# DEPARTMENT OF DEVELOPMENTAL AND MOLECULAR BIOLOGY

The department consists of the laboratories of Drs. Alissa Baker, Nick Baker, Teresa Bowman, Dianne Cox, Ana Maria Cuervo, Meelad Dawlaty, Sofia de Oliveira, Antonio Di Cristofano, Carolina Eliscovich, Ellen Fraint, Andreas Jenny, David Loeb, Anne Müsch, Michael Ross, Aditi Shastri, Rajat Singh, Nicholas Sibinga, Richard Stanley, Amit Verma, Duncan Wilson and Fajun Yang. Research interests in the Department cover four major areas: (1) Cell determination, regulation and dysregulation in blood, heart, kidney, muscle, nervous and reproductive systems development (Drs. N. Baker, Bowman, Chen, Dawlaty, Eliscovitch, Jenny, Riascos Bernal, Ross, Sibinga, Stanley, Verma and Wolkoff); (2) Signal transduction pathways regulating cellular function or developmental interactions (Drs. N. Baker, Cox, Jenny, Müsch, Stanley and Yang); (3) Regulation in normal and neoplastic cells (Drs. A. Baker, Bowman, Cox, de Oliveira, Di Cristofano, Fraint, Loeb, Shastri and Verma); and (4) Protein synthesis, processing, targeting, intracellular vesicle trafficking, metabolism and autophagy (Drs. Cuervo, Jenny, Müsch, Singh and Wilson). Faculty frequently collaborate on projects within and among these areas.

The strength of the Department derives from our outstanding group of PhD and MD/PhD graduate students, who have been encouraged to, and accepted the challenge of, working along-side of our faculty in all aspects of department life. We have weekly departmental work-in-progress (Fridays at noon) and theme-focused journal clubs (in the morning) that all graduate students participate in, along with post-doctoral fellows and our faculty. In these meetings, we enjoy not only superb presentations but also probing questioning-answering periods in an atmosphere of learning, appreciating, and being critical and caring at the same time. A cherished asset of the department is our spirit of cooperation, which makes being a member of DMB a fruitful and pleasant experience that you will take with you after you finish your Degrees.

The Department's outside speakers' seminar program contains regularly scheduled Memorial and "student-invited speaker" seminars. Students and post-doctoral fellows poll and vote on their most favorite speakers every year and the students serve as hosts at the seminars.

The department retreats are another highlight of our departmental life. The retreats have been held in a mountain lodge in the Shawangunk Mountains in upstate New York, Mystic Seaport in Connecticut, Long Island Aquarium and Exhibition Center, Riverhead, NY and Montauk on the east tip of Long Island, among many others. Last year's retreat in November was at Edith Macy. In addition to formal presentations of research findings and strategies at these retreats, we discuss ways of improving the function and atmosphere of the department as well as finding time to socialize!

Besides the scientific events, we are just as proud of our recreational activities, including our BBQ and picnic at Glen Island at the beginning of the school year, and the joyful but competitive presentations of skits by the students and the faculty at our Holidays parties, when students and faculty try to outwit each other!


## DR. NICHOLAS E. BAKER

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**Cell competition, translation, and neuronal development**

One of the most effective ways to find the genes required for new processes is the genetic approach of identifying mutations that disrupt the process. We use *Drosophila* and more recently mice to address newly discovered mechanisms of growth regulation.

Cell competition Cell competition results from differences between cells, and does not occur between similar cells. Our studies show that one function of cell competition is selectively removing cells that have become aneuploid, or acquired other large-scale genetic changes. Since aneuploidy is found in nearly all cancers, is a hallmark of aging, and is responsible for birth defects and most spontaneous miscarriage, cell competition may be important in preventing cancer, birth defects and age-related diseases. We used fruitflies to identify genes that are required for cell competition Our current goals include the molecular characterization of the cell competition pathway, including how differences between cells are recognized, how cell competition and the tumor suppressor p53 are related, and how changes in cell competition might increase or be exploited to decrease cancer incidence.

Regulation of translation Ribosomes are essential for growth. Their biogenesis and assembly are regulated, both during growth and in neurodegenerative disease. Ribosomal proteins are affected in several human diseases and also appear to act as tumor suppressors for multiple cancers. How ribosomal proteins act as tumor suppressors is not yet clear or affect neurological disease is not yet clear. Our laboratory discovered that cell competition involves novel signaling pathways that are activated by defects in ribosome assembly, and that defects in ribosome assembly surprisingly cause transcriptional responses. We are interested in the molecular signaling mechanisms activated by ribosomes, and their potential roles in cancer and neurological diseases.

Neural cell fate determination Proneural bHLH proteins are the transcriptional master regulators for most neuronal differentiation and important in neuronal reprogramming strategies. Their activities appear to be highly regulated. Our studies use genetic screening in *Drosophila*, modern genome resequencing methods and multidisciplinary studies to characterize how proneural bHLH proteins are regulated in neuronal fate determination. Surprisingly, defects in proneural bHLH genes act in part through non-apoptotic caspase-dependent processes that appear to control neuronal cell fate specification. Homologs of many of these genes are implicated in schizophrenia, axon and dendrite patterning, suggesting that transcriptional control of non-apoptotic caspase signaling may be relevant to brain diseases.

**Recent publications and preprints**


Kiparaki, M., Khan, C., Folgado Marco, V., Chuen, J., and Baker, N.E. The transcription factor Xrp1 orchestrates both reduced translation and cell competition upon defective ribosome assembly or function. BioRxiv https://doi.org/10.1101/2021.07.12.452023

Folgado-Marco, V., Ames, K., Chuen, J., Gritsman, K., and Baker, N.E. Haploinsufficiency of the essential gene RpS12 causes defects in erythropoiesis and hematopoietic stem cell maintenance. *Elife*, under revision. *BioRxiv* https://doi.org/10.1101/2021.05.04.442585

Ji, Z., Chuen, J. Kipakai, M., and Baker, N.E. (2021) Cell competition removes segmental aneuploid cells from *Drosophila* imaginal disc-derived tissues based on ribosomal protein gene dose. *Elife*, **10**:e61172.

Quiquand, M., Rimesso, G., Qiao, N., Suo, S., Zhou, C., Slattery, M., White, K.P., Han, J.J., and Baker, N.E. (2021) New regulators of *Drosophila* eye development identified from temporal transcriptome changes. *Genetics*, **217(4)**: iyab007.doi: 10.1093/genetics/iyab007.

Baker, N.E. Emerging mechanisms of cell competition. (2020) *Nature Reviews Genetics,* **21**; 683-697.

Blanco, J., Cooper, J.C., and Baker, N.E. (2020) Roles of C/EBP class bZip proteins in the growth and cell competition of Rp ("Minute") mutants in Drosophila (2020). *Elife,* **9** 9:e50535

Ji, Z., Kiparaki, M., Folgado, V., Kumar, A., Blanco, J. Rimesso, G., Liu, Y., Zheng, D., and Baker, N.E. (2019) *Drosophila* RpS12 controls translation, growth, and cell competition through Xrp1. *PLoS Genetics,* **15**(12):e1008513.

Wang, L.-H. and Baker, N.E. (2019) Salvador-Warts-Hippo pathway regulates sensory organ development via caspase-dependent non-apoptotic signaling. *Cell Death & Disease* **10** 669.

Li, K. and Baker, N.E. (2019) Transcriptional and post-transcriptional regulation of *extra macrochaetae* during *Drosophila* adult peripheral neurogenesis. *Dev Biol* **449**: 41-51.

Baker, N.E., Kiparaki, M., and Khan, C. (2019) A potential link between p53, cell competition and ribosomopathy in mammals and in *Drosophila*. *Dev Biol* **446**: 17-19.

Lee, C.H., Kiparaki, M., Blanco, J., Folgado, V., Ji, Z., Kumar, A., Rimesso, G., and Baker, N.E. (2018) A regulatory response to ribosomal protein mutations controls translation, growth, and cell competition. *Dev Cell*, **46**, 456-469.

Baker, N.E., and Brown, N.L. (2018) All in the family: neuronal diversity and proneural bHLH genes. *Development*, **145:** dev159426.

Li, K., and Baker, N.E. (2018) Regulation of the *Drosophila* ID protein Extra Macrochaetae by proneural dimerization partners. *Elife* **7**: e33967.

Kale, A., Ji, Z., Kiparaki, M., Rimesso, G., Flibotte, S., and Baker, N.E. (2018) Ribosomal protein S12e has a distinct function in cell competition. *Dev Cell* **44**, 42-55.


## DR. TERESA BOWMAN

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## Bowman Laboratory

Hematopoietic stem cells (HSCs) are one of the most widely utilized stem cell populations in the clinic today. Our research focuses on identifying the regulation of normal and malignant HSCs. Our studies combine the advantages of zebrafish and mammalian models to explore the development and genetic regulation of HSC formation and regeneration. Zebrafish offer powerful genetic pliability, easily accessible *in vivo* imaging, numerous transplantation assays, and screening capabilities. We aim to identify factors that are critical in the HSC regenerative response, which can be used to inform therapeutic strategies to improve treatments for patients with hematologic diseases.

