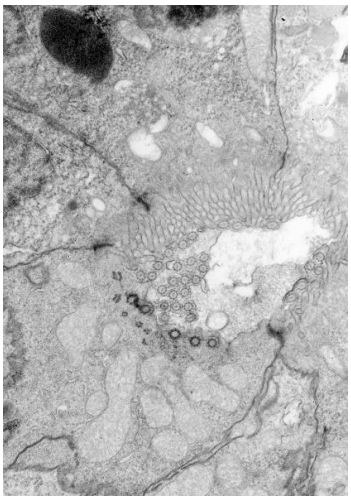
ROTATION PROJECTS AVAILABLE IN THE CBMP PROGRAM IN 2016/17

Gerry Apodaca, Ph.D. Professor of Medicine gla6@pitt.edu

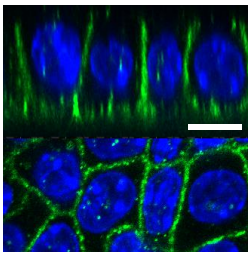
Role of uroplakins in urinary tract development and congenital anomalies of the kidney and urinary tract (CAKUT):



CAKUT are developmental disorders that occur in 1 out of every 500 live births, yet the cellular or molecular basis of these malformations is not well understood. One gene targeted in CAKUT is *UPK3a*, which encodes the type I transmembrane protein uroplakin 3a (UPK3a). Substitution of a Pro residue for Leu (P273L) in the cytoplasmic domain of human UPK3a leads to renal adysplasia and other urinary tract defects. To better understand why UPK3a expression is important, we have examined the function of Upk3l (aka UPK3d), the UPK3a-like ortholog in zebrafish. Upk3l is expressed at the apical surfaces of the pronephric tubule-associated epithelial cells that form the zebrafish larval urinary tract (i.e., pronephros). Strikingly, loss of Upk3l expression leads to altered epithelial differentiation, including the aberrant expression and distribution of polarity proteins, as well as defects in morphogenesis, including loss of apical microvilli.

Current projects in the lab include determining whether UPK3a/Upk3l interacts with Par polarity proteins, identifying how polarity proteins contribute to the formation of the microvillar brush border, and defining whether the P273L mutation of UPK3a causes disease as a result of aberrant and/or defective trafficking of this protein

Michael Butterworth, Ph.D. Assistant Professor of Cell Biology michael7@pitt.edu



- *In the Kidney*

We investigate the regulation of microRNAs in the kidney by hormones, and the mechanisms by which microRNAs alter ion transport. Current projects seek to understand miRNA regulation by several hormones including insulin and aldosterone. We employ a range of bioinformatics approaches to identify miRNA targets and then investigate the molecular mechanisms that these targets use to alter the activity of sodium transporters and channels in the kidney. MiRNA links to diseases like diabetes and chronic kidney disease is a new focus in the lab.

- *In the Airway*

The epithelial sodium channel (ENaC) is responsible for the movement of sodium and water, across epithelial membranes. ENaC activity is increased by the action of proteases which cleave its extracellular loops to activate the channel. A proposed role for ENaC in the human airway is the maintenance of airway fluid homeostasis and regulation of airway surface liquid (ASL) height. One hypothesis to account for decreased ASL height and airway dehydration in cystic fibrosis is inappropriate upregulation of ENaC due to an imbalance in protease activity. Bacterial proteases may therefore contribute to virulence by increasing proteolytic activation of ENaC and reducing mucociliary clearance, which would facilitate colonization. Studies aim to investigate the mechanisms that underlie this activation.

