Yasmine Abdelaal (Supervisor: D.Godt). Generating a recombinant line for clonal analysis of the function of *traffic jam* in migration of the border cell cluster in *Drosophila* ovaries.

Ariba Alam (Supervisor: I. Zovkic). Elevated H2A.Z in the Prefrontal Cortex of TgCRND8 mice at Plasticity-Related Genes Associated with Impaired Transcription and Memory Deficits in Alzheimer's Disease.

Alzheimer's disease (AD) is a complex neurodegenerative disorder that can appear between early and late life. A hallmark of AD is the accumulation of amyloid plaques and neurofibrillary tangles in the brain, as well as profound memory deficits. Epigenetic factors, such as post-translational modifications of histones, have been implicated in AD because of their crucial role in memory formation. Existing studies have focused solely on DNA methylation and histone acetylation, whereas a potential role for histone variants in AD has never been investigated. Here, we tested the hypothesis that histone H2A.Z, a variant of histone H2A and a newly identified memory suppressor, is elevated in AD, thus contributing to AD-related memory deficits. Using qPCR, we showed that mRNA levels of *H2afz,* a gene encoding histone H2A.Z are elevated in the prefrontal cortex (PFC) of TgCRND8 mouse model of AD compared to wild-type (WT) littermates. Using chromatin immunoprecipitation (ChIP), we confirmed that H2A.Z binding is elevated at plasticity-related genes in AD and that impaired learning-induced H2A.Z exchange is associated with impaired transcription. These data suggest that histone H2A.Z accumulation may be a novel contributor to memory deficits in AD and implicate histone variant exchange as a novel category of epigenetic regulation in AD.

Sarah Armitage (Supervisor: S. Plotnikov). Prickle1, a core component of Planar Cell Polarity complex, regulates cancer cell migration through two distinct mechanisms.

Cell migration is vital to embryogenesis, immune responses, and tissue homeostasis, but also to cancer. During these processes, cells are required to migrate directionally to specific locations within a tissue. Directional migration is particularly important for cancer invasion allowing tumor cells to migrate towards the circulatory or lymphatic system. Directional migration requires the cells to polarize in the direction of movement, mediated by local activation of two Rho family GTPases, Rac1 and RhoA. Activity of the GTPases is regulated by GTPase-activating proteins (GAPs) and guanine nucleotide exchange factor (GEFs). However, it is unknown what regulates the GEFs and GAPs. Here we hypothesized a role for planar cell polarity pathway in the regulation of cancer cell migration. To test this we analyzed how Prickle1 depletion affects spreading and migration of MDA-231 breast cancer cells. We found that Prickle1 suppresses cell edge protrusion by locally down-regulating Rho GTPases. By live-cell imaging we showed that Prickle1 is essential for polarization and efficient migration of single cells, but is dispensable for directional migration in a wound-healing assay. We showed that depletion of Prickle1 decreases velocity of collective cell migration by suppressing focal adhesion dynamics. Together, these data demonstrate that Prickle1 regulates cell migration by either establishing cell polarity or promoting focal adhesion turnover.

Jagdeep Bal (Supervisor: S. Plotnikov). The effect of formins knockdowns in MEF cells and its effects on mechanosensing.

Formins (formin homology proteins) are a family of proteins that are major actin nucleators in eukaryotic cells. Formins are involved in numerous cellular functions, including cell polarity, cell migration, and cytokinesis. Multiple isoforms exist, some of which are involved with actin filament elongation. Mechanosensing also plays a role in the cytoskeleton structure of a cell by recognizing the stiffness of the extracellular environment leading to remodelling of the actin cytoskeleton. If and how formins are involved with mechanosensing in remodelling the cytoskeleton seems to be a question of great importance. Through MEF cells treated with Blebbistatin, CK666, and SMIFH2, knockdown of all formins were shown to be important in cell mechanosensing. Through treatment of MEF with specific siRNA's of different isoforms, Fhod1, Fmn1, Fmnl1 formins were shown not to be involved with mechanosensing, while Diap1, Fhdc1 and non targeting SiRNA were shown to be possibly involved with how a cell is able to remodel its cytoskeleton.

Brian Burt (supervisor: M. Ringuette). Production of intracellular reactive oxygen species and thermal characteristics of cold atmospheric plasma for differential targeting of ovarian cancer cells.

Cytoreductive surgery is the most effective primary treatment for ovarian cancer; however, surgically intractable disease prevents optimal debulking in many patients. Approaches that would target metastatic lesions while preserving adjacent healthy tissue are needed. Cold atmospheric plasma (CAP) recently emerged as a promising technology for selective targeting of malignant cells, including ovarian cancer cells, by generating reactive oxygen and nitrogen species (ROS and RNS) to trigger cell death. The clinical implementation of CAP would require an ability to treat large areas of tissue and necessitates consideration of thermal properties and characterization of ROS generation.

We constructed a prototype CAP device to determine 1) the thermal characteristics and ROS generated, and 2) if a tissue lavage pretreated with CAP increases intracellular ROS. Intracellular fluorescent dyes were used to measure intracellular ROS accumulation in vertebrate neural tissue as a surrogate for peritoneum. Direct application of the CAP plume to tissue resulted in a 6-fold increase in the rate of ROS accumulation over baseline. Application of CAP to the artificial cerebrospinal fluid (aCSF) 2.5 cm upstream of the tissue increased both aCSF and intracellular ROS concentrations. Thermal assessment revealed that CAP delivers energy to an area 3 cm from the torch at a rate of 200 J min^{-1}, resulting in evaporative cooling of the aCSF.