**RNA regulation of tissue homeostasis**: Proper mRNA processing is critical to HSC function, but which components are involved is largely unknown. Recent identification of somatic mutations in spliceosomal components in patients with myelodysplastic syndrome (MDS) demonstrated how deregulation of splicing could lead to a disease state. We are combining zebrafish genetics and genomic techniques to determine how mutations in spliceosomal factors result in aberrant hematopoiesis. Zebrafish is well-suited for this project as mutants in a subset of splicing factors display hematopoietic stem cell or differentiation defects. We have performed chemical modifier screens for factors that enhance or suppress defects in a zebrafish spliceosomal mutant, identifying tissue-selective roles for spliceosomal components in DNA damage and inflammatory signaling. Determining the contextual specificity of interactions between splicing and these processes will help reveal how mutations in general machinery could elicit specific phenotypic outcomes *in vivo*.

Through these studies, we also demonstrated that embryonic neurons and HSPC formation are especially dependent on proper homeostasis of R-loops, non-canonical nucleic acid structures comprised of RNA:DNA hybrids and ssDNA. R-loops are thought to normally aid in cellular processes such as mitochondrial DNA replication and transcriptional regulation, but excessive or mis-localized R-loops promote genomic instability and inflammation. Not all R-loops are equal, so our next steps are to uncover which R-loops and the underlying mechanism that drives hematopoietic and neuronal dysfunction. These findings can be therapeutically exploited to treat R-loop-associated diseases as we gain a deeper mechanistic understanding of how R-loop imbalance causes disruption to tissue homeostasis.

**Deciphering the development of HSC regenerative capacity:** Rapid hematopoietic recovery following myeloablative treatments or post hematopoietic cell transplantation is critical to minimize complications from infection, bleeding, or anemia. In order to find additional pathways involved in HSC regeneration, we plan to take advantage of the optical transparency and screening capabilities afforded in the zebrafish. We have developed numerous novel approaches to examine self-renewal and differentiation capacities in developing zebrafish and into adulthood via hematopoietic cell transplantation, targeted cell ablation, and lineage tracing. The goal is to identify novel regulators of HSC regeneration using genetics and chemical screening in the zebrafish. Ultimately, we aim to find new ways to expand adult HSCs and guide improved *de novo* generation and expansion of functional HSCs from pluripotent stem cells.

Lab website: https://sites.google.com/view/tvbowmanlab/

**Selected Publications**


Fraint E, Feliz Norberto M, and Bowman TV[#]. A Novel Conditioning-Free Hematopoietic Stem Cell Transplantation Model in Zebrafish. *Blood Advances,* 2020; 4(24):6189-98. PMCID: PMC7756993.

Weinreb JT, Ghazale N, Pradhan K, Gupta V, Potts KS, Tricomi B, Daniels NJ, Padgett RA, De Oliveira S, Verma AK, and Bowman TV[#]. Excessive R-loops Trigger an Inflammatory Cascade Leading to Aberrant HSPC Expansion. *Developmental Cell,* 2021; 56(5):627-640 e5. PMID: 33651979.

Weinreb JT, Gupta V, Sharvit E, Weil R, and Bowman TV[#]. Ddx41 inhibition of DNA damage signaling permits erythroid progenitor expansion in zebrafish. *Haematologica*, 2021; doi:10.3324/haematol.2020.257246. PMID: 33763998.

**Selected Reviews**

Nik S and Bowman TV[#]. Splicing Factors in Neurodegeneration. *WIREs RNA,* 2019; 10(4): e1532. PMID: 30895702.

Fraint E*, Ulloa BA*, Feliz Norberto M, Potts KS[#], and Bowman TV[#]. Advances in Pre-clinical Hematopoietic Stem Cell Models and Possible Implications for Improving Therapeutic Transplantation. *Stem Cells Translational Medicine*, 2021; 10(3):337-345. PMCID: PMC7900582.

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## Vascular Calcification in Patients with Chronic Kidney Disease

Research description

Thirty million people in the United States are estimated to have chronic kidney disease (CKD). In patients with CKD, arterial calcification (pathological deposition of calcium phosphate salts into the arterial walls) is common, with a prevalence approaching 80%, and may contribute to their high cardiovascular mortality. The long-term goal of our research program is to understand the pathophysiology of arterial calcification and to develop new treatment strategies for it.

Our research program includes:

1) the role of calciprotein particles (CPPs) in the development of arterial calcification. CPPs are nanoparticles that are composed of calcium phosphate crystals and calcification inhibitors. They are thought to mediate the transport and clearance of excess calcium and phosphate in circulation.
    a. We developed a new, microplate -based assay that uses dynamic light scattering to measure both the size of secondary CPPs and transformation time. With the assay, we investigate the role of CPPs in arterial calcification using human vascular cells and CKD patients.
    b. To assess arterial calcification in patients with CKD patients, we are collaborating with Nuclear Medicine and Cardiology to $^{18}$F-sodium fluoride perform positron-emission tomography (PET) with computed tomography (CT). $^{18}$F-sodium fluoride PET has higher sensitivity and can detect nascent micro-calcification that is under the threshold of computed tomography, which is routinely used in research.
2) the role of cellular interaction in arterial calcification. Studying the paracrine signaling between endothelial cells and vascular smooth muscle cells will help identify novel therapeutic targets that attenuate vascular calcification without adverse effects on bone mineralization.

# MACROPHAGE PHAGOCYTOSIS AND MOTILITY

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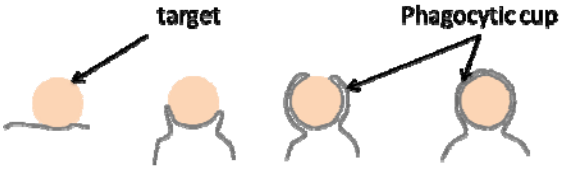
**Macrophage Phagocytosis and Motility**

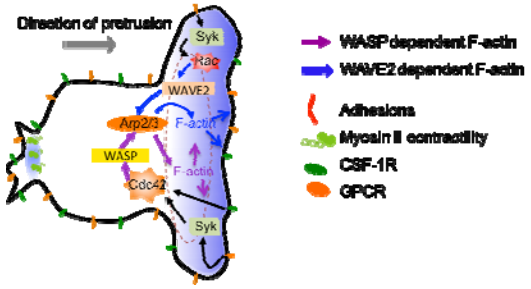
*Macrophages play important roles in host defense against invading micro-organisms and they are also key players in initiating and maintaining an immune response. However, macrophages can also play negative roles, such as in chronic inflammatory disease. Also, tumor-associated macrophages (TAMs), which are present in large numbers in many tumors, appear to play an important role in promoting the progression of solid tumors to an invasive, metastatic phenotype. Macrophages are therefore a prime target for therapies, but it is important to elucidate the mechanisms by which they are recruited to and activated in tissues.*

**Studying the molecular mechanisms of phagocytosis**




Among their many roles, macrophages are best known for their striking ability to engulf a large number of big (>0.5µm) particles that are very diverse in size and shape in a process called phagocytosis. Phagocytosis is important in many situations such as the clearance of pathogen and particles (bacteria, yeast, pollen and pollutants). Crucially, phagocytosis may also be a major player of cancer immunity by mediating the engulfment and killing of cancer cells. Phagocytosis requires actin assembly, pseudopod extension, and phagosome closure (Figure 1). Actin polymerization in response to particle binding requires the activation of members of the Rho GTPases, either Rac or Cdc42 for Fc gamma Receptor-mediated phagocytosis or Rho in the case of CR3-mediated phagocytosis. Both Rac and Cdc42 regulate the cytoskeleton in part through the activation of the Wiskott Aldrich Syndrome/ WASP verprolin-homologous (WASP/WAVE) family of proteins. RhoG, another family member, is also important for phagocytosis but the precise role of RhoG is currently unknown. We are exploring the roles of these signaling pathways as well as those regulating the myosin family of molecular motors in phagocytosis. We are employing a novel technique called traction force microscopy to understand the roles of these factors in the protrusive forces needed to engulf the diverse particles of various sizes and stiffnesses found in nature.

**Studying the molecular mechanisms of chemotaxis**



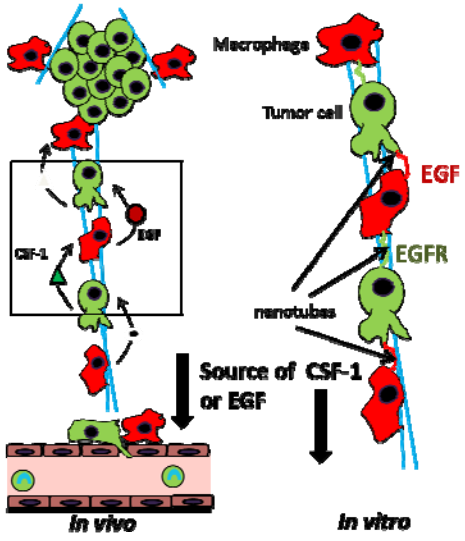

The directed movement of cells in response to chemoattractants involves several complex, interrelated processes, including directional or chemotactic sensing, polarity, and motility. These processes are mediated by complex, interacting signaling pathways that appear to have many similarities but yet have distinct

characteristics depending on the chemoattractant and receptor. Many of the signaling cascades utilized for phagocytosis are also required for chemotaxis yet they result in the appearance of different structures (Figure 2). We are currently dissecting the signaling pathways required for macrophage chemotaxis towards:

1. CSF-1, a growth factor for macrophage survival and differentiation produced by many tumors and found in high concentrations in arthritic joints;
2. Chemokines that direct monocyte recruitment to different tissues.

