**Yuchen, Cai (Supervisor: D. Godt)**. A cell lineage analysis was conducted for the germline stem niche in the *Drosophila* ovary using the twin-spot MARCM technique.

**Myung Soo Chang (Supervisor: M. Ringuette)**. Parameter establishment of cold-atmospheric plasma (CAP) device and evaluation of bioefficacy of CAP on normal *Drosophila melanogaster* growth and survival.

It is known that cold atmospheric plasma (CAP) contains and generates reactive oxygen and nitrogen species (RONS) and is being studied for possible medical use in wound healing and surgical treatment of cancer in the future. Particularly, CAP exhibits potential in treatment of premalignant epithelial neoplasia and cancer. This is known to occur by increasing oxidative stress in cells which has been exposed to the CAP plume, leading to triggering cell death pathways selectively in neoplastic and malignant cells while preserving normal cells. Therefore, it is important that the CAP therapy method is developed to target abnormal cells while minimizing damage on normal cells. In order to study the effect of CAP on normal cells, preliminary tests to serve as the basis of development of CAP therapy have been carried out. Several parameters such as temperature, flow rate and application distance, of CAP device developed by the Department of Mechanical and Industrial Engineering at University of Toronto has been tested out. Also, *Drosophila melanogaster* larvae were exposed by the CAP plume in various conditions in order to evaluate the bioefficacy of the plasma on growth and survival of normal organism.

**Alamjeet Chauhan (Supervisor: M. Woodin)**. The KCC2 C-terminal domain is not required for KCC2:GluA1 and KCC2:GluK2 interactions.

The potassium chloride (K+/Cl-) co-transporter, KCC2, is a 12 pass transmembrane protein, and it is exclusively expressed in the mature central nervous system. KCC2 maintains low intracellular Cl- concentration via active Cl- extrusion, and promotes GABAA mediated fast synaptic inhibition. Deficits in KCC2 expression in mature neurons can lead to numerous neurological disorders such as epileptic seizures and autism. Recent studies have shown that KCC2 coexists in a functional complex with the kainate receptor subunit, GluK2, and that KCC2:GluK2 interaction regulates the function as well as the expression of KCC2 in the membrane. Furthermore, emerging evidence indicates that other ionotropic glutamate receptors, such as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors interact with KCC2. There is a physical protein-protein interaction between KCC2 and the AMPA receptor subunit, GluA1 as well as between KCC2 and GluK2; however, the KCC2 domain that is involved in the interaction with kainate and AMPA receptor subunits is still under investigation. To determine how KCC2 interacts with GluA1 and GluK2, we need to identify the KCC2 region (N-terminus, C-terminus, or transmembrane domain) that is involved in the interaction. I found that the KCC2 C-terminal domain is not required for KCC2:GluK2 and KCC2:GluA1 interaction. This finding indicates that the KCC2 N-terminal or transmembrane domains are important for the interaction between KCC2, kainate, and AMPA receptor subunits.

**Xuran Chu (Supervisor: D. Christendat)**. Phenotypic recovery of *skl1* plastid biogenesis defective mutant in *Arabidopsis thaliana*.

Shikimate kinase (SK) is an important enzyme involved in the shikimate pathway required for plant growth and survival. Shikimate kinase-like 1 (SKL1) is a SK gene duplicate that arose very early in the evolution of land plants including moss and ferns. Previous studies show that SKL1 has evolved a novel function distinct from SK. The TDNA knockout line of SLK1 in Arabidopsis shows albino phenotype, which is caused by a defect in plastid biogenesis. DEX is a strong synthetic glucocorticoid, which can be used to induce gene transcription by binding to the receptors in cells. It is widely used in plant biology research. My goal was to rescue the albino phenotype using a DEX-inducible system, whereby upon the application of DEX, SKL1 transcription is initiated. The SKL1 expression system was transformed in the homozygous mutant background. Basta supplemented plates were used to select for plants successfully transformed with the DEX-inducible construct.

We have successfully grown progeny of heterozygous *skl1* mutant line with the DEX expression construct and screen them on MS agar plate supplemented with; Basta, Basta and DEX and DEX. Survival ratios on each conditions suggest that the albino plants are being rescued on plates that have DEX. Further genetic analysis will be required to confirm that the WT phenotype plants are in fact SKL1 KO plants rescued by DEX.