CAP transiently increases intracellular ROS concentration in normal tissue when the plume is directed at or upstream of the tissue. This evidence indicates that CAP treatment of a tissue lavage would be effective in increasing intracellular ROS, which presents an attractive option for accessing peritoneal tumor deposits. Since the lavage media undergoes evaporative cooling, tissues should be protected from thermal stress. Further studies will explore a differential impact on malignant tissue.

Te Chen (Supervisor: N. Provart). ePlant Pathway: An interactive ePlant module for pathway data visualization and analysis.

When it comes to exploring biological databases for system biology research, two main challenges remain: poor data integration across different databases creates difficulties to analyze and understand

data; limited data visualization and interaction capabilities hinder effective data searching and utilization.

Several solutions have been implemented to overcome these issues such as the ePlant in this project. ePlant is a multi-scale system biology exploration tool for model plant *Arabidopsis thaliana*. It integrates large-scale datasets in various biological scales through a unified user-centered visualization interface. ePlant allows user to visualize the effects of a gene through the gene sequence, biochemical properties and interactions of the gene product, as well as the expression pattern in the physiological and geographical level.

However, viewing metabolic pathways, an essential functionality for understanding gene functions, has not been integrated to ePlant. Therefore, this project aims to build a pathway module that can effectively fuse Reactome data (a species-specific pathway database) into ePlant. By incorporating other most advanced visualization tools and databases such as Cytoscape.js and Araport, this pathway module along with ePlant can provide the most accessible way to visualize and analyze *Arabidopsis thaliana* data.

Yi (Andy) Chen (Supervisor: E. Nambara). Investigation of Abscisic Acid 2's (ABA2) function in abscisic acid (ABA) signalling and biosynthesis pathway.

Abscisic acid (ABA) is an important phytohormone that regulates many aspects of plant's life including embryo development and seed germination. Recent result in the Nambara lab suggested that abscisic acid 2 (ABA2), a protein that function in ABA biosynthesis pathway, play a role in the ABA signaling pathway as well. It is important to validate ABA2's role in ABA signaling and to identify the amino acid regions that are important for ABA2 functions. Ten different mutated *aba2* alleles were transformed into *Saccharomyces cerevisiae*. The protein's ability to function in the biosynthesis and signaling pathway of ABA were assessed using yeast-2-hybrid. We also transformed *Arabidopsis thaliana* with different *aba2* alleles to see their effect *in planta.* As of the moment, we have identified one potential amino acid region that is essential for ABA2's function in ABA signaling.

Elizabeth Cho (Supervisor: L. Buck). Identification and Quantification of GABAergic cells in cerebral cortex of anoxia-tolerant Western painted turtles (*Chrysemys picta belli*).

Western painted turtles (*Chrysemys picta belli*) are exceptionally adaptive creatures that have evolved an ability to endure 4-5 months under ice in complete absence of oxygen. Accompanying this robust anoxia-tolerance is the reliance on metabolic substrates in the body for energy consumption. In order to maintain a reduced metabolic rate, the turtle have adapted ways to reduce excitatory (NMDA/AMPA) signalling and increase inhibitory (GABAergic) signalling in the brain. The source of GABA is likely stellate interneurons as these neurons are known to regulate mammal pyramidal neurons through the release of GABA; however the response of turtle stellate interneurons to anoxia has never been measured. The objective of this study was to perform immunohistochemical techniques to identify the stellate interneurons as GABAergic and to determine their proximity and distribution to pyramidal neurons. GABAergic neurons were mostly found in the subellular and to a higher degree, the molecular layer of the turtle dorsal cortex. Compared to pyramidal neurons, GABAergic cells were less abundant, ranging

from 4% to 23% of all neurons (10X magnification). This finding can help target stellate interneurons for electrophysiological base measurements.

Lily Huang (Supervisor: A. Bruce). Characterizing the role of Rab25b during Zebrafish development.

During zebrafish embryonic development, epiboly, an essential morphogenetic event, occurs. Epiboly is characterized as the spreading and thinning of a multilayered cell sheet, which engulfs the yolk just before gastrulation occurs. For this process to occur normally, it has been suggested that specific zygotic genes play important roles in epiboly initiation. One gene known to be up-regulated at the beginning of epiboly and expressed in the outer epithelium of the embryo is *rab25a*. This gene encodes a small GTPase and knock-down studies suggest that it is essential for normal epiboly. Since *rab25a* is known to be important in epiboly, it is proposed that *rab25b*, a closely related gene, may have a similar function. In order to investigate this possibility, *rab25b* was cloned from cDNA, and whole-mount in situ hybridizations were performed to determine the spatial and temporal expression pattern of *rab25b*. To confirm the findings of the whole-mount in situ hybridizations, RT-PCR was also performed. From these experiments, evidence suggests that *rab25b* is similar to *rab25a* in terms of spatial and temporal expression patterns in the embryo. However for the exact function of the *rab25b*, both gain and loss of function experiments must be performed in the future.

Daewoo Hwang (Supervisor: T. Harris). Identifying phosphatases that reverse the effects of aPKC during early embryogenesis in *Drosophila*.