**Determining the role of macrophages in the tumor microenvironment**

It is now increasingly recognized that the tissue microenvironment plays a critical role in tumor progression, but most studies on tumor metastasis involve the use of endpoint assays or *in vitro* studies on cell lines. It appears that macrophages and tumor cells




participate in a paracrine interaction, with the tumor cells secreting CSF-1 and macrophages secreting EGF, but the precise roles of this paracrine interaction in tumor metastasis are unknown. We have developed a number of *in vitro* assays that reconstitute paracrine interaction between macrophage and carcinoma cells that mimic *in vivo* interactions of macrophages and tumor cells in the tumor microenvironment that have been observed by intravital imaging using multiphoton microscopy (Dovas et al.,*J. Microscopy* 2012). We are currently using these assays to understand the roles of both soluble factors and direct interaction between macrophages and tumor cells through tunneling nanotubes to mediate long distance signaling to promote tumor metastasis (Figure 3).

**Development of tools for live cell imaging**

In order to determine the role of individual molecules in macrophage functions it is essential to understanding the timing and localization of specific protein involved. We have been actively involved in the generation of new probes for single live cell imaging including photoconvertible actin probes that label various structures in live cells. We have also developed and employed biosensors to monitor localized protein activity in live cells, including the recent work in collaboration with Dr. Louis Hodgson on the development of isoform specific single chain RhoGTPase biosensors.

**Selected Publications (Students in bold)**

**Abou-Kheir, W.**, Gevrey, J-C., Issac, B.M., Yamaguchi, H., and Cox, D. (2005) A WAVE2/Abi1 complex mediates CSF-1-induced F-actin rich membrane protrusions and migration in macrophages. *J. Cell Science* 118:5369-5379.

**Abou-Kheir, W.G**., Isaac, B., Yamaguchi H., and Cox, D. (2008) Membrane targeting of WAVE2 is not sufficient for WAVE2 dependent actin polymerization: a role for IRSp53 in mediating the interaction between Rac and WAVE2. *J. Cell Science* 121:379-90.

**Luo, Y**., Isaac, B.M., Casadevall, A., and Cox D. (2009) Macrophage phagocytosis suppresses F-actin enriched membrane protrusions stimulated by CX3CL1 and CSF-1. *Infect. and Immun.* 77:4487-4495.

Isaac, B.M.*, **Ishihara, D.*, Nusblat, L.M.**, Gevrey, J-C, Dovas A., Condeelis, J., and Cox, D. (2010) N-WASP has the Ability to Compensate for the Loss of WASP in Macrophage Podosome Formation and Function. *Exp. Cell Res.* 316:3406-16. *co-first

**Nusblat, L.M**., Dovas, A., and Cox, D. (2011) The essential role of N-WASP in podosome-

mediated matrix degradation. *Eur. J. Cell Biol.* 90:205-212.

**Ishihara, D.**, Dovas A., Park, H., Isaac, B.M., and Cox, D. (2012) The chemotactic defect in Wiskott-Aldrich Syndrome macrophages is due to reduced persistence of directional protrusions. *PLoS One* 7(1):e30033

**Ishihara, D.***, Dovas, A.*, Hernandez, L., Pozzuto, M., Wyckoff, J., Segall, J., Condeelis, J., Bresnick, B., and Cox, D. (2013) Wiskott-Aldrich syndrome protein regulates leukocyte-dependent breast cancer metastasis. *Cell Reports* 4:429-436 *co-first authors

Rougerie, P., **Miskolci, V.** and Cox, D. (2013) Generation of membrane structures during phagocytosis and chemotaxis of macrophages: role and regulation of the actin cytoskeleton. *Immunol. Reviews*. 256:222-239.

Park, H., Dovas, A., **Hanna, S.**, Cougoule, C, Marridoneau-Parini, I., and Cox, D. (2014) Tyrosine phosphorylation of Wiskott-Aldrich Syndrome protein (WASP) by Hck regulates Macrophage Function. *J. Biol. Chem.* 289(11):7897-906.

**Hanna, S.*, Miskolci, V.***, Cox, D.# and Hodgson, L.# (2014) Development of a new single-chain genetically encoded Cdc42 FRET biosensor. *PLoS One* 9(5):e96469. *co-first authors, #co-corresponding authors

**Miskolci, V.,** Hodgson, L., Cox, D., and Vancurova, I. (2014) Western Analysis of Intracellular Interleukin-8 in Human Mononuclear Leukocytes. Methods in Molecular Biology: Cytokines. 1172:285-93.

**Miskolci, V.,** Spiering, D., Cox, D., and Hodgson, L. (2014) A mix-and-measure assay for determining the activation status of endogenous Cdc42 in cell lysates. Methods in Molecular Biology: Cytokines. 1172:173-84.

Wu, B.*, **Miskolci, V.***, Donnelly, S.K., Cox, D., Singer, R.H.[%] and Hodgson L.[%] (2015) Synonymous modification of repeated sequences in retroviral reporters. *Genes Dev.* 29 (8):876-86. *co-first authors

McCoy-Simandle, K., **Hanna, S.**, and Cox, D. (2015) Exosomes and nanotubes: control of immune cell communication. *Internat. J. Biochem. Cell Biol.* 71:44-54. PMID:26704468

**Miskolci, V.**, Wu, B., Moshfegh, Y., Cox, D.*, and Hodgson L.* (2016) Optical tools to study the isoform-specific roles of small GTPases in immune cells. *J. Immunol.* 96:3479-93. *co-corresponding authors

**Miskolci, V.**, Hodgson, L., Cox, D. (2016) Using FRET-based biosensors to probe Rho GTPase activation during phagocytosis. *Methods in Molecular Biology: Phagocytosis and Phagosome Maturation*. 1519:125-143. PMCID: PMC5116239

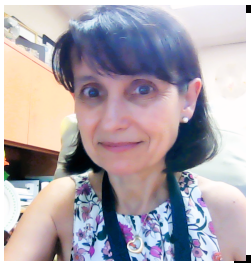
**Leung, E**., Xue, A., Wang, Y., Rougerie, P., Sharma, V., Eddy, R., Cox, D., and J. Condeelis (2017) Blood vessel endothelium - directed tumor cell streaming in breast tumors requires the HGF/C-Met signaling pathway. *Oncogene* 36(19):2680-2692. PMID:PMC5426963

**Hanna, S.***, McCoy-Simandle, K.*, Miskolci, V., Guo, P., Cammer, M., Hodgson, L., and Cox, D. (2017) The Role of Rho-GTPases and actin polymerization during Macrophage Tunneling Nanotube Biogenesis. *Sci. Reports* 17;7(1):8547. PMCID:PMC5561213 *co-first.

**Hanna, S.J**., McCoy-Simandle, K., Leung, E., Genna, A., Condeelis, J., Cox, D. (2019) Tunneling Nanotubes, a Novel Mode of Tumor Cell-Macrophage Communication in Tumor Cell Invasion. J. Cell Sci. Feb 11;132(3). pii: jcs223321. doi: 10.1242/jcs.223321. PMID:30659112.

**Carter, K.P.**, Hanna, S., Genna, A., Lewis, D., Segall., Cox, D. (2019) Macrophage induced tumor cell tunneling nanotubes enhance tumor cell 3D invasion. Cancer reports 2(9). 2(6):e1213. doi: 10.1002/cnr2.1213. PMCID: PMC7254960

**Mighty, J**., Sauma, S., **Hanna, S.**, Muntzel, M., Molina, H., Cox, D., Redenti, S. (2020) Analysis of adult neural retina extracellular vesicle release, cell to cell RNA transport and proteomic cargo. Investigative Ophthalmology & Visual Science. 61(2):30. doi: 10.1167/iovs.61.2.30. PMCID: PMC7326611



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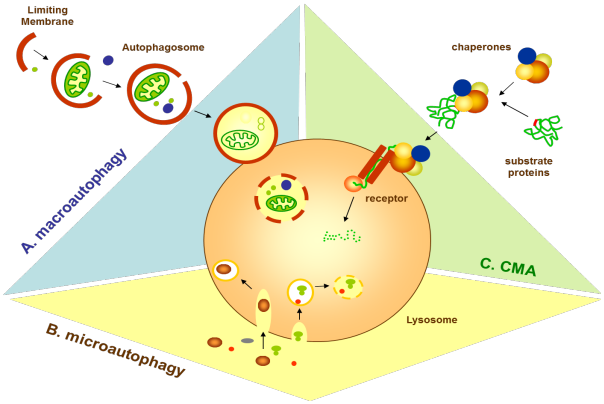
**Autophagy in Aging and Age-related diseases**

The **main focus** of our laboratory is understanding how proteins are transported into lysosomes for their degradation (*autophagy*) and how impaired autophagy contributes to aging and age-related diseases. A common feature of aging cells is the accumulation of abnormal or damaged proteins in their cytosol that impaird cellular function. Protein accumulation results, at least in part, from impaired protein degradation with age. We have previously identified the activity of a selective pathway for the degradation of cytosolic proteins in lysosomes known as chaperone-mediated autophagy (CMA). **The main goal of our researc**h is to identify the defect(s) that lead to the decreased activity of CMA and other forms of selective autophagy with age and in age related pathologies, and to determine if the correction of these defects in old cells leads to an improvement in cellular function.

