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SCHOOL of MEDICINE

Department of Cell Biology
invites you to the inaugural

RISING STARS IN CELL BIOLOGY SYMPOSIUM

A day of science talks, posters, and networking with cell biologists in the mid-Atlantic region.

APRIL 30, 2022

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KEYNOTE BY DR. KANDICE TANNER OF THE NCI

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About the Rising Stars Symposium

Organised by the IDEA team, the Rising Stars in Cell Biology Symposium is an opportunity for early career scientists (undergraduates, post baccalaureates, PhD students and postdoctoral fellows) from diverse backgrounds within the greater Baltimore area to present their research and network with peers. This symposium will include talks and poster sessions for trainees to exchange ideas and feedback with peers and senior scientists. We hope you enjoy the symposium.

About the IDEA Team

The IDEA Team is a group of graduate students, postdoctoral fellows, and faculty from the Department of Cell Biology at the Johns Hopkins School of Medicine. We recognize the need to work continually to foster a welcoming and inclusive environment within our department, and it is our mission to develop and implement initiates that promote diversity, equity, and inclusion at our own institution and in science more broadly.

Participating Institutions

George Mason University
George Washington University
Howard University
James Madison University
Johns Hopkins University
Morgan State University
National Institutes of Health
Towson University
University of Delaware
University of Maryland
University of Maryland, Baltimore County

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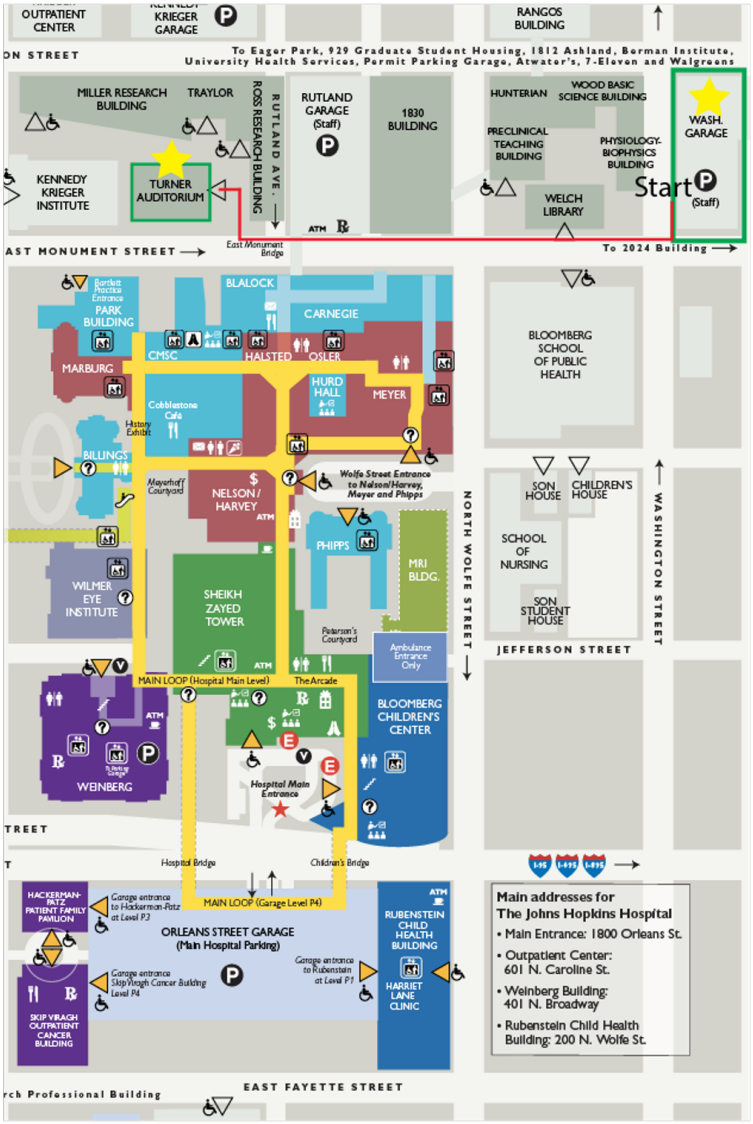
Shigeki Watanabe, PhD
Sumana Raychaudhuri, PhD
Tatsat Banerjee

Map

Registration: Apr 30th, 8AM at Turner Concourse (left gold star on map), located on ground floor

Conference Location: 720 Rutland Ave
Baltimore, MD 21205

Parking Location: Washington Street Garage (right gold star on map)
701 N Washington St
Baltimore, MD 21205



Schedule of Events

Time	Event	Location
8:00-8:45AM	Registration, Breakfast Set up for poster session I	Turner Concourse
9:00-10:00AM	Welcome & Introduction : Abhijit (Abhi) Deb Roy - JHU Keynote Address: Kandice Tanner - NCI, NIH *Microenvironment regulation of metastasis*	Tilghman Auditorium
10:00-10:15AM	Coffee Break	
10:15-11:00AM	Scientific Session I Moderator : Molly Gordon - JHU Naresh Kumar Meena - NIH *Liver-directed and systemic AAV gene transfer approaches for Pompe disease therapy* [link] Adam Winter - JHU *Expression and regulation of Tdrd5l in the Drosophila germline* [link] Mara Grace - JHU *The role of ESCRTs in signaling within the testis stem cell niche* [link]	Tilghman Auditorium
11:00AM-12:30PM	Poster Session I	Turner Concourse
12:30-1:30PM	Lunch & Networking Set up for poster session II	MRB Prefunction Area Turner Concourse
1:30-2:15PM	Scientific Session II Moderator : Alexis Tomaszewski - JHU Chaitali Khan - NIH *Tumor suppressive role of cell competition in eliminating cells with genomic damage* [link] Kishore K. Mahalingan - NIH *Cisplatin resistance in ovarian cancer cells can be mediated through cis- and trans-effects on microtubule dynamics* [link] Austin Dellafosse - Howard University *Evaluating the effect of partial loss of ATXN1 on the expression of BACE1 in the mouse brain* [link]	Tilghman Auditorium

2:15-3:45PM	Poster Session II	Turner Concourse
3:45-4:30PM	Scientific Session III Moderator : James McCann - JHU Matthew Eason - UMD *Obscurin-deficient breast epithelia generate secreted factors to prime a vascular smooth muscle cell-dependent pre-metastatic microenvironment* [link] Michelle Baird - NIH *Upregulation of lamin B receptor in metastatic melanoma mediates loss of nuclear envelope integrity during confinement* [link] Junior West - JHU *Tight junction proteins restrict aggressive cancer cell behavior* [link]	Tilghman Auditorium
4:30-5:30PM	Happy Hours & Networking	MRB Prefunction Area

Poster Session I

Poster #	Presenting Author	Poster Title
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A2	Ankita Jha	Front-rear polarity establishment during bleb-based cancer cell migration
A3	Kishore M. Mahalingan	Cisplatin resistance in ovarian cancer cells can be mediated through cis- and trans-effects on microtubule dynamics
A4	Claudia Hernandez-Chavez	Comparative Genomics of Bifidobacterium Strains Isolated From Early Preterm Infants With Improved Leaky Gut
A5	Tony Yao	Elucidating structure and isoform-specificity of a novel PI3K p85 endocytic role
A6	Azmath Fathima	Quantum Sensing in Cell Biology
A7	Diana M Abraham	Comparing the impact of Busulfan conditioning on clonal patterning with total body irradiation following rhesus macaque autologous transplantation
A8	Isaiah Roberts	The impact of astrocytes on tumor cell dormancy versus proliferation in brain metastatic breast cancer spheroids
A9	Carla Lopez	Impaired Development of Enteric Glia in the Premature Bowel Contributes to Dysmotility and the Development of Necrotizing Enterocolitis via Exaggerated TLR4 Activation
A10	Gordon Sun	Modeling mitochondrial network dynamics in protein aggregate inheritance
A11	Shantel Angstadt	Uncovering a myosin regulator in pancreatic tumor cell mechanics and behavior
A12	Kelvin Fadojutimi	Characterization of the Gag/RNA Interactions That Nucleate HIV-1 Viral Assembly
A13	Jenna Clements	Investigation of HDAC11 in Hepatocellular Carcinoma
A14	Aya Kutbi	A Meta-Analysis Study on the Effects of Breastfeeding Practices on the Gut Microbiome in the MAL-ED Cohort Study
A15	Langston Locke	Identifying and Verifying Variants in Breast Cancer Genes
A16	Stephanie Myers	Targeting the sterol regulatory element-binding protein (SREBP) pathway in pancreatic ductal adenocarcinoma
A17	Armaan Jamal	Calcium Triggered Compound Fusion at Ribbon Synapses During Exocytosis
A18	Haoyuan Jing	Mechanics of ultrafast coupling between exocytosis and endocytosis at neuronal synapses
A19	Matthew Eason	Obscurin-deficient Breast Epithelia Generate Secreted Factors to Prime a Vascular Smooth Muscle Cell-dependent Pre-metastatic Microenvironment
A20	Elodie Henriet	Triple negative breast tumors contain heterogeneous cancer cells expressing distinct KRAS-dependent molecular invasion programs
A21	Austin Dellafosse	Evaluating the effect of partial loss of ATXN1 on the expression of BACE1 in the mouse brain

Poster Session II

Poster #	Presenting Author	Poster Title
B1	Dhiman S. Pal	Spatiotemporally precise optogenetics reveal Ras-Akt1 regulate polarity and migration in human neutrophils
B2	Malikeya Chaudhary	Effects of VEGF inducer GS4012 on blood vessel formation and regeneration in larval axolotl tails
B3	Amilcar Perez	Single molecule tracking reveals a relationship between bacterial FtsWI dynamics and cell division progression.
B4	Andrew M Gausepohl	Characterizing the Relative Abundance and Spatial Distribution of Lipids in SIV-Infected Rhesus Macaque Brain
B5	Jonathan Kuhn	Cytoskeletal feedback controls directed migration signaling networks
B6	Farid Shahid	Development of molecular sensors and actuators to elucidate PI3K signaling
B7	Sarah Syed	Identifying the Role of Microglia Trogocytosis in Maintaining Excitatory Synapses
B8	Marco A Alfonzo-Mendez	Mechanisms of growth factor and adhesion receptors crosstalk by clathrin lattices
B9	Cristian Saez-Gonzalez	The role of acetylated microtubules in cell migration
B10	Siran Tian	mRNA structure regulates complementary sequences to instruct homotypic mRNA self-assembly
B11	Eleana Parajon	Mechanoresponsive Adaptability in Pancreatic Cancer Cells and Tissue
B12	Longhua Hu	FtsZ treadmilling dynamics spatiotemporally regulates sPG synthesis in bacterial cell division
B13	Oliver Valera	Modeling translational bursting at the single-mRNA level
B14	Claire Charpentier	The cranial mesenchyme as a potential driver of neural tube closure
B15	Xingyao Wang	An active, self-organized molecular organization of BAR-domain protein, Pacsin2/Syndapin2
B16	Mark Allan C. Jacob	Cortical dynamics feedback into metabolic activity and mechanisms
B17	Saki Takayanagi	Identifying the missing link between skeleton and skin: Pacin2/Syndapin2 regulates cell motility via dynamically instable subcellular organization consisting of actin and membranes
B18	Michelle Baird	Upregulation of lamin B receptor in metastatic melanoma mediates loss of nuclear envelope integrity during confinement
B19	Arjun Kanjarpane	Elucidating interactions between the HIV-1 RRE and the viral proteins Rev and Gag
B20	Jeeun Song	Evolutionary Expansion of the MAPK Signaling Network and the Origin of Animal Multicellularity
B21	Fairine Ahmed	Design of Stable Antibody: HIV-1 RNA Complex Suitable for Structural Studies

All Abstracts

A Meta-Analysis Study on the Effects of Breastfeeding Practices on the Gut Microbiome in the MAL-ED Cohort Study

Presenting Author : Aya Kutbi
Aya Kutbi, Jacquelyn Meisel, Douglas Dluzen, Mihai Pop2, YueJin Li

Keywords: microbiome; Gut microbiome; 16S sequencing; Breastfeeding; diet

Background: Studying the human gut microbiome is critical to understanding microbiota homeostasis and disease predisposition. Gut microbiome plays an important role in childhood development and its dysbiosis has been associated with many diseases.
Objective: This study aims to understand how breastfeeding practices affect the gut microbiome in the MAL-ED sub-cohort study.
Methods: We analyzed 16S rRNA microbiome data of 777 stool samples of Peruvian children aged 6, 12, 18, and 24 months. The samples were classified into Exclusive Breastfeeding group (EB) and Breastfeeding with Solid/Liquid Food group (BSLF). Faith Phylogenetic Diversity and Pielou's Evenness measurements were employed to examine Alpha diversity. Weighted UniFrac phylogenetic distance followed by PERMANOVA tests was used to measure Beta diversity. Log-ratio based method, ANCOM, was applied to identify the differential abundance of taxa between groups.
Results: Alpha Diversity measurements showed that the EB group had less rich microbial diversity at 18 and 24 months and less even microbial distribution at 12 and 18 months, as compared to the BSLF group (P<0.004 in all analyses). Breastfeeding groups presented significantly different microbial compositions between age groups. The Genera Coprococcus, Lactobacillus, Roseburia, Megasphaera belonging to the phylum Firmicutes, and the genus Bifidobacterium belonging to the phylum Actinobacteria are the most differentially abundant taxa between the two groups. Notably, significant differences in the microbial richness, evenness, dissimilarities, and taxa were detected among age groups with exclusive breastfeeding.
Conclusion: Breastfeeding practices affect the gut microbiome in children under 2 years old. During early child development, the gut microbiome also changes with age.

An active, self-organized molecular organization of BAR-domain protein, Pacsin2/Syndapin2

Presenting Author : Xingyao Wang
Helen (Di) Wu, XingYao Wang, Takanari Inoue

Keywords: Mast cells; BAR protein; Pacsin2/Syndapin2: membrane organization

F-BAR domain-containing proteins coordinate cell morphological changes by actively bridging actin cytoskeleton and plasma membranes (PM). We recently observed that Pacsin2 (Syndapin2), an F-BAR domain-containing protein, exhibited a unique patchy ring-like organization at the PM in mast cells. How this micron-scale subcellular organization arises from an assembly of a nanometer-scale protein remains unknown. Pacsin2 rings were closely associated with the PM and F-actin patches at basal surface of resting RBL-2H3 mast cells. Investigating the spatiotemporal dynamics of Pacsin2 could help us advance in understanding a principle of how membrane curvature, tension, and F-actin organization coordinate with each other. Toward this end, an RBL mast cell line expressing fluorescently labelled Pacsin2 was subjected to total internal fluorescence microscopy (TIRF) to visualize Pacsin2 dynamics before and after cell activation. We found that Pacsin2 forms distinct punctate units which appear to self-arrange to form ring-like structure. In addition, in RBL cells pre-treated with anti-DNP IgE followed by simulation with DNP-BSA, Pacsin2 rings shrink and eventually disappear as cells undergo remodeling with membrane ruffles and spreading. Pacsin2 then reappears and rings reform at random locations. The cell activation indicated a strong positive correlation with the duration of ring dissipation. Notably, higher PM signal was also observed in the rings before and after cell activation, suggesting the presence of significant membrane mass. Besides the above qualitative characterization, we quantified spatiotemporal kinetics and dynamics of Pacsin2 rings, including ring formation/dissipation rate, movements, inter-ring distance, based on which we are currently building a robust physical model to deduce molecular principles governing the self-organized phenotypic patterns, specifically focusing on feedback interplays among membrane tension, curvature sensing, and actin polymerization.

Calcium Triggered Compound Fusion at Ribbon Synapses During Exocytosis

Presenting Author : Armaan Jamal
Armaan Jamal, Sebastian Markert, Shigeki Watanabe

Keywords: Compound Fusion; Ribbon Synapses; Retina; Exocytosis

Exocytosis in photoreceptors involves fusion of vesicles with the plasma membrane at the base of a specialized proteinaceous electron-dense structure known as a synaptic ribbon. The molecular mechanisms underlying exocytosis at ribbon synapses are not well understood. Research suggests a compound fusion model where vesicles at ribbon synapses undergo homotypic fusion forming larger vesicles prior to fusion with the plasma membrane. But how this process is mediated remains unclear. Ca2+ triggers several forms of exocytosis in different types of eukaryotic cells through activation of the SNARE complex. Here, we report that, like typical synaptic exocytosis, compound fusion is mediated by Ca2+ influx. To examine the role of calcium in compound fusion, we performed vesicle purification experiments from Mus musculus (C57BL/6 WT) retina. Brain tissue was also used as a control. Retinas and brain tissue were dissected, homogenized, and ultracentrifuged. The supernatant was incubated in 2mM Ca2+ for 5 minutes then vesicles from the Ca2+samples and controls containing no Ca2 were isolated using magnetic beads coupled with rho1D4 monoclonal antibody. Vesicles were negatively stained and viewed under TEM. Vesicle sizes of the Ca2+ and control samples were determined via blind analysis using ImageJ. We found that in the retina samples, exogenous Ca2+ caused a significant increase in vesicle size when viewed under TEM. In the retina Ca2+ samples, vesicle sizes ranged from 30nm to 250nm in diameter. This effect was not seen in retina controls containing no exogenous Ca2+ where vesicle sizes ranged from 30nm to 60nm in diameter. Additionally, large vesicles were not seen in any of the brain samples. Our research supports the compound fusion model of vesicle exocytosis at ribbon synapses. We suggest that in retina, the larger vesicles formed during exocytosis are a result of smaller vesicles fusing with each other and that Ca2+ has a significant role in this process.

Characterization of the Gag/RNA Interactions That Nucleate HIV-1 Viral Assembly

Eleana At the early stage of HIV-1 assembly, two copies of the viral RNA genome (gRNA) are selectively packaged by the major structural protein Gag, which consists of multiple domains including the N-terminal myristoylated matrix (MA), capsid (CA), and nucleocapsid (NC). The molecular mechanisms that direct the selective genome packaging during Gag assembly remain poorly understood. Our research focuses on characterizing Gag/gRNA interactions using a variety of biochemical tools, such as Electrophoretic Mobility Shift Assays (EMSAs) and Nuclear Magnetic Resonance (NMR). Our results uncovered that Gag proteins assemble as dimers on the RNA packaging signal (ψ), a 155 nt region of gRNA responsible for selective packaging. Competitive EMSA experiments further revealed that both the dimerization of Gag through the CA-CA dimerization and the N-terminal myristoylation are essential for the selective binding of Gag to ψ. In addition, previous and current NMR data suggest that the N-terminal myristoyl group mediates Gag trimerization through the MA domain which is likely promoted by the Gag dimerization. These results collectively suggest an assembly model that ψ-binding promotes Gag dimerization and further triggers myristoyl-mediated Gag trimerization, which results in the formation of a Gag/ψ complex nucleating the following massive Gag assembly. This study will further our understanding of HIV-1 Gag assembly and selective genome packaging, which may lead to the development of novel antiviral therapeutics.

Characterizing the Relative Abundance and Spatial Distribution of Lipids in SIV-Infected Rhesus Macaque Brain

Presenting Author : Andrew M. Gausepohl
Andrew M. Gausepohl, Cory J. White, Dionna W. Williams

Keywords: HIV; SIV; brain; lipid metabolism; MALDI-IMS

The ability of the brain to meet its high energetic demand is disrupted by human immunodeficiency virus (HIV) infection, contributing to the development of neurocognitive deficits. While it is known that HIV can disrupt this energetic balance, the specific contribution of changes in lipid metabolism to these deficits remains unknown. This study serves to characterize lipid metabolic changes in the brain, due to HIV, to better understand how they contribute to this deficit. We hypothesize that the abundance and distribution of lipids critical for fatty acid oxidation (FAO) and membrane structure will be altered in the brain by HIV and that these changes will be distinct from peripheral tissue. To test this, we infected rhesus macaques with simian-immunodeficiency virus (SIV) and suppressed the virus using antiretroviral therapies. Brain and kidney tissues were collected and analyzed using matrix assisted laser desorption ionization imaging mass spectrometry (MALDI-IMS) which shows the relative abundance and positions of different ionized lipid species within each sample. The mass charge ratio (M/Z) of the ions were used to identify acylcarnitines (ACs), a critical FAO intermediate, and phospholipids necessary for proper membrane structure. AC levels are generally elevated in brain regions compared to kidney. AC species also display unique spatial restriction patterns within brain regions. Phosphatidylcholine (PC), a membrane phospholipid, levels are also elevated in brain regions compared to kidney. As M/Z increases, some brain regions show a decline in PCs while others maintain relatively constant levels. These results provide evidence that differences in abundance and localization of ACs and phospholipids exist not only between brain and peripheral organs but also between brain structures in SIV-infected macaques. The extent of variation in these lipid species suggest that changes to lipid metabolism may be occurring in the brains of SIV-infected rhesus macaques.

Cisplatin resistance in ovarian cancer cells can be mediated through cis- and trans-effects on microtubule dynamics

Presenting Author : Kishore K. Mahalingan
Kishore K. Mahalingan, Sachi Horibata, Ruchi Patel, Jordan Hotz, Yu Fan, Keith MacRenaris, Daud Meerzaman, Michael M. Gottesman and Antonina Roll-Mecak

Keywords: Ovarian Cancer; Cisplatin resistance; Microtubule dynamics; Tubulin; TPPP

Cisplatin based chemotherapeutics are a mainstay in cancer treatment, but resistance limits their therapeutic potential. Although cytotoxicity of cisplatin has long been associated with DNA damage, an RNA seq analysis of cisplatin-sensitive, -resistant, and re-sensitized ovarian cancer cells identified many changes in genes associated with the microtubule cytoskeleton including overexpression of the microtubule-stabilizing tubulin polymerization promoting protein 3 (TPPP3), that strongly correlate with drug resistance. We hypothesized that cisplatin cytotoxicity partly comes from microtubule destabilization and that drug resistance can be mediated through changes in microtubule dynamics. We found that clinical concentrations of cisplatin destabilize microtubule ends and increase the frequency with which microtubules depolymerize. Cisplatin-resistant cells counteract the destabilizing effects of cisplatin by over-expressing TPPP3 which synergizes with changes in tubulin isotype repertoire for maximal stabilization of the microtubule cytoskeleton. TPPP3 depletion restores partial cisplatin sensitivity and increases microtubule dynamics in-vivo. Lastly, we find that cancer patients with lower TPPP3 expression have improved therapeutic outcome with cisplatin treatment. The direct effects of cisplatin on microtubule stability as well as the involvement of microtubule dynamics effectors in cisplatin resistance has important implications for cisplatin and paclitaxel combination chemotherapy as well as cisplatin associated neuropathies.

Claudin proteins restrict invasive and metastatic cell behaviors in murine models of breast cancer

Presenting Author : Kate Cho
Kate Cho, Junior West, Andrew Ewald

Keywords: Claudin; Tight junction; Collective invasion; Metastasis; 3D organoids

Breast cancer is the most common cancer diagnosed and second leading cause of death in women. Transcriptomic analyses have identified 'Claudin-Low' as an additional subtype of breast cancer, with low expression of tight junction genes Claudin 3, 4, 7, Occludin, and E-cadherin as a key defining feature. While clinical and genomic studies have shown that Claudin-low is an aggressive subtype with poor prognosis, it remains unclear whether loss of these proteins is directly responsible for the aggressive cancer cell behaviors involved in metastatic disease. We hypothesized that Claudin proteins suppress invasive and metastatic cancer cell behaviors. As majority of Claudin-low tumors tend to be triple-negative, C3-1-TA-g basal mouse model of breast cancer was used to examine Claudin expression profile in primary tumor and using 3D organotypic assay. To test our hypothesis, CRISPR gene-editing was used to deplete Claudin 3 and Claudin 7 in 3D organotypic assay. Upon individual knockout of Claudin 3 and Claudin 7 using CRISPR gene-editing, breast cancer organoids grown in 3D culture showed elevated invasive ability and increased organoid area. Given that invasion promotes metastasis, we also hypothesized that the loss of Claudin expression would result in increased metastatic potential. To test this, Claudin 3 and Claudin 7 depleted organoids were orthotopically transplanted in immunocompetent mice. Mice transplanted with Claudin7-knockdown organoids showed striking increase in metastasis to the lungs as compared to control. Altogether, our data indicates that Claudin 3 and 7 restrict invasive and metastatic cell behavior in breast cancer progression.

Comparative Genomics of Bifidobacterium Strains Isolated From Early Preterm Infants With Improved Leaky Gut

Presenting Author : Claudia Hernandez-chavez
Hernandez-Chavez Claudia, Lwin Hnin Wai, Sundararajan Sripriya, Viscardi Rose M., Ravel Jacques, Ma Bing

Keywords: gut microbiome; leaky gut; bacteria; bioinformatics; genomes

"Leaky gut" or intestinal barrier immaturity with elevated intestinal permeability (IP), is the proximate cause of multiple conditions including life-threatening necrotizing enterocolitis (NEC), which has a mortality rate of 30-50% in preterm infants. Previous research has identified the microbial biomarker Bifidobacterium strains being associated with improved intestinal permeability, therefore demonstrating significantly improved intestinal barrier functions in preterm infants. However, this potential probiotic of Bifidobacterium strains affecting intestinal maturity has not been previously characterized. In this study, we aim to identify the genome content underlying the functional role that Bifidobacterium plays in the postnatal maturation of the intestinal barrier. To achieve this, we will cultivate Bifidobacterium in 200 preterm infants (25-33 weeks of gestation) with low or high intestinal permeability and characterize their genomic content along with their carbohydrate utilization activities. We have observed a unique and expanded carbohydrate utilization capability of novel Bifidobacterium strains, indicating strong human milk oligosaccharides (HMOs) assimilation capabilities. The characterization of the genome content will warrant future development of probiotics prophylaxis and potentially serve as a screening target to assess intestinal barrier function, thus preventing and/or reducing the severity of leaky gut-associated morbidities and mortality in newborns.

Comparing the impact of Busulfan conditioning on clonal patterning with total body irradiation following rhesus macaque autologous transplantation

Presenting Author : Diana M. Abraham
Diana M. Abraham, Richard J. Lozano, Xavi Guitart, Ryland Mortlock, Diego A. Espinoza, Xing Fan, So Gun Hong, Chuanfeng Wu, Cynthia E. Dunbar

Keywords: Clonal Hematopoiesis; Rhesus Macaque; Busulfan Conditioning; Hematopoietic Stem and Progenitor Cell Gene Therapies; Lentiviral Barcode

The clonal dynamics following hematopoietic stem cell transplantation with busulfan (Bu) conditioning are of great interest to the development of hematopoietic stem and progenitor cell (HSPC) gene therapies. Compared to total body irradiation (TBI), Bu conditioning has reduced toxicity, rendering Bu as a more tolerable and clinically relevant conditioning approach. In this study, we genetically barcoded autologous HSPCs to investigate the impact of Bu conditioning on hematopoietic reconstitution in a rhesus macaque model. We observed distinct clonal patterns in three animals conditioned with Bu post-transplantation. Two of the three animals demonstrated overall vector marking levels less than 4% while a third animal demonstrated marking greater than 15%. Tracking clonal dynamics, we observed initial engraftment by unilineage clones, which were replaced by multilineage clones by 1-2 months posttransplant in all three animals. However, significant variation in clonal patterns among the three animals occurred by 9 months posttransplant. We also analyzed the clonal geographic distribution of bone marrow HSPCs at several timepoints post transplantation. Compared to TBI, Bu-conditioned monkeys displayed more rapid clonal mixing, with CD34+ HSPC clones found in both left and right sites as early as 3.5 months post-transplant. In our previous studies with TBI, we reported a distinct NK clonal pattern consisting of NK-biased massively expanded clones, suggesting peripheral mature NK self-renewal. The NK clonal pattern following Bu-conditioning in all three monkeys was polyclonal and correlated with other lineages, without NK-bias. In conclusion, these results suggest Bu conditioning regimens can variably impact the marrow niche, resulting in marked differences in clonal engraftment patterns, with implications for HSPC gene therapies.

Cytoskeletal feedback controls directed migration signaling networks

Presenting Author : Jonathan Kuhn
Jonathan Kuhn and Peter Devreotes

Keywords: Cell migration; Cell signaling; Microscopy; Actin; Myosin

In many eukaryotic cells, motility requires the coordination of a polymerizing actin network and a contractile actomyosin network. To move in a specific direction the cell must create a single actin polymerization front and an actomyosin back. A signaling cascade governed by the small GTPase Ras controls the size, location, and kinetics of these force-producing cytoskeletal components. While there is evidence that the cytoskeleton can also influence the behavior of signaling proteins like Ras, the causes and consequences of this feedback are largely unknown. To determine how feedback from the cytoskeleton can control upstream networks, we designed a system to tune Myosin activity in a migrating cell and observed the effects on cell shape and Ras activation. Globally disassembling contractile myosin filaments in a cell undergoing amoeboid migration causes an increase in the number of cell fronts and the amount of activated Ras. Additionally, cells with lower Myosin contractility are more sensitive to chemical stimuli which serve as guidance cues for this signaling network. Finally, local disassembly of Myosin bipolar thick filaments at the cell back relieves polarity and suppression of protrusion formation. Together, our data suggest that Myosin is acts as a negative regulator of Ras that helps the cell maintain front-back polarity during migration.

Design of Stable Antibody: HIV-1 RNA Complex Suitable for Structural Studies

Presenting Author : Fairine Ahmed
Fairine Ahmed, Dr. Nele Hollmann, Dr. Michael Summers

Keywords: Fab BL3-6; Core Encapsidation Signal; RNA; Antibody; HIV

Determining macromolecular structures of the Human Immunodeficiency Virus (HIV-1) is vital in developing therapeutic strategies to eradicate the illness. Currently, many large structures of RNA have not been solved; thus, hindering our understanding of the biological roles of RNA in viral assembly. Thus, my role was to develop a method to characterize RNA constructs with Cryo-electron microscopy (cryo-EM), a high-resolution imaging technique. Prior studies confirmed the structure of the Core Encapsidation Signal (CES), a segment on RNA that provides the minimum required signal for genomic packaging. Therefore, we can use CES to create target RNA and compare it to the solution structure. In this study, I am using a recombinantly expressed antibody, Fab BL3-6, as a chaperone that is characterized to recognize and bind to RNA sequences. My goal is to dimerize CES constructs and bind them with Fab BL3-6 to create clear cryo-EM samples. One construct (CCCC) has an antibody binding site that will bind with another construct (GGGG) that lacks the site; the dimerization tests the efficiency of the chaperone on large RNA structures under cryo-EM. The constructs were labeled with different fluorophores (Cy3 and Cy5) by a 3' end ligation reaction. I monitored the assembly capacity using an electrophoretic mobility shift assay (EMSA). With various RNA sequence ratios (0 to 2), I observed the constructs' binding affinity.

Thus far, we confirmed the binding possibility of CCCC with GGGG at a 0.5 to 1 concentration. These results display a homogeneous sample with minimal unbound RNA.

To continue understanding the hybridization capacity of the constructs through fluorophore-labeled oligonucleotides experiments, we can create homogenous samples of the RNA-antibody complexes to achieve a stable structure. The chaperone tags specific sites, enabling distinctions among the varying orientations through which a clear structure can be determined.

Development of molecular sensors and actuators to elucidate PI3K signaling

Presenting Author : Farid Shahid
Farid Shahid, Eesha Yadav, Abhijit Deb Roy, Lakshmi Srinivasan, Justin Nwafor, Sandra Gabelli, Takanari Inoue

Keywords: Synthetic biology; Phospholipids; Cytoskeleton; Cell migration; Nanobodies

Phosphoinositide 3-kinase (PI3K) is a kinase that regulates fundamental cell functions such as proliferation, migration, and survival and defects in PI3K regulation lead to cancer pathogenesis. Despite intense studies, a detailed mechanism of PI3K regulation at subcellular scale remains understudied due to lack of molecular tools to spatially and temporally control PI3K activity. To address this, we aimed to develop molecular tools to spatiotemporally and reversibly sense and control PI3K activity to elucidate its subcellular regulation. We generated a series of single chain nanobodies against PI3K, of which three specific nanobodies (namely nb14, nb16 and nb30) showed binding to PI3K in biochemical assays. To examine how these nanobodies affected PI3K signaling, we used a Rapamycin-based chemical induced dimerization system (CID) to rapidly induce translocation of cytosolic nanobodies to the plasma membrane. We characterized the effects of such recruitment on cellular morphology using live cell fluorescence microscopy. Here, we report that recruitment of nb30 to the plasma membrane in HeLa cells induced membrane ruffling, protrusions, and cell spreading, which is consistent with PI3K activation. We observed similar, but subdued, morphological changes on recruiting nb14. On the other hand, recruitment of nb16 did not have any morphological changes. Based on these observations, we propose that nb30 may be used to actuate PI3K activity, whereas nb16 may be used as a PI3K biosensor in future studies. In future, we will further characterize the binding of nb16 and nb30 with p85 and p110, which heterodimerize to compose PI3K. We will use nb30 to develop an optogenetic tool to spatiotemporally regulate PI3K activity. We will use nb16 to elucidate how PI3K activity is regulated in migrating cells. These molecular tools will help in developing live cell-based drug screening approaches to target PI3K-signaling pathways.

Effects of VEGF inducer GS4012 on blood vessel formation and regeneration in larval axolotl tails

Presenting Author : Malikeya Chaudhary
Dr. Renee Dickie, Malikeya Chaudhary

Keywords: Vascular Endothelial Growth Factor; Angiogenesis; Vascularization; Tissues growth and repair

Axolotls (Ambystoma mexicanum) are a type of salamander known for their remarkable capacity to regrow appendages, making them an excellent model system for regeneration studies. The drug GS4012 is a vascular endothelial growth factor (VEGF) inducer. This study explores the processes of angiogenesis and regeneration in larval axolotls that were treated with GS4012 after the amputation of their tail tips. It was hypothesized that the axolotls in the experimental group would have faster tail regrowth after amputation than those in the control group (n=4/group). Experimental animals were treated with GS4012 for one week, followed by a washout period. The tail tips of all subjects were imaged five, seven, and thirteen days after amputation to observe morphology and quantify the regeneration process. Some of the axolotls were used for histological analysis. Treatment with GS4012 did not reduce survivorship compared to the control group. Vessels were observed in the regrown area of the tail in one animal in the treatment group and none in the controls. At 5 days post amputation, the length and area of tail tips were statistically significantly different (P<0.05) between treatment and control group which suggests that growth in the length and area of the tail tips of the treatment group was slightly faster than the control group. Future studies will include a larger sample of animals to better assess this. These investigations are expected to ultimately give insight into the role of VEGF in tissue repair.

Elucidating interactions between the HIV-1 RRE and the viral proteins Rev and Gag

Presenting Author : Arjun Kanjarpane
Arjun Kanjarpane; Lucia Rodriguez; Jan Marchant, PhD; Michael F. Summers, PhD

Keywords: HIV-1; RNA-Protein Interactions; Viral Genome Nuclear Exportation; Isothermal Titration Calorimetry

The human immunodeficiency virus (HIV) is a retrovirus that can cause acquired immunodeficiency syndrome. Although multiple pharmaceutical options exist to control HIV, medication compliance, feasibility of medicine acquisition, and viral mutations present ongoing issues. Thus, future drug targets require an increased biochemical and structural understanding of viral components and processes. To help facilitate the export of the viral genome from a host cell's nucleus to the cytoplasm, the HIV-1 genome codes for Rev, an RNA-binding protein translated in the early phases of viral replication. Later, Rev re-enters the nucleus via its nuclear localization sequence and binds to the Rev Response Element (RRE), found on incompletely spliced viral RNAs. This complex is then exported into the cytoplasm where the RNA is made available for packaging and translation. The RRE also interacts with the nucleocapsid domain of the structural viral protein Gag. However, the Rev and Gag binding sites on the RRE have only been partially determined, and the biological importance of the Gag interaction is unclear. Therefore, this project seeks to provide a detailed characterization of the interactions between sections of the RRE, Rev, and Gag. We initially focused on an RRE construct comprising a part of Stem I, which has been shown to bind both Rev and Gag. We use a peptide containing the RNA-binding, arginine-rich motif of Rev, and a protein containing the nucleocapsid domain of Gag. We have also focused on Stem II, which contains the highest-affinity Rev binding site, and its interactions with the Rev peptide. We will describe construct design, sample preparation, and characterization by electrophoretic mobility shift assays and isothermal titration calorimetry. With a concrete understanding of RRE-protein interactions, it may be possible to identify conserved drug targets for future pharmaceuticals.

Elucidating structure and isoform-specificity of a novel PI3K p85 endocytic role

Presenting Author : Tony Yao
Tony Yao, Hideaki Matsubayashi, Takanari Inoue

Keywords: Phosphatidylinositol 3-Kinase; Endocytosis; Chemically Induced Dimerization; iSH2; p85

Endocytosis is a critical cell process responsible for the internalization of external cargoes and membrane-bound proteins. Current work (Matsubayashi et al., unpublished) discovered a new potential link between endocytosis and phosphatidylinositol 3-kinase (PI3K). The iSH2 domain of the regulatory subunit of PI3K, p85, was found to form endocytic punctate structures when recruited to the plasma membrane. As the PI3K/Akt pathway regulates essential cell functions including cell survival, migration, and cell cycle progression, this previously unknown link to endocytosis may have important implications for cell physiology. However, much about this novel behavior is still unknown.

This work helps map this new role to iSH2 structure, and probes into the potential function and the evolutionary history of iSH2 puncta production. Using chemically-induced dimerization, I characterized the spatiotemporal dynamics of iSH2 puncta, identified a 46 amino acid region capable of this behavior, and found evidence that this region may be functionally segregated from the iSH2 domain's canonical role: the binding of the catalytic PI3K subunit.

It was previously observed that only p85β, and not p85α, iSH2 could produce puncta. This work finds that while the full length of p85α iSH2 cannot form puncta, a truncated region of 52 amino acids can, indicating that an upstream region of p85α iSH2 may inhibit endocytic puncta formation.

Though the physiological relevance of this behavior remained elusive, the putative AP2-binding motif hypothesized to be responsible for this behavior was found to be conserved only in mammals and bony fish.

This work furthers our understanding of the structural basis of this novel behavior. As the PI3K/Akt pathway is among the most frequently altered pathways in cancer, and is critical for proper cell function, investigating this phenomenon may open new ways of targeting PI3K in cancer and disease.

Evaluating the effect of partial loss of ATXN1 on the expression of BACE1 in the mouse brain

Presenting Author : Austin Dellafosse
Austin Dellafosse, Merin John, Dr. Maria Cvetonovic, Juao Rosa

Keywords: ATXN 1; BACE-1 protein; Alzheimer's disease

According to the amyloid-hypothesis, amyloid-plaque accumulation is a driving factor of Alzheimer's Disease (AD) pathogenesis. These plaques are formed when the β-Secretase 1 (BACE1) enzyme catalyzes the cleavage of the Amyloid Precursor Protein (APP). Previous studies have shown BACE1 expression increases in mice with a total knockout of ATAXIN-1 (ATXN1)—a gene linked to late onset AD. However, a better pathological analogue would be a partial loss of ATXN1, yet the effect of a partial loss remains unknown. My project determines the effects of partial loss of ATXN1 on BACE1 protein expression, and I demonstrate a consistent trend of increased BACE1 levels in western blotting and immunofluorescence staining when comparing wild-type (WT) mice against heterozygous ATXN1 (het) mice. Western blot quantification revealed a higher BACE1 protein expression in the het mice, with the greatest localized differences seen in the hippocampus. Additionally, immunofluorescent staining showed a significant increase in BACE1 intensity in both the hilus and CA3 areas of the hippocampus in het mice. These results indicate a likely pathway where a partial loss of ATXN1 is sufficient to increase BACE1 levels, increasing Aβ accumulation, demyelination of neurons, and increasing the risk of AD.

Acknowledgement: This work was supported by the University of Minnesota and R25 NS083059.

Expression and regulation of Tudor domain containing protein 5-like in the Drosophila germline

Presenting Author : Adam Winter
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Keywords: Sexual dimorphism; Germline gene regulation; Sex determination; Developmental genetics

In sexually reproducing organisms, the successful production of either eggs or sperm depends on the germ cells receiving proper sexual identity information during development. In D. melanogaster, the protein Tudor domain containing protein 5-like (Tdrd5l) has been shown to promote male identity in the germline, but the early expression and regulation of Tdrd5l remains poorly understood. We hypothesized that expression of Tdrd5l will be male-biased in early stages of development and that it will be post-transcriptionally repressed in females. To investigate these hypotheses, we used an endogenously epitope (Flag)-tagged allele of tdrd5l and conducted immunostaining of Drosophila gonads at different stages of development. We also conducted various RNAi experiments, manipulations of the tdrd5l gene, and masculinization of the female soma to investigate regulatory mechanisms acting on tdrd5l. We found clear male-biased expression of Tdrd5l in germ cells beginning at the L3 stage. We also found that early Tdrd5l expression is directly repressed by the RNA binding protein sex-lethal (Sxl) in the female ovary. Additionally, we found evidence of transcriptional regulation of Tdrd5l and evidence that signals from the male soma promote tdrd5l expression. Overall, we found that both cell-autonomous and non-autonomous signals contribute to the sexually dimorphic expression of Tdrd5l in the Drosophila germline.

Front-rear polarity establishment during bleb-based in cancer cell migration

Presenting Author : Ankita Jha
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Keywords: cell migration; polarity; bleb; metastasis; receptors

Cancer cells with high contractility are prone to blebbing and when confined in the absence of integrin based cell-substrate adhesion, exhibit "leader bleb-based" migration characterized by a highly polarized morphology with a long, stable leader bleb leading the direction of migration especially during metastasis. However, the mechanisms mediating the establishment and maintenance of the extreme cell polarization are not known. We sought to test the hypothesis, that polarization is mediated by the segregation of signaling components on the plasma membrane especially growth factor receptors. To test this, I performed time-lapse imaging of highly metastatic melanoma cells under 3μm vertical confinement. In the absence of integrin signaling, EGFR signaling is required for bleb stability and hence polarity during bleb-based migration. EGFR exhibited a strong gradient with accumulation at the base of the bleb compared to the bleb tip. Proteins on the outer and inner leaflet of the plasma membrane are distributed uniformly showing lack of any membrane flow from tip to the base of the bleb. With super resolution live imaging of actin and manipulations, we show receptor gradients are established due to strong corralling by membrane proximal actin, immobile picket proteins like CD44 and rapid protein turnover on the back of the bleb compared to the tip of the bleb not by advection due to fast actin retrograde flow. Stronger membrane-cortex link at the back enhances the actin corralling. With fluorescence anisotropy measurement we show that EGFR activity is high in the back of the bleb. This asymmetry results surprisingly in a reverse front-rear polarity signature compared to adhesion based cell migration with RhoA/ PIP2 in the front and Rac/PIP3 in the back of the bleb. With this work, I elucidate novel mechanisms of protrusion formation and front-rear polarity establishment during less understood bleb-based migration and front-rear polarity establishment.

FtsZ treadmilling dynamics spatiotemporally regulates sPG synthesis in bacterial cell division

Presenting Author : Longhua Hu
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Keywords: FtsZ treadmilling; sPG synthesis; Brownian ratchet mechanism; two-track model; bacterial cell division

Cell division in E. coli is mediated by a large protein complex called the divisome. FtsZ, a tubulin homolog, is one of the most essential divisome components and forms a contractile ring-like structure at midcell. The treadmilling of FtsZ filaments drives the directional movement of the enzymes such as FtsW that synthesize the septum peptidoglycan (sPG) via a Brownian ratchet mechanism. However, it is not well understood how the FtsZ treadmilling process spatiotemporally regulates sPG synthesis. We developed a computational model incorporating FtsZ treadmilling dynamics, FtsW movement as well as its activation to study the spatiotemporal coordination of sPG synthesis. We tuned model parameters utilizing experimental data and performed computer simulations of the model system. We analyzed the motion trajectories of FtsW molecules and also quantified the smoothness of the sPG density spatial profile. Our results showed how the treadmilling process can facilitate sPG spatial smoothness. Our model provides insights into how FtsZ treadmilling dynamics controls the spatial distribution of active sPG-synthesis enzymes to promote efficient sPG synthesis in bacterial cell division.

Identifying and Verifying Variants in Breast Cancer Genes

Presenting Author : Langston Locke
Langston Locke, Mwangala Akamandisa, Katherine Nathanson

Keywords: breast cancer; sanger sequencing; breast cancer susceptibility genes; pathogenic mutations; genomic medicine

According to the World Health Organization, in 2021, breast cancer is the most frequent form of cancer in the world, affecting nearly 13% of all women within their lifetime. A woman's risk of breast cancer diagnosis increases significantly (up to 72%) due to inherited mutations in breast cancer susceptibility genes, such as BRCA1, BRCA2, and MLH1. Genetic variations in these genes are responsible for almost 10% of breast cancer cases. Furthermore, variants found in noncoding regions of DNA are also known to be associated with numerous cancers. In this study, I used sanger sequencing as a verification tool in confirming the presence of known pathogenic mutations in breast cancer genes detected through next generation sequencing. Through this, I have verified that these mutations are credited for various frameshifts and substitutions in these patients' genomes and likely to contribute to disease. Firstly, various scientific studies and papers were carefully selected and analyzed to identify pathogenic mutations in the noncoding regions of breast cancer susceptibility genes. Next, primers were designed to segment the amplicon for nine samples that would be amplified through the polymerase chain reaction. Furthermore, amplicon size was validated through a gel, which separated the fragments of interest by size. Lastly, by using exosap, Big Dye terminator, and centrifuge columns, and a series of reactions were performed on the PCR products before being placed into a Sanger sequencing machine. Once taken out of the sanger sequencing machine, a DNA sequence was obtained and analyzed to authenticate the presence of the variant of interest.

In conclusion, sanger sequencing confirmed that five out of nine samples used in this study possessed pathogenic variants that were initially identified through next generation sequencing. It was also deduced that the intronic regions of breast cancer susceptibility genes were very likely to cause disease, despite not being expressed.

Identifying the missing link between skeleton and skin: Pacin2/Syndapin2 regulates cell motility via dynamically instable subcellular organization consisting of actin and membranes

Presenting Author : Saki Takayanagi
Helen Di Wu, Saki Takayanagi, Takanari Inoue

Keywords: Pacsin2/Syndapin2; F-BAR protein; F-actin; mast cell; chemotaxis

A coordinated remodeling of cellular skeletons (actin filaments) and their skins (plasma membrane, PM) is crucial for innate immune functions that involve dramatic morphological changes such as chemotaxis, phagocytosis and degranulation. To enable a sensitive reaction to an environmental cue in a form of the timely membrane deformation, signal transduction pathways need to cooperate with actin cytoskeleton. However, how this mechano-chemical coupling is achieved at a molecular level remains largely unknown. Family members of BAR domain-containing proteins can curve the PM through BAR domains. These proteins often contain other domains such as an SH3 domain that binds to actin regulators such as N-WASP and dynamin, making these proteins a great candidate for linking actin remodeling to PM deformation.

In this work, we combine live-cell fluorescence imaging, genetics and chemical genetics approaches to reveal a molecular mechanism of a F-BAR protein, namely Pacsin2 (Syndapin2), in forming a unique subcellular organization of several microns at the PM and its significance on actin filament reorganization. More specifically, using total internal fluorescent microscopy, we found Pacsin2 forms a ring-like structure at the PM inner leaflet in RBL-2H3 mast cell lines. These Pacsin2 rings create inward membrane folds enriched with F-actins. Upon mast cell activation with antigen ligands, the Pacsin2 rings temporarily dissipate and then reappear albeit in a different form; a traveling wave. Interestingly, the Pacsin2 wave is in an anti-phase of F-actin and N-WASP. By conducting loss-of-function of the Pacsin2 gene, we uncovered that Pacsin2 is a negative regulator of cell motility by reducing cell velocity during chemotaxis. Therefore, dynamic instability of the Pacsin2 organization, in particular transition between ring-like structure in the resting state and traveling wave during activation, may be at the basis of highly cooperative immune cell functions.

Identifying the Role of Microglia Trogocytosis in Maintaining Excitatory Synapses

Presenting Author : Sarah Syed
Sarah Syed, Jacqueline Griswold, Shigeki Watanabe

Keywords: microglia; synapse trogocytosis; phagocytosis; debris accumulation; confocal live-cell imaging

Microglia, glial cells in the central nervous system, play an essential role in shaping neural circuits and removing pathogens in the cellular environment. Recently, microglia have also been implicated in neurodegenerative diseases as they become activated in the presence of large protein aggregates. However, specific neuron-microglia interactions are still not well understood which leads to continued uncertainty about the role of microglia in disease. Recent evidence suggests that microglia remove synapses through trogocytosis, the process of immune cells removing a small portion of another cell. Current studies have suggested a model where trogocytosis is triggered by synaptic silence or dysfunction. However, preliminary data from the Watanabe Lab suggest that microglia trogocytosis occurs at regions of synaptic debris accumulation as a novel mechanism of synaptic maintenance. We tested these two models by using confocal live-cell imaging to visualize trogocytic events in a primary microglia culture system with purified synapses. Because a synaptic feeding workflow has not been established previously, we developed a novel workflow for visualizing and quantifying microglia trogocytic capacity. Our preliminary findings indicate that our primary microglia retain their phagocytic capacity, suggesting that the culture system we used is not inhibiting of microglia trogocytosis. We have since used our developed protocol to test microglia phagocytic preference for lipids classically associated with either inducing or inhibiting phagocytosis. Preliminary data indicate that microglia prefer phosphatidylserine and avoid sialic acid. Moving forward we will use our culture system to test if microglia trogocytosis is induced by synaptic debris accumulation. Understanding the role of microglia trogocytosis in synapses will help advance our understanding of neuronal health and degeneration and influence the future of therapeutic treatments for neurodegenerative diseases.

Impaired Development of Enteric Glia in the Premature Bowel Contributes to Dysmotility and the Development of Necrotizing Enterocolitis via Exaggerated TLR4 Activation

Presenting Author : Carla M. Lopez
Carla M. Lopez, Mark L. Kovler, Andres J. Gonzales Salazar, William B. Fulton, Peng Lu, Yukihiro Yamaguchi, Maame Sampah, Asuka Ishiyama, Raheel Ahmad, Thomas Prindle, Sanxia Wang, Hongpeng Jia, Chhinder P. Sodhi, David J. Hackam

Keywords: Necrotizing Enterocolitis; Pediatric Surgery; TLR4

Purpose: Necrotizing enterocolitis (NEC) develops in premature infants after exaggerated bacterial Toll-like receptor 4 (TLR4) signaling, through mechanisms that remain unknown. Intestinal motility in premature infants is impaired, suggesting a NEC link. We hypothesized that the premature intestine displays impaired motility due to enteric glia loss, leading to exaggerated TLR4 signaling and NEC.
Methods: Intestinal motility was measured via FITC-dextran and x-ray in mice treated with pro-kinetic agents (Metoclopramide and Cisapride). TLR4 signaling was achieved through lipopolysaccharide(LPS) or NEC-bacteria injection. Neurons (Tubb3+) and glia (Sox10+) were quantified in mouse and human ilea. NEC was induced via formula feeding and hypoxia in wild-type and enteric glial-(Sox10iDtr)-deficient newborn mice. Human intestine was obtained from NEC and stoma-closure controls.
Results: Newborn mouse intestine revealed abundant neurons but significantly reduced enteric glia, and significantly reduced motility (day 0-10: 50±5% vs. day 11-15: 75±11.5%, p<0.05), and dilation on X-ray that could be reversed with Metoclopramide and Cisapride. TLR4 activation via LPS and NEC-bacteria in mice with dysmotility induced hyper-inflammation that was reversed by prokinetic agents (p<0.001). In support of a role for enteric glia in NEC, enteric glia-deficient mice revealed severe dysmotility (WT=6+1.5, Sox10iDtr=3.7+1.2, p<0.05), increased LPS-TLR4 signaling (Tnfa, WT=15+4.3, Sox10iDtr=35+12,) and severe NEC (Tnfa, WT=10+2.1, Sox10iDtr=27+7.5, p<0.001). Strikingly, mouse and human NEC intestine showed reduced enteric glia compared to controls, while administration of prokinetics improved motility, reduced TLR4 signaling and reversed NEC in neonatal mice.
Conclusion: Impaired enteric glia development leads to impaired motility, leading to exaggerated TLR4 signaling and NEC that can be reversed by prokinetic agents, suggesting new motility-based approaches for prevention of NEC.

Insights Into SiaDw Enzyme Reaction Mechanism Through Molecular Modeling

Presenting Author : John-Paul Akinbami
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Keywords: Capsular polysaccharide; Galactosyltransferase; Sialyltransferase; Neisseria meningitidis serogroup W; Glycosyltransferases

Neisseria meningitidis (Nm) is a Gram negative diplococcus that causes bacterial meningitis and sepsis. The extracellular capsule polysaccharides are virulence factors that are essential in the survival of Nm by providing a barrier that hinders attacks from the immune system. Serogroup W, which is one of the leading causes of meningitis worldwide, consists of a repeating galactosyl-sialic acid disaccharide. This study characterizes SiaDw, a capsule polymerase, to understand the mechanism of function. SiaDw structure contains an N-terminal galactosyltransferase domain (aa 1-399) and a sialyltransferase domain (aa 763-1037) and intervening domain (aa 400-762) of unknown function. We aim to study the structure of the individual components of the glycosyltransferase domains and the adjoining stretch of amino acids. To model SiaDw, the Robetta server was used and the resulting models were validated with Molprobity. Motifs that could potentially influence function were characterized by running the HMMER program to identify sequence homologs while alignment was conducted with Clustal Omega. Schrödinger software packages used include: binding pocket identification (SiteMap); and docking of ligand (Glide). The computational studies generated models and identified conserved sequences with functionally important domains. Further, binding pockets for both the galactosyl-tranferase and sialyl-transferase domains were identified, including motifs that are consistent with biochemical and homology studies reported in literature. Substrate sugars were docked to the respective active sites with Glide and docking scores suggest plausible interaction poses. In conclusion, this computational approach provides a foundation for further experimentation to understand the mechanism of function of SiaDw. Mechanistic knowledge will inform future studies using the enzyme as a tool for the bioengineering of novel polysaccharides and aid in the design of therapeutic agents.

Investigation of HDAC11 in Hepatocellular Carcinoma

Presenting Author : Jenna Clements
Jenna Clements, Than Tu Ho, Hening Lin, Edward Seto

Keywords: Cancer; HDAC11; Post translational modification; RNA splicing

Histone deacetylase (HDAC) enzymes are established epigenetic targets in cancers. In liver cancers, high HDAC11 expression is significantly correlated with patients' poor survival. HDAC11 is a unique histone deacetylase enzyme with an efficient alternative function that removes long chain lysine fatty acylation (de-KFA) from non-histone proteins. Using a new small molecule HDAC11 inhibitor (HDAC11i), we illustrate that HDAC11i causes decreased colony formation in hepatocellular carcinoma cell lines (HCC) without significant effects on cell viability. We hypothesize that this phenotype is due to HDAC11i induced alternative splicing of the cancer stem cell (CSC) marker CD44, as HDA11i causes a decrease in CD44v9 and an increase in CD44v5. Thus, HDAC11 as a therapeutic target in liver cancers is a promising avenue of investigation.

Liver-directed and systemic AAV gene transfer approaches for Pompe disease therapy

Presenting Author : Naresh Kumar Meena
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Keywords: Autophagy; Gene Therapy; AAV; Pompe disease; Lysosomal storage disorder

Gene therapy for Pompe disease, a severe neuromuscular disorder, has moved to clinical reality. The disease is caused by a deficiency of acid alpha-glucosidase (GAA), the enzyme which is uniquely responsible for lysosomal glycogen breakdown. Gene therapy is a welcome development because the current enzyme replacement therapy (ERT) with recombinant human GAA (rhGAA) has fallen short of its goal – reversing skeletal muscle pathology. Generally, two Gene therapy strategies have been explored: liver directed and systemic AAV gene transfer. The liver-targeted approach harnesses the ability of the liver to produce and secrete hGAA into the circulation, thus providing a steady supply of the enzyme for cross-correction in other tissues. The systemic method employs a ubiquitous promoter to drive the transgene expression and distribution among multiple tissues. Here, we compared these two approaches in head-to-head experiments in 3.5-month-old Gaa knockout mice. The animals received a single injection of either vector, and skeletal muscle was analyzed five weeks after treatment. At high doses (5.0 × 1013 vg/kg) both vectors reduced the accumulated glycogen by >90%, reversed lysosomal enlargement, and eliminated autophagic buildup. In contrast, there was a significant difference between the two treatments at a lower dose (2.5 × 1013 vg/kg). All measured outcomes - GAA activity, histological analysis, glycogen levels, lysosomal size, and the extent of autophagic accumulation – were much better following systemic compared to liver-directed gene transfer. Systemic delivery resulted in significantly higher levels of GAA activity in muscle on both high and low doses, indicating that the cellular uptake of the tagged enzyme is much more efficient compared to the liver-secreted enzyme, which is structurally similar to rhGAA. Furthermore, the systemic approach resulted in complete reversal of muscle pathology whereas liver-directed did not.

Mechanics of ultrafast coupling between exocytosis and endocytosis at neuronal synapses

Presenting Author : Haoyuan Jing
Haoyuan Jing, Tyler Ogunmowo, Grant Kusick, Shigeki Watanabe, Jian Liu

Keywords: endocytosis; exocytosis; neuronal snapses; active zone; actin

To maintain the surface area of synapses constant, the excess membranes added by synaptic vesicle exocytosis are removed as fast as 100 ms following a single action potential and tens of seconds after high-frequency firing. Although exocytosis and endocytosis are coordinated in space and time, the coupling mechanism is not well understood. Combining theory and experiments, we report a membrane-compression model to explain exo-endocytosis coupling at synapses. We show that actin filaments are organized in a ring, surrounding an active zone where exocytosis occurs. This organization keeps the active zone area constant despite the flux of membranes. Flattening of the exocytic vesicles compresses the active zone membrane, resulting in the rapid formation of endocytic membrane pits at the interface between an active zone and an actin ring due to the area conservation. Consistent with this model, disruption of filamentous actin and myosin II results in failure in the initiation of ultrafast endocytosis. Furthermore, a successful coupling requires more than two vesicles to fuse simultaneously or sequentially within 10 ms. Ultrafast endocytosis fails when the second fusion event is blocked by EGTA or in a mutant lacking a calcium sensor Doc2. The timescale of this endocytic membrane pit formation is variable, ranging from milliseconds to tens of seconds, depending on the frequency of exocytosis and the size of active zones. The membrane-compression mechanism may thus define a general principle of exo-endocytosis coupling.

Mechanisms of growth factor and adhesion receptors crosstalk by clathrin lattices

Presenting Author : Marco A. Alfonzo-méndez
Marco A. Alfonzo-Méndez, Kem A. Sochacki, Marie-Paule Strub, Justin W. Taraska

Keywords: endocytosis; growth factors; cell adhesion; crosstalk; integrins

The crosstalk between growth factor and adhesion receptors is key for cell growth and migration. In pathological settings, these receptors are drivers of cancer. Yet, how growth and adhesion signals are spatially organized and integrated is poorly understood. Here we use quantitative fluorescence and electron microscopy to reveal a mechanism where flat clathrin lattices partition and activate growth factor signals via a coordinated response that involves crosstalk between epidermal growth factor receptor (EGFR) and the adhesion receptor Beta5-integrin. We show that ligand-activated EGFR, Grb2, Src, and Beta5-integrin are captured by clathrin coated-structures at the plasma membrane. Clathrin structures dramatically grow in response to EGF into large flat plaques and provide a signaling platform that link EGFR and Beta5-integrin through Src-mediated phosphorylation. Disrupting this EGFR/Src/Beta5-integrin axis prevents both clathrin plaque growth and dampens receptor signaling. Our study reveals a reciprocal regulation between clathrin lattices and two different receptor systems to coordinate and enhance signaling. These findings have broad implications for the regulation of growth factor signaling, adhesion, and endocytosis.

Modeling mitochondrial network dynamics in protein aggregate inheritance

Presenting Author : Gordon
Gordon Sun, Christine Hwang, Tony Jung, Jian Liu, Rong Li

Keywords: mitochondria; neurodegeneration; yeast

Contrary to their spheroid representation in biology textbooks, mitochondria are found to form intricate reticulated structures, comprised of individual mitochondrion. Throughout the lifespan of an eukaryotic cell, these mitochondria undergo frequent restructuring through fission and fusion to form network structures, which are thought to aid in efficient distribution of mitochondria around the cytoplasm while maintaining network health through known mechanisms such as genetic complementation.

Prior studies have found aggregation prone proteins in the mitochondria capable of driving mitochondrial dysfunction–a common pathological mechanism in the etiology of neurodegenerative disorders. Similarly using yeast as a model organism, we observe accumulation of protein deposits in mitochondria interfering with normal mitochondrial function and structural remodeling dynamics

We hereby report a comprehensive characterization of the impact of protein deposit buildup in driving mitochondrial structural remodeling and loss of function. Buildup of protein aggregates results in a general slowdown in remodeling processes, with pronounced fission/fusion bias in both afflicted and unafflicted mitochondria. We observe and characterize targeted mitochondrial remodeling around protein deposits which facilitate multiple aggregate consolidation and limit inheritance of aggregate afflicted mitochondria. We construct a cellular model of mitochondrial network remodeling and aggregate diffusion to further demonstrate the importance of inner membrane fission/fusion machinery in enforcing homogenization of the mitochondrial matrix content, which consequently drives aggregate consolidation.

Modeling translational bursting at the single-mRNA level

Presenting Author : Oliver Valera
Oliver Valera, Nathan Livingston, Jiwoong Kwon, Bin Wu, Jian Liu

Keywords: mRNA; translation; bursting; stochastic simulation; single-molecule imaging

Gene expression is critical determinant of cellular behavior, identity, and fate. A crucial step of this process is mRNA translation, which exhibits complex, highly stochastic spatiotemporal dynamics that are currently not well understood at the single-molecule level. Translational dysfunction serves as a common source of disease and developmental disorder, rendering further investigation to be of immense biomedical interest. Here we studied the behavior of single mRNAs in live cells using a modified SINAPs (Single Molecule Imaging of Nascent Peptides) technique. While tracking the translation of individual mRNAs, we observed pulse-like, bursting behavior in which the mRNAs appeared to oscillate between translational activity and inactivity. We hypothesized that this bursting behavior may be a result of the mRNAs switching between multiple intrinsic, molecular states with distinct translational characteristics. Using stochastic simulation, we investigated the dynamics of this behavior, and explored potential models of multi-state mRNA translation. We were unable to capture the observed bursting behavior with a single-state model of constitutive ribosomal initiation. Instead, we found that a simple, two-state stochastic switching model for mRNA translation was able to recapitulate the observed bursting behavior, as well as predict the experimentally verifiable dependence of burst duration on open reading frame (ORF) length. Further experimentation would be required to identify the underlying mechanism of any state-switching activity, and a more complex model may be required to reconcile experimental observations resulting from pharmacological intervention or modification of untranslated structures (5'UTR). Altogether our findings suggest that mRNA molecules may utilize multiple molecular states with distinct translational properties, providing additional cellular control over the tightly regulated, yet highly stochastic pipeline of gene expression.

mRNA structure regulates complementary sequences to instruct homotypic mRNA self-assembly

Presenting Author : Siran Tian
Siran Tian, Ziqing Ye, Silvi Rouskin, Tatjana Trcek

Keywords: RNA granule; phase separation; germ granule; condensates; RNA structure

Biomolecular condensates spatiotemporally control the post-transcriptional regulation of mRNAs in cells. It has been proposed that within the condensates, mRNAs interact with each other in trans through base stacking, non-Watson-Crick interactions, as well as sequence-specific Watson-Crick base pairing (WCBP). Previous observations have shown that mRNAs can self-assemble into either homotypic clusters, composed of the same transcripts, or heterotypic clusters, composed of different transcripts. However, how different trans mRNA:mRNA interactions contribute to the composition of mRNA assemblies in vivo is unclear. Here we study the mechanism of homotypic mRNA self-assembly in Drosophila germ granules. We hypothesize that mRNA structure instructs homotypic mRNA self-assembly by regulating complementary sequences. We computationally searched for complementary sequences that could potentially drive homotypic mRNA self-assembly through trans WCBP. Surprisingly, we found that all mRNAs are replete with such sequences and many of them are shared among different mRNAs. Thus, every mRNA is inherently multivalent and has the potential for heterotypic interaction with diverse mRNAs via trans WCBP. We then applied DMS-MaPseq to predict the secondary structure of the germ granule mRNAs in vivo. We found that most complementary sequences are embedded in the secondary structure and thus unavailable for trans WCBP. Also, different germ granule mRNAs are strongly devoid of identical, exposed complementary sequences, suggesting that heterotypic trans WCBP could be prevented by mRNA structure. Our in vivo and in vitro experiments show that counterintuitively, non-sequence-specific mRNA:mRNA interactions primarily drive homotypic mRNA self-assembly, while trans WCBP is the main player in heterotypic mRNA self-assembly. Collectively, our data suggest that the global property of the mRNA, which includes the mRNA structure, generates the specificity of homotypic mRNA assembly in vivo.

Obscurin-deficient Breast Epithelia Generate Secreted Factors to Prime a Vascular Smooth Muscle Cell-dependent Pre-metastatic Microenvironment

Presenting Author : Matthew Eason
Matthew Eason, Talia Guardia, Derek Osorio Luciano, and Aikaterini Kontrogianni-Konstantopoulos, PhD

Keywords: obscurin; pre-metastatic microenvironment; vascular smooth muscle cell; extracellular matrix; metastasis

New therapies targeting metastatic spread are greatly needed to improve breast cancer patient survival. The lung pre-metastatic niche (PMN), primed by vascular smooth muscle cell (vSMC) extracellular matrix (ECM) generation, is essential to breast cancer metastasis. When PMN formation is blocked, metastasis is dramatically reduced. Previous work has shown that obscurin (encoded by OBSCN), a 800kDa scaffolding protein located at the cell membrane in breast epithelial cells, is a potent metastasis suppressor in breast cancer. Higher grade breast cancer biopsies have reduced obscurin expression compared to lower grade biopsies. Mechanistically, reduced obscurin expression in breast epithelial cells increases survival via apoptosis evasion, decreases RhoA-signaling resulting in increased actin dynamics, microtentacle formation and cell migration, and upregulates the PI3K/Akt axis, mediating epithelial-mesenchymal transition (EMT) and stemness. Downstream transcriptional targets of PI3K/Akt-activated transcription factors are known mediators of vSMC ECM deposition and subsequent PMN formation. As a result, we hypothesized that the sole loss of obscurin in breast epithelia was sufficient to trigger the release of secreted factors that prime vSMC-dependent pre-metastatic microenvironment formation. We report that secreted factors generated by obscurin-deficient breast epithelial cells markedly enhance vSMC proliferation, migration, and invasion. Furthermore, vSMCs treated with secreted factors from obscurin-depleted breast epithelial cells upregulate fibronectin and collagen 1a1 mRNA and protein expression, as well as deposition into the ECM. Importantly, this fibronectin/collagen 1a1 enriched matrix is strongly chemoattractive and adhesive, driving enhanced cell spreading and microcluster formation that colocalize with fibronectin and collagen 1a1 fibers, and enhances obscurin-deficient breast epithelial cell chemoresistance.

Prevention of chromosome instability by tinkering with a DNA helicase

Presenting Author : Molly Gordon
Molly Gordon, Gordon Sun, Jin Zhu, Rong Li

Keywords: Chromosome instability; Rrm3; Shugoshin

Cells must equally segregate their genomes to ensure fidelity across generation. Cancers are known to have increased rates of chromosome mis-segregation, also referred to as chromosome instability (CIN). CIN promote metastasis and lead to poor prognosis. As such, it is imperative to understand how cells typically maintain low levels of genomic instability, as this information can be leveraged to prevent diseases progression or even onset. Historically, studies have focused on characterizing CIN-inducing mutations leading to a well-characterized list in both yeast and humans, but little progress has been made to identify pathways inhibiting instability. We have identified an interesting trend in which deletion of an accessory DNA helicase, Rrm3, suppresses high CIN caused by a wide range of genetic perturbations. Although this mutant has altered cell cycle dynamics via activation of canonical regulatory checkpoints, CIN suppression still occurs in the absence of the DNA damage response or spindle assembly checkpoint. A distribution of cohesin at from pericentromere to possible sites of rDNA in the rrm3δ mutant lead us to uncover a dramatic increase in shugoshin abundance in the rrm3δ mutant. Evidence suggests this increased shugoshin or a function pathway to recruit shugoshin to chromatin is required for rrm3δ-dependent CIN suppression.

Quantum Sensing in Cell Biology

Presenting Author : Azmath Fathima
Azmath Fathima, Jacob Curtis, Amber Robinson, Peker Milas, James Wachira, Birol Ozturk

Keywords: Quantum sensing; ODMR; Brain cells; Nanodiamond; Confocal imaging

Quantum sensing with defects in diamond and wide-bandgap semiconductors is an emerging field that enables detection of extremely small temperature changes, host material strain, and magnetic and electric fields. Action potential detection in cardiac tissue and in whole organisms has previously been demonstrated using nitrogen vacancy (NV) defects in bulk diamond crystals. We hypothesized that nanodiamonds (NDs) with NV defects can serve as effective fluorescent markers, as they do not bleach under laser illumination like conventional fluorescent dyes. We performed confocal fluorescence imaging using 60nm and 140nm NDs to test this hypothesis. We also hypothesized that NV NDs can be used to detect action potentials at the single-cell level by sensing magnetic fields induced by the action potentials. To test this, optically detected magnetic resonance (ODMR) experiments were conducted with the NV NDs.

Here we report our results on the confocal imaging of NV NDs in cath.a-differentiated (CAD) mouse brain cells . 60 nm NDs were shown to diffuse into cells within 30 minutes with no additional surface modification. In contrast, 140 nm NDs were observed to mostly remain localized on the cell surface. CAD cells with NDs added (ND+cells), when fixed between two coverslips, exhibited enhanced photoluminescence (PL) signals due to better confinement of the NDs, compared to ND+cells that were allowed to freely circulate within 300-microliter chamber slide wells. NV ND photoluminescence signals did not fade over the course of 5-hour-long imaging studies. ODMR technique was used to detect millitesla-level magnetic fields with 140 nm NDs in solution. Finally, The PL spectrum of 140 nm NDs was obtained in ND+cell solutions as a first step in the detection of action potentials. In summary, NDs were shown to be effective, non-bleaching fluorescent markers in mouse brain cells, with further potential for use in the detection of action potentials.

Rapid Inducible Decay of RNA (RIDR) reveals roles of processing bodies

Presenting Author : Lauren Blake
Lauren Blake, Yang Liu, Bin Wu

Keywords: RNA decay; chemically inducible dimerization; processing bodies; single molecule

Inducible modulation of RNA transcription, translation or degradation is important for probing biological mechanisms and developing therapeutic tools. Existing technologies such as translation inhibition, and CRISPR or RNA interference, can exert global expression regulation, but may lack the transcript specificity or spatiotemporal precision that is required to induce rapid responses and capture transient biological processes. We devised a general method to inducibly tether RNA binding proteins to target RNAs to influence their fate. Specifically, we established a rapid inducible decay of RNA (RIDR) technology by recruiting an RNA decay factor to the target mRNA. RNA decay is pivotal in regulating the abundance of specific mRNAs. RIDR accelerates decay of specific RNAs from the timescale of hours to minutes. The fast and synchronous RNA decay induced by RIDR enabled previously unattainable direct visualization of RNA decay dynamics in cells. We applied RIDR to endogenous β-actin RNA and observed rapid appearance and gradual dissolution of RNA granules. Immunofluorescence staining of known membraneless organelles revealed that these RNA granules colocalized with processing bodies (p-bodies). We measured the RNA recruitment and decay kinetics in p-bodies by fitting a mathematical model of compartmentalized RNA counts in single cells as a function of time. This allowed us to determine the decay kinetics of specific RNAs in p-bodies for the first time. To elucidate the role of p-bodies in RNA decay, we knocked down specific p-body constituent proteins or RNA degradation enzymes. We observed distinct recruitment and decay kinetics in p-bodies. Together, RIDR provides a valuable and generalizable tool to study the spatial and temporal RNA decay mechanisms in cells.

Single molecule tracking reveals a relationship between bacterial FtsWI dynamics and cell division progression.

Presenting Author : Amilcar Perez
Amilcar Perez, Joshua McCausland, Jie Xiao

Keywords: Bacteria; Cytoskeleton; FtsZ; Peptidoglycan synthesis; Single Molecule Imaging

In E. coli, cell division occurs at the midcell via initial assembly of the division apparatus into a septal ring and subsequent septal ring closure. The septal ring contains FtsZ, FtsW (glycosyltransferase), FtsI (transpeptidase), and many other proteins. Together FtsW and FtsI form a protein complex (FtsWI) that is essential for making septal peptidoglycan (sPG) required to divide cells. Recently, single molecules studies in cells demonstrated processive motions of FtsWI in the septal ring. FtsWI processivity can be powered by their own sPG synthesis activity, where this movement occurs within the "sPG track". Alternatively, FtsWI processivity can be driven by FtsZ treadmilling where FtsWI are inactive; where this movement occurs on the "Z-track". It is unknown how FtsWI transitions between these two tracks, and if this is coordinated with different division stages of E. coli cell cycle. Given the essential role of FtsZ treadmilling in pre-constricting cells, we hypothesize that the Z-track is the main mode of synthase dynamics in the initial assembly of the septal ring stages while the sPG track is the main mode of synthase dynamics in the later stage. To address this, we performed single molecule tracking studies where we tracked the dynamics of FtsI or FtsW in vertically oriented E. coli cells. This method allowed for tracking of the processive dynamics of FtsWI throughout the whole septal ring. Using ZapA as a marker for division stage, we showed that the distribution of FtsWI on the Z-track predominates the early division stages whereas FtsWI switch onto the sPG track in later division stages. Analyzing transitions between the sPG track and the Z-track revealed an intermediate "immobile" state suggesting the requirement of additional inputs such as lipid II PG substrate or protein-protein interactions. Altogether this data shows that E. coli spatio-temporally separates active populations of FtsWI to integrate this as a function of cell division progression.

Spatiotemporally precise optogenetics reveal Ras-Akt1 regulate polarity and migration in human neutrophils

Presenting Author : Dhiman S. Pal
Dhiman S. Pal, Tatsat Banerjee, Peter N. Devreotes

Keywords: chemotaxis; signaling networks; lymphocytes; blood cancers

In recent years, Ras GTPases have been investigated extensively in neutrophil development and its various functions such as ROS formation, NET release, and tissue infiltration. However, their role in immune cell motility is still unexplored. Although Ras mediates multiple signaling pathways which control mammalian chemotaxis, there is no conclusive evidence to show Ras activity on the plasma membrane directly regulates cell migration. To prove this, we employed a spatiotemporally precise, cryptochrome-based optogenetic system in the migratory HL-60 human neutrophils to recruit GTP-bound or constitutively active Ras to the cell membrane. K- or H-Ras recruitment during global illumination experiments promoted actin polymerization at the 'front' or leading edge resulting in sustained F-actin protrusions and increased random motility in neutrophils. These findings were corroborated by transient recruitment of GTP-bound Ras to 'back' or inactive regions of the cell membrane which led to polarity breaking and localized protrusion generation. Orthogonally, activating endogenous H-Ras by recruiting RasGRP4, a physiologically relevant RasGEF in neutrophils, showed similar results. Next, pharmacological inhibition studies suggested that these cytoskeletal effects of Ras activation are predominantly mediated through mTORC2 pathway, and not PI3K. These findings prompted us to investigate a crucial downstream Ras-mTORC2 effector, Akt1, whose role in cell migration is still unidentified. Global or localized activation of Akt1 on the membrane showed that it is a positive regulator of F-actin polymerization, protrusive activity, and migration. Interestingly, we also discovered that Akt1 activation occurs via a yet uncharacterized PI3K-independent mechanism in neutrophils. Altogether, our study provides the first mechanistic evidence of Ras-Akt1 in mammalian migration and highlights their importance as a therapeutic target for immune cell migration disorders.

The cranial mesenchyme as a potential driver of neural tube closure

Presenting Author : Claire Charpentier
Claire Charpentier and Irene Zohn

Keywords: development; mouse embryogenesis; neural tube closure; live imaging; cranial mesenchyme

Neural tube closure occurs when the flat neural plate rolls into a tube via neural fold elevation during formation of the central nervous system. In the cranial region, this process is driven by cellular movements and shape changes within the neural plate and the underlying cranial mesenchyme (CM). While the pathways that shape the neural plate are well characterized, how morphogenesis of the CM contributes to neural tube closure remains poorly understood. The CM consists of cells that originate from neural crest (NC-CM) and paraxial mesoderm (PM-CM) and both of these lineages are implicated as key mediators of neural fold elevation. We hypothesize that the expansion of the CM is a driving force for neural fold elevation. To test this hypothesis, our studies analyze cell movements and lineage relationships that occur during normal and abnormal neural fold elevation.

We use a newly developed SiMView adaptive light-sheet microscope to image in vivo mice embryo development to determine the trajectory of CM cell movement and tissue expansion during neural tube closure. Additionally, we use a Hectd1 null mouse mutant with failure in CM expansion to test the requirement for expansion on CM morphogenesis and neural fold elevation. Preliminary studies indicate that Hectd1 is required in the NC-CM for neural fold elevation, yet the PM-CM also fails to expand in the Hectd1 mutant. To test the hypothesis that the NC-CM is causing the failure of neural tube closure, we use genetic lineage tracing strategies and in vitro CM explant assays. Our live imaging studies indicate that cells move in a dorsal lateral direction during neural fold elevation consistent with cell movement driving elevation. Explant assays suggest that PM-CM and NC-CM cells are the cells migrating in wild type embryos. Future experiments will explore how CM movements differ during abnormal neural tube formation using light-sheet microscopy and gene expression changes using RNA sequencing experiments.

The impact of astrocytes on tumor cell dormancy versus proliferation in brain metastatic breast cancer spheroids

Presenting Author : Isaiah Roberts
Isaiah Roberts, Raghu Vamsi Kondapaneni, Rachel Warren, Shreyas S. Rao

Keywords: Dormancy; spheroid; brain metastatic breast cancer; astrocyte; proliferation

While great strides have been made in the fight against cancer through primary, adjuvant, and palliative treatments, research has shown that dormancy, a state that cancer cells undergo where they do not proliferate, allows cancer cells to resist treatments. The dormancy time can fluctuate year to year and the signals that enable the cancer cells to leave the dormant state and proliferate are currently not well understood, particularly in the context of brain metastatic breast cancer. Herein, we examined the impact of astrocytes (supporting cells of the brain) on the phenotype of brain metastatic breast cancer cells in a co-culture spheroid model. In this model, we employed brain metastatic breast cancer cells and astrocytes in ratios of 1:1, 1:4, and 1:9 with cancer cells only serving as controls and cultured them for a period of 7 days. Among the 3 co-culture ratios tested, 1:4 and 1:1 culture condition showed an increasing trend in proliferation as measured via spheroid area. The spheroid area for 1:9 condition decreased initially and stayed constant over the period of 7 days. Consistent with this observation, we found that the percentage of Ki67 (a proliferation marker) cells was significantly lower in the 1:9 condition compared to the other conditions. In future studies, we will further characterize the tumor cell phenotype, as well as their response to drug therapies. Such models provide useful tools to study cell-cell interactions and how such interactions impact the dormant phenotype. Ultimately, an improved understanding of dormancy may lead to the development of new therapeutic strategies.

The role of acetylated microtubules in cell migration

Presenting Author : Cristian J. Saez-gonzalez
Abhijit Deb Roy, Cristian J. Saez-Gonzalez, Takanari Inoue

Keywords: cell migration; microtubules; acetylation; focal adhesions; front-and-back polarity

Cell migration is essential for many physiological processes including embryonic development, wound healing, and immune responses. Cells achieve efficient directional migration through two major steps: front-and-back polarization and re-arrangement of interactions between the cell and extracellular matrix (ECM). Cells adhere to the ECM using multiprotein complexes called focal adhesions (FAs) with which the cells exert forces to push or pull themselves to migrate. Cell polarity and migration are regulated in part by the dynamic reorganization of FAs and microtubules (MT) network. However, it remains unknown if there is a central regulator that orchestrates these distinct processes. Our lab has recently found that cells lacking α-tubulin acetyltransferase 1 (αTAT1), the sole mediator of microtubule (MT) acetylation, display defects in FAs and front-and-back polarity. These results suggest a universal role of acetylated MTs in cell migration. Here, I hypothesize that MT acetylation is a master regulator of directional cell migration by regulating FA dynamics and front-and-back polarity. To test this, I combined live-cell microscopy with pharmacological and genetic perturbations to evaluate their effects on cell migration. Our results show that cells with an αTAT1 knock-out (KO) showed increased motility during random cell migration and defects in directional migration during chemotaxis. In addition, while immunostaining of vinculin, a marker for FAs, showed a decreased signal in cells lacking αTAT1, western blot showed a similar expression of other FA proteins between our WT and αTAT1 KO cells. These results suggest that defects in FAs in cells lacking acetylated MT, are a consequence of FAs formation, and not due to differences in expression. Collectively, this data demonstrate a novel role of acetylated MTs as a regulator of directed cell migration by regulating front-and-back polarity and FAs dynamics.

The role of ESCRTs in signaling within the testis stem cell niche

Presenting Author : Mara Grace
Mara Grace, Erika Matunis

Keywords: stem cells; cell biology; endocytosis; cell signaling; Drosophila

Adult stem cells are crucial for regeneration, tissue repair after injury, and developmental processes such as spermatogenesis. Stem cells exist in a dynamic microenvironment termed the niche that provides signals to ensure the maintenance and self-renewal of the adult stem cell population. An appreciation of the dynamic communication between stem cells and their niche is vital to understand processes such as reproduction, oncogenesis, aging, development, and regeneration. Here I use the testis of Drosophila melanogaster as a model to investigate the role of endocytosis, and the ESCRT complexes specifically, in signaling within the stem cell niche. Endocytosis regulates a myriad of signaling pathways as well as cellular communication. The ESCRT complexes are involved in a variety of cellular processes, such as multivesicular body formation and particle budding, and are considered endocytic tumor suppressor genes due to their role in signal attenuation. Knockdown of ESCRTs in somatic stem cells causes niche cells to become significantly enlarged and exhibit abnormal morphology. Furthermore, Unpaired, a ligand for the JAK-STAT pathway normally secreted by niche cells, accumulates within the cytoplasm of niche cells upon ESCRT knockdown in somatic stem cells. As knockdown of ESCRTs in somatic stem cells affects the morphology of niche cells, this suggests that ESCRTs mediate signaling from somatic stem cells back to their niche to prevent niche hypertrophy. I am further exploring the role of additional pathways in modulating this signaling. A deeper understanding of the signaling dynamics within the Drosophila testis stem cell niche will have further implications for stem cell niches in other tissues and organisms as well as processes such as regeneration and renewal.

Tight junction proteins restrict aggressive cancer cell behaviors

Presenting Author : Junior West
Junior West, Andrew Ewald

Keywords: Tight Junction; Breast Cancer; Organoids; Metastasis; Cell Biology

Breast cancer is the most common cancer diagnosed among women. Metastasis of breast cancer cells to distant organs is responsible for most breast cancer related deaths, yet our understanding of this process remains limited. Highly metastatic cells generally display decreased expression of tight junction proteins, and this phenomenon is a defining feature of the 'Claudin-low' subtype of breast cancer. Normally, tight junction proteins are known to promote proper intercellular adhesion and epithelial barrier function in healthy epithelial cells. We hypothesize that expression of tight junction proteins is required to promote normal tissue architecture, that they suppress metastatic cell behaviors, and that their loss directly promotes breast cancer metastasis. Using a genetically engineered mouse model (GEMM) of breast cancer, we have observed a spontaneous decrease in TJ mRNA expression during tumor growth in vitro and in vivo. Localization of tight junction proteins is also disrupted in cells found within hyperplastic lesions. Using 3D organotypic culture assays we demonstrated that knockdown of tight junction proteins generally results in increased growth and invasion of mammary organoids. Specifically, we have observed a 2-fold increase in organoid size and average organoid growth rate after depleting two tight junction proteins, ZO1 or ZO2. We have also observed increased invasion of epithelial cells into the surrounding matrix and loss of normal ductal architecture upon ZO1 or ZO2 depletion. These results indicate a role for these proteins in restricting proliferative, invasive, and metastasis of cancer cells. Taken together, our results support a model whereby cancer cells spontaneously turn off tight junction expression during tumor progression and that this promotes aggressive cancer cell behaviors.

Triple negative breast tumors contain heterogeneous cancer cells expressing distinct KRAS-dependent molecular invasion programs

Presenting Author : Elodie Henriet
Henriet E, Knutsdottir H, Grasset EM, Dunworth M, Haynes M, Bader JS, Ewald AJ

Keywords: Organoids; Heterogeneity; TNBC; Invasion; Kras

Inter-patient and intra-tumor heterogeneity complicate the identification of predictive biomarkers and effective treatments of basal-triple negative breast cancer (b-TNBC). Invasion is the initiating event in metastasis and is known to occur by both single and collective mechanisms. We cultured primary organoids from a b-TNBC genetically engineered mouse model (GEMM; C3(1)-TAg) in 3D collagen I gels to characterize their invasive behavior. We observed that organoids from the same tumor presented different invasive phenotypes that we classified as non-invasive, collective invasive and disseminative. The relative proportion of these phenotypes varied between mice indicating that our assay recapitulated both inter- and intra-tumor heterogeneity. To identify the molecular regulators driving these invasive phenotypes we developed a workflow in which we isolated individual organoids from the collagen gels based on their invasive behavior, pooled organoids of the same phenotype, extracted RNA and performed RNA-seq. We then validated our top targets by assessing their requirement for invasion using shRNA knockdown. Strikingly, Kras was required for both collective and disseminative phenotypes. As Kras cannot be easily targeted by small molecules, we inhibited signaling nodes up and downstream of Kras. We performed a drug screen on both upstream growth factor receptors and downstream pathways. We found that EGFR, MAPK and PI3K/Akt signaling were involved in invasion by promoting both collective and disseminative phenotypes. In conclusion, we demonstrated that different cancer cells in the same b-TNBC tumor can simultaneously express different metastatic molecular programs and we identified Kras as an essential regulator of both collective and single cell dissemination.

Tumor suppressive role of cell competition in eliminating cells with genomic damage

Presenting Author : Chaitali Khan
Chaitali Khan and Nicholas E Baker

Keywords: Cell Competition; p53; Genomic damage; Drosophila; Tumor suppression

Cell competition is a process of eliminating relatively unfit cells from chimeric tissues. Differences in p53 activity underlie many instances of cell competition in mammals, but no comparable role has been seen for p53 levels in Drosophila. The transcription factor Xrp1 is an effector of cell competition in Drosophila, and is also a p53 target gene induced upon DNA damage. We report that Xrp1 mediates multiple functions of p53 in the DNA damage response, mediating p53-dependent gene transcription and DNA damage-induced apoptosis. Differences in either Xrp1 or p53 activity, occurring between wild type and mutant cells experiencing mild genotoxic stress, both resulted in cell competition. Unexpectedly, cell competition due to differential p53 activity did not require Xrp1 but was enhanced by it. We show that p53, and to a lesser extent Xrp1 limit the accumulation of genomically-damaged cells after DNA damage, and propose that genomic damage enhances cellular growth and promotes cell competition of wild type cells.

Upregulation of lamin B receptor in metastatic melanoma mediates loss of nuclear envelope integrity during confinement

Presenting Author : Michelle Baird
Michelle A. Baird, Alexander X. Cartagena-Rivera, Cayla Jewett, Mehdi Pirooznia, Robert S. Fischer, and Clare M. Waterman

Keywords: melanoma metastasis; confined migration; Lamin B Receptor; nuclear deformability

Metastatic melanoma (MM) is characterized by its mutational heterogeneity and aggressive metastatic spread. During metastasis, MM cells migrate through diverse microenvironments, including regions of tissue confinement, resulting in nuclear deformation. This leads to loss of nuclear envelope (NE) integrity and DNA damage. We hypothesize that during metastatic progression, expression levels of NE genes are altered, facilitating nuclear deformability and NE fragility, mediating an increase in genetic heterogeneity within the population. To test this, we performed bioinformatic analysis of RNA-seq data sets from patient samples of MM and benign nevi, revealing NE proteins upregulated in metastatic disease. Performing a targeted siRNA-based screen using a PDMS confinement device to assay for nuclear fragility, we found reduction of lamin B receptor (LBR) dramatically reduced NE fragility in MM cells, and ectopic overexpression of LBR was sufficient to increase NE fragility in benign melanocytes. Utilizing functional protein domain truncations and point mutations in LBR, we found the cholesterol synthase activity of LBR was specifically required for increased NE fragility, independent of LBRs additional roles tethering heterochromatin and lamins to the NE. Additionally, we found that reduction of LBR in MM cells results in a reorganization of cholesterol in the NE. Thus, LBR generated cholesterol in the NE promotes NE fragility. To determine if LBR-mediated NE fragility was correlated with increased nuclear deformability, we assayed NE mechanics with atomic force microscopy. We found that reduction of LBR results in an increase in nuclear stiffness and a decrease in deformability, while LBR overexpression in benign melanocytes results in an increase in nuclear deformability. These results show that upregulation of LBR in MM plays dual roles in reducing nuclear deformability and increasing NE rupture, specifically through alterations in NE cholesterol organization.

Mechanoresponsive Adaptability in Pancreatic Cancer Cells and Tissue

Presenting Author : Eleana Parajon
Eleana Parajon, Douglas N. Robinson

Keywords: Non-muscle myosin IIC; Alpha-actinin 4; Pancreatic cancer; Mechanoadaptability; Mechanosensing machinery

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive cancer characterized by early systemic dissemination and poor patient prognosis. Our lab identified a PDAC program of upregulation of key highly mechanoresponsive cytoskeletal proteins that accumulate in response to mechanical stress. Cancer cells are highly adaptive and must continuously adjust to their ever-changing environment, including nutrient and oxygen availability, stiffness of the local matrix, and the neighboring cells. The mechanoresponsive proteins compose an adaptability program that allows cells to respond to alterations in many of these parameters. In humans, this mechanoresponsive machinery encompasses nonmuscle myosin II proteins (NMIIA, NMIIB, and NMIIC), alpha-actinins (ACTN4, but not ACTN1) and filamin (FLNB with FLNA to a much smaller extent). However, it is still unclear how the mechanoresponsive cytoskeletal machinery drives PDAC dissemination and metastasis. NMIIA, NMIIC, ACTN4, and FLNB, are expressed at low levels in normal pancreatic ductal epithelia but become highly expressed in PDAC. I hypothesize that NMIIC and ACTN4 help promote cancer cell dissemination and invasion. I am utilizing a three-dimensional, reconstituted tissue model with engineered cells where I am altering the adaptability program, starting with NMIIC and ACTN4. I will also utilize the small molecule 4-HAP that works through NMIIC, promoting its over-assembly and reducing cancer cell dissemination. I will also investigate how these engineered cells influence neighboring cell behaviors and tissue mechanics. This effort will decipher how alterations in these proteins impact cell growth, shape, motility, and invasiveness and will guide future strategies for manipulating these proteins towards a therapeutic end.

Targeting the sterol regulatory element-binding protein (SREBP) pathway in pancreatic ductal adenocarcinoma

Presenting Author : Stephanie Myers
Stephanie Myers, Meredith McGuire, Wei Shao, Chune Liu, Theodore Ewachiw, Zeshaan Rasheed, William Matsui, Toni Seppala, Richard Burkhart, Peter Espenshade

Keywords: Lipids; SREBP; SCAP; Pancreatic cancer; Synergy

Background: Pancreatic ductal adenocarcinoma (PDAC) is a very aggressive tumor with limited diagnostic and therapeutic options. Due to its proliferative nature and desmoplastic stroma, tumor cells are challenged with meeting a high demand for lipids in a hypoxic, lipid-poor environment. Cancer cells respond to this demand through sterol regulatory element-binding proteins (SREBPs), which are master transcriptional regulators of lipid homeostasis that require SREBP cleavage activating protein (SCAP) during signaling.

Methods: Using four patient-derived PDAC cell lines, SCAP was knocked out. All cell lines were utilized in functional growth assays in lipid-variable conditions, subcutaneous xenograft, and orthotopic xenograft experiments. A well-established PDAC mouse model, LSL-KrasG12D/+; LSL-Trp53R172H/+; Pdx-1 Cre (KPC), was utilized, and KPC mice lacking Scap in one or both alleles were generated. In all cell lines, the following FDA-approved drugs were applied individually and in combination: Dipyridamole (DP), Fluvastatin, and Simvastatin.

Results: In lipid-poor conditions, SCAP knockout cells showed significantly reduced cell growth. In tumor xenograft models, SCAP knockout cells exhibited reduced tumor growth and tumor volume. KPC mice with a heterozygous loss of Scap exhibited a significantly increased median survival time. In combination, DP with either statin demonstrates synergy in lipid-poor conditions. Furthermore, DP with Fluvastatin increased apoptosis in lipid-poor conditions.

Conclusions and impact: Loss of SCAP in PDAC tumor cells alters the growth capability both in vitro and in vivo. Heterozygous loss of SCAP in the KPC mouse model significantly increased survival. Finally, dipyridamole works in synergy with statins to alter growth of tumor cells and enhances apoptosis in lipid-poor conditions. These findings suggest that targeting the SREBP pathway has significant therapeutic potential in pancreatic cancer.

Cortical dynamics feedback into metabolic activity and mechanisms

Presenting Author : Mark Allan C. Jacob
Mark Allan C. Jacob, Douglas N. Robinson

Keywords: Cytoskeleton; Dictyostelium Discoideum; Feedback Control; Metabolism; non-muscle myosin II

The cytoskeleton is an energy intensive system responsible for many cellular processes such as cell motility, morphology, and division. Accordingly, this demand for energy applies pressure on metabolic pathways to drive energy production to fuel cytoskeletal dynamics. Feed forward systems, like AMPK signaling, modulate cytoskeletal proteins like non-muscle myosin II (NMII) through phosphorylation – reducing cytoskeletal dynamics while alleviating metabolic strain. However, feedback systems where cortical cytoskeletal dynamics influence ATP producing pathways, have yet to be described. Based on genetic screens and proteomic studies done in Dictyostelium discoideum, our lab has shown mitochondrial proteins to associate with NMII as either genetic suppressors or interactors, such as ancA, mmsdh, and cyc1. We hypothesize that NMII assembly and function feedback and regulate glycolysis and oxidative phosphorylation (OxPhos) in Dictyostelium discoideum. To test this, NMII knockouts and constructs were generated to be measured in Agilent's Seahorse Analyzer platform. This platform will determine ATP production attributed to either OxPhos or glycolysis. We further consider that NMII may not necessarily be the only protein to be involved in the feedback system. Therefore, we will evaluate other cytoskeletal proteins such as Cortexillin I, a cooperative actin-bundling interactor of NMII, to test if they interact in the feedback system. These results will lay the foundation of cytoskeletal impact on metabolic activity. Following this assessment of metabolic activity, we will likely incorporate a metabolomics approach to characterize the mechanisms and elements involved in cortical dynamics that relay to glycolytic and OxPhos drive. Collectively, we aim to define if and how a feedback system derived from cortical activity, ultimately impacts metabolism.

Uncovering a myosin regulator in pancreatic tumor cell mechanics and behavior

Presenting Author : Shantel Angstadt
Shantel Angstadt, Qingfeng Zhu, Elizabeth M. Jaffee, Douglas N. Robinson, Robert A. Anders

Keywords: PDAC; non-muscle myosin II; MYPT1; myosin phosphatase; cortical mechanics

Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest cancers as the third leading cause of cancer related deaths with a 5-year survival rate of 10.8%. A critical need is to develop new PDAC-specific prognostic and therapeutic strategies. To this end, we are investigating the role of the myosin phosphatase targeting subunit 1 (MYPT1). MYPT1 was originally implicated in PDAC through a clinical trial where it was found to be upregulated in patient PDAC cells and established PDAC cell lines. MYPT1 is a component of the myosin phosphatase complex that dephosphorylates the regulatory light chain of non-muscle myosin II (NMII) to promote disassembly. NMII assembles into bipolar thick filaments, which are the functional units within the cytoskeleton that are largely responsible for cell shape changes and have been linked to the altered cell mechanics associated with cancer transformation and metastasis. As a major regulator of NMII, we hypothesize MYPT1 has implications in cancer cell shape control, especially during cell division, migration, and invasion. To elucidate MYPT1's function in PDAC, we developed an in vitro model system composed of engineered cell lines representing each stage of PDAC (noncancerous ductal epithelium, primary tumor-derived, and metastatic site-derived) with MYPT1 knocked out via CRISPR. Each of the cell lines was then characterized for alterations in NMII-mediated cortical mechanics and oncogenic behavior. Thus far, we have demonstrated loss of MYPT1 to increase regulatory light chain phosphorylation, drive NMII paralog C assembly, and regulate cell surface tension. Interestingly, MYPT1-mediated surface tension antagonizes cell shape change ability as observed through proliferation, migration, and invasion data. Using the case of MYPT1, we aim to provide insight for investigations into the targetability of PDAC mechanobiology with the ultimate goal of increasing its 5-year survival rate.

Evolutionary Expansion of the MAPK Signaling Network and the Origin of Animal Multicellularity

Presenting Author : Jeeun Song
Jeeun Song, Amy Peterson, EJ Huang, Michael Pokrass, Gabriel Bever, Sergi Regot

Keywords: animal multicellularity; MAPK network; embryonic compaction; evolution; single-cell imaging

Individual cells and multicellular tissues require fundamentally disparate processes to ensure survival and homeostasis. Many of these processes, from yeast osmoregulation to plant apoptosis, are controlled by the ubiquitous three-tier Mitogen Activated Protein Kinase (MAPK) network. Because multicellular organisms must prioritize tissue homeostasis over individual cell survival, multicellular evolution necessitated profound changes in signaling-mediated control of cell behavior. Recent studies suggest that rather than de novo creation of new gene families, pre-existing pathways in unicellular organisms were duplicated then co-opted for tissue-level control. Nevertheless, molecular pathway changes that propelled the emergence of multicellular animals remain a poorly understood. Using a multidisciplinary approach, we show that phylogenetically distinct groups of MAP3Ks have distinct contributions to unicellular and multicellular fitness. Network-wide single-cell characterization of the downstream outputs of each MAP3K reveal that TKLs preferentially activate the MAPK JNK, whereas STEs preferentially activate p38. Phylogenetic analysis of eukaryotic MAPKs and MAP2Ks reveals that a concurrent divergence of JNK and its cognate MAP2Ks from an ancestral module occurred at the origin of animal multicellularity. The importance of this evolutionary expansion is supported by embryological parallels in the transition from unicellular to multicellular physiology. We show that the TKL-JNK signaling axis is activated during mouse embryo compaction and essential for further development as a multicellular collective. Based on our interdisciplinary study, we propose that the emergence of the JNK pathway provided a necessary platform for the origin of animal multicellularity.

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