Epithelial tissue is one of the four basic animal tissues and its main functions are secretion, selective absorption, and protection. Epithelial cell polarity is fundamental for the proper function of epithelial cells. The PAR complex has been previously shown to be a key regulator of epithelial apical polarity. One protein of this complex in particular, aPKC, is a serine-threonine protein kinase that has been shown to be important in proper epithelial cell development. aPKC- mutants are known to produce punctas of E-cadherin around epithelial cells during early embryogenesis. A phosphatase that specifically reverses the effects of aPKC has not been extensively researched. Here, we report 3 genes that may encode for such phosphatases. A genetic screen was performed to generate 33 double shRNA (short hairpin RNA) lines for aPKC and the candidate gene (CG). 9 CG's showed an improvement in hatch rate of embryos in 24 hour collections (+ 48 hours at 25^0C) and underwent further experiments. The cuticle preparations of the 9 CG's were revealed to be mostly scraps, similar to the control (mch-; aPKC-), suggesting that there may be a threshold for rescuing aPKC- defects. The 9CG's were stained for DE-cad and Dlg and 3 CG's showed rescue of the punctated DE-cadherin phenotype, suggesting that there may be a stage-specific relationship between the phosphatase and aPKC. These phosphatases may play an important role in regulating epithelial cell polarity during early *Drosophila* embryogenesis by modulating the effects of aPKC.

Do Hee Kim (Supervisor: D. Guttman). Functional characterization of host-associated loci in *Pseudomonas syringae*.

Most strains of pathogenic bacteria can infect a limited number of hosts; a phenomenon known as host specificity. *Pseudomonas syringae* is one such pathogen that, as a species, can cause disease on a broad

range of plant hosts, while specific strains have a narrow host range. In an attempt to identify gene(s) contributing to this specificity, I worked with a collection of 386 *P. syringae* strains isolated from a wide variety of hosts with sequenced genomes. Sixteen of these strains were isolated from hosts in the *Brassicaceae* family. Twenty-eight genes were found to be present in all 16 *Brassicaceae* isolates while being at very low prevalence in the rest of the *P. syringae* strains. We hypothesize that these 28 genes that are statistically over-represented in the *Brassicaceae* isolates, may be essential for the ability of *P. syringae* to infect and grow to high density in Brassicaceous hosts. I tested this using a strain of *P. syringae* (PmaES4326) originally isolated off of a radish plant, but which has also been shown to be highly virulent to *Arabidopsis thaliana* (both plants are in the *Brassicaceae*). I created knockouts of some of the 28 putative host-specific genes through homologous recombination, and then compared the growth of the knockout strain to the wild-type strain on *A. thaliana*. We have only tested one of the selected mutants *in planta* and observed no difference in virulence compared to the wild type. Since not every candidate gene may play a role in conferring an increase in *in planta* growth, further trials need to be conducted and additional mutants must be tested.

Gyu-Tae Kim (Supervisor: D. Lovejoy). Interaction of Teneurin C-terminal associated peptides (TCAP-1) and Latrophilin (LPHN) in skeletal muscle metabolism.

Teneurin C-terminal associated peptides (TCAP) is encoded in the terminal axon among the four vertebrate teneurin proteins. Introduced and highly conserved since Metazoan evolution, TCAPs bind to latrophilin (LPHN) (adhesion G-protein coupled receptor (GPCR)) and play a role in signalling pathways involved with stress-associated behaviour and physiology. Out of four TCAPs, TCAP-1 is also known to be present and has a functional role in skeletal muscle fibres, enhancing glucose metabolism of an organism. For the purpose of this course, the following four leg muscle compartments have been observed in rats: Quadriceps, Soleus, Gastrocnemius, and Tibialis. Using fludeoxyglucose F-18, PET scanner was used to collect images four days after TCAP injection. Collected images were processed using software, AMIDE, to calculate glucose uptake. Region of Interest (ROI) was measured using 3D-Freehand drawing, with a set absolute max threshold of 0.00179387 and a thickness of 0.388192 mm. The TCAP injected group showed a higher amount of glucose uptake compared to the control, and negative control group. Furthermore, different muscle groups showed varying amounts of glucose uptake due to their muscle fibre composition, Type I and/or II muscle fibres. Thus TCAP-1 and LPHN binding increases glucose uptake in skeletal muscle, essential in determining the overall metabolism of an organism.

Julia Kitaygorodsky (Supervisor: T. Harris). Liquid Facets, an Endocytosis Adaptor, and Fat Facets, a Deubiquitinating Enzyme, in the Early *Drosophila* Embryo.

The interplay between endocytosis and the actin cytoskeleton are crucial to the developing embryo. During cell formation, endocytic events promoted by cytohesin Arf-GEF Steppke antagonize actomyosin networks. Restraining of the cytoskeleton contributes to plasma membrane ingression, forming furrows that surround and separate dividing nuclei at the periphery of the embryo. In *Drosophila*, actomyosin network repression is aided by the cytohesin adaptor Stepping stone (Sstn), which contains the deubiquitinating enzyme fat facets (faf). Loss of Sstn or faf result in abnormal membrane expansion and organization during cellularization. Liquid facets (lqf), a substrate of faf, is an adaptor protein for

clathrin-dependent endocytosis at the cell membrane. Coordination of endocytosis and ubiquitination in early development is not yet understood.