**Chaperone-mediated autophagy** (CMA) and **endosomal-microautophagy (eMI)** are responsible for the degradation of as much as 30% of cytosolic proteins. CMA is mainly activated under conditions of stress, such as nutrient deprivation and oxidative stress, whereas eMI is inhibited by nutrient deprivation. Substrate proteins are selectively recognized by cytosolic chaperones (hsc70 and cochaperones) that stimulate their binding either to a glycoprotein receptor in the lysosomal membrane (LAMP-2A) (in the case of CMA) or to lipids in the membrane of late endosomes (in the case of eMI).

Our current efforts are directed to:

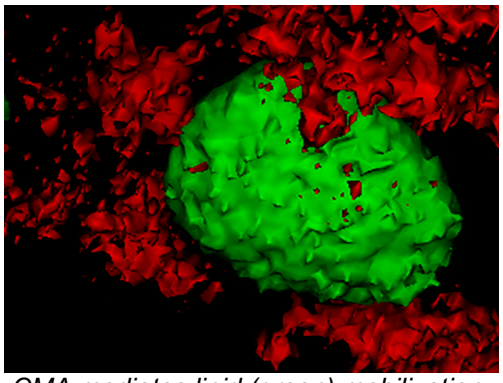
1. **Characterization of the different components involved in chaperone-mediated autophagy and endosomal microautophagy.-** We can isolate intact lysosomes and late endosomes from several tissues (liver, kidney and spleen) of rodents. For the identification of new CMA and eMI components we have developed a photoswitchable reporter (for CMA) and a split-venus based reporter (for eMI). We are using cells with the reporters, genome-wide CRISPRi screen, immunochemical approaches and a organelle proteomic approaches to complete the molecular dissection of these two pathways.




*Autophagic pathways in mammalian cells*

**2 Understanding the consequences of the age-related defect in chaperone-mediated autophagy and endosomal-microautophagy.-** We have generate conditional and inducible transgenic mouse models incompetent for CMA in different tissues and are investigating possible tissue-dependence differences in the requirements for functional CMA. We are also analyzing the cellular response to CMA blockage and the different compensatory mechanisms elicited. We have found that blockage of CMA leads to important alterations in cellular quality control and cellular metabolism and deficiencies in the response to different stressors. Similarly, we have initiated CRISPRi screens to identify eMI components and their changes in aging.

**3. Consequences of impaired autophagy in age related-disorders**.- We have been analyzing changes in autophagy in three main groups of age-related diseases: neurodegeneration, metabolic disorders and cancer. By combining metabolic assays, cellular fractionation procedures and our in vitro lysosomal transport assays in different animal and cellular models of these diseases, we have found that autophagy malfunctions in conditions such as diabetes, obesity and also that the age-related decline in autophagy could be an early step in oncogenic transformation. We have identified a primary defect in CMA in some familial forms of Parkinson's disease and of taupathies such as Alzheimer's disease. We are interested in identifying the primary defect, the compensatory mechanisms elicited and developing approaches to modulate autophagy with therapeutic purposes in age-related disorders



*CMA mediates lipid (green) mobilization.*

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**Epigenetic regulation of stem cells, development and cancer**

We seek to understand the epigenetic mechanisms governing the biology of stem cells, development and cancer. We study how the DNA methylation and demethylation machineries reshape the epigenome and regulate stem cell biology during development and in disease. We integrate mouse genetics with cellular, molecular, biochemical and bioinformatics approaches to define epigenetic pathways and mechanisms regulating stem cell specification, self-renewal, pluripotency and multipotency. We focus on the Tet family of DNA dioxygenases (Tet1, Tet2 and Tet3) which promote DNA demethylation by converting 5-methylcytosine (5mC) to 5-hdyroxy-methylcytosine (5hmC) and other derivatives. Tet enzymes also partner with chromatin regulatory complexes to promote gene activation and repression independent of their catalytic activity. We study how these dual functions of Tets regulate gene expression, and define their molecular and biological requirements in development and their implications in human diseases like cancer.

- *Embryonic stem cell (ESC) biology:* We study the enzymatic dependent and independent roles of Tets in gene regulation in ESCs and dissect their biological significance in pluripotency and development. We have identified noncatalytic roles of Tet1 in partnering with Sin3a and PRC2 for H3K27 modification. This is essential for establishing bivalency at developmental genes and is critical for silencing mesodermal and trophectodermal genes as well as cell cycle progression in ESCs.

- *Embryonic lineage specification and development:* We study how Tet enzymes regulate lineage specification and organogenesis during post gastrulation development with an interest in the hematopoietic and neural lineages. We have implicated Tets in activating hematopoietic and neural genes during embryogenesis.

- *Hematopoietic stem cells (HSCs) and cancer:* We investigate how Tet2, which is commonly mutated in human blood malignancies, regulates HSCs. We have identified enzymatic and nonenzymatic requirements for Tet2 in regulating the myeloid and lymphoid lineages, respectively.

Our work defines novel mechanisms of epigenetic regulation by Tet enzymes and 5hmC in development. It has implications in identifying new markers and targets for stem cell applications and for treatment of diseases.

For more details on our research please visit our lab website: https://www.dawlatylaboratory.com

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**Deciphering how diet and aging impact neutrophil biology and their role in disease**

The overall goal of my research is to understand how the inflammatory response modulates disease progression and outcome, with focused on neutrophils. We are interested in increasing our understanding of neutrophil biology, how these innate immune cells are impacted by metabolic dysregulation related with overnutrition or aging and how such effect impairs their response in different inflammatory context (injury, cancer, infection). Our unique research using a whole-animal approach considers the complex and intricated cellular and molecular mechanism involved in inflammation, which is only possible using the small vertebrate animal model, the zebrafish. My lab is currently focused on developing projects that will help answer some big open questions:

i) **Injury:** How do neutrophils decide which injuries go first in polytraumatic injury? Are neutrophils reprogrammed in diferent injured tissues? Can neutrophils spread inflammation and contribute to development of secondary complications such as infections, poor outcome, and death? How does metainflammation impact such mechanisms?

ii) **Liver:** How does neutrophil population evolve in NAFLD? Can we use neutrophils' pro-resolution capacity to block and revert NAFLD? How do neutrophils regulate liver immune landscape?

iii) **Cancer:** How do diet and aging impact neutrophil function and response to therapy in cancer? How can we activate neutrophil cytotoxic function to enhance efficacy of immunotherapies?

**Publications|**

**a.** de Oliveira S*, Rosowski EE*, Huttenlocher A. (2016) Neutrophil migration in infection and wound repair: going forward in reverse. Nat Rev Immunol. 16(6):378-91. * These authors contributed equally.

**b.** de Oliveira S*, Houseright RA, Graves AL, Golenberg N, Korte BJ, Miskolci V and Huttenlocher A*. (2019) Macrophages contribute to early progression in a zebrafish model of NAFLD/NASH-associated hepatocellular carcinoma. *Journal of Hepatology.* 70(4):710-721. *These authors are co correspondents.

**c.** de Oliveira S*, Houseright RA, Korte BJ and Huttenlocher A*. (2020) DnaJ-PKAc fusion induces liver inflammation in a zebrafish model of Fibrolamellar Carcinoma. *Disease Models & Mechanisms.* pii: dmm.042564. doi: 10.1242/dmm.042564. *These authors are co-correspondents.

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# Dissecting and targeting oncogenic pathways in thyroid cancer

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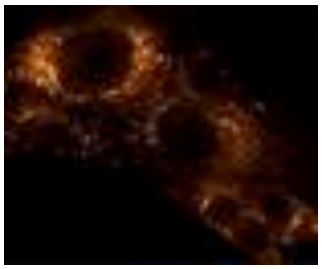
The main focus of my laboratory is the PI3K signaling pathway, and the role it plays in epithelial transformation and tumor progression. We utilize genetically engineered mouse models to i) identify and deconstruct the signaling cascades controlled by PI3K and altered during early neoplastic transformation, ii) understand through which signaling nodes the PI3K cascade intersects with other growth-promoting pathways, and iii) design and test in vivo innovative, rationally designed therapeutic approaches for aggressive cancer.
We utilize the thyroid gland as our main model system. The thyroid is a central endocrine hub where a complex array of signals in the form of hormones, growth factors, and environmental cues are integrated to govern thyroid growth and activity. As such, the thyroid represents an ideal model system to study the interaction between these signals in normal homeostasis as well as in the context of neoplastic transformation.

## Current research areas:

- **Beyond AKT: additional PI3K-regulated pathways in thyrocyte transformation.**



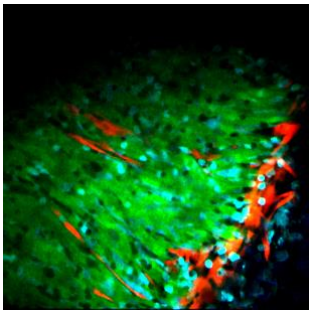
PI3K pathway activation, via mutation/amplification of *PIK3CA*, or *PTEN* mutation/silencing, is a frequent event in thyroid cancer. However, our *in vivo* data suggest that the linear PI3K-PDK1-AKT pathway is not sufficient to drive thyroid transformation, and that additional PDK1-dependent pathways, including a novel one leading to activation of SGK1, are absolutely required for tumor development and thus may represent valid therapeutic targets.

- **Overcoming adaptive resistance to PI3K inhibitors in thyroid cancer.**

Intrinsic, acquired, and adaptive resistance are common consequences of cancer cell exposure to targeted inhibitors. Indeed, we have found that while PI3K inhibitors reduce the growth rate of anaplastic thyroid cancer (ATC) cells *in vitro* and in allografts, this effect is short-lived and these cells invariably become resistant. Our objective is to characterize *in vivo* the cell autonomous and non-autonomous mechanisms through which $PI3K^{active}$ thyroid tumor cells develop adaptive resistance to PI3K inhibitors, and to test the efficacy of combination therapies targeting key mediators of resistance.

- **Therapeutic apoptosis in aggressive thyroid carcinomas.**

The recent discovery of a “trigger site” in BAX that can be targeted by a small molecule, leading to BAX activation and oligomerization, allows us to design pro-apoptotic therapeutic approaches that bypass the need for chemo- and radiotherapy-dependent BIM and BID induction, one of the key



limitations of current cytotoxic therapies. We are testing the ability of a novel BAX activator developed by the Gavathiotis laboratory to directly induce apoptosis in a large panel of human ATC cell lines representing all the major driver oncogenic events. Activated BAX can be directly bound and sequestered by BCL2 and BCL-XL, limiting its ability to oligomerize and induce mitochondrial permeabilization. The extent of apoptosis induced by activated BAX, thus, is likely to be dictated by the balance between BAX levels and the expression of anti-apoptotic BCL-2 proteins. We are thus testing the ability of specific inhibitors of BCL2-family proteins to sensitize ATC cells to the killing activity of the BAX activator.

- **mTOR activation as a key hub for RAS-mediated transformation.**

NRAS-mutant ATC often shows concomitant dysregulation of the PTEN/PI3K/AKT pathway. In the mouse, *Nras*$^{Q61R}$ alone is not sufficient to transform thyroid epithelial cells. Concomitant *Pten* deletion and *Tp53* inactivation, instead, lead to the development of aggressive ATC. We have thus developed a novel *Nras*/*Pten*/*Tp53* mouse model that recapitulates the genetic and histological features of ATC. Using this unique model, we are defining the role of mTOR as a key signaling node that allows full RAS transforming activity.

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**Cell biology of RNA in the liver**

Our research program focuses on how gene expression is regulated in living cells in the native context of the liver tissue. We develop microscopy technology using fluorescent-labeled probes to *in situ* visualize single molecules of mRNA within cells and tissue with high spatiotemporal resolution.

The liver is the central coordinator of many metabolic events and carries out a wide range of essential functions required for health and homeostasis. This organ is unique in its incredible capacity to regenerate from injury. Although hepatocytes turn over slowly, the liver displays the ability to recover its original mass within 7-10 days after surgical resection of as much as 70% in experimental models such as mice and rats. A similar process of regeneration is seen in humans. However, although this process of regeneration has been studied for many years, the fundamental mechanisms by which regeneration is initiated and later terminated remain unknown. Our understanding of the molecular and cellular processes controlling cell state changes in the liver during health and disease is far from complete. Our goal is to explore gene expression programs that drive mouse liver regeneration after partial hepatectomy and elucidate failures of these pathways in liver pathologies in the spatial context of the liver tissue using imaging technologies with single-cell and single-molecule sensitivity.

In the liver, hepatocyte function is linked to liver architecture; and liver zonation controls metabolic events along the porto-central axis of the liver lobule. We also study heterogeneity in gene expression of metabolic genes during the feeding to fasting transition in mice. Our goal is to determine the individual cells and molecular events that are dysregulated in metabolic liver diseases such as diabetes.

Single-molecule Fluorescence in situ Hybridization (smFISH) method that preserves the *in vivo* characteristics of the liver will allow us to find novel aspects of the spatial organization of the hepatocytes. This imaging technology applied to liver tissue will demonstrate what an individual hepatocyte *is doing* in a format that maintains the morphology of the tissue and might provide the basis for determining which individual cells are dysregulated in disease.

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***Key Words:*** *autophagy, eMI, lysosome, proteostasis, spermatogenesis, development, Drosophila, genetics, cancer, Rho kinase, signaling, Parkinson, neurodegenration*

**A genetic model for Endosomal Microautophagy**

Aging-associated diseases are an increasing socio-economic burden despite efforts to improve healthspan. Pathologies that cause degeneration of the nervous system are particularly devastating, and in many cases are associated with decline in proteostasis and lysosomal malfunction. Prime examples are Parkinson and Alzheimer's disease that are characterized by accumulation of insoluble protein aggregates that lead to neuronal decay. Autophagy delivers cytosolic components to lysosomes for degradation and is thus essential for cellular homeostasis and to cope with different stressors. Macroautophagy (MA) can selectively degrade organelles or aggregated proteins, but selective degradation of single proteins has only been described for Chaperone-mediated autophagy (CMA) and endosomal Microautophagy (eMI). These two autophagic pathways, described to date only in mammals, are specific for proteins containing KFERQ-related targeting motifs. In collaboration with the Cuervo lab, we have established a genetic model for eMI in *Drosophila in vivo*. We are thus for the first time able to perform genetic screens for regulatory components of eMI, this only recently identified form of autophagy about which barely anything is known.

Lysosomes are also important regulatory hubs that integrate nutritional signals and participate in lipid metabolism. We have recently characterized *Drosophila* Lamp1, a bona fide homolog of the mammalian LAMP1/2. *Lamp1* deficiency results in an increase in the number of acidic organelles in the fat body, strongly suggesting defects in the regulation of the pH of the endolysosomal system. Furthermore, *Lamp1* mutant larvae have elevated levels of sterols and diacylglycerols, indicating functions of Lamp1 in lipid transport beyond sterols. Significantly, these phenotypes are similar to loss of Glucocerebrosidase 1, the gene causing Gaucher disease and a major risk factor for Parkinson. Indeed, *Lamp1* mutations enhance fly PD models!

**Rho kinase and its effector Cmb in spermiogenesis**

During development, genetic and molecular programs control the differentiation of various cell types and orchestrate their morphogenetic behaviors to form organs with specific functions. Organogenesis requires the coordination of cell polarity, cellular movement, and cell shape, driven by intercellular signaling and the tissue-specific interpretations of these signals. Traditionally, Rho kinase (Rok) functions as effector of the non-canonical Wnt/Frizzled PCP pathway during gastrulation and neural tube formation. In a systematic, genome-wide screen, we have identified the previously uncharacterized Combover, an intrinsically disordered protein as novel Rok substrate. Significantly, our follow up studies have identified a novel and unanticipated role of Rok and Cmb during spermiogenesis. We currently address how, downstream of Rok, Combover orchestrates the transition between axoneme elongation and sperm individualization by coordinating the actin and microtubule cytoskeletons with the plasma membrane, thus ensuring proper resolution of the syncytial spermatids into functional sperm that are encapsulated by their own plasma membrane. The biomedical significance of sperm individualization is further exemplified by the presence of multiciliate spermatozoa and spermatids with unresorbed cytoplasm in infertile men.

It is our goal to use *Drosophila* as model system to address fundamental questions that are relevant for development and disease in general.

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Dr. David Loeb – Research Summary

The primary focus of our laboratory is sarcoma metastasis. Sarcomas are malignant tumors arising from bone, muscle, and other connective tissue. Refinements in the use of chemotherapy, radiation, and surgery have resulted in significant improvements in the survival of children, adolescents, and young adults diagnosed with localized osteosarcoma and Ewing sarcoma, the two most common sarcomas in this age group. In contrast, patients diagnosed with metastatic sarcomas have the same grim prognosis today as those diagnosed 30 years ago. Since most cancer-related deaths are due to metastatic disease, future improvements in patient survival depend upon a deeper understanding of the biology of metastasis.

Our group developed a novel animal model of sarcoma metastasis involving orthotopic implantation of patient-derived xenografts followed by amputation of the tumor-bearing limb. Mice treated in this manner develop spontaneous distant metastases and ultimately die from metastatic disease. This model more faithfully reflects the clinical course of sarcoma patients than traditional models of metastasis, such as tail vein injection. We have leveraged this model to fuel a number of research projects.

The Wnt signaling pathway has been implicated in a variety of processes in normal development and in cancer biology, including processes vital to metastasis such as invasion, motility, and stem cell function. Utilizing pharmaceutical reagents being developed for clinical use, we have investigated the role of this important signaling pathway in sarcoma metastasis. Interestingly, we found that activation of canonical Wnt signaling promotes the differentiation and inhibits metastasis of osteosarcoma, while inhibition of a noncanonical Wnt signaling pathway inhibits Ewing sarcoma metastasis. Ongoing work is dissecting the molecular details on Wnt signaling in these tumor types, as well as investigating the seemingly contradictory roles of this pathway in these related tumors. These studies are being expanded into a zebrafish model of Ewing sarcoma that will also enable us to study how the EWS-FLI1 oncoprotein that drives Ewing sarcomagenesis affects Wnt signaling both in the tumor and in other developmental processes.

In related work, we have used both our mouse models and patient blood samples to begin to explore the biology of circulating tumor cells, based on the hypothesis that these cells, the presumed agents of metastasis, have a distinct biology that can be understood at a molecular level and targeted therapeutically. In partnership with a biotech company from Maryland, we have developed a low pressure filtration procedure to isolate circulating tumor cells with minimal manipulation, allowing us to quantify these cells and analyze them at the molecular level. Preliminary data from single cell RNA-seq analysis of these cells has revealed important signaling pathways that will be the subject of future investigations.

Our mouse model also revealed an important role for the tumor microenvironment in regulating sarcoma metastatic potential. We observed that patient-derived xenografts implanted in an orthotopic location undergo spontaneous distant metastasis, while the same xenograft implanted subcutaneously does not. This difference in metastatic potential correlated with a difference in the nature of the macrophage infiltrate in the tumors, suggesting a possible role for the immune system in mediating sarcoma metastasis. Our laboratory is currently engaged in a number of collaborative efforts to understand the interactions between sarcomas and the immune system, as well as exploring ways in which this interaction might be modulated pharmacologically for therapeutic benefit.

Also, based on our longstanding interest in radiopharmaceuticals, we are also exploring how radiation affects the interaction between sarcomas and the immune system.

Another area of active investigation is the role of a specific RNA helicase, DDX3, in sarcoma biology. We discovered that DDX3 is expressed at high levels in a variety of sarcoma subtypes, including Ewing sarcoma, that DDX3 expression is important to the survival and proliferation of these cells, and that genetic or pharmacologic inhibition of DDX3 is toxic to Ewing sarcoma. Interestingly, proteomic analysis of cells after inhibition of DDX3 implicated this molecule in DNA damage repair. This observation led to our discovery that a small molecule DDX3 inhibitor potentiates the effect of ionizing radiation on DDX3-expressing sarcomas, but not on sarcomas with low levels of DDX3. Ongoing work is focused on understanding this phenomenon at the molecular level, as well as exploring other mechanisms by which DDX3 plays a role in sarcoma growth and metastasis, including DNA damage repair and regulation of stress granule formation.

One of the most intriguing characteristics of Ewing sarcoma is that although it is the second most common bone tumor in adolescents and young adults of European descent, it is almost never seen in individuals of African or East Asian descent. We have developed a bank of mesenchymal stem cells (MSC) from umbilical cords collected from individuals of varied ancestry and will be using this tool to investigate the molecular basis for this discrepancy. This library of MSC with molecularly defined ancestry will be a powerful tool for exploring the genetic basis for other health care discrepancies that appear to be based on ethnicity and can be studied in MSC.

Selected Publications

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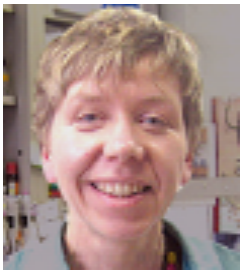

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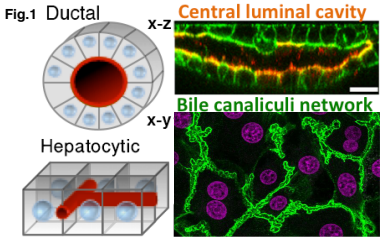
**How Cell adhesions shape epithelial tissues**

The liver is our largest metabolic organ. It produces proteins, lipids, clotting factors and glycogen while dispensing bile and detoxifying xenobiotics. To transport these different substances, a sophisticated network of liver venules, capillaries and interstitial conduits has evolved. An essential feature of this network are the lumen-forming epithelia that give rise to two major liver cell populations: (1) hepatocytes - the main parenchymal cell type, and (2) bile duct cells. Both acquire radically different polarity phenotypes adapted to their different functions (Fig.): Bile duct cells, which form simple conduits for bile, organize like other tubular epithelia around a central lumen. Hepatocytes, by contrast form single-cell cords, aligned along blood vessels on either side and with a capillary-like luminal network (bile canaliculi) running between them. This organization facilitates their extensive bi-directional molecular exchange with the blood, while allowing bile acid excretion into the bile canaliculi for drainage into bile ducts. Common liver diseases such as cirrhosis and hepatocellular carcinoma present with defects in hepatocyte polarity, which range from discontinuities in the bile canalicular network to a switch to ductal organization. These distortions impair metabolic functions and cause cytotoxic interstitial bile accumulation (cholestasis), making it imperative to understand their molecular basis. Yet, how hepatocytes obtain their unique morphological phenotype and why it becomes disrupted in disease remains largely unknown. To tackle these questions, we developed a unique tissue culture model in which the polarity phenotype can be switched from ductal to hepatocytic. It allowed us to identify Extracellular Matrix (ECM)- and Cell-cell adhesion signaling as critical determinants for the hepatocytic versus ductal polarity decision. We are now utilizing cell biological approaches in primary hepatocyte cultures to elucidate the mechanisms underlying the roles of these adhesion systems for bile canaliculi formation. This includes modeling cirrhosis-associated ECM and integrin expression changes in primary hepatocytes to reveal critical morphological mechanisms affecting hepatocyte polarization in liver disease.



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**Molecular Mechanisms of Cardiovascular Disease**

Cardiovascular disease is the number one cause of death worldwide. The overall goal of my laboratory is to improve the molecular understanding of smooth muscle and endothelial cell biology in both vascular homeostasis and cardiovascular disease. The knowledge derived from our NIH-funded research program may serve as basis for novel strategies to prevent or treat cardiovascular disease. Our laboratory is also committed to mentor the next generation of biomedical scientists and to foster diversity and inclusion in science.

We are tackling significant gaps of knowledge in vascular biology including:

- The pathophysiology of atherosclerotic plaque erosion, the second most common cause of acute coronary syndrome, i.e., unstable angina and acute myocardial infarction.
- How mitochondrial mechanisms and cell metabolic processes affect the phenotype of endothelial cells during re-endothelialization, atherosclerosis development, and atherosclerotic plaque erosion.
- The potential role of atypical cadherins such as FAT1 in atherosclerosis development and plaque stability.
- How smooth muscle cell metabolism, including mitochondrial respiration, contributes to artery formation and the arterial response to injury or disease.
- The functional interaction between the canonical Wnt and sphingosine-1-phosphate signaling pathways in vascular remodeling.

To address our scientific questions, we generate and study genetically modified mice, use different mouse models of vascular remodeling or disease, analyze human tissue samples, and evaluate primary mouse and human vascular cells in culture. We also employ tissue clearing and light sheet fluorescence microscopy to visualize arteries in three dimensions. For our mechanistic studies, we used transcriptomics, proteomics, and metabolomics analyses, and other tools from molecular and cellular biology and biochemistry.

**Michael Ross, MD**

The major focus of research in the Ross laboratory is to identify novel mechanisms of kidney injury occurring in HIV-positive persons and his laboratory uses in vitro and murine models to generate new strategies to prevent and treat kidney diseases. Dr. Ross also works with members of the International Network for Strategic Initiatives in Global HIV Treatment (INSIGHT) to perform research on kidney disease in the context of large international HIV treatment trials.

**Ongoing NIH-funded projects:**

**Mechanisms by which antiretroviral medications protect kidneys from HIV- and diabetes-induced injury:**

Though antiretroviral therapy is efficacious in preventing and treating HIV-induced kidney disease, the mechanisms by which these medications protect the kidney from the deleterious effects of HIV are poorly understood. We have exciting data demonstrating that HIV protease inhibitors protect the kidneys from HIV via affects that are independent of blocking HIV protease and recent data from our lab demonstrates that these medications protect mice from diabetic kidney disease, the most common cause of kidney disease in developed countries. We are performing studies using transgenic animal models and molecular and genomic techniques to identify novel pathways by which antiretroviral medications protect the kidneys from injury and disease.

**The role of APOL1 polymorphisms in promoting HIV-induced kidney injury:**

Polymorphisms in the APOL1 gene account for most of the excess risk of African-Americans to non-diabetic kidney disease and HIV-associated kidney disease in particular. In our studies, we are using genetically modified human kidney cells to perform innovative proteomic, and genomic studies to identify novel mechanisms by APOL1 genetic variants predispose to HIV-induced kidney injury.

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***Key Words: Cardiovascular disease, inflammation, growth regulation, metabolism, mouse models***

**CONTROL OF VASCULAR CELL GROWTH, DIFFERENTIATION, AND INJURY RESPONSE**

Vascular disease is the greatest single cause of morbidity and mortality in developed societies. Pathogenesis stems from interactions between circulating inflammatory cells, the endothelium that lines the vasculature, vascular smooth muscle cells (SMCs) in the arterial wall, and systemic metabolism. We seek to identify new factors and pathways that regulate disease-associated functions of relevant cell types and tissues. We are also interested in areas of biological overlap with cancer – specifically, to understand molecular mechanisms that control cell proliferation, migration, differentiation or fate, and metabolism.

The complex pathogenesis of vascular disease encompasses developmental pathways, innate and acquired immune responses, and response to physical stresses, perturbed blood flow, and biochemical stimuli. Our general approach is to characterize such responses at the molecular level, in cell culture, and in mouse models that reflect specific types of vascular injury, including atherosclerosis, restenosis after angioplasty, vein graft disease, transplant-associated arteriosclerosis, and integrated metabolism. One project focuses on the atypical cadherin FAT1, an emerging tumor suppressor that is also involved in vascular pathogenesis. We found that FAT1 interacts with beta-catenin, the downstream mediator of Wnt signaling, a core developmental pathway that regulates many aspects of metazoan embryogenesis. Interestingly, beta-catenin-mediated signaling in SMCs is required for vascular wall assembly during development, and in vascular injury response in the adult; FAT1 controls SMC growth, metabolism, and differentiation in injured adult vessels. The effects of FAT1 on mitochondrial function have led us to studies of cellular metabolism, specifically respiration and mitochondrial complex I, in SMC growth and migration. Yet another project involves the allograft inflammatory factor-1 (AIF1, aka Iba1), a 17kD EF-hand protein expressed primarily in activated macrophages and microglia. We found that AIF1 has important effects on macrophage function, promoting immune activation and specific gene expression; interestingly, impaired catecholamine metabolism in AIF1-deficient macrophages causes hyperactivation of brown adipose tissues, increasing calorie utilization and conferring resistance to obesity in mice.

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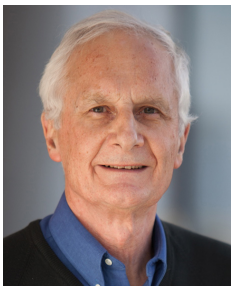

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**Growth Factors and Signaling in Development: The colony stimulating factor-1 receptor**

The major focus of my laboratory has been to identify colony stimulating factor-1 (CSF-1), its 3 isoforms and its receptor (CSF-1R), and to define their developmental and physiological roles using biochemical, cell biological and mouse genetic approaches. We have shown that CSF-1 and the CSF-1R regulate the production of macrophages, osteoclasts, microglia, Langerhans cells, Paneth cells and neural progenitor cells and play an important role in the development of leukemia and of several inflammatory diseases. We have shown that CSF-1 and the CSF-1R, via their regulation of tumor-associated macrophages, enhance solid tumor progression. We have developed novel biochemical and genetic approaches to CSF-1R signal transduction to analyze CSF-1R structure/function, identifying and elucidating the function of several downstream signaling molecules, most recently, MAYP/PSTPIP2, in which we have described mutations that lead to an autoinflammatory disease in mice, involving macrophages and osteoclasts. In work relevant to our more recent focus on the nervous system, we have shown 1) the complete dependence of microglial development and maintenance on the CSF-1R, 2) that a second ligand for the CSF-1R, interleukin-34 (IL-34), similarly activates the CSF-1R, but has a more restricted tissue distribution than CSF-1, with primary expression in brain and skin, 3) that via the CSF-1R, CSF-1 and IL-34 directly suppress neural progenitor cell (NPC) self-renewal and enhance NPC survival and differentiation, 4) that IL-34, but not CSF-1, also acts via an additional receptor, protein tyrosine phosphatase zeta, which is also expressed on NPC, but not on microglia, 5) that miR-21 is induced by macrophage CSF-1R activation to suppress the macrophage M1 (inflammatory) phenotype and enhance the M2 (trophic) phenotype and 6) that the *Csf1r*$^{+/-}$ mouse is a model of CSF-1 receptor related leukoencephalopathy (CRL), a dementia in man caused by dominant inactivating mutations in the kinase domain of the *Csf1r* gene, formerly known as adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) and most often misdiagnosed as Alzheimer's disease. This mouse faithfully reproduces the symptoms and pathology of CRL and our very recent studies have elucidated the mechanisms underlying CRL development and identified prophylactic targets.

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***Key Words: cytokines, hematopoiesis, epigenetics, mds, leukemia, esophageal cancer, pancreatic cancer***


1. **Targeting signal transduction in hematologic malignancies**: Cytokines play important roles in the regulation of normal hematopoiesis and a balance between the actions of hematopoietic growth factors and myelosuppressive factors is required for optimal production of different hematopoietic cell lineages. We study the role of MAP kinases in the regulation of hematopoiesis and have shown that the p38 MAPK signaling pathway is the dominant cytokine regulated inhibitory pathway in human hematopoiesis and is overactivated in MDS. The myelodysplastic syndromes (MDS) are collections of heterogeneous hematologic diseases characterized by refractory cytopenias due to ineffective hematopoiesis. These preleukemic disorders are common causes of anemia in the elderly and are rapidly increasing in incidence. We have also demonstrated that the TGF-□ pathways is overactivated in MDS. We are trying to study the molecular mechanisms that lead to the activation of these pathways in MDS and are using small molecule inhibitors in mouse models to target these pathways.

2. **Epigenomic analysis of tumors**: We are using high throughput assays to analyze DNA methylation across the genome and have optimized these assays to work with low amounts of DNA. We have successfully used these assays in conducting an integrative analysis of epigenetic and genetic alterations in MDS and esophageal cancer. We are now exploring alterations in DNA methylation in pancreatic cancer, myeloproliferative neoplasms and renal cell cancer.

3. **Clinical studies in Myelodysplastic syndromes**: We have a "center of excellence" clinic for patients with Myelodysplatic syndromes. We offer a variety of clinical trials for these patients (www.mdstreatment.com).

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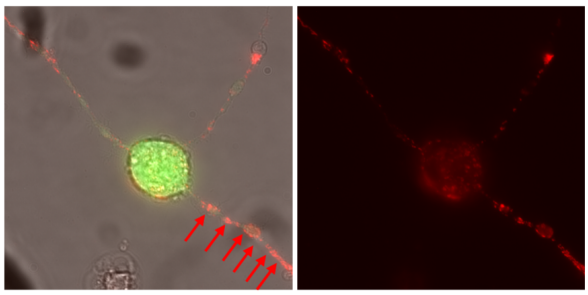
# Herpes simplex virus – a pathogen of the human nervous system

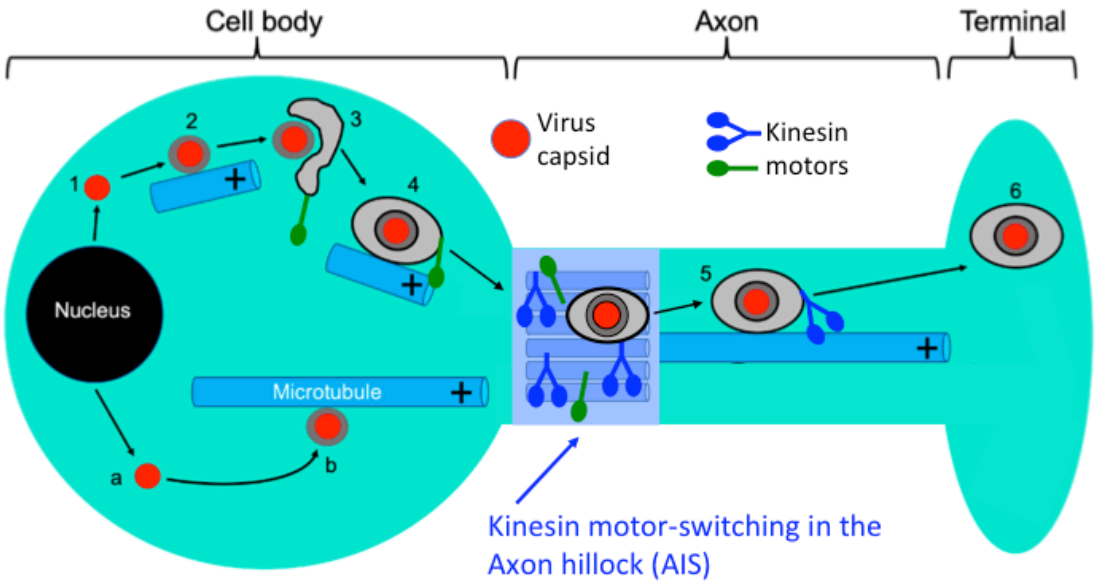
## Herpes simplex virus

- Major role in Alzheimer's disease, as many as 50% of AD cases.
- Chronic, lifelong infection. Life-threatening in the immunocompromised.
- Painful oral and genital lesions, associated psychosocial issues.
- Keratitis, encephalitis, herpes labialis.
- Neonatal infection can cause high fatality rates, or long-term neurological defects.
- Increases risk of HIV infection/transmission.

## We model HSV (●) trafficking in primary sensory neurons and cultured neuronal cell lines

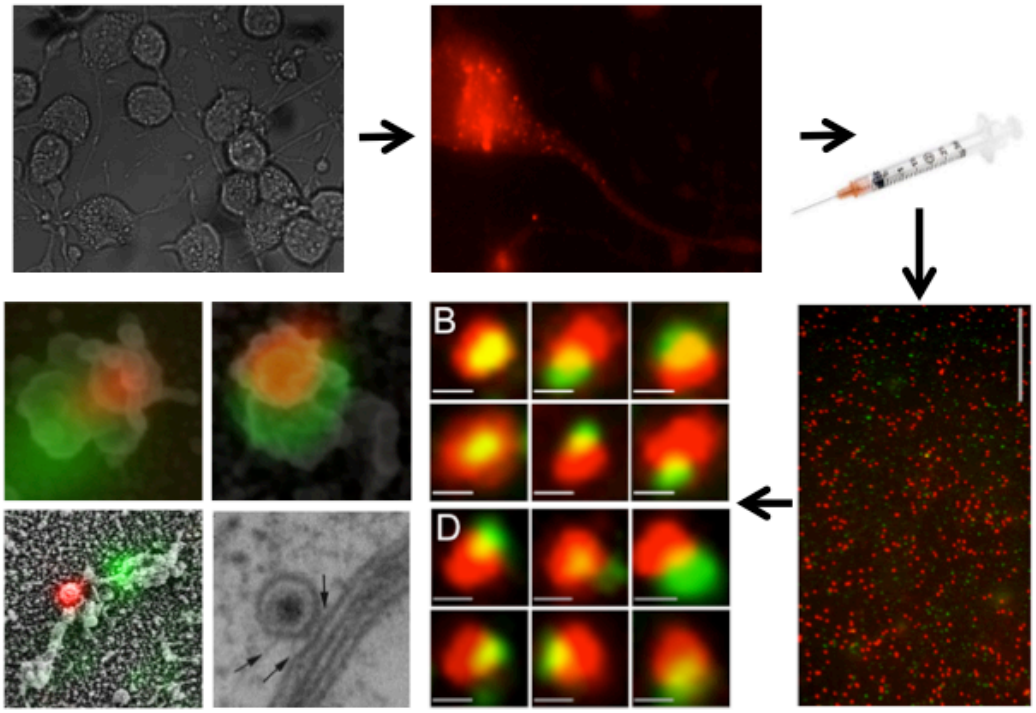


## To cause disease HSV must replicate and traffic within the human nervous system. We are dissecting the molecular details of how this is accomplished



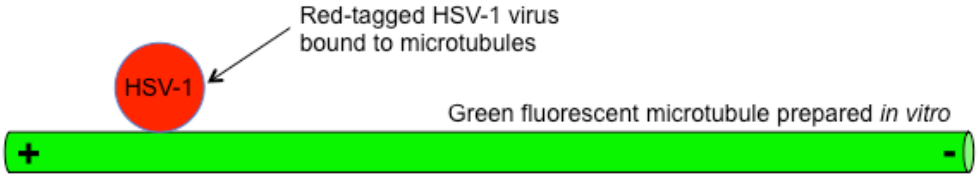

Capsids leave nucleus (1), bud into cellular organelles (3) and traffic down the axon (5) to the nerve terminal (6) to cross the synapse and spread to the brain or to other individuals

One approach is to isolate viruses or mutant viruses from infected neurons, purify them and perform state of the art imaging analysis to analyze their structure

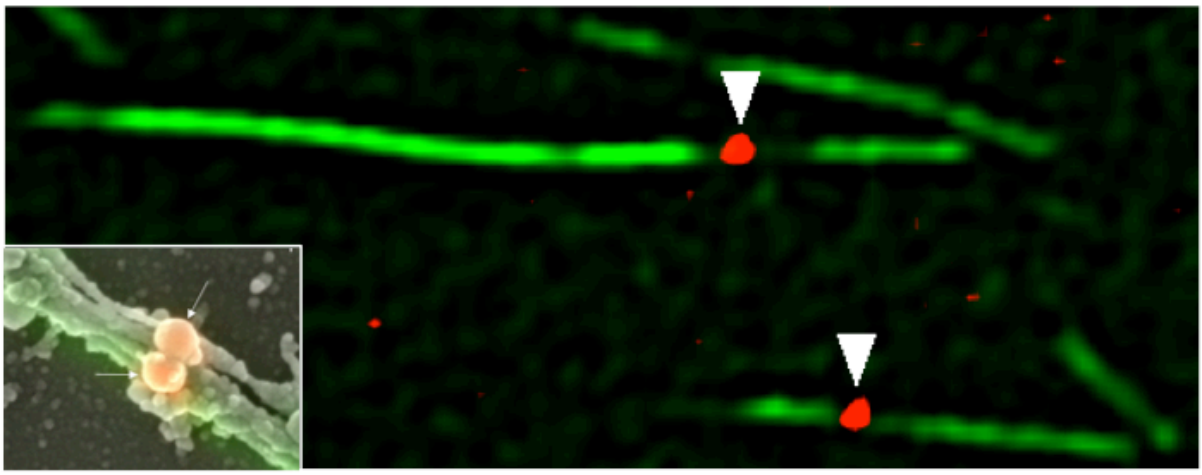



Kharkwal et al. J. Virol. 2015, Kharkwal et al. J. Virol. 2016

We also study transport of viruses along axons in a biochemical system that reproduces the process on the surface of a microscope slide!



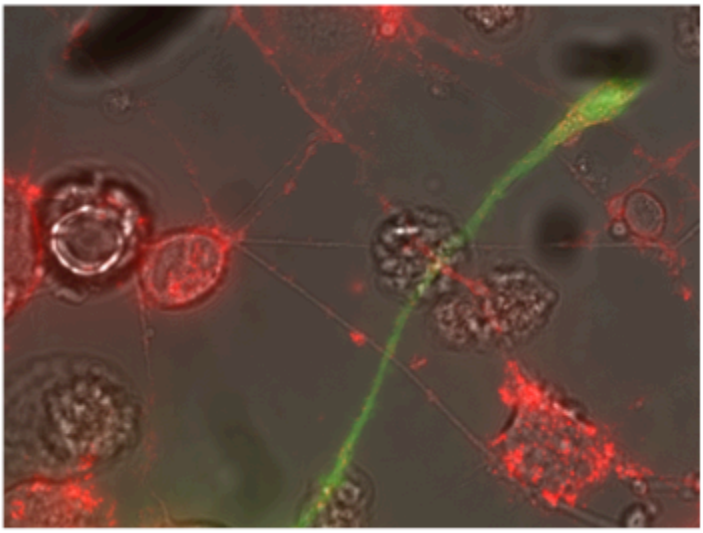

(With thanks to Dr. John Murray and Dr. Allan Wolkoff)



Diwaker et al. 2020

## In summary

- HSV is a life-long pathogen of the human nervous system, and responsible for many diseases.
- We are using biochemistry, cell biology, genetics and sophisticated imaging to study how this virus manipulates the nervous system.
- Molecular dissection of virus-host neuron interactions teaches us about neuronal cell biology and reveals new viral drug targets.



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# DR. FAJUN YANG

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## *Key Words: gene expression, lipid metabolism, metabolic syndrome, obesity*

Dysregulation of lipid homeostasis is common in major human diseases, including type 2 diabetes, obesity, fatty liver diseases, atherosclerosis and some types of cancer. Our laboratory is interested in the transcriptional control of lipid metabolism. In addition to DNA-binding transcription factors, gene expression in eukaryotic cells often requires a series of transcriptional cofactors. We use biochemical and genetic approaches to study the physiological and pathophysiological functions and regulation of the network of transcription factors and their cofactors in the liver (hepatocytes) and adipose tissues (white, brown and beige adipocytes). A focus of our work is the multi-subunit Mediator complex, which is a highly conserved transcriptional cofactor. The mammalian Mediator is one of the cofactors for the sterol regulatory element - binding proteins (SREBP), the master regulators of both fatty acid/triglyceride and cholesterol biosynthesis. SREBP proteins are synthesized as inactive precursors that are tethered to the endoplasmic reticulum membrane. When lipid biosynthesis is demanded, SREBP precursors are proteolytically processed to mature forms of transcription factors that migrate into the nucleus, interact with cofactors including the Mediator complex, and activate transcription of target genes, which encode the rate-limiting enzymes in synthesizing fatty acids/triglycerides and cholesterol. Through the protein-protein interactions of different subunits with SREBP as well as other transcription factors, the Mediator complex critically regulates lipid metabolism. Moreover, dynamic remodeling of the Mediator complex conveys the metabolic signals to transcriptional outputs, providing another layer of regulation of gene expression. With the ultimate goal of identifying novel targets for preventing or treating metabolic syndrome (including type 2 diabetes, obesity, fatty liver disease, hyperlipidemia and hypertension), our research will advance our understanding of how lipid homeostasis is regulated at the molecular level.

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**Yang F**[*], Vought BW[*], Satterlee JS, Walker AK, Sun ZY, Watts JL, DeBeaumont R, Saito RM, Hyberts SG, Yang S, Macol C, Lyer L, Tjian R, van den Heuvel S, Hart AC, Wagner G, Näär AM. An ARC/Mediator subunit required for SREBP control of cholesterol and lipid homeostasis. ***Nature***, 2006, 442(7103): 700-7004. ([*]Equal contribution)