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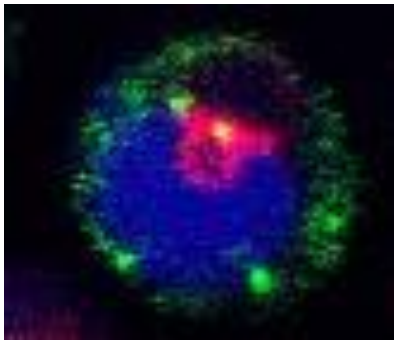


Our goal is to understand how members of the dynamin-related protein family catalyze membrane fusion and fission events throughout the cell. Dynamin itself is well understood: it assembles into helical structures around the necks of endocytosing vesicles and, in response to GTP hydrolysis, undergoes a concerted conformational change that results in membrane scission. Other members of the family are responsible for fusion and fission events at many other cellular membranes, including the mitochondrial membranes. We use Vps1, a yeast-specific member of the family, to examine the role of dynamin-related proteins in the membrane deformations required for yeast to respond to starvation stress. To this end, we use high throughput genomics, cell biology, biochemistry and structural biology.

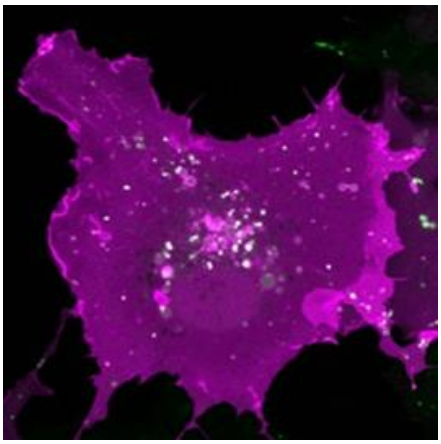
Arjumand Ghazi, Ph.D. Assist. Professor of Pediatrict arjumand.ghazi@chp.edu

1. miRNAs and small RNAs that are used as signals to communicate aging status between organismal issues.
- This project involves examining the role of miRNAs that are produced by the acivity of lipid-regulating transcription factors in neurons and how they influence aging and lipid metabolism in other tissues in the worm *C. elegans*

2. The balance of lipid breakdown and synthesis in metabolism and aging
- This project will use a combination of molecular genetic, genomic and imaging approaches to understand how the simultaneous enhancement of lipid production and breakdown is coordinated in animals, and how this promotes a long healthy life.

Gerry Hammond, Ph.D. Assist. Professor of Cell Biology ghammond@pitt.edu

The tightly regulated flux of materials and signals across an exquisitely organized plasma membrane (PM) is essential for healthy cellular function. Although typically less than 5% of the total PM, inositol lipids are key players in directing this careful choreography, regulating each PM process individually and constraining activity to the PM. Many bacterial and viral pathogens actively take over inositol lipid metabolism to hijack cellular function, and severe genetic diseases sometimes stem from an inability to properly control inositol lipid levels, which ultimately disrupts PM function. Our lab focuses on basic questions concerning how cells organize these lipids in healthy cells; how the cell controls this organization; and crucially, how its disruption leads to disease. We use a variety of state-of-the-art high resolution imaging and chemical genetic approaches to tackle these questions in single, living cells. These techniques allow us to monitor cellular regulation in real time, and to model diseases by actively controlling lipid organization.



Ossama Kashlan, Ph.D. Assist. Professor of Medicine, obk2@pitt.edu

My lab is interested in how extracellular factors affect the structure, function, and dynamics of the epithelial Na+ channel (ENaC). Acid sensing ion channel 1 (ASIC1) is the only member of the gene family whose structure is resolved. We recently generated a hybrid or chimeric channel, where we swapped the most diverse domain in the protein family from the ENaC alpha subunit into ASIC1. The primary purpose of this maneuver was to crystallize and determine the structure of this ENaC domain. However, we also found that this chimeric channel has unique properties. One project could be work on the purification of the chimeric channel which we are now growing up in an insect cell line. Another project could be to characterize the functional properties of the chimeric channel, which has the potential to inform both ENaC and ASIC1 function.

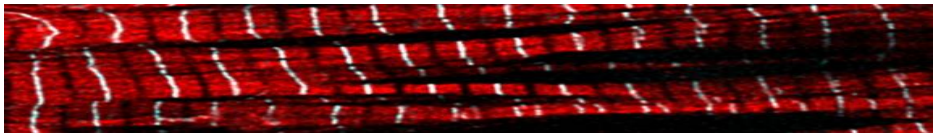
Thomas Kleyman, MD Professor of Medicine and Cell Biology kleyman@pitt.edu

The Kleyman laboratory focuses on studies of epithelial Na^+ channels (ENaC) and large conductance Ca^{2+}-activated K^+ (BK) channels that are found in epithelia lining the aldosterone sensitive distal nephron and airways. Recent work has focused on the identification of sites within ENaC's extracellular domain that have key roles in the modulation of channel activity in response to extracellular factors, including Na^+, shear stress, and proteases. We have identified novel functional human ENaC variants, and are assessing how these variants affect renal Na^+ handling and blood pressure in rodent models. ENaC are expressed in vascular endothelial cells, and we examining their role in modulating vascular tone in response to changes in laminar shear stress. We are examining mechanisms by which mechanical forces and changes in dietary K^+ regulate BK channels. We are particularly interested in defining the roles of members of the WNK (with no lysine (K)) kinase family in facilitating adaptive changes in BK channel expression in response to changes in dietary K^+.

Adam Kwiatkowski, Ph.D. Assist. Professor of Cell Biology adamkwi@pitt.edu

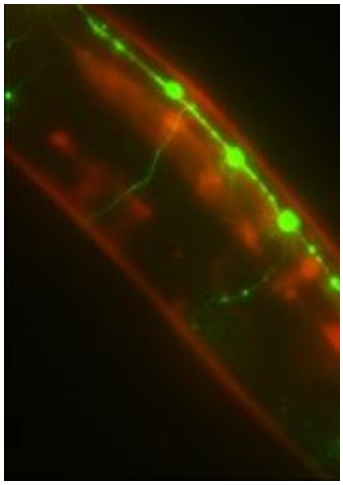
A long-term objective of the Kwiatkowski Lab is to gain a deep mechanistic understanding of cardiomyocyte adhesion and cytoskeletal organization at the intercalated disc. Our approach is to define mechanisms of cell-cell adhesion, and downstream regulation of actin and intermediate filament organization, by the cadherin/catenin family of adhesion proteins. This is an important biomedical problem because mutations in cell adhesion and cytoskeletal proteins at the intercalated disc are linked to cardiomyopathies.

The Kwiatkowski Lab uses a combination of protein biochemistry, cell biology and light and electron microscopy to study cell adhesion and cytoskeletal proteins at the molecular and cellular levels. Potential rotation projects using cultured primary cardiomyocytes and combining advanced live cell fluorescence microscopy with biochemistry include: 1) Defining mechanisms of mechanotransduction through the actin-binding protein α-catenin at cardiomyocyte cell-cell junctions; 2) Determining the role of α-catenin ligands, particularly afadin, in anchoring the actomyosin network to cell-cell adhesions; and 3) Defining the molecular pathways the direct and coordinate initial cell junction assembly in neonatal cardiomyocytes.



Todd Lamitina, Ph.D. Associate Professor of Pediatrics todd.lamitina@chp.edu

Ageing is the #1 risk factor for most human diseases, including many neurodegenerative conditions. It is now clear that the ageing process is under genetic regulation and that manipula- tion of ageing genes can slow the onset and reduce the severity of age-related disease pathophysiology. Most ageing genes also regulate the response to environmental stress, suggesting an important relationship between ageing and stress. Our lab studies the cell biological and physiological responses to stress using the small roundworm C. elegans. Using this system, we explore how stress responses might be leveraged to treat ageing and age-related diseases. We also use C. elegans to develop new models for age-related human diseases.



Currently, we are developing a C. elegans model of Amyotrophic Lateral Sclerosis, or ALS, and utilizing in vivo approaches to investigate the role of ageing and ageing pathways in disease pathogenesis.

Aleksander Rajkovic, M.D., Ph.D. Professor rajkovic@upmc.edu

Research topics:

- Genetic causes of premature ovarian failure
- Transcriptional regulation of oogenesis
- Uterine leiomyoma formation
- Ovarian function and aging in general

My scientific focus has been on the genetics of reproductive tract development. My laboratory has utilized mouse transgenic models to discover novel transcriptional regulators which play an important role in male and female gonadal and reproductive tract development. We also conduct genomic studies, utilizing chromosomal microarrays and whole genome sequencing to identify mutations that cause human reproductive pathologies. We use animal models to model mutations and their effect on the gonadal and reproductive tract development. Our studies are currently focused on the ovary, testes and uterine development and genetics of infertility and uterine tumors.

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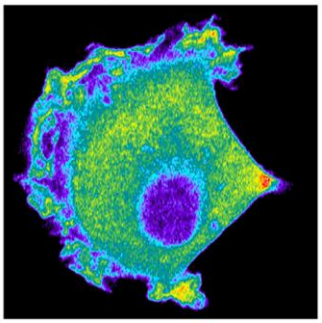

Directed cell migration plays an important role in embryonic development, wound healing, angiogenesis, immune response, cancer invasion and metastasis. Dynamic reorganization of actin cytoskeleton, a key aspect of cell migration, is regulated by the concerted actions of various classes of actin-binding proteins (ABPs), and some of these ABPs are fundamental drivers of actin-based cell motility. Altered expressions and activities of fundamental drivers of cell migration are correlated with aberrant cell motility in pathologic scenarios. Our main research efforts are to 1) gain novel insights on how dysregulation of fundamental drivers of cell migration contributes to metastatic progression of solid cancers, 2) study fundamental aspects of angiogenesis, and 3) develop translational strategies (through virtual screening of compound library followed by functional assays and unbiased small molecule screening) exploiting the pathways of dysregulation as means to suppress metastatic phenotype of cancer cells and angiogenesis-dependent pathology. We are also studying how myocardin family transcriptional cofactors regulate gene expression, endothelial cell migration and angiogenesis through its SRF-dependent and –independent actions. For these studies, we utilize a variety of experimental approaches including RNAi, 2D gel electrophoresis, mouse models of cancer, in vitro and in vivo angiogenesis assays, functional genomics and proteomics, live cell imaging, computational-based identification of protein-protein interaction inhibitors and small molecule screening.

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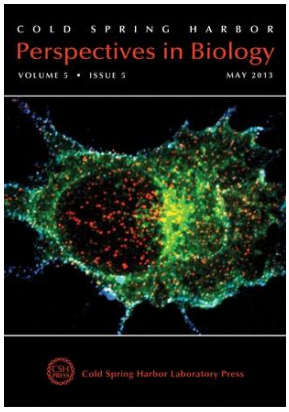

EGF receptor signaling in time and space: This project involves analysis of subcellular localization and dynamics of signaling proteins participating in signaling by epidermal growth factor (EGF) receptor in living cells. Endogenous H-Ras and K-Ras proteins will be labeled with fluorescent proteins by novel genome-editing techniques, such as using TALEn and CRISP, in cells derived from cancer patients (head-and-neck carcinoma). Multidimensional fluorescence microscopy imaging of living cells, image analysis, fluorescence recovery after photobleaching, FRET and other techniques will be used to analyze dynamics of endogenously labeled Ras proteins. These data will be used to generate the computational spatiotemporal model of EGF receptor signaling.

Mechanisms of EGF receptor endocytosis in cancer cells:
This project involves analysis of the molecular mechanisms of epidermal growth factor (EGF) receptor endocytosis and intracellular sorting in head-and-neck cancer cells. Using high-resolution fluorescence microscopy, FRET, biochemical measurements of endocytosis rates, RNA interference, high-throughput screening, mass-spectrometry, general biochemical and cell biology techniques, the mechanisms of endocytosis and sorting will be studied in cultured cells and in tumor tissue in vivo. The role of endocytosis in the regulation of tumorigenic signaling by EGF receptor will be also investigated

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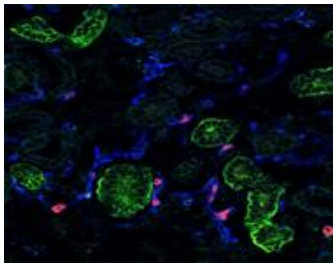
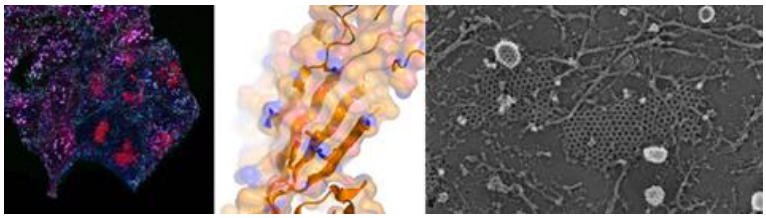


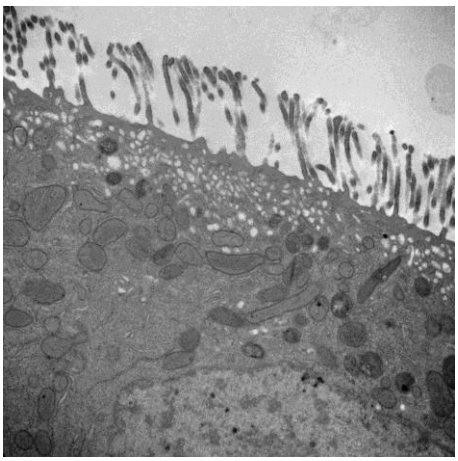
Dr. Sims-Lucas' research program interrogates the role of blood flow, oxygenation and endothelial progenitors in patterning developmental organs with a particular focus on the kidney and lung during development and disease. Formation of the vasculature is a critical developmental process that requires tightly regulated cellular and molecular processes. The developing kidney is a very vascular organ that receives approximately 20% of the total cardiac output. The peritubular capillaries surround the nephron tubules and play a critical role in reabsorption and electrolyte balance. Little is known about the various origins of the peritubular capillary network and the role of the peritubular capillaries in normal development and also during disease processes. His laboratory identified an endothelial progenitor in the kidney that is critical for kidney development and also limiting postnatal injury. Dr. Sims-Lucas' robust program also interrogates the role of oxygenation and blood flow in patterning the formation of the kidney. His research is funded by an NIH grant as well as grants from the Diabetes Complications Consortium and by the Vascular Medicine Institute.

Linton Traub, Ph.D. Associate Professor of Cell Biology traub@pitt.edu

We study aspects of protein trafficking from the eukaryotic cell surface. Clathrin-mediated endocytosis is an ancient and fundamentally important process that is linked to human health and disease. Currently we focus on a group of pioneer proteins that cooperate to begin assembly a clathrin-coated vesicle in about a minute. Projects range from using gene-editing techniques to inactivate relevant genes in cultured cells and zebrafish, to biochemical reconstitution assays with purified proteins and mutant forms. The consequences of pioneer protein gene-inactivation in human haploid HAP1 cells also need to be examined using proteomics. Experience with and training in molecular biology, biochemistry, cell-based assays, and zebrafish developmental biology are all available.



Ora A Weisz, Ph.D. Professor of Medicine weisz@pitt.edu



In humans, the entire plasma volume is filtered into the kidneys approximately every half hour. Most of the water, salt, glucose, and protein that enters the ultrafiltrate is reabsorbed by cells lining the proximal tubules of kidney nephrons.

Research in the Weisz lab focuses broadly on understanding how membrane traffic in proximal tubule cells responds to physiologic cues to maintain kidney function. Our team is unraveling the mechanisms by which newly synthesized proteins are sorted and delivered to the appropriate plasma membrane domains of differentiated kidney cells. Additionally, we have been generating new *in vitro* and *ex vivo* systems, including disease models, with which to unravel how proximal tubule cells in the kidney alter their endocytic and ion transport capacity in response to changes in tubular flow and the accompanying fluid shear stress.

Our studies have direct implications for the understanding and treatment of genetic and other disorders that result in tubular proteinuria and eventually lead to kidney failure, including Lowe syndrome and sickle cell disease.