Short hairpin RNA (shRNA) targeting liquid facets and fat facets sequences were generated and tested for evidence of abnormal development by hatch rate calculation, quantification of defects in the secreted cuticle, and immunostaining. The shRNA constructs were expressed using a maternal triple driver (MTD) and the UAS-GAL4 system. The faf-shRNA resulted in severe early defects with abnormal membrane expansion and arrangement. Two lqf-shRNA lines demonstrated increased lethality and cuticle defects, but imaging did not reflect lower lqf levels in the embryos. This suggests potential off-target effects, or a weak effect from the shRNA, so defect is accumulated only at a later stage of development. Future assessment of stronger lqf knockdowns, and lqf and faf knockdowns in combination may provide further insight into the role of endocytosis and ubiquitination in the *Drosophila* embryo, with implications for mammalian development.

Jacqueline Law (Supervisor: K. Yip). The Role of Epstein-Barr Virus in Nasopharyngeal Carcinoma.

Joanne Lee (Supervisor: D. Guttman). Dissecting *Arabidopsis* protein-protein interactions underlying immune responses triggered by secreted effectors of the bacterial pathogen *Pseudomonas syringae.*

The *Pseudomonas syringae* 'effector' protein HopZ1a is a secreted acetyltransferase that can enhance virulence in susceptible plant hosts or induce immunity in resistant plant hosts. Previous research from the Guttman lab has identified two *Arabidopsis* proteins that are necessary to elicit an immune response in resistant *Arabidopsis* ecotypes: (1) ZED1 is directly modified by the bacterial HopZ1a effector, while (2) ZAR1 is a resistance protein that monitors ZED1 and presumably triggers the immune response upon recognition of ZED1 modification.

ZED1 is a pseudokinase whose gene is localized in a genomic cluster of seven other closely-related genes that also encode kinase-like proteins. These proteins are called ZED1-related kinases (ZRKs). As pseudokinases, ZED1 and ZRKs may play a role in recognition of the HopZ1a effector. Other *Arabidopsis* proteins including the true kinase PBS1 have been implicated in immunity against unrelated bacterial pathogens (e.g. *Xanthomonas spp.*). We hypothesized that HopZ1a can either promote or inhibit interactions between kinases/pseudokinases and the resistance protein ZAR1, resulting in immune activation.

To investigate this hypothesis, we compared "two-hybrid" and "three-hybrid" interaction assays in yeast. We first established basal interactions in yeast using select prey plasmids and an existing array of bait plasmids. We then contrasted these findings with yeast "three-hybrid" interactions obtained by screening the same prey plasmids against the bait array, but in the presence of chromosomally-integrated *hopZ1a* or a catalytically inactive mutant.

Surprisingly, we find that basal interactions between ZAR1 and ZED1/ZRKs/PBS1 are quite weak, compared to previously well-characterized positive controls. Nonetheless, results from our assay suggest that wild-type HopZ1a, but not a catalytically inactive mutant, is able to disrupt interactions between sub-domains of ZAR1. In contrast, a novel allele of an unrelated *P. syringae* effector ($HopF2^{PacM}$) has the opposite effect, promoting interactions between ZAR1 sub-domains. These results

suggest that distinct effectors are recognized by different molecular mechanisms, despite sharing common signaling components.

Karthik Natarajan (Supervisor: U. Tepass). Generating a MiMIC EGFP-FLaSH-StrepII-3×Flag protein multi-tag for Cad87A and Expanded genes in *Drosophila melanogaster.*

In order to understand the mechanisms underlying the establishment and maintenance of cell polarity, adhesion, and growth in epithelial tissues, the function of proteins expressed during development needs to be elucidated. Minos mediated integration cassette (MiMIC) is a transposon which allows unbiased insertion into any region of a gene in *Drosophila melanogaster*. Within the transposon is a pre-existing replaceable DNA sequence which allows for the insertion of any sequence of interest. In this project, MiMIC was used to insert a protein multi-tag sequence, EGFP-FLaSH-StrepII-3×Flag, into two endogenous proteins known to be involved in the regulation of epithelial cell polarity and growth, Cad87A and Expanded. Within this multi-tag sequence, the EGFP and FLaSH tags allow for high resolution imaging of protein expression through techniques such as scanning electron microscopy. The StrepII and Flag tags allow for quantification of protein expression, and protein purification to characterize structure and function. The created lines were then imaged to determine expression of these two genes. The tools generated in this work will allow determining protein dynamics and turnover *in vivo*. They will also allow controlled temporal and spatial protein knockdown by using the GAL4/UAS system to induce RNA interference against EGFP, as well as other available genetic tools in *Drosophila*.

Dang Ngyuen (Supervisor: M. AbouHaidar). Expression of Human Hepatitis C virus (HCV) Core and Envelope Proteins in *Arabidopsis thaliana.*

HCV is a worldwide health concern for millions of people worldwide as it is a major cause of liver cirrhosis and liver cancer in humans. However, there is currently no commercially available vaccine for Hepatitis C. Plant-derived vaccines have been successfully produced and proven effective against viral agents of infectious diseases such as Hepatitis B virus and Human Papillomavirus. In this study, we utilized *A. thaliana* as a fast-growing heterologous system to transgenically express the structural proteins of HCV, namely the capsid protein (C) and two envelope proteins (E1 and E2), with the end goal of manufacturing HCV antigens for vaccination. These transgene-derived structural proteins are expressed as a polyprotein similarly to the one synthesized from HCV genome in the HCV infected mammalian system. Our goal is to examine whether the transformed plant cells can synthesize and subsequently process the viral polyprotein corresponding to the equivalent HCV replication events in the mammalian hepatocytes. Here, we showed that HCV structural polypeptide can be stably expressed and cleaved into the correct-length core and envelope proteins in *A. thaliana*. Future studies will investigate other post-translational modifications of these plant-derived viral proteins such as glycosylation and disulfide bond isomerization as well as their potential to form HCV virus-like particles.

Maha Noor (Supervisor: D. Godt). Fate map study of the germline stem cell niche in the ovary of Drosophila.

Alborz Noorani (Supervisor: D. Guttman). Comparative genomics of the *Burkholderia* genus with emphasis on the *Burkholderia cepacia complex.*

The *Burkholderia* genus consists of versatile organisms with the ability to adapt to diverse ecological niches. This genus includes a group called the *Burkholderia cepacia complex* (Bcc). Bcc encompasses opportunistic human pathogens which can have lethal effects when infecting cystic fibrosis or immune-compromised patients. Despite their importance, our current understanding of these pathogens is limited to a few important genes. This project aims to explore the genetic complexity and functional diversity of the genus *Burkholderia* by comparing whole genome sequences of several *Burkholderia* strains. We collected the genomes of 88 strains of this genus from public databases, including 42 Bcc and 46 other strains. Furthermore, ortholog analysis was performed to compare the genetic content of these strains. We identified 51049 protein families in the pan-genome of the *Burkholderia* genus, including 814 core families and 50235 flexible families. We used the concatenated alignment of the core genes to reconstruct the phylogenetic relationship between all *Burkholderia* strains. We found that the core genome of Bcc was approximately 5% of the size of the entire *Burkholderia* pan-genome. Also, 70% of the genes in the Bcc core genome are unique to that group. Among the genes unique to Bcc, we found genetic factors associated with virulence such as fliH, pilA and motoB which are related to invasion, adherence and motility respectively.

Hokyun Park (Supervisor: T. Harris). Investigating the mechanisms behind ubiquitination of Rho1 in early development of *Drosophila* embryos.

RhoGTPases are molecular switches that mediate activity of other effector proteins, which are involved in cell shape formation, migration and division. In the context of cell division, Rho localizes along cleavage furrow and trigger formation of contractile ring through effector molecules such as ROCK and formins to promote cytokinesis. It has been shown that some of the RhoGTPase such as Rac1, RhoA and Cdc42 can be ubiquitinated. Previous papers have demonstrated that Rac1 linked to mono-ubiquitin at N-terminus was available at endosomal structure suggesting ubiquitin-mediated endocytosis has occurred. However, whether other RhoGTPases can be endocytosed and exact mechanisms behind endocytosis is not yet shown. In my project, I investigated whether ubiquitination of Rho1 from drosophila, which is homologous to mammalian RhoA, trigger endocytosis of itself. Different Rho1 constructs with mutations at sites previously shown to be important for ubiquitination and mono-ubiquitin fused at N-terminus of Rho1 has been exogenously expressed to evaluate their localizations and activity at plasma membrane. Activity of Rho1 at the plasma membrane depends on the lysines known to be ubiquitinated. The level of Rho1 with mutation at sites known to be ubiquitinated had highest level of Rho1 at the plasma membrane. This is consistent with role for lysine in endocytosis. However, the collective activity of mutated Rho1 was lower than just Rho1 itself.

Van Phan (Supervisor: K. Yoshioka). The Arabidopsis Cyclic Nucleotide-Gated Ion Channel AtCNCG2 has a role in Auxin Homeostasis.

The loss-of-function *AtCNGC2* mutant *dnd1 (defense no death1)* in *Arabidopsis thaliana* (Arabidopsis) shows impaired autoimmune hypersensitive response, a characteristic response of effector-triggered resistance. To understand *dnd1*-mediated immunity signaling, Arabidopsis mutant *repressor of defense no death1* (*rdd1*), which suppresses *dnd1*-mediated phenotypes, was identified. Recently, *RDD1* has

shown to encode a biosynthesis gene for the plant hormone auxin. However, the relationship between auxin and cyclic nucleotide gated ion channels (CNGCs), ligand-gated, non-selective cation channels implicated in Ca^{2+} signaling and involved in plant processes such as development, pathogen defense and immunity, has not been elucidated. We hypothesize that *AtCNCG2* is involved in mediating auxin homeostasis or signaling in Arabidopsis. To investigate this point, we can use an artificial auxin-sensitive DR5::GUS reporter system introduced into wild type and *dnd1/cncg2* mutants. Based on the previous analysis, it is predicted that auxin signaling will be decreased in *dnd1/cngc2* mutants, where Ca^{2+} channel activity is required for auxin transport. Thus, we predict that *AtCNGC2*-dependent Ca^{2+} signaling is involved in auxin homeostasis in Arabidopsis. This data will open up future direction for exploring the potential mechanism of calcium-induced signal transduction in auxin.

Pamela Psarianos (Supervisor: K. Yip). Reversal of radiation fibrosis using adipose-derived stem cells.

Radiation fibrosis (RF) is a late side effect of radiotherapy (RT) that is associated with increased morbidity. Reversing this condition may lead to a substantial improvement in the quality of life of RT patients. Recent studies have shown evidence that RF may be reversed via epigenetic modifications. Moreover, adipose-derived stem cells (ADSCs) have been implicated in the repair of tissue damage, and may be of clinical significance to patients suffering from fibrosis. The role that ADSCs play in the reversal of RF was explored *in vitro* and *in vivo*. Radiation fibrosis models were established and then treated with ADSCs. Collagen deposition assessed as the primary indicator of fibrosis, measured through trichrome staining, qRT-PCR, as well as Western blotting. It was found that ADSCs decreased the amount of collagen both *in vivo* and *in vitro*. We are currently seeking to determine the mechanism of action of ADSCs, focusing on the DNA methylation machinery. The results obtained in this study could have significant implications in the treatment of fibrosis and the quality of life of cancer patients.

Purohit, Priyank (Supervisor: N. Provart). Multi-track RNA-Seq Browser for Visualization of Global Patterns of Gene Expression in *Arabidopsis thaliana*.

The development of next-generation sequencing technologies has lead to a significant reduction in the cost to obtain "–omics data." As this technology has improved, researchers have been able to generate vast amount of transcriptomics data by conducting RNA-Seq experiments to better understand an organism's biology by analyzing the global gene expression patterns. In addition to generating biological big data, many tools to analyze and visualize these datasets have also been developed to allow researchers to make inferences. Here we present a novel multi-track RNA-Seq browser that shows the mapping coverage, and an electronic fluorescent pictographic (eFP) image that serve as visual representations of expression levels of a particular gene under different growth conditions and developmental stages. The tool also presents details of each RNA-Seq experiment, and it is capable of performing statistical analysis in form of Reads Per Kilobase per Million reads mapped (RPKM) and Pearson Correlation Coefficient (PCC) to make unbiased relative comparisons. This multi-track RNA-Seq browser will be the Canadian contribution to the Arabidopsis Information Portal/Araport.org initiative, which consists of tools and datasets for plant biology researchers using *Arabidopsis thaliana* as the model organism.

Tags: Arabidopsis thaliana, RNA-Seq, biological big data, Araport, gene expression, transcriptome.

Shuyue Qiao (Supervisor: A. Bruce). Development of constructs for imaging early zebrafish morphogenesis.

A major question in developmental biology is how cell shapes and cell movements are coordinated to give rise to the adult vertebrate body plan. Research in the Bruce lab focuses on the process of epiboly or the thinning and spreading of a multilayer of cells in the zebrafish embryo as a model system for cell rearrangement. Epiboly is the first coordinated cell movement during zebrafish development. However the exact mechanism for cell rearrangements during epiboly remains unclear. To understand the dynamics of cell shape changes and movements during epiboly, live imaging is essential and for effective live imaging, constructs encoding fluorescently tagged proteins that label different parts of the cell are needed. It is also useful to be able to do double labeling. We have a number of GFP labeled constructs that highlight the cell membrane and the actin cytoskeleton, such as the EB3-GFP constructs. However we lack red fluorescent constructs. The focus of this project was to test whether tdtomato would be a good red fluorescent protein to use in our studies. Tdtomato and EB3-tdtomato, which labels polymerizing plus end of microtubules, were cloned into an expression vector. Tdtomato RNA was transcribed *in vitro* and microinjected into 1-cell stage embryos, which were then examined by fluorescent microscopy including confocal microscopy. The results suggest that tdtomato could be a useful marker but not for studying zebrafish epiboly movement in the yolk cell, where it forms aggregates. EB3-tdtomato could not be tested because the plasmid appeared to be toxic to *E. coli*, preventing enough plasmid to be recovered to generate RNA. For the next step, other red fluorescent protein could be tested to find the optimal one for studying yolk cell movements in epiboly.

Rajiv Rampersaud (Supervisor: D. Goring). Investigating self-incompatibility signaling proteins APK1A and APK1B in *Arabidopsis thaliana*.

In the Brassicaceae family of plants, the self-incompatibility (SI) pathway is a conserved trait which reduces inbreeding depression. In *Brassica* and *Arabidopsis* species, this pathway is initiated when S-locus Cys-Rich/ S-locus Protein 11 (SCR/SP11) from the pollen grain bind to S-Receptor Kinase (SRK) on the stigmatic papillae. This activates SRK by autophosphorylation, leading to the phosphorylation of Armadillo Repeat Containing 1 (ARC1). ARC1 ubiquitinates Exo70A1, a component of the exocyst complex required for pollen hydration. This process causes self-pollen rejection. In *Brassica* species, M-Locus Protein Kinase (MLPK) is a Receptor-Like Cytoplasmic Kinase that positively regulates the SI pathway by binding to SRK. APK1A and APK1B are orthologues of *Brassica* MLPK in *Arabidopsis* species; however, their function in *Arabidopsis* is largely unknown. In this project, we investigated the roles of the *APK1A* and *APK1B* genes in self-incompatibility by testing T-DNA knockout mutants for both genes in self-incompatible *A. thaliana* transformed with the *SCRb-SRKb-ARC1* transgenes. Self-pollen rejection was observed by aniline blue staining of pistils and measuring seed counts. Transgenic *SCRb-SRKb-ARC1* plants with a wild-type copy of either the *APK1A* or *APK1B* genes did not significantly show differences in seed counts or pollen tube penetration when compared to transgenic double homozygous *apk1a/apk1b* mutant sibs. Thus, this data suggests that the *APK1A* and *APK1B* genes are not required for the *Arabidopsis thaliana* self-incompatibility response.

Zheng Song (Supervisor: K. Yoshioka). Investigation of Ca^{2+} flux mediated by Arabidopsis cyclic nucleotide-gated ion channels (CNGCs).

Ca^{2+} signaling is critical to plant immunity; however, the channels involved are poorly characterized. Cyclic nucleotide-gated channels (CNGCs) are non-specific, Ca^{2+}-permeable cation channels. Plant CNGCs are hypothesized to be negatively regulated by the Ca^{2+} sensor calmodulin (CaM), and previous work has suggested a single CaM-domain (CaMBD) overlaps with the cyclic nucleotide-binding domain (CNBD) of the cytosolic C-termini of plant CNGCs. However, recently the Yoshioka laboratory showed that the Arabidopsis isoform AtCNGC12 that possesses multiple previously unidentified CaMBDs at cytosolic N- and C-termini, reminiscent of animal CNGCs and unlike any plant channel studied to date. My project focuses on the analysis of Ca^{2+} flux in via wildtype or various CaMBD mutants of CNGC12 using fluorescent genetically-encoded Ca^{2+} indicators including 35S:GCaMP3 and YC6.5 as a probe. The CNGC12 mutants used for treatments on transgenic N. Benth and N. nicotania and N. Benth and N. nicotania wild type were cpr22 (CNGC11/12), cpr22 (IQ^{mut}), cpr22(CT^{mut}), $CNGC12^{wt}$, NT^{mut}, IQ^{mut}, CT^{mut} and $CNGC12^{8del}$ (Δ32-39). The insert of 35S:GCaMP3 plasmid and YC6.5 plasmid was genotyped via DNA extraction of the plant tissue and PCR. The visual analysis of Ca^{2+} flux was performed via florescence microscopy, while semi-quantitative measurements was made using a fluorescent plate-reader. This analysis enabled us to reveal the important information regarding the regulation of CNGCs by CaM as related to downstream Ca^{2+} flux.

Amanda Stojcevski (Supervisor: J. Peever), Immunohistochemical staining of c-Fos-mediated hM3Dq expression in a transgenic mouse model.

Designer Receptors Exclusively Activated by Designer Drug (DREADDs) are derived from mutated muscarinic receptors and bind clozapine-*N*-oxide (CNO), an otherwise pharmacologically inert molecule. The hM3Dq DREADD is derived from the M3 muscarinic receptor and promotes cellular activation upon binding of CNO. Therefore, hM3Dq receptors can be used to understand the physiological and behavioural effects of activating different neuronal populations. Recently, a transgenic mouse model was developed to allow expression of hM3Dq receptors in cells that express c-Fos, an immediate-early gene that is expressed in cells activated past their baseline level (Kovács, 1998). In this model, c-Fos promotes expression of the Tetracycline Transactivator (tTA) protein, which binds to its promotor on the Tet-Operon upstream of the hM3Dq gene, resulting in hM3Dq expression. Doxycycline (dox) blocks the binding of the tTA to the Tet system, and therefore inhibits hM3Dq expression. This allows for a way to control the time window in which hM3Dq can be expressed. It was my goal to create an immunohistochemical protocol that would allow us to stain for hM3Dq in this transgenic mouse model. To do this, we took a group of animals off dox food for 30 days to allow an excess of hM3Dq expression. In these animals, we found hM3Dq expression in the amygdala, which was not present in wild type controls. Now that we are able to stain for hM3Dq in this mouse model, it can allow us to see which neuronal populations are linked to important behaviours, such as the generation of REM sleep.

Aren Thomasian (Supervisor: J. Peever). Characterizing the role of the Sub-Coeruleus in the rapid eye movement (REM) sleep circuit using immunohistochemistry and fluorescence in situ hybridization.

Glutamatergic neurons of the SubCoeruleus (SubC) have been hypothesized to regulate REM sleep and its features, including muscle paralysis. Recent advances supporting this hypothesis have shown that

optogenetic stimulation of the region increases REM sleep duration, and chemogenetic activation produces a state resembling cataplexy- the involuntary loss of muscle tone during wakefulness. This study aimed to contextualize these behavioral results by quantifying the degree of activation of glutamatergic cells at the level of the SubC using both chemogenetic and optogenetic techniques. Transgenic mice expressing Cre-recombinase at excitatory glutamatergic neuronal cell bodies were injected with an adeno-associated virus infused with either chemogenetic or optogenetic transgenes. Immunohistochemical staining was performed for cFos, a neuronal marker of activity, as well as for the mCherry fluorophore conjugated onto the chemogenetic receptors to determine the level of cellular activation. Fluorescent in situ hybridization for the vGluT2 (vesicular glutamate transporter 2) mRNA as well as staining for the eYFP (enhanced yellow fluorescent protein) fluorophore conjugated onto the optogenetic receptor was used to determine specificity in targeting glutamatergic cells of the SubC. The number of cells expressing the fluorophore alone (either mCherry or eYFP) and those double-labeled (either mCherry+/cFos+ or eYFP+/vGluT2+) were quantified in each condition. When the chemogenetic receptor was activated using clozapine-N-oxide (CNO), an otherwise biologically inert ligand, 77±7% of the mCherry+ cells within the SubC were double-labeled for cFos+/mCherry+, significantly more than controls injected with saline alone (22±6%), as well as those injected with the ligand (i.e. CNO) without the presence of the chemogenetic receptor (13±4%) (ANOVA, $p<0.001$, $n=4$). In addition, all cells in the SubC expressing eYFP were found to be glutamatergic (i.e. vGluT2+, $n=2$). These findings confirm the accuracy of our methodology, and that the glutamatergic cells of the SubC are being significantly activated, leading to major behavioral alterations.

Daphne Vijayakumar (Supervisor: J. Peever). Anatomical connection between the hypoglossal motor nucleus and the A6 region in the context of respiratory long-term facilitation.

The brainstem contains the respiratory centre which controls the rhythmic contractions of multiple respiratory muscles to regulate breathing. The neural circuitry controlling the inspiratory and expiratory muscles are dynamic, they not only respond reflexively to mechanical and chemical feedback, but also learn to adapt to previous stimuli. One mechanism that allows for learning and adaptation is plasticity. Long-term facilitation (LTF), a type of respiratory plasticity, is a progressive and sustained increase in respiratory motor output onto motor neurons that then drives the inspiratory muscles such as genioglossus and diaphragm, following repeated respiratory challenges. LTF is of particular interest in the clinical manifestation of obstructive sleep apnea (OSA). Obstructive apneas have shown to elicit LTF which is central to increasing the hypoglossal motor neuron activity and strengthening the genioglossus muscle tone. The objective of this study was to elucidate the neural circuitry which mediates LTF by determining whether there exists a direct anatomical connection between the hypoglossal motor nucleus (Mo12) and the tyrosine hydroxylase positive (TH+) cells of the A6 region. This was done using the retrograde tracer, cholera toxin subunit B (CTB), and immunohistochemical methods. The A5 cell group was used as a control region which has known projections to the Mo12. Overall, the data shows that there exists a direct anatomical connection between the A6 region and the Mo12, the motor pool which innervates the genioglossus muscle. These findings are significant in order to develop effective pharmacological therapeutics for OSA patients.

Limin Wang (supervisor: U. Tepass). Analysis of the role of M-region of alpha-catenin in adhesion junction organization and cell movement during oogenesis using *Drosophila melanogaster*.

Alpha-catenin is a core component of cadherin-catenin complex (CCC), which is essential for mediating effective adhesion between epithelial cells. The N-terminal region of α-catenin binds to β-catenin whereas the C-terminal region binds directly to F-actin. It is not clear how α-catenin links the cadherin-catenin complex (CCC) to the actin cytoskeleton. Recent studies have shown that the central region of α-catenin (M-region) is a mechanosensory module. The M region consists of three domains, the M1, M2, and M3 domains. When actomyosin contraction is applied, the M region of α-catenin undergoes conformational change and exposes M1, which is a binding site for actin-binding protein, vinculin. Association with vinculin and other actin-binding proteins is expected to strengthen the linkage between CCC and the actin cytoskeleton.

To determine the function of the M-region, we expressed deletion constructs such as α-catΔM lacking the M-region, and various α-catNT-N-M* constructs that express M-domains in cells lacking endogenous α-catenin. Further, we scored the ability of these constructs in maintaining follicular epithelium integrity and border cell migration during oogenesis. A-catenin mutant cells lose epithelial integrity and accumulate abnormal clusters of cytoskeletal protein, α-Spectrin. In addition, mutant border cell clusters fail to migrate. Our results showed that α-catΔM could only partially rescue α-catenin mutant phenotypes suggesting that M-region is required for α-catenin function. We also found that the various M-domains of α-catenin do not rescue α-catenin phenotypes during oogenesis. Our data suggest that these constructs are not sufficient to replace endogenous α-catenin in these tissues.

Nili Yuen (Supervisor: T. Harris). How endocytic motifs affect the function of DE-cadherin in the *Drosophila* embryo.

Mammalian cell culture studies have shown that the juxtamembrane domain (JMD) in E-cadherin contains signals that are important for trafficking. However, the roles of these signals *in vivo* are still unclear. To investigate these signals and specifically, if ubiquitination of this domain plays a role in cadherin trafficking in the *Drosophila* embryo, various constructs with mutations in the JMD were expressed. These include lysine substitutions, tyrosine substitutions and the deletion of the entire domain. The degree of hatching was construct-specific, and all constructs displayed similar lethal cuticle morphologies. All constructs also showed similar levels of cadherin at the plasma membrane. The wild-type control embryos and lysine mutants both showed cadherin accumulation within multiple small puncta in each cell, and the JMD mutants showed accumulation in 1-2 large puncta in each cell. Altogether, my results suggest that overexpression of cadherin leads to embryonic lethality, and that the JMD may contain signals that are important for cadherin trafficking. However, lysine residues within the JMD may not be critical for cadherin trafficking.

Alexandra Zimmer (Supervisor: J. Mitchell). Characterization of a distal Sox2 enhancer cluster found in human embryonic stem cells.

SOX2 is one of the core transcription factors needed to maintain stem cell pluripotency. Novel enhancer regions have been identified that are involved in regulating SOX2 expression in mouse embryonic stem (ES) cells. These enhancers can be classified as either proximal (SRR1, SRR2 and SRR18) or distal (SRR107

and SRR111) Sox2-regulatory regions (SRR). The two distal SRRs form what is known as the Sox2-control region (SCR). The SCR is bound by 10 different transcription factors including OCT4, NANOG and SOX2. *In silico* analysis identified a distal region in human ES cells that is similar to the SCR found in mice ES cells. This distal region is composed of two clusters of transcription factor binding sites. In this study, luciferase reporter assays evaluated the ability of the human SCR clusters to enhance *SOX2* transcription jointly and in isolation. Point mutations were created for each of the putative transcription factor binding sites in the human SCR to further enrich our analysis of how *SOX2* transcription is regulated.