diequez	Anne Müsch	Albert Einstein College of Medicine
Ellen Ezratty	Elaine Fuchs	Rockefeller University
Jeremy Fagan	Andreas Jenny	Albert Einstein College of Medicine
Dawn Fernandez	Anne Müsch	Albert Einstein College of Medicine
Allison Gilder	Michael Caplan	Yale University
Keiji Itoh	Sergei Sokol	Mount Sinai School of Medicine
Kyeongmi Kim	Sergei Sokol	Mount Sinai School of Medicine
Diana Klompstra	Jeremy Nance	New York University
Xin Li	Florence Marlow	Albert Einstein College of Medicine
Adrian Santos-Ledo	Andreas Jenny	Albert Einstein College of Medicine
Sandra Veronica Lopez	David Spray	Albert Einstein College of Medicine
Sam Maung	Andreas Jenny	Albert Einstein College of Medicine
Jeannette Nachbar	Anne Müsch	Albert Einstein College of Medicine
Rashmi Nanjundappa	Mon Mon Myat	Weill Cornell Medical College
Olga Ossipova	Sergei Sokol	Mount Sinai School of Medicine
Kimberly Reidy	Kimberly Reidy	Albert Einstein College of Medicine
Pramodh Seneviratne	Geri Kreitzer	Weill Cornell Medical College
William Smith	Geri Kreitzer	Weill Cornell Medical College
Sozanne Solmaz	Gunter Blobel	Rockefeller University
Sunandini Sridhar	Anna Maria Cuervo	Albert Einstein College of Medicine
Emily Stoops	Michael Caplan	Yale University
Randy Stout	David Spray	Albert Einstein College of Medicine
Aleksandr Treyer	Anne Müsch	Albert Einstein College of Medicine
Ann Wehman	Jeremy Nance	New York University
Hua-Xing Wei	Pamela Stanley	Albert Einstein College of Medicine
Yuliya Zilberman	Jeremy Nance	New York University
Hong Zhu	Chi-Wing Chow	Albert Einstein College of Medicine

KIF17 activity is regulated by its C-terminal tail domain and by EB1

Bipul R Acharya, Jessica Morgan and Geri Kreitzer

Department of Cell and Developmental Biology, Joan and Stanford I. Weill Medical College, Cornell University New York, NY 10065, USA.

KIF17 is a kinesin-2 family, plus-end directed microtubule (MT) motor that interacts with EB1 and APC and contributes to MT stabilization and polarization in epithelial cells. To gain insight into the mechanism by which KIF17 participates in MT stabilization, and to elucidate the functional significance of its interaction with EB1, we analyzed KIF17 activities in the absence or presence of EB1 *in vitro*. Purified, full-length EB1 enhanced the steady-state MT-stimulated ATPase activity of KIF17 motor domain 2.0 fold and increased the number of motility events as observed in MT gliding assays. This EB1-mediated increase in ATPase and MT gliding activity was abrogated by addition of purified KIF17 Tail domain, consistent with a role of head-tail interactions in regulating kinesin activity. Interestingly, we found EB1 and the KIF17 tail domain compete for binding to the KIF17 catalytic motor domain. In two *in vitro* assays, the KIF17 catalytic domain was sufficient to protect MTs from depolymerization. This MT stabilizing activity was also enhanced by EB1 but was unaffected by the KIF17 tail domain. Consistent with *in vitro* MT stabilization assays, we also found that overexpressed KIF17 motor domain was sufficient to stabilize MTs in MDCK epithelial cells. As expected, the KIF17 motor domain co-sedimented with MTs. Unexpectedly however, we also found that the KIF17 tail domain co-sediments with MTs and indeed, bound with higher affinity to MTs than the motor domain. This suggests that the KIF17 tail, in addition to regulating the ATPase activity of the motor, may also contribute to binding of the holoenzyme to MTs. Furthermore, the KIF17 tail domain also interacts with soluble tubulin heterodimers, suggesting this kinesin could potentially regulate MT dynamics by dual mechanisms in cells. In support of this, we also found that expression of the KIF17 tail domain in cells transiently attenuated MT regrowth after washout of MT depolymerizing drugs, possibly by sequestering tubulin dimers. The emerging role of various kinesins in disease raises the possibility of using them as targets for treatment and therapy but very little is known about the intrinsic biochemical properties of each kinesin contribute to determining patterns of motility and patterns of microtubule dynamics within eukaryotic cells. Our result suggested some unique control of KIF17 motor and tail domain over MT growth and stabilization needs further research to explore its novel regulation in microtubule dynamics.

AS160: a new Na,K-ATPase partner that regulates the trafficking of the sodium pump in response to energy depletion and renal ischemia.

Alves, DS¹, Thulin G², Loffing, J³, Kashgarian M² and Caplan MJ¹.

¹ Department of Cellular and Molecular Physiology. Yale University.

² Department of Pathology. Yale University.

³ Institute of Anatomy. University of Zurich.

The Na,K-ATPase is the major active transport protein found in the plasma membranes of most epithelial cell types. The Na,K-ATPase appears to be located in two different cellular pools. The major sodium pump pool is located at the plasma membrane, while the other pool resides in cytoplasmic vesicular compartments which can translocate to the plasma membrane in response to physiological stimuli. We have identified AS160 as a new Na,K-ATPase partner. AS160 is a Rab-GTPase-activating protein (Rab-GAP) that regulates the trafficking of the glucose transporter 4 (GLUT4) in response to insulin and muscle contraction. We have shown that AS160 interacts with the large cytoplasmic NP domain of the α -subunit of the Na,K-ATPase. We also demonstrated that the knockdown of AS160 increases the level of cellular Na,K-ATPase activity, suggesting that AS160 modulates the activity of the enzyme. In the present study we characterized aspects of the physiological role of the interaction between AS160 and Na,K-ATPase. We find that AS160 is required for the intracellular accumulation of the Na, K-ATPase that occurs in response to energy depletion in cultured epithelial cells. The Rab-GAP activity of AS160 is negatively regulated by phosphorylation at S558. We find that, following energy depletion, AS160 is no longer phosphorylated at S588, suggesting that the activation of AS160 by dephosphorylation may play a role in energy depletion-induced Na,K-ATPase accumulation in cytoplasmic compartments. In intact animals ischemic kidney injury has been shown to induce internalization of the Na,K-ATPase from the plasma membranes of

renal epithelial cells. We assessed the distribution of the Na,K-ATPase following renal ischemia and reperfusion in kidneys from wild type and AS160 knockout mice. We observed a substantial decrease in the injury-induced cytoplasmic accumulation of the Na,K-ATPase in the renal epithelial cells of the AS160 knockout mice as compared to those of the wild type controls. In conclusion, we propose that AS160 plays a key role in regulating the redistribution of the Na,K-ATPase that occurs in response to energy depletion and renal ischemia.

Complex N-glycans are Essential for Spermatogenesis

Frank Batista and Pamela Stanley

Dept. Cell Biology, Albert Einstein College Medicine, New York, NY, USA

Complex N-glycans are generated in the Golgi following the action of MGAT1 (GlcNAcT-I), the enzyme that transfers GlcNAc to initiate complex N-glycan synthesis. This class of glycans is essential for life. Mouse embryos that lack MGAT1 cannot survive beyond embryonic day 9.5. We have investigated the role of MGAT1 during spermatogenesis using conditional deletion in spermatogonia via a testis-specific *Cre* recombinase transgene driven by the *Stra8* promoter (*Stra8-iCre*). The deletion efficiency of MGAT1 floxed alleles was 100%, as shown by transmission of the deleted allele by heterozygous mice. Males in which the synthesis of complex N-glycans was blocked by deletion of *Mgat1*, did not produce sperm. Sertoli cells and spermatocytes appeared normal on histological analyses, while most spermatids formed giant multinucleated cells associated with increased apoptosis. The phenotype became visible during the first wave of spermatogenesis at 22-26 days post-partum, when spermatids begin to appear. The mutant mice failed to produce complex N-glycans as shown by lectin histochemistry of testis sections using L-PHA and GSA. Western-blot analyses using a monoclonal antibody against basigin, an N-glycoprotein expressed in elongated spermatids, combined with PNGase F or Endo H digestions, confirmed that complex N-glycans were not detectable in mutant mice, and revealed that a small fraction of basigin in wild-type mice remained modified with high-mannose glycans. This high mannose fraction is consistent with the presence of an inhibitor of MGAT1 that is expressed in spermatocytes. The inhibitor is a Golgi glycoprotein termed GlcNAcT-I Inhibitory Protein (GnT1IP). A membrane bound form (GnT1IP-L) is the active and specific inhibitor and it is expressed in spermatocytes of adult mice. We are investigating the hypothesis that down-regulation of complex N-glycans in spermatocytes is required for their interactions with Sertoli cells and the progression of spermatogenesis.

Supported by grant RO1 30645 to PS.

Protein sorting in the direct and indirect apical targeting pathways: simultaneous sorting of apical and basolateral cargo in the transcytotic pathway.

Lázaro-Dié́guez F, Treyer A, Müsch A.

Department of Developmental and Molecular Biology, Albert Einstein College of Medicine, Bronx, NY, USA.

Epithelial cells employ two strategies for apical/luminal protein transport in the secretory pathway. Columnar-epithelial cells, such as those of the kidney, utilize predominantly the direct route where proteins are transported from the *trans*-Golgi (TGN) network to the apical membrane. By contrast, single-spanning membrane proteins in hepatic cells are instead targeted from the TGN to the basolateral surface and delivered to the luminal domain (bile canaliculus) upon endocytosis in a process referred to as transcytosis (i.e. indirect targeting). Here we have visualized the sorting and trafficking of apical (DPPIV) and basolateral (VSV-G) cargo. Live dual-confocal cell imaging reveals simultaneous TGN sorting of apical and basolateral cargo in primary cultures of hepatocytes and hepatic WIFB9 cells in a different domains of common tubulovesicular structures, while in columnar, kidney-derived MDCK cells both proteins are segregated into independent transport carriers. Unexpectedly, the fusion events of the apical and basolateral carriers were observed by dual-TIRF microscopy in different domains of the plasma membrane in WIFB9 cells. Further analysis of the hepatic common tubulovesicular structures by immuno-EM and STORM superresolution microscopy shows the segregation of the

apical and basolateral carriers. Interestingly the formation of the common tubulovesicular structures was prevented by microtubule depolymerization. These findings suggest that the direct and indirect apical pathways differ in their ability to segregate apical from basolateral proteins at the level of the TGN and the putative sorting of apical and basolateral cargo in independent carriers through a common pool of microtubules in hepatic cells.

An Emerging Role for Arl/Arf Family GTPases in Regulation of Cilia Function during Notch Signaling and Epidermal Differentiation

Ezratty, EJ; Stokes, N and Fuchs, E.

The Rockefeller University, NYC, 10065

The primary cilium is a microtubule-based cellular “antenna” that can sense the extracellular environment, transmit developmental signals, and influence cell-cycle progression. Primary cilia play at least two temporally and spatially distinct roles in balancing growth and differentiation during skin development: a novel, early role in epidermis, whose morphogenesis relies upon Notch signaling; and a later role in hair follicles, reliant upon Sonic Hedgehog signaling (Ezratty *et al* Cell 2011). We’ve found that ciliogenesis requires proper epidermal polarity and matrix adhesion, but occurs upstream of Wnt, Shh and Notch-mediated fate specification. Employing reagents to eliminate cilia in cultured primary mouse keratinocytes (1°MKs) and embryos, we show that Kinesin II and Ift74 ciliary-mutants display altered stratification, differentiation and Notch-signaling. Interestingly, epidermal Notch-receptor and Notch-processing-enzymes co-localize to ciliary structures at sites of induced Notch-signaling, and ciliary-mutants show diminished nuclear Notch-intracellular-domain and Notch-reporter activity. The molecular mechanisms underlying cilia-mediated control of Notch signaling during epidermal morphogenesis are unknown, but localization of Notch components to ciliary structures suggests that spatial and temporal activation of Notch signaling could be directly modulated by primary cilia. We examined a panel of ciliopathy-related small GTPases that have been implicated in ciliary targeting or polarized exocytosis to the basal body and used shRNA-mediated knockdown (KD) to ascertain their potential function during epidermal morphogenesis. KD of Arf/Arl GTPase family members reduced both Notch reporter activity and induction of differentiation in 1°MKs. We utilized ultrasound guided *in utero* injection of lentivirus to KD a subset of Arf/Arl family GTPases in developing epidermis, and found that Notch components were mislocalized to specific intracellular structures and epidermal differentiation was subsequently diminished. These data suggest that GTPase-dependent polarized exocytosis may be required for the localization of Notch signaling components to the basal body/cilia, where they could function to directly influence the temporal and spatial regulation of Notch signaling during epidermal differentiation. Time-lapse imaging studies are being employed to characterize the dynamics of Notch components during ciliogenesis and to directly determine whether targeting of Notch components to ciliary structures is required for proper epidermal morphogenesis.

Characterization of CG10732, a Novel *Drosophila* Rho Kinase Substrate Candidate and its Potential Role in Planar Cell Polarity Signaling

Jeremy K.Fagan¹ Qiheng Lu², Paul Adler² & Andreas Jenny^{1, 3}

1. Department of Developmental and Molecular Biology, Albert Einstein College of Medicine, Bronx NY 10461

2. Department of Biology, University of Virginia, Charlottesville, VA 22903

3. Department of Genetics, Albert Einstein College of Medicine, Bronx, NY 10461

Cellular polarization is essential for nutrient transport, cell-cell communication and other cellular processes including spindle orientation during cell division, cell migration and cell differentiation. In addition to apical-basal polarity, polarity across the plane of an epithelium is a fundamental phenomenon required for the formation of complex tissues. This phenomenon is known as Planar Cell Polarity (PCP) and is controlled by the non-canonical Wnt signaling pathway. While some are known, most downstream effectors of the PCP pathway remain elusive. Particularly, it is not understood how

Rho kinase, a known PCP effector also implicated in tumor cell migration, exerts its function. In a genome-wide Rho kinase substrate screen we identified CG10732 as a novel candidate Rho Kinase substrate. RNAi knockdown of CG10732 in the *Drosophila* wing yielded a multiple wing hair phenotype, indicative of a defect in planar cell polarity signaling. Using mass spectrometry, we have identified two sites of Rho Kinase phosphorylation and site-directed mutagenesis has confirmed that these sites are bona-fide sites of Drok phosphorylation. Using a Yeast-2-Hybrid approach, we have identified the planar cell polarity effector Multiple wing hair (Mwh) as a CG10732 interacting protein. Co-immunoprecipitation will confirm this interesting protein-protein interaction. The generation of a CG10732 homozygous viable null mutant via homologous recombination was successfully achieved. However, we were not able to phenocopy the initial multiple wing hair RNAi phenotype. Excitedly, the generation of UAS overexpression lines of both isoforms of CG10732(RA and RB) have been successfully generated with a strong multiple wing hair phenotype observed when combined to wing specific Gal4 drivers. Future work will be dedicated to the combination of the CG10732 knockout with *drok* and *mwh* mutant alleles and genetic interaction assays with PCP effector mutant alleles and PCP overexpression lines in combination with these newly derived UAS-CG10732 transgenic flies, respectively.

Identification of novel Par1b substrates that regulate cell-extracellular matrix signaling

D. M. Fernandez, and A. Müsch

Department of Developmental and Molecular Biology, Albert Einstein College of Medicine

The serine/threonine kinase Par1b is well known for its role in regulating epithelial cell polarity and morphology. Recent evidence suggests that Par1b mediates its function as polarity determinant in part by regulating cell-extracellular matrix (ECM) signaling, which includes the process of cell spreading. How Par1b regulates ECM signaling mechanisms remains to be explored in full detail. We conducted an unbiased screen for novel Par1b substrates in polarized kidney-derived MDCK cells. The identification strategy was based on a chemical genetics approach in which the ATP-binding pocket of a kinase was altered to accommodate bulky ATP-analogues that when offered as phosphate donor in complex mixtures can only be utilized by the engineered kinase to selectively phosphorylate its substrates. The ATP-analogues contain a chemical affinity tag that is transferred onto the substrates and allows their isolation along with the identification of the phosphorylation sites by LC-MS/MS analysis. Using this approach, we have identified a total of 58 novel candidate substrates, as well as 5 known substrates. According to their gene ontology association, more than half belonged to categories, which are known to regulate Cell-ECM interactions. From those novel candidates, we have validated 18 as Par1b substrates. We are currently exploring how regulation of these substrates by Par1b effects cell spreading.

Role of Calcineurin in Polycystin protein trafficking to the primary cilium in LLCPK cells

Allison Gilder¹, Hannah C. Chapin³, Michael J. Caplan^{1,2}

¹ Department of Cell Biology, Yale University School of Medicine

² Department of Cellular and Molecular Physiology, Yale University School of Medicine

³ Department of Biochemistry, University of Washington

Pathological mutations in PKD1 and PKD2 cause Autosomal Dominant Polycystic Kidney Disease (ADPKD). PKD1 and PKD2 code for Polycystin 1 (PC1) and Polycystin 2 (PC2), respectively. PC1 and PC2 traffic to the primary cilium in epithelial cells, including LLCPK cells. They assemble with one another to form a complex that may mediate aspects of ciliary signaling. It has been shown that, in both ciliated and non-ciliated cell lines, expression of PC2 is necessary in order to permit PC1 to traffic to the cell surface membrane. To identify potential regulatory partners of PC1, we conducted a genome wide siRNA knockdown screening study in HEK 293T cells. This analysis suggested that knockdown of the calcium dependant protein phosphatase, calcineurin, decreased PC1 cell surface localization. Interestingly, in *c. elegans*, dephosphorylation of PC2 by calcineurin has been shown to be necessary for PC2 trafficking to the male sensory neuronal cilia. We find that inhibition of calcineurin through Cyclosporine A treatment in LLCPK cells produced a decrease of PC1 and PC2 accumulation in the primary cilium. In addition, Cyclosporine A-treated zebrafish embryos develop a curly tail phenotype that is characteristic of zebrafish in which

functional expression of PKD1 a/b or PKD2 has been disrupted. We hypothesize that calcineurin may play a role in transport of the PC1/PC2 complex to the primary cilium, perhaps by mediating the dephosphorylation of PC2.

GEF-H1 regulates myosin function and morphogenetic movements during neural tube closure

Keiji Itoh and Sergei Y. Sokol

Department of Developmental and Regenerative Biology, Mount Sinai School of Medicine, New York

Neural tube defects are among the most common developmental abnormalities in vertebrates, yet the molecular processes involved in neural tube closure are poorly understood. Among molecules widely implicated in the control of cell shape and behavior are small GTPases of the Rho family. Here we show that GEF-H1/Lfc, a guanine nucleotide exchange factor for RhoA, acts to regulate vertebrate neural tube closure. Depletion of GEF-H1 with specific antisense morpholino oligonucleotides (MO) caused defective apical constriction and dorsal convergence in *Xenopus* embryos. Supporting these observations, Sox2-positive neural plate remained wider at the injected side. Moreover, both the ectodermal and mesodermal layers were significantly thicker at the injected side, consistent with a novel role for radial intercalation (epiboly) in dorsal convergence. These effects were rescued by human GEF-H1 RNA. Strikingly, neural tube closed more readily in neuroectoderm overexpressing active GEF-H1, suggesting that GEF-H1 promotes the speed and/or efficiency of neural fold formation. Ectopic GEF-H1 triggered vigorous apical constriction in embryonic ectoderm that was accompanied by Myosin II light chain phosphorylation and required Rho associated kinase (ROCK). The apical constriction caused by GEF-H1 was suppressed by the polarity kinase PAR-1/MARK, suggesting a molecular link between epithelial polarity proteins and apical constriction. These findings demonstrate a role for epiboly in dorsal convergence and reveal a specific function for GEF-H1 in morphogenetic processes that accompany neural tube closure.

Rab11 Regulates Planar Polarity and Migratory Behavior of Multiciliated Cells in *Xenopus* Embryonic Epidermis

Kyeongmi Kim¹, Blue B. Lake¹, Tomomi Haremaki², Daniel C. Weinstein², and Sergei Y. Sokol¹

¹Department of Developmental and Regenerative Biology, Mount Sinai School of Medicine, New York, New York

²Department of Biology, Queens College, CUNY, Flushing, New York

Background: *Xenopus* embryonic skin is composed of the superficial layer with defined apicobasal polarity and the inner layer lacking the apical domain. Multiciliated cells (MCCs) originate in the inner layer of the epidermal ectoderm and subsequently migrate to the surface. How MCCs acquire the apicobasal polarity and intercalate into the superficial layer during neurulation is largely unknown. As Rab11-dependent vesicle trafficking has been implicated in ciliary membrane assembly and in apical domain formation in epithelial cells, we assessed the involvement of Rab11 in MCC development. Results: Here we report that Rab11 is specifically enriched and becomes apically polarized in skin MCCs. Interference with Rab11 function by overexpression of a dominant negative mutant or injection of a specific morpholino oligonucleotide inhibited MCC intercalation into the superficial layer. Dominant negative Rab11-expressing MCC precursors revealed intrinsic apicobasal polarity, characterized by the apical domain, which is not normally observed in inner layer cells. Despite the presence of the apical domain, the cells with inhibited Rab11 function were randomly oriented relative to the plane of the tissue, thereby demonstrating a defect in planar polarity. Conclusions: These results establish a requirement for Rab11 in MCC development and support a two-step model, in which the initial polarization of MCC precursors is critical for their integration into the superficial cell layer.

Identifying mechanisms of contact-mediated cell polarization

Diana Klompstra, Dorian Anderson, and Jeremy Nance

Developmental Genetics Program, Skirball Institute, NYU School of Medicine

During gastrulation, cells move to a position in the embryo that is appropriate for the type of tissue or organ that they will form. The directional movements of gastrulation are facilitated by a polarity that early embryonic cells acquire that allows them to asymmetrically localize cytoskeletal components. The polarity of early embryonic cells is determined by cell-cell contacts, which restrict the PAR polarity proteins PAR-3, PAR-6, and PKC-3 to contact-free surfaces. The goal of my project is to determine how cell contacts induce the PAR protein asymmetries that polarize early embryonic cells, preparing them for gastrulation.

We previously identified the RhoGAP protein PAC-1 as an upstream regulator that is required to exclude PAR proteins from contacted surfaces of early embryonic cells. PAC-1 itself is recruited specifically to sites of cell contact, and directs PAR protein asymmetries by inhibiting the Rho GTPase CDC-42. How PAC-1 is able to sense where contacts are located and localize specifically to these sites is unknown. We identified an N-terminal fragment of PAC-1 that is sufficient for localization to cell contacts, and showed that localization of this fragment depends on HMR-1/E-cadherin. We show that HMP-1/ α -catenin and JAC-1/p120-catenin that interact with the HMR-1/E-cadherin cytoplasmic tail function redundantly to recruit the PAC-1 N-terminal domain. We also identified a novel adaptor protein that functions to physically link the PAC-1 N-terminus to the cadherin-catenin complex. In contrast to the PAC-1 N-terminus, full-length PAC-1 can localize to cell contacts when HMR-1/E-cadherin is removed, indicating that a redundant signal functions with HMR-1/E-cadherin to recruit PAC-1 to contacts. These findings provide insight into how a polarity regulator is spatially segregated to a subdomain of the cortex to polarize cells.

The adhesion GPCR Gpr125 modulates Dishevelled distribution and planar cell polarity signaling

Xin Li^{1, 5}, Diane S. Sepich², Mingwei Ni³, Heidi E. Hamm^{1, 4}, Florence L. Marlow^{5*} & Lilianna Solnica-Krezel^{1,6*}

¹Neuroscience Graduate Program, Vanderbilt University, Nashville, TN 37232, USA; ²Department of Developmental Biology, Washington University School of Medicine, St. Louis, MO 63110, USA; ³Department of Surgery, New York Hospital Medical Center of Queens, Flushing, NY 11355, USA; ⁴Department of Pharmacology, Vanderbilt University, Nashville, TN 37232, USA; ⁵Department of Developmental and Molecular Biology, Albert Einstein College of Medicine, Bronx, NY 10461, USA; ⁶Department of Biological Sciences, Vanderbilt University, Nashville, TN 37232, USA

During vertebrate gastrulation, Wnt/planar cell polarity (PCP) signaling orchestrates polarized cell behaviors underlying convergence and extension (C&E) movements to narrow embryonic tissues mediolaterally and lengthen them anteroposteriorly. Here, we identified Gpr125, an adhesion G protein-coupled receptor (GPCR), as a novel component of the Wnt/PCP signaling system. Excess Gpr125 impaired C&E movements and the underlying cell polarity. Reduced Gpr125 function exacerbated the C&E and facial branchiomotor neuron (FBMN) migration defects of embryos with reduced Wnt/PCP signaling. At the molecular level, Gpr125 recruited Dishevelled (Dvl) to the cell membrane, a prerequisite for Wnt/PCP activation. Intriguingly, Gpr125 clustered with Dvl in discrete membrane subdomains, suggesting Dvl-mediated PCP supramolecular complex formation recently reported in *Drosophila*, is a conserved mechanism in vertebrates. In addition, Gpr125 intracellular domain directly interacted with Dvl in pull-down assays. Our study reveals a role for Gpr125 in PCP mediated processes and provides mechanistic insight into PCP signaling.

Identification of Rho Kinase Substrates and their Role During Zebrafish Morphogenesis

Santos-Ledo A^{1,2}, Marlow FL¹, Jenny A^{1,2}

¹Developmental and Molecular Biology Department. ²Department of Genetics.

The Wnt/Planar Cell Polarity (Wnt/PCP) pathway is one of the main regulators of cell polarity and motility. Defective PCP signaling causes developmental defects including a shortened body axis and neural tube closure defects in vertebrates. Rho kinase (Rok) regulates cell behaviors underlying motility during PCP and in other contexts; however, the effectors that Rok utilizes are largely unknown.

The aim of this work is to analyze the expression pattern and roles of one of the Rok substrates identified in flies: Frl (Fmnl in vertebrates). Among the candidates identified, Fmnl is promising since overexpression of *Drosophila* Frl causes phenotype reminiscent of defective PCP signaling in fish. Fmnl belongs to the Formin family, catalysts of linear actin polymerization and also modulators of microtubule activity *in vitro* but unknown roles *in vivo*. In zebrafish, we have identified five *fmdl* homologs with distinct and overlapping expression patterns during embryogenesis. For example, *fmdl2a* is robustly expressed at the neurectoderm boundary and within the hatching gland at the end of the gastrulation. Later, individual *fmdls* show both distinct and overlapping domains of expression in regions such as the visual and vascular system.

Interference with *fmdl2a* by morpholino injection induces expansion of dorsal markers (*chordin*, *cyclops* and *bozozok*) and concomitant loss of ventral structures during early development, phenotypes that can be rescued by RNA injection, which suggest modulation of Wnt or BMP signaling. On the other hand, interference with *fmdl3* induces slight ventralization of embryos, vascular edema and later causes tail blisters, which may be a consequence of altered cell movements during gastrulation or defective development of the vascular system.

Our studies will advance our understanding of Fmnl functions, which may extend beyond pure regulation of cytoskeletal dynamics. This project may also provide a basis to clarify the distinct roles of each Fmnl during vertebrate development.

Blood Brain Barrier model to test permeability of nanoparticles designed to target brain tumors

Sandra Veronica Lopez, Eddie Ham, Parimala Nacharaju, Joel Friedman, and David C. Spray

Dominick P. Purpura Dept. of Neuroscience, Department of Physiology & Biophysics. Albert Einstein College of Medicine, Bronx, NY 10461

The Blood brain barrier (BBB) separates the extracellular fluid environment of the central nervous system (CNS) from the circulating blood. The main structural barrier is comprised by the continuous endothelium of the capillaries of the brain and the spinal cord. Adjacent endothelial cells form tight junctions, which are complex interactions between transmembrane proteins (e.i. ZO-1, occludin, claudin) that restrict the paracellular passage of polar molecules from the blood to the CNS interstitial space. In addition, several other transporters and enzymes are key to maintain homeostasis of the brain. A few of these transport proteins are expressed differentially at the luminal side compared to the abluminal side, and there is unidirectional transport for influx or efflux of solutes to and from the *interstitium*. The close association between endothelial cells and astrocytes at the basal side is responsible for the formation of a high resistance (low permeability) BBB. Targeted drug delivery to the brain is challenging and transport of molecules into the parenchyma is hard to predict theoretically because of the presence of cellular influx and efflux mechanisms, the half-life of the compound, activation and change of conformation, the lipid solubility, the in-lieu metabolic activity and the shape and charge among other variables. We have developed an in-vitro model of the BBB coculturing endothelial cells and astrocytes on opposite sides of culture inserts that has allowed us to evaluate BBB permeability of nanoparticles suitable to target brain tumors. We have found that the neutral nanoparticles perform better than the positively charged nanoparticles and we are now planning to test if these results translate to the in-vivo setting.

Identifying Frl as a putative Rho Kinase substrate and down stream effector protein of the non-canonical Wnt PCP pathway in *Drosophila*

Sam Maung and Andreas Jenny

Department of Developmental and Molecular Biology, Albert Einstein College of Medicine

Planar cell polarity (PCP) is an evolutionarily conserved signaling mechanism, which provides cells directional information within the X-Y plane. This is distinct from apical-basal polarity but nonetheless essential for developmental processes such as convergence & extension and neural tube closure in vertebrate embryogenesis.

PCP signaling is one of two distinct Wnt signaling pathways; the canonical and the non-canonical PCP pathway. Both pathways require secreted Wnts as ligands for the receptor protein Frizzled (Fz) and recruitment of the cytoplasmic phosphoprotein Dishevelled (Dsh/Dvl). Once bound to Fz the Wnt signal can travel down the β -catenin dependent canonical pathway in the presence of a secondary receptor LRP5/6.

In contrast, PCP signaling does not require the LRP co-receptor. Instead, the signal is transduced via Rho family GTPases to a JNK-type MAPkinase cascade and Rho Kinase to elicit a transcriptional and cytoskeletal response, respectively.

To study down stream effector proteins of PCP signaling we have focused on finding novel substrates of Rho Kinase. An *in vitro* kinase assay has identified potential Rock substrates including the 'Formin related protein in leukocytes' Frl.

Frl contains a GTPase binding domain and multiple formin homology domains. We are currently investigating the functional activity of these domains with respect to the small GTPases Rho, Rac and Cdc42. We are conducting genetic interaction assays with Frl dominant negative and aim to create null alleles of Frl using P-element excisions and snp mapping in addition to direct targeting of the gene using TALE nucleases. We aim to fully characterize the null allele of Frl in order to explore its potential role as a Rho kinase substrate and down stream effector of PCP signaling.

The Role of KifC3 in Mitosis

Jeannette Nachbar and Anne MÜsch

Department of Developmental and Molecular Biology, Albert Einstein College of Medicine

KifC3 is a minus end-directed microtubule motor¹. Previous work on this protein from other laboratories has implicated KifC3 in Golgi localization and targeting of proteins to the apical surface in polarized epithelial cells^{1, 2}. In addition overexpression of KifC3 in breast cancer cells led to resistance to chemotherapeutic agents³. Interestingly, the velocity of KifC3 is similar to other mitotic, as opposed to cargo, motors. This fact, coupled with its centrosomal localization in polarized MDCK cells, led us to study KifC3's potential role in mitosis in HeLa cells. We have specifically localized endogenous and recombinant KifC3 to the centrosome in HeLa cells. To assess KifC3's role in mitosis we expressed wild-type KifC3 (WT-KifC3) or a dominant negative version of the protein (DN-KifC3) in HeLa cells and imaged cells live during mitosis. Interestingly, cells transfected with DN-KifC3 displayed longer intercellular bridge formation during late telophase relative to cells transfected with WT-KifC3. The question then became as to how KifC3, which is normally localized to the centrosome in interphase cells, could affect central spindle formation in telophase at a point distal to normal KifC3 localization. Indeed, data from our live imaging experiments revealed that a population of KifC3, independent from the pool at the centrosome, can traverse the central spindle. We therefore hypothesized that this pool of KifC3 can affect the length of the central spindle, and perhaps influence the progression to abscission, by interacting with other components known to travel to the midbody during late stages of telophase. FIP3 is a component of endosomal vesicles which traffic to the midbody region during telophase and has been hypothesized to be important for priming the area for abscission⁴. KifC3 and FIP3 co-localized at a number of different areas in the cell during late stages of mitosis. In early telophase, both proteins were visualized at the centrosomes and flank the central spindle. Later in telophase, KifC3 and FIP3 move internal to the intercellular bridge prior to abscission. Preliminary results indicate that expression of DN-KifC3 may reduce the efficiency of FIP3 delivery to the intercellular bridge during late telophase, which may in turn perturb abscission and explain the extended bridge phenotype.

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Role of Klarsicht, a KASH domain protein, in collective migration of the *Drosophila* embryonic salivary gland

Rashmi Nanjundappa¹, Michael Welte² and Monn Monn Myat¹

¹Department of Cell and Developmental Biology, Weill Cornell Medical College, 1300 York Avenue, New York, NY 10065.

²Department of Biology, University of Rochester, Hutchison 317, Rochester, NY 14627.

Salivary glands (SGs) of the *Drosophila* embryo consist of a pair of elongated secretory tubes that are connected to the larval mouth by the duct tubes. SG cells invaginate from the embryo surface to form a tubular structure followed by collective migration of the tube along the surrounding mesoderm. During SG migration, distal cells contact the overlying mesoderm in an integrin-dependent manner and turn to migrate posteriorly while the proximal cells rearrange and follow the distal cells. We report on the role of Klarsicht (Klar), a KASH domain protein in collective migration of the SG.

In *klar* mutant embryos that lacks the KASH domain, the distal SG cells contact the surrounding mesoderm and turn posteriorly but proximal SG cells failed to turn. We found that failure of proximal SGs to turn posteriorly in *klar* mutant embryos is due to failure of proximal SG cells to rearrange. *Klar* mutant SG cells have reduced stable microtubules (MTs), suggesting that *klar* normally controls cell rearrangement during SG migration through its effects on the MT cytoskeleton. We're currently testing whether *klar* controls MT stability through Spastin.

Neural crest specification by non-canonical Wnt signaling and the polarity protein PAR-1

Olga Ossipova and Sergei Sokol

Department of Developmental and Regenerative Biology, Mount Sinai School of Medicine, New York, NY, USA 10029

Olga.Ossipova@mssm.edu

Neural crest develops from epithelial progenitors that undergo epithelial-mesenchymal transition at the neural plate border. A question arises whether proteins that coordinate cell polarity are involved in this process. Here we use gain- and loss-of-function approaches to demonstrate that noncanonical Wnt11/Wnt11R are essential regulators of premigratory neural crest in *Xenopus* embryos. We also show that the polarity kinase PAR-1 plays an essential role in neural crest formation and is regulated by Wnt signals. PAR-1 interacts with and likely acts by antagonizing ROCK and Myosin II, because inhibitors of Rho kinase or Myosin II promote neural crest fate in vivo. These results uncover novel roles for noncanonical Wnt signaling and PAR-1 in neural crest specification and reveal a novel connection between cell polarity and cell fate.

Podocyte Deletion of LKB1 Results in Increased Severity of Diabetic Nephropathy in Mice

Kimberly J. Reidy, MD, Zhongfang Du, MD, Katalin Susztak, MD, PhD.

Pediatrics/ Nephrology, Albert Einstein College of Medicine, Bronx, NY; Medicine/ Nephrology, University of Pennsylvania, Philadelphia, PA.

Background: LKB1 (also known as Par4 or stk11) is a serine-threonine kinase expressed in the developing kidney and in podocytes. LKB1 is a key regulator of cell polarity and metabolism. Recently LKB1 has been shown to play a role in regulation of AMPK kinase and cellular metabolism, suggesting it may play a role in cellular response to the diabetic

milieu. In vitro studies showed decreased activation of LKB1 in podocytes exposed to high glucose. As LKB1 is a critical integrator of polarity and metabolism, we hypothesized that LKB1 contributes to maintenance of podocyte structure, and may be protective in the setting of metabolic stress. Methods: Conditional deletion of LKB1 in podocytes was achieved by generating NPHS2-Cre:LKB1^{flox/flox} mice. Diabetes was induced by intraperitoneal streptozotocin (STZ) injection (50mg/kg) for 5 days in five week old male mice. Controls were non-diabetic (vehicle injected) NPHS2-Cre:LKB1^{flox/flox} and LKB1^{flox/flox} male mice as well as diabetic (STZ injected) LKB1^{flox/flox} littermates. Severity of diabetic nephropathy was assessed by urine albumin:creatinine ratios, and histologic examination of 14-20 week old male using light and electron microscopy. Results: Nondiabetic mice with podocyte deletion of LKB1 were indistinguishable from controls on light microscopy, and appear to have normal polarity, with majority of foot process intact and normal distribution of nephrin and *Pard3* expression. Control diabetic mice developed only mild mesangial expansion and mild albuminuria, diabetic mice with podocyte deletion of LKB1 developed nephrotic range albuminuria, and Kimmelstein-Wilson like glomerular lesions. This was associated with decreased nephron number as assessed by WT-1 staining. Conclusions: Podocyte LKB1 deletion results in severe diabetic nephropathy, suggesting that podocyte LKB1 contributes to podocyte survival and maintenance of the glomerular filtration barrier in the diabetic milieu.

Modulation of ERR α Nuclear Localization by KIF17

Pramodh Seneviratne and Geri Kreitzer

Weill-Cornell Medical College

Kinesins are a family of microtubule stimulated ATPases that transport a variety of cellular cargoes within the cell. Among the kinesins, KIF17 belongs to the kinesin 2 family and is a plus directed motor protein that regulates diverse cellular processes. To identify novel KIF17 cargo proteins we carried out a yeast-2-hybrid screen on normal epithelial cells using the C-terminal tail domain of KIF17 as bait. Iterative rounds of screening showed that, clones encoding the orphan nuclear receptor ERR α were the most abundant. Exogenous and endogenous co-immunoprecipitation experiments showed co-precipitation of both KIF17 and ERR α confirming the yeast-2-hybrid results. Therefore KIF17 represents a novel ERR α interacting protein. ERR alpha is a ligand-independent, orphan nuclear receptor that modulates cellular responses to estrogen by competing with ER for binding to estrogen response elements (ERRE) on target DNA and regulatory co-activators or co-repressors. Based on the documented activity of KIF17 in nucleo-cytoplasmic shuttling, we hypothesized that KIF17 regulates ERR α activity by controlling its sub-cellular distribution and interaction with promoter elements on target genes.

To determine the effect of KIF17 on the localization of ERR α we carried out cDNA microinjection of fluorescently tagged KIF17 constructs with GFP-tagged ERR α . Microinjection followed by time lapse live cell imaging of ERR α alone in both ER(-) and ER(+) cell lines showed its accumulation within the nucleus of the cells. The mechanism of transport of ERR α to the nucleus has so far not been established. However immunostaining of ERR α and KIF17 shows their localization in close proximity to microtubules as well as within cells. KIF17 contains 2 regions important for nuclear localization. Co-injection of nuclear localization defective KIF17 mutants leads to the disruption of ERR α nuclear localization in some cell types but not others, suggesting KIF17 mediated ER α localization is cell type specific. Using a Luciferase reporter assay we determined that expression of KIF17 nuclear localization defective mutants leads to a reduction of endogenous ERR α binding with its binding element ERRE in these cells.

Collectively our data provide evidence for the interaction of KIF17 with ERR α and that perturbation of KIF17 nuclear localization with dominant negative constructs in leads to accumulation of ERR α in the cytoplasm. Additional characterization of KIF17 functions with ERR α and, ultimately, targeted inhibition of ERR α localization could serve as a novel strategy for breast cancer treatment.

Regulation of vesicle transport to the apical membrane by kinesins in epithelial cells

William Smith and Geri Kreitzer

Dept. of Cell and Developmental Biology, Weill Cornell Graduate School of Medical Sciences

Epithelial tissue function is required for maintaining homeostasis of the body, acting as a barrier to outside environments and allowing for the unidirectional transport of molecules. To establish this barrier, epithelial cells must polarize to create an apical domain and a basolateral domain. These domains differ in function by maintaining a unique lipid and protein composition. Previous work in the lab has characterized the transport of the p75 neurotrophin receptor (p75), which is targeted apically in polarized Madin-Darby Canine Kidney (MDCK) cells. Post-Golgi transport of p75 to the cell surface occurs along microtubules by different kinesin motor proteins in non-polarized and polarized cells. In non-polarized cells, the kinesin-3 family members Kif1A and Kif1B β mediate post-Golgi transport. In polarized cells, the kinesin-1 family member Kif5B mediates post-Golgi transport. These kinesins are expressed in both non-polarized and polarized cells, so this switch in kinesin use must be regulated. Currently, what regulates these kinesin-vesicle interactions is unknown. We hypothesize that these kinesin-vesicle interactions are regulated by three potential mechanisms: kinesin interaction with different Rab GTPases, kinesin interaction with different adaptor proteins, and kinesin autoregulation. To identify new trafficking regulators, we plan to compare the proteomes of different post-Golgi kinesin cargoes. We have begun by isolating post-Golgi vesicles from non-polarized and polarized cells. Cell homogenates are fractionated on a density gradient and identified by known organelle markers. We then isolate kinesin cargoes by using vesicle affinity for bead-immobilized Kif1A or Kif5B tails. p75-GFP containing vesicles act as a control for kinesin specificity. These isolates will be analyzed by mass spectrometry to identify differences between non-polarized and polarized kinesin-specific cargoes. Proteomes can then be compared for similarities and differences to identify potential trafficking regulators.

A flexible mid-plane ring in the nuclear pore complex

Sozanne R. Solmaz, Radha Chauhan, Günter Blobel and Ivo Melcak

Laboratory of Cell Biology, HHMI at the Rockefeller University, 1230 York Av, New York NY 10065

The central element of the nuclear pore complex (NPC) is a remarkably versatile transport channel that allows bidirectional nucleo-cytoplasmic transport of substrates ranging greatly in size, from single proteins, to ribosomal subunits and viruses. Transport is accompanied by large structural changes, especially in the transport channel, which is thought to consist of the three nucleoporins (nups) Nup62, Nup54 and Nup58, but its structure is elusive. By mapping interactions between the channel nups, we identified two distinct interacting domains, Nup54•Nup58 and Nup54•Nup62, and determined their crystal structures (1). Nup62•Nup54 forms a triple-helix, whereas the structure of Nup54•Nup58 is a higher-order oligomer, in which Nup54 exists in two distinct conformations. Comparison of the Nup54•Nup58 complex with the structures of the Nup58 and Nup54 homo-oligomers (2,3) reveals large conformational changes as well as crucial residues for the inter-conversion between these states (3). Based on our structural and biochemical data, we pieced together the architecture of the transport channel. Its centerpiece is a highly flexible Nup54•Nup58 mid-plane ring that can undergo large-scale rearrangements, yielding huge diameter changes from ~20 to ~45 nm (1). Nup62•Nup54 triple helices project alternately up and down from either side of the mid-plane ring and form nucleoplasmic and cytoplasmic entries. We propose that the FG-repeat regions of the channel nups act as sensors for transport demands and that binding events of transport receptor cargo complexes to these regions modulate the channel diameter. The diameter changes may enable rapid adjustments of transport activity and allow the NPC to accommodate cargo of various sizes.

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The lipid kinase PI4KIII β regulates lysosomal efflux

Sunandini Sridhar and Ana Maria Cuervo

Albert Einstein College of Medicine, Bronx, NY

Exit of selective lysosomal content via tubular carriers occurs in conditions such as lysosome re-formation, lysosomal secretion, or antigen presentation in immune cells. However, the determinants governing formation and vectorial transport of these tubular carriers remain largely uncharacterized. Our aim is to investigate if the phosphoinositide PI(4)P regulates spatio-temporal sorting events directly at the lysosomal membrane and thus contributes to maintain lysosomal identity and function.

Using live cell video microscopy in cells knocked down for the PI-4-Kinase isoform PI4KIII β , we have found enhanced formation of long tubulated structures positive for LAMP1. We demonstrate that LAMP1 tubules arise from vesicles positive for lysosomal markers and that both, constitutive lysosomal components and cargo could be identified along the tubules. Transfection with PI4KIII β reverted this phenotype, but only when its kinase activity was preserved, suggesting that PI(4)P is the effector of PI4KIII β function at lysosomes. Using different biochemical procedures, we have identified a lysosome-associated active form of PI4KIII β that we propose modulates lysosomal exit. Proteomic analysis of vesicles positive for PI4KIII β confirmed the presence of this kinase in lysosomal component carriers and allowed us to identify the coat-forming proteins and adaptors recruited to lysosomes in a PI4KIII β -dependent manner. Lastly, we have demonstrated the physiological relevance of this new lysosomal function of PI4KIII β in two different contexts dependent on proper lysosomal sorting: antigen presentation and adaptation to starvation/refeeding; we found that dendritic cells deficient for PI4KIII β fail to properly sort MHC class II molecules from lysosomes to the plasma membrane and that the regulated sorting of lysosomal proteins into elongated tubular carriers elicited upon refeeding, is also abolished in cells deficient in PI4KIII β .

We show that PI4KIII β regulates lysosomal content exit by directly localizing to this compartment. We propose that PI4KIII β and its product PI(4)P mediate the lysosomal recruitment of the molecular components required for cargo sorting and carrier formation, which makes PI4KIII β a novel regulator of lysosome identity.

Biosynthetic sorting of the sodium pump: Visualization of the segregation of newly synthesized epithelial Na,K-ATPase from apically directed proteins

Emily Stoops, Glen Farr, Michael J Caplan.

Cellular & Molecular Physiology, Yale University, New Haven, CT

Regulation of transport protein function involves many mechanisms, including endocytosis, recycling and biosynthetic sorting. The Na,K-ATPase generates the ion gradients that drive most transepithelial transport. In almost every polarized epithelial cell type, the Na,K-ATPase is restricted to the basolateral domain of the plasma membrane. To study the regulation of Na,K-ATPase trafficking and its separation from apical cargo, we have created a stable MDCK cell line expressing a SNAP-tagged version of Na,K-ATPase and a CLIP-tagged version of gp135, a glycoprotein that traffics to the apical membrane. The SNAP and CLIP tags are modified forms of a DNA repair protein that react with benzylguanine (SNAP) and benzylcytosine (CLIP) substrates, resulting in covalent linkage of the benzyl group to an active thiol residue within the tag. Using a block/pulse method and SNAP/CLIP-specific blocking reagents, newly synthesized cohorts of SNAP- and CLIP-tagged protein can be visualized by confocal and electron microscopy, or subjected to biochemical analysis to reveal time-dependent protein-protein interactions. Our data suggest that Na,K-ATPase and gp135 pursue the same postsynthetic trafficking pathway through the trans-Golgi network, after which they appear to segregate from one another. Kinetic analysis reveals that Na,K-ATPase traffics through the biosynthetic pathway more rapidly than the apical gp135.

Organization of the gap junction Nexus: Looking beyond intercellular communication to roles in tissue organization and function

Randy F. Stout, Jr., and David C. Spray

Albert Einstein College of Medicine, Dominick Purpura Dept. of Neuroscience, Bronx, NY, USA

Gap junction channels formed by connexin-family proteins are the most prominent form of direct cytoplasmic connection between cells in tissue types such as the brain and heart. Gap junctions are the primary component of a complex protein structure termed the Nexus that varies in a regulated way according to cell type, developmental stage, and cellular activity state. The channel-functions of gap junctions are just one important role that these multi-protein structures undertake. They interact with the cytoskeleton and other types of cellular junctions in reciprocal and regulated ways. This cytoskeletal interaction has been proposed to both help determine the establishment and maintenance of gap junctions and in turn, through cellular organizing roles of gap junctions, to regulate processes such as traffic and release of cytokines, cellular adhesion, and organization of the cytoskeleton. We recently found new roles for the Connexin43 (Cx43) C-terminus (CT) in how gap junctions are organized within the Nexus using optical experimental techniques on connexins and associated proteins. We found that truncation of the CT leads to destabilization of the Nexus and changes in the mobility of Cx43 not yet incorporated into gap junction plaque structures. We went on to test how these experimentally induced changes to the Nexus affect the membrane surrounding the Cx43-based channels. These findings will improve knowledge of how the Nexus works to establish and maintain tissue organization because the CT of Cx43 undergoes changes in secondary structure (and possibly proteolytic cleavage) in a regulated manner within the human brain and heart. This contributes to understanding of how improper assembly and/or maintenance of the Nexus might lead to human diseases such as epilepsy, chronic pain, and cardiac myopathy.

Deciphering protein sorting itineraries at the trans-Golgi network of polarized epithelial cells

A. Treyer¹, F. Lazaro-Diequez¹, D. Cohen¹, A. Müsch¹

¹Developmental and Molecular Biology, Albert Einstein College of Medicine, Bronx, NY

The trans-Golgi network (TGN) is thought of as the diverging point for the sorting of apical and basolateral proteins into their subsequent itineraries through the rest of the secretory pathway. However, how the membrane structure or lipid and protein composition of the TGN provides for the specific sorting of such itineraries remains unclear. Our preliminary studies, which rely on analysis of fluorescently labeled, cargo protein-enriched, fractionated TGN membranes show that much of the apical and basolateral cargo mass is independent of either the enzymatic trans-Golgi cisternae (sialyltransferase) or the cycling TGN (TGN38) markers, indicating that most of the cargo that accumulates in the TGN delineates an independent functional compartment that is essentially devoid of known compartmental markers. Furthermore, our studies show that ~70% of apical DPPIV and basolateral VSVG cargo proteins are present in distinct TGN-entities after a cargo accumulation step at 20°C. This indicates that a significant segregation of apical and basolateral proteins has occurred within the 20°C compartment, but additional protein sorting step(s) must occur to result in the release of distinct apical and basolateral carriers in MDCK cells. Currently, we are attempting to elucidate the mechanisms and the stringencies of the “coarse” and “fine” sorting events for the different cargo types in the TGN.

The lipid flippase TAT-5 inhibits the budding of extracellular vesicles from the surface of *C. elegans* embryos

Ann M Wehman, Ph.D and Jeremy Nance, Ph.D.

Developmental Genetics Program, Skirball Institute, NYU School of Medicine

Cells release extracellular vesicles (ECVs) that can influence differentiation, modulate the immune response, promote coagulation, and induce metastasis. Many ECVs form by budding outwards from the plasma membrane, but the molecules that regulate budding are unknown. In ECVs, the outer leaflet of the membrane bilayer contains aminophospholipids that are normally sequestered to the inner leaflet of the plasma membrane, suggesting a potential role for lipid asymmetry in ECV budding.

We show that loss of the conserved lipid flippase TAT-5 causes the large-scale shedding of ECVs and disrupts cell adhesion and morphogenesis in *C. elegans* embryos. ECVs accumulate between cells disrupting the structure of cell-cell contacts. TAT-5 localizes to the plasma membrane and its loss results in phosphatidylethanolamine (PE) exposure on cell surfaces, suggesting that TAT-5 maintains plasma membrane PE asymmetry. Since viruses also bud from the surface of cells, we tested whether viral budding regulators also regulate ECV budding. We show that RAB-11 and endosomal sorting complex required for transport (ESCRT) proteins are enriched at the plasma membrane in *tat-5* embryos and are required for ECV production.

TAT-5 provides a molecular link between loss of PE asymmetry and the dynamic budding of vesicles from the plasma membrane, supporting the hypothesis that lipid asymmetry regulates budding. Our results also suggest that viral budding and ECV budding may share common molecular mechanisms.

Mammalian GDP-Fucose Transporters Required for Notch Signaling

Hua-Xing Wei, Linchao Lu and Pamela Stanley

Dept. Cell Biology, Albert Einstein College Medicine, New York NY, 10461

Notch signaling is important during embryonic development and in cell fate decisions during somitogenesis. Notch receptors must be modified by O-fucose glycans on epidermal growth factor-like (EGF) repeats in their extracellular domain for optimal signal induction by canonical Notch ligands to be achieved. *Slc35c1* is the only known GDP-fucose transporter. However, mice and humans lacking *Slc35c1*, do not have an obvious Notch mutant phenotype. In addition, *Slc35c1* null fibroblasts transfer O-fucose to Notch and exhibit robust Notch signaling. Nevertheless, when we examined *Slc35c1* null embryos at embryonic day 18.5, skeletal defects were observed indicating defective Notch signaling during somitogenesis. *Slc35c2* is a GDP-fucose transporter related gene, that we showed promotes Notch1 O-fucosylation and is required for optimal Notch signaling. We generated *Slc35c2*^{-/-} mice by deletion of exon4 of the *Slc35c2* gene. Western blot analysis showed that *Slc35c2* was not expressed in *Slc35c2*^{-/-} testis or *Slc35c2*^{-/-} mouse embryonic fibroblasts. No apparent abnormalities were observed in *Slc35c2*^{-/-} mice at birth, and both male and female homozygous mutants were fertile. In contrast to *Slc35c1*^{-/-} embryos, *Slc35c2*^{-/-} embryos had no significant skeletal defects. We therefore crossed *Slc35c1*^{+/-} mice with *Slc35c2*^{+/-} or *Slc35c2*^{-/-} mice to obtain *Slc35c1*^{-/-}*Slc35c2*^{-/-} double knockout mice. Double knockout mice are born, but at a lower ratio than expected. Preliminary results indicate that skeletal defects in *Slc35c1*^{-/-}*Slc35c2*^{-/-} double knockout embryos are similar to *Slc35c1*^{-/-}*Slc35c2*^{+/+} embryos. They have thoracic and lumbar skeletal defects in several vertebrae, but exhibit only mild rib and tail defects. In contrast, mice that are unable to add GlcNAc to O-fucose on Notch have severe thoracic, lumbar and rib defects, and no tail. Therefore, mice lacking the known and putative GDP-Fuc transporter activities of mammals must nevertheless be adding O-fucose to Notch in the secretory pathway, providing strong evidence for the existence of at least one other GDP-Fuc transporter important for the O-fucosylation of Notch.

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A novel role of Rho GTPase PAC-1 in *C. elegans* epithelial adherens junctions.

Yuliya Zilberman and Jeremy Nance

Developmental Genetics Program, Skirball Institute, NYU School of Medicine

PAC-1 is a Rho GTPase activating protein (RhoGAP) that mediates the contact-induced polarization of blastomeres in the *C. elegans* early embryo (Anderson DC, et al., 2008). PAC-1 is recruited to cell-cell contacts and inhibits cdc42 activity at these sites, causing the redistribution of PAR polarity proteins to the contact-free surface. We found that PAC-1 localized to the apical Cadherin/catenin junctions in embryonic epithelial tissues, which first form later during development. *pac-1* mutant epithelia develop normally, but *pac-1* mutations enhance the lethality of *hmp-1/α-catenin* hypomorphic mutants. *hmp-1* and *pac-1* regulate elongation, when the epidermis contracts to squeeze the elliptical embryo into a worm shape; while many *hmp-1* mutants complete elongation, *pac-1; hmp-1* double mutants arrest during elongation and their epidermis ruptures. We show that PAC-1 localizes to adherens junctions together with HMP-1, and that the RhoGAP activity of PAC-1 is required for its function. We hypothesize that PAC-1 regulates the strength of adherens junctions by regulating CDC-42 or another RhoGTPase associated with the cadherin-catenin complex.

Mutation of SIMPLE in Charcot-Marie-Tooth 1C Alters Production of Exosomes

Hong Zhu¹, Sara Guariglia¹, Raymond Y.L. Yu, Wenjing Li, Deborah Brancho, Hector Peinado, David Lyden, James Salzer, Craig Bennett, and Chi-Wing Chow*

Department of Molecular Pharmacology, Albert Einstein College of Medicine

Charcot-Marie-Tooth (CMT) disease is an inherited neurological disorder. Mutations in protein SIMPLE accounts for the rare autosomal dominant demyelination in type 1C CMT patients (CMT1C). Understanding the molecular basis of CMT1C pathogenesis is impeded, in part, by the perplexity for the role of SIMPLE, an evolutionarily conserved protein expressed in multiple cell types. Here, we show that SIMPLE plays a role in the production of exosomes, which are *nanovesicles* secreted extracellularly. We find that SIMPLE resides within the intraluminal vesicles of multivesicular bodies and inside exosomes. Expression of SIMPLE increases the production of exosomes. Conversely, endogenous SIMPLE carrying CMT1C mutation in primary mouse embryonic fibroblasts shows reduced secretion of exosomes, in part, due to improper formation of MVBs. CMT1C patient B cells also show similar defects in exosomes and MVBs. Upon CMT1C mutation, SIMPLE exhibits increased binding of ESCRT0 protein Hrs, which is mislocalized and displays elevated punta formation in CMT1C MEFs or B cells. Together, these data indicate that SIMPLE regulates the production of exosomes *via* modulating the formation of MVBs. Dysregulated endosomal trafficking and changes in the landscape of exosome-mediated intercellular communications may place an overwhelming burden on the nervous systems and, account for the CMT1C molecular pathogenesis.