**Victoria Chuen (Supervisor: J. Peever)**. A biochemical approach to defining the subcoeruleus (SubC).

The subcoeruleus (SubC) is widely hypothesized to be critical for the generation of rapid eye movement (REM) sleep and muscle atonia, a physiological hallmark of the sleep state. Previous studies have identified REM-on neurons in the SubC as primarily glutamatergic. However, as the most abundant excitatory transmitter within the central nervous system, glutamate does not serve as an ideal biochemical marker for this particular region as it is not exclusive to the SubC. As such, the goal of this project was to investigate whether any genes are uniquely expressed within the SubC and to better characterize the region. Using the Allen Brain Atlas, it was found that *DBH* and *MAO A* mRNA are strongly expressed within the SubC region. Since these genes are typically expressed in noradrenergic neurons, immunological staining for tyrosine hydroxylase (TH), an enzyme required for the synthesis of noradrenaline, was performed to characterize the presence and quantity of noradrenergic neurons in the SubC.

**Eugenia Daradur (Supervisor: K. Yoshioka)**. Investigating the role of the *YUCCA* family in the suppression of *dnd1* (*cngc2*) – mediated phenotypes.

The *Arabidopsis thaliana defense, no death (dnd1)* mutant is a rare loss-of-function autoimmune mutant that was identified by its reduced ability to produce a hypersensitive response (HR). *dnd1* also exhibits phenotypes such as high levels of endogenous salicylic acid (SA), increased expression of pathogenesis-related genes (PR genes), spontaneous cell death, dwarf morphology and hypersensitivity to Ca2+. It was identified that *DND1* encodes *CYCLIC NUCLEOTIDE-GATED ION CHANNEL2 (CNGC2)* which is a non-selective ion channel that has been postulated to act as a Ca2+ channel in *A. thaliana*. Previously, in a screen for suppressors of a *cngc2* knockout line, which displays phenotypes as *dnd1*, the mutant *rdd1* was identified as a suppressor of all *dnd1 (cngc2)* related phenotypes, except Ca2+ hypersensitivity (Chin et al., 2013). Current data suggests that *RDD1* encodes *YUCCA6* (*YUC6*), which is a biosynthesis gene for the plant hormone, auxin. On account that *cngc2* *yuc6* loss-of-function double mutant displayed the same phenotype as *cngc2* *rdd1* double mutant, it was suggested that *rdd1* is a loss-of-function mutant, as well. Since *YUC6* belongs to a family of 11 members and some *YUCCA* family members are functionally redundant, it was hypothesized that the loss-of-function mutants of closely related *YUC* genes might also suppress *dnd1* (*cngc2*)-mediated phenotypes. Data obtained so far focuses on the most closely related *YUC6* family member, *YUC2*, as a possible suppressor of *dnd1* (*cngc2*). Moreover, for several other *YUC* family members, *YUC1*, *7* and *11*, *cngc2* *yuc* homozygous double mutants were identified and will be tested for the same function. The results of this project will provide novel insights into the connection between *DND1* (*CNGC2*) and auxin signaling.

**Samantha Del Borrello (Supervisor: U. Tepass)**. Use of the MiMIC system in Drosophila to fluorescently tag endogenous apical cell adhesion proteins.

In the current project, the Minos-mediated integration casette (MiMIC) system developed by Hugo Bellen’s lab in 2011 was used to create 3 transgenic Drosophila melanogaster strains through recombinase-mediated cassette exchange (RMCE). The apical cell adhesion proteins *Stardust (sdt)*, α-*catenin (α-cat)* and the FERM domain protein *Okapi (oka)* were tagged with fluorescent markers to allow for live imaging of endogenous protein expression. *Sdt* and *oka* were successfully tagged with GFP, resulting in five successful independent integration events that are homozygous viable for the *sdt* gene. In all embryos, *sdt::GFP* was found to colocalize with the known interacting protein *Crumbs (crb)* at cell-cell contacts in the ectoderm. The third protein, *α-cat* was successfully tagged with KillerRed and three independent strains of *α-cat:KillerRed* were created as homozygous stocks. *α-cat* is thought to act as a mechanosensitive anchor between the apical cell junctions and the acto-myosin cytoskeleton, regulating the recruitment of actin regulators to the apical cell junctions during morphogenesis and wound repair. The novel *α-cat:KillerRed* fly lines will allow the selective inactivation of α-Catenin in single or multiple cell-cell contacts in normal epithelia, expanding the opportunity to address the role of *α-cat* in processes like wound repair, cell rearrangements and cell shape changes where regulated tension is applied to junctions. In addition, the acute down-regulation of α-Cat will allow testing its role in the recruitment and maintenance of multiple actin regulators at the adherens junctions.

**Zeal Desai (Supervisor: S. Varmuza)**. Identification of proteins anchored by SFMBT2 in trophoblast cells.

Extraembryonic tissues (placenta and yolk sac) are critical for survival of mammalian embryos. They derive mainly from a specialized cell type called trophoblast. A trophoblast specific chromatin protein called SFMBT2 (Scm-Like With Four Mbt Domains 2) is required to maintain the trophoblast progenitor cell pool in order to make a functional placenta. SFMBT2 is a polycomb group protein, and functions as part of a multi-subunit silencing complex. Currently the other subunits within the complex are unknown. The aim of this study was to build cellular tools that will aid in the identification of the other components of the complex anchored by SFMBT2 in trophoblast cells. This was to be accomplished by constructing trophoblast stem cell lines expressing an inducible 3X FLAG-tagged SFMBT2 transgene. The proteins extracted from cells expressing the transgene, would have been subjected to co-immunoprecipitation with an anti-FLAG antibody to be extracted and identified by mass spectrometry. To construct a 3X FLAG-tagged SFMBT2 transgene, three different protocols were used 1) Gibson cloning 2) annealing of primers and restriction enzyme digestion 3) Oligonucleotide preparation followed by annealing and ligation of oligonucleotides. After attempting all three protocols, none of them showed successful cloning results of the 3X Flag tag into the long Sfmbt2 plasmid. The cloning technique was ineffective and was thus dismissed. The study was then directed towards looking for targets of SFMBT2 proteins by analyzing the ChIP-seq data obtained by Priscilla Tang from Varmuza Lab. The data was analyzed to find transcription factors in the mouse genome that are conserved in other mammalian genomes. Using the intragenic peak regions from the ChIP-seq data a bioinformatics analysis was performed to check for conservation within the mouse, rat and human genome. The conserved transcription factors were then inquired for any expression in differentiated and undifferentiated human trophoblast cells.

**Jennifer Duan (Supervisor: D. Desveaux)**. Enhancing plant immunity: A targeted forward genetics screen.

The Gram-negative phytophatogen *Pseudomonas syringae* infects a wide range of agronomically important crops, including rice and tomato. *P. syringae* employs a type III secretion system to inject effector proteins into plant host cells. Effectors are pathogen virulence proteins that are utilized by the pathogen to suppress the plant’s immune system. In turn, these effectors may also be recognized by the plant, and elicit an immune response known as effector-triggered immunity (ETI). ETI is a very rapid immune response and often culminates in localized cell death known as the hypersensitive response. Over thirty different families of effectors exist and this diversity allows *P. syringae* to infect numerous plant species. One such effector family is the HopF family, which plays a role in both beans and the model plant organism, *Arabidopsis thaliana*. HopF2PtoT1 and AvrRpm2PsvNCPPB3335 both belong to the HopF family, and both elicit a weak ETI response in *A. thaliana*. These phenotypes make it difficult to study these effectors and their molecular mechanisms. Through pathogen growth assays, both have been identified to cause a significant decrease in pathogen growth, suggesting host recognition triggering an immune response. This study aims to enhance this weak ETI response using a forward genetics screen with EMS mutagenized *A. thaliana* plants. Plants with this enhanced resistance phenotype will be utilized to map the affected genes, and elucidate the underlying mechanisms of increased resistance.

**Nicole Fogel (Supervisor: J. Kim)**. Perisomatic inhibitory interneurons of the cortex and hippocampus: characterizing the distribution of CCK-GABA versus PV-GABA neurons as a way to determine specific behavioural functions of CCK-GABA neurons.

Inhibitory basket cells powerfully shape neuronal activity and behavior through perisomatic inhibition. Parvalbumin (PV) and cholecocystokinin (CCK)-expressing GABA neurons are the two major types of basket cells that are thought to be different from one another functionally, due to differences in structural and electrophysiological properties. Distinct features of PV- and CCK-GABA neurons indicate that they likely have specialized functions in controlling network activities and behaviours. However, the functional relevance of CCK-GABA neurons is unclear, and the size and distribution of CCK-GABA neuron networks within the brain is not well understood. Here, we characterized the distribution of CCK-GABA neurons, identified brain regions where CCK-GABA neurons were present in large numbers to form functionally significant networks, and compared CCK-GABA neuron distribution to that of other GABA neurons (e.g. PV-GABA). Using an intersectional genetic approach to restrict reporter protein expression to specific neurons, it was found that CCK-GABA neurons are numerous in the hippocampus, ventromedial frontal cortex, ventral temporal cortex, and secondary areas. Surprisingly, our findings showed that this CCK-GABA proportion was larger than other models have predicted (about 20-30% compared to about 10%). This study is the first comprehensive, systematic assessment of CCK-GABA neuron distribution in the forebrain. Understanding the distribution of CCK-GABA neurons may provide important clues to the functions of these cells, shedding light on the role of these cells in pathologies of the nervous system. Future studies can employ *in vivo* optogenetic neuron manipulation techniques to investigate specific behavioural functions of CCK-GABA neurons in freely behaving mice.

**Shreyas Harita (Supervisor: M. Woodin)**. Evaluating the effect of phosphorylation state and protein interactions on the stability and motility of KCC2 at the cell membrane.

One of the most essential factors underlying synaptic transmission is the molecular composition and organization of synapses. In the central nervous system (CNS), the majority of fast inhibitory neurotransmission is mediated by γ-aminobutyric acid (GABA) and the GABA type A receptor (GABAAR). The potassium-chloride cotransporter 2 (KCC2) is a K+/Cl- co-transporter which plays many roles in regulating the physiology of neurons within the CNS. KCC2 has a significant influence on the polarity and efficacy of the chloride-permeable GABAAR and glycine receptor (GlyR) as it controls the intra-neuronal chloride homeostasis. This study examined two different aspects of KCC2 behaviour at the cell membrane in two separate parts. The first part of the study evaluated the stability of KCC2 at the cell membrane. The density of KCC2 puncta was determined by analyzing images obtained from total internal reflection fluorescent (TIRF) microscopy, using the Volocity™ software. The results of this experiment contradicted the proposed hypothesis as the number of KCC2 puncta which were stable was higher in the phosphate buffered saline (PBS) than the PMA (PKC activator) or Gö6983 (PKC inhibitor) conditions. The second part of the study looked at the motility of KCC2 at the cell membrane and how KCC2-GluK2 interaction affected KCC2 motility. A series of images was obtained using TIRF microscopy. Particle tracking, using the ImageJ software, revealed that GluK2 interacts with KCC2 and restricts its motility when compared to the control (PBS) condition. This study demonstrated that KCC2 stability and motility at the cell membrane is influenced and increased by its phosphorylation state and by the interaction of KCC2 with other proteins such as GluK2.

**Alan Hsieh (Supervisor: D. Lovejoy)**. Teneurin C-terminal associated peptide (TCAP) expression in *Ciona intestinalis*.

Teneurin C-terminal associated peptide (TCAP) belongs to a newly discovered class of four peptides, found in many metazoans. Past research finding have demonstrated that it plays a major role in modulating corticotropin releasing-factor (CRF) to inhibit the stress response and it promotes digestive metabolism. Recently, the structure of TCAP has been determined in the vase tunicate, *Ciona intestinalis*, a close evolutionary ancestor of vertebrates. This project determined the expression of TCAP in *C. intestinalis* tissues using reverse transcription polymerase chain reaction (RT-PCR). In addition, a synthetic form of the endogenous *C. intestinalis* TCAP hormone was utilized to investigate its effect on general behavior and feeding response. This project supports TCAP as an important neuroendocrine hormone in *C. intestinalis.*

**Alex Hsieh (Supervisor: J. Kim)**. Chronic CB1 receptor activation and its effects on CCK+ GABA neurons and working memory

Chronic cannabis use during adolescence has been associated with enduring cognitive differences in adulthood. Delta-9-tetrahydrocannabinol, the primary psychoactive component of cannabis, partly exerts its effects as an agonist on CB1 receptors. CB1 receptors are preferentially expressed on the axon terminals of CCK-positive GABA interneurons. However, the long-lasting behavioural and molecular alterations in this subclass of cells following repeated drug administration are relatively unexplored. In the present experiment, a dual recombinase-responsive mouse line was used to selectively label CCK-positive GABA cells with GFP and CCK-negative GABA cells with mCherry. These mice were repeatedly administered WIN 55,212-2, a potent CB1 receptor agonist, during adolescence and tested during adulthood to measure differences in recognition memory and cell density counts for CCK neurons. This project provides important insight into how molecular changes can alter behavioural mechanisms induced by repeated cannabis use.

**Shen Hsu (Supervisor: D. McMillen)**. Simulation on 8 basic integral controller motifs and their combinations for details in homeostasis under perturbations.

Homeostasis in regulatory networks is crucial for maintaining proper biological functions under unstable environments. To sustain a certain concentration of a particular molecule, this molecular can be involved in a two components negative feedback as a controlled variable. The range of variation in kinetic reactions between these two components, in which range homeostatic feature is possible, is important for understanding how in gene across species can lead to similar response to the environment. With different compositions of improvement or inhibition on formation or removal of each two components, 8 different possible negative feedback motifs can be raised. The simulations of each motif are based on the reaction rates of each component to predict the behaviour of steady status. The combinations of two different motifs are simulated when perturbations are influencing the concentrations. The simulations show a consistent steady status of controlled variable within a wide range of perturbations.

**Syed Saad Husainie (Supervisor: D. Godt)**. A member of the protein kinase C family, PKCδ, regulates collective cell migration in Drosophila oogenesis.

**Sepehr Jamali (Supervisor: M. Ringuette)**. Functional analysis of SPARC in *Drosophila melanogaster*.

*SPARC* is an evolutionary conserved extracellular matrix glycoprotein. It has been shown SPARC presence is necessary for proper basal lamina (BL) formation in Drosophila melanogaster and its deficiency is embryonically lethal. In order to rescue embryonic lethal SPARC deficient flies, five drosophila constructs were made; human SPARC, Drosophila SPARC, Mutated Collagen binding domain, mutated disulfide bridge and deleted signal peptide domain. By crossing those to the SPARC deficient lines the ability of each of those constructs to rescue is revealed and the functional importance of different SPARC domains is analysed.

**Sophia Kim (Supervisor: A. Bruce)**. Investigation of heart defects in *eomesodermin* mutant embryos.

The transcription factor Eomesodermin A (Eomesa) has been implicated in zebrafish epithelial-to-mesenchymal transition, dorsal-ventral patterning, mesoderm migration, endoderm specification, and epiboly. Eomesodermin is highly conserved in vertebrates and has been implicated in heart development in mouse and frog. In the mouse, Eomes has been shown to control expression of Mesp1, the master regulator of cardiovascular cell fate. However, the role of Eomesa in zebrafish heart development has not yet been studied. Zebrafish are an excellent model to study cardiogenesis as embryos are externally fertilized, transparent, and do not require a functional cardiovascular system for survival during embryogenesis. For this study, whole mount in situ hybridization was used to examine heart development in wild type and eomesa mutant embryos of zebrafish. Digoxigenin labelled riboprobes for heart specific genes *cmlc2*, *hand2*, *mef2cb*, *vmhc*, and *nkx2*.5 were synthesized. To study the defects from loss of Eomesa function, maternal-zygotic *eomesa* (MZ*eomesa*) mutants as well as embryos injected with *eomesa*-eng RNA were used. The *eomesa*-eng RNA injected embryos provided insight as they acted as a dominant negative construct to block Eomesa function. MZ*eomesa* mutants often had their heart tube developing on the opposite side of the midline. The *eomesa*-eng RNA injected mutants displayed heart defects that showed incomplete cardiac fission or improper cardiac migration. These results suggest that Eomesa does play a role in zebrafish heart development by affecting organization and movement during development.

**Martin Musiol (Supervisor: S. Plotnikov)**. Regulation of cancer cell migration by the planar cell polarity complex.

From conception until death, cell migration plays a crucial role in embryonic development, immune responses, and wound repair, as well as in several disease states including cancer. The molecular mechanisms of migration are complex and rely upon highly regulated interplay between intracellular signaling networks, cytoskeletal structures, and integrin-based focal adhesions. To date, several signaling pathways including Rho family GTPases have been identified as master regulators of cell migration. Deregulation of these pathways, which act as molecular switches that control the organization and dynamics of the actin cytoskeleton at the edges of motile cells, was shown to result in abnormal migratory patterns commonly observed in cancerous cells. However, much less is known about molecular mechanisms that regulate activity of Rho GTPases across migrating cells. Recently we found that planar cell polarity (PCP) signaling, which is primarily responsible for maintaining polarity in epithelial cells, regulates Rho GTPase activity in cancer cells. This link implicates PCP signalling could play a pivotal role in regulating the molecular mechanisms of migration. In this study, immunostaining techniques, pharmacological perturbation, and quantitative cell imaging were used to determine the molecular mechanisms employed by PCP proteins to regulate cell migration. We found that breast cancer cells with decreased expression of key PCP proteins are more circular relative to untreated controls, indicating that PCP signaling is involved in regulation of cell shape. By quantitative image analysis we demonstrated that reduction of PCP protein expression had a dramatic effect on actin cytoskeleton causing abnormally large lamellipodia, which resulted in a loss of cell polarity and suppression of migratory ability. Additionally, interfering with PCP protein expression caused an increase in focal adhesion stability, which further reduced the migratory ability of cells. Thus, the results suggest that PCP signaling governs cancer cell migration by regulating actin cytoskeleton and focal adhesion dynamics.

**Dang Nguyen (Supervisor: D. Desveaux)**. The effects of temperature and humidity on Effector-Triggered Immunity (ETI) in *Arabidopsis thaliana*

*Pseudomonas syringae* is a biotrophic plant pathogen which carries a diverse repertoire of effector proteins – the majority of which function as virulence factors. These effectors are injected into plant cells via a widely-conserved type III secretion system (T3SS). Type III secreted effectors (T3SEs) are essential for bacterial pathogenicity and survival, as they suppress plant immune responses by disrupting signaling pathways and cellular processes. Recognition of T3SEs by nucleotide-binding rich repeat (NLR) proteins coded by resistance (R) genes in *Arabidopsis thaliana* launch an effector-triggered-immunity (ETI) response. This ETI response manifests as localized programmed cell death called the hypersensitive response (HR) to prevent further invasion and extensive colonization by the pathogen. Environmental effects on R-gene mediated defense responses remain somewhat unclear. In order to evaluate the general effects of both temperature and humidity on ETI, we studied three effectors that are recognized via different R-gene mediated signaling pathways. We investigated the effect of high humidity and high temperature on the ETI response induced by HopZ1a – a T3SE that elicits immunity independent of the salicylic acid (SA) signaling pathway, which was previously thought to be required in all ETI responses. Additionally, we evaluated two effectors that elicit SA-dependent ETI responses – AvrRpt2 and AvrRpm1. We observed a general suppression of the ETI-induced HR by high humidity and high temperature. High humidity (95%) could delay HR not only in HopZ1a-treated plants, but also in plants treated with AvrRpt2 and AvrRpm1. In addition, high temperature can suppress HR. These results suggest both elevated temperature and humidity may interfere with a conserved element of the ETI signaling pathway emphasizing the effect of environment on plant immunity.

**Avery Noonan (Supervisor: P. McCourt)**. Genetic screening in Arabidopsis uncovers Strigolactone insensitive mutants

Strigolactones (SL) are terpenoid-based plant hormones involved in regulating shoot architecture, as well as symbiotic inter-species interactions between plants and soil fungi. They are of particular interest as they relate to the parasitic lifecycle of the weed *Striga hermonthica*, a plant that has a significant impact on agricultural productivity throughout Africa, Asia and Australia. SLs cause germination in *Striga*, however the molecular mechanism of SL perception in *Striga* is not yet well understood. It is believed that HTL (KAI2) is the SL receptor that is responsible for the germination response to SLs in *Striga*. SL mimics GR24 and 5850825 have different chemical structures, but both initiate *Striga* germination in an HTL dependent manner. Biochemical evidence suggests that both SL mimics can bind directly to HTL, but the binding site for each chemical is still unknown. In order to map these binding sites, we used a forward genetic approach. Since *Striga* is not genetically tractable, *Arabidopsis thaliana* was used as a model organism. Approximately 100 000 randomly mutagenized seeds were plated on 5850825 to screen for resistance to the effects of SLs on early seedling development. Selected mutants were grown to a second generation to confirm the heritability of their insensitivity. This process identified 11 mutants that are insensitive to both GR24 and 5850825 that resemble loss of function alleles in *HTL*. Amplification and sequencing of missense mutations in *HTL* may give insight into the binding sites for GR24 and 5850825, increasing our knowledge of the molecular details of SL perception.

**Rachel Youjin Oh (Supervisor: D. Godt)**. Analysis of interactions between Traffic jam and Piwi in gonad morphogenesis.

**Yoon Woo Park (Supervisor: T. Harris)**. Examining how Arf G protein signaling affects epithelia.

The epithelium is a prevailing and important aspect of all multicellular, eukaryotic organisms. Functions of the epithelium include structure, protection, and selective secretion and absorption among other known functions. The epithelium is composed of epithelial cells, each with its own individual plasma membrane. Each cell may undergo endocytosis which is a process by which cells internalize and regulate molecules from the plasma membrane. Here, we investigate the importance of endocytosis in epithelial regulation and its phenotypic consequence on the cuticles of drosophila larvae through the use of the ARF family G proteins which regulate membrane trafficking, and the ARF-GAP, ASAP, which catalyzes GTP exchange and hydrolysis of the ARF-GTP complex. We first crossed transgenic lines carrying UAS-ARF1/ARF6/ARL4 wild-type, GTP-locked, GDP-locked, and/or fast-cycling constructs with the daughterless-GAL4 driver strain to overexpress the ARF and ARL constructs and determine its impact on embryogenesis and cuticles of drosophila larvae. Screening for embryonic lethal mutations indicate that the overexpression of ARF1 GTP-locked and GDP-locked constructs causes significant lethality, and that the rate of embryonic lethality in the overexpression of ARF1 GTP-locked construct is greater than the overexpression of ARF1 GDP-locked construct. Next, we compared and quantified the embryonic phenotypes of each lethal ARF1 constructs. In these cuticle analysis studies, both ARF1 GTP-locked and GDP-locked constructs had near-homogenous phenotypes of total cuticle disruption and normal cuticle formation, respectively; the GDP-locked construct showed a minor phenotype characterized by disruption in the organs of the posterior end in addition to the disruption of the medial and lateral embryo. To develop a precise ASAP deletion line, BAC recombineering was used following the p-element excision of ASAP to rescue the local deletion of the flanking genes.

**Sara Pintwala (Supervisor: J. Peever)**. Characterization of the hypothalamic A11 cell group: relevant genetic expression and functional connectivity in the narcolepsy-cataplexy paradigm.

The neurotransmitter dopamine (DA) is associated with arousal in many areas of the brain. Innervation of the spinal cord by the neurotransmitter DA is believed to arise from a small group of cells in the diencephalon called the A11 region. The A11 region is of particular interest in the clinical application of narcolepsy. This chronic neurological disorder affects systems involving sleep and wake regulation, resulting in excessive daytime sleepiness. Specifically, narcolepsy involves the loss of orexin/hypocretin (HCRT) producing neurons within the lateral hypothalamus (LH) or mutations of the 1 or 2 HCRT receptor. Cataplexy is a major symptom of narcolepsy and includes the loss of muscle tone, muscle atonia, in any or all-skeletal muscles except the diaphragm. The A11 region is of significance in the narcolepsy-cataplexy paradigm, as inhibition of DAergic cells in this region results in increased intensity of cataplexy episodes. The Allen Brain Atlas was first used to survey the A11 region to confirm the presence of the necessary enzymatic machinery to produce DA. It was then our objective to establish a functional circuit between HCRT –producing cells of the LH and A11 using the retrograde tracer cholera toxin subunit B. Overall, the data reveals that the cells of the A11 are capable of producing DA in mice and that there exists a direct connection between this region and the LH. These findings are of paramount importance in our understanding of the molecular, functional mechanisms of narcolepsy and for clinical application.

**Farrah Rajab (Supervisor: A. Bruce)**. Characterizing the role of Eomesodermin in cell adhesion

During early zebrafish development, embryonic cells at the animal pole undergo multiple cell divisions to form the blastoderm, which sits on top of the vegetally located yolk cell. Upon completion of the blastula stage, zebrafish embryos begin gastrulation which results in the formation of the embryo’s three germ layers: ectoderm, mesoderm, and endoderm. Epiboly, characterized by the thinning and spreading of the multilayer of blastoderm cells to engulf the yolk, is the first step in zebrafish gastrulation. *eomesodermin a* (*eomesa*) is a maternally and zygotically expressed T-box gene in zebrafish, which plays a role in early embryonic development. Maternal-zygotic *eomesa* mutant embryos (MZ*eomesa*), which fail to initiate epiboly normally, are often used to study the regulation of epiboly. To test the effect of Eomesa on the adhesive properties of embryonic cells during epiboly, blastoderm dissociation was performed on MZ*eomesa* mutant embryos and on wild-type embryos injected with an *eomesa*-engrailed repressor construct that acts a dominant-negative to block Eomesa function. Blastoderm cells from the *eomesa*-eng injected embryos dissociated faster and to a greater extent than cells from the uninjected, and GFP injected control embryos, suggesting that the functioning of T-box genes is necessary for embryonic cells to maintain normal adhesive properties. Blastoderm cells of MZ*eomesa* mutant embryos also dissociated faster and to a greater extent than cells from the control embryos, but dissociated more slowly than cells of the *eomesa*-eng injected embryos. These findings suggest that the epiboly delay observed in MZ*eomesa* mutant embryos may be due to reduced expression of cell adhesion genes at the onset of gastrulation leading to aberrant cell-cell adhesion and the inability of cells to undergo the necessary rearrangements for epiboly initiation.

**Austyn Roseborough (Supervisor: A. Bruce)**. Generation of actin labelling tools for the study of zebrafish epiboly.

Zebrafish development involves many coordinated cell movements and rearrangements. One such movement, epiboly, involves the spreading and thinning of enveloping layer cells over the yolk just prior to the onset of gastrulation. The mechanics and molecules behind this large cellular movement have yet to be fully elucidated and require further study. As an important determinant of cell mobility, actin makes for a useful molecule when studying epiboly. Labeling and studying actin rearrangements may aid in understanding the cellular movements underlying epiboly. While various actin labelling tools exist, their reliabiliy and accuracy in zebrafish requires further testing. Therefore, this project involved generating two actin labelling tools, Utrophin and F-tractin, in order to optimize their usage in studying zebrafish epiboly.

**Hannah Samuels (Supervisor: J. Mitchell)** GATA-1 binding site alteration influences reporter gene expression in murine erythroleukemia (MEL) cells.

The *Pim1* proto-oncogene plays an important role in erythropoiesis, but the mechanisms underlying its transcriptional regulation have not yet been delineated. Two regulatory regions in proximity to *Pim1* were previously identified using luciferase assays conducted on MEL cells. The region upstream of *Pim1*, Reg3, has been shown to decrease luciferase reporter gene expression, while the downstream region, Enh5, enhances its expression. Both regions are bound by GATA-1, a transcription factor that drives erythropoiesis. The goal of this study was to determine whether known GATA-1 bound sites drive the regulatory activity observed in the previous luciferase assays. This was achieved by mutating select GATA-1 binding sites within these regions using site-directed mutagenesis (SDM) on pJET1.2 plasmids already containing either the Reg3 or Enh5 region.

The Reg3 region contains one GATA-1 binding site, and the Enh5 region contains three GATA-1 binding sites. Using SDM to mutate each binding site individually, four new mutant constructs were created. The mutated regulatory regions were cloned into pGL4.23 vectors containing the *Pim1* minimal promoter and the luciferase reporter gene in either the forward or reverse orientation.

MEL cells were then transfected with the mutated constructs, with the original non-mutated constructs and with the pGL4.23 vectors lacking either regulatory region acting as controls. Dual luciferase assays were performed, and relative luciferase activity was observed and compared between constructs. The results obtained indicate that mutating the GATA-1 binding site within Reg3 enhances luciferase activity. In addition, while mutating the 2nd binding site within Enh5 seems to have no effect on enhancer activity, mutations in the 1st and 3rd GATA-1 binding sites in Enh5 seem to decrease enhancer activity. Thus, it is likely that these genomic sequences are important for conferring the regulatory activity to putative *Pim1* enhancers.

**Darren Tan (Supervisor: U. Tepass)**. Identification and characterization of epithelial cell polarity proteins in *Drosophila Melanogaster*.

The asymmetrical architecture of epithelial cells that outline the cavities and surfaces of the body is critical for animal development. The polarized organization of the epithelial cell plasma membrane into apical and basolateral domains is necessary for their specialized. Disruption of apical-basal cell polarity can lead to a large number of pathologies such as the formation of cystic kidneys, retinal degeneration and tumorigenesis. Therefore, it is crucial to identify the key players and mechanisms involved in establishing and maintaining this apical-basal polarity. Extensive research in this field has identified a number of key players involved in cell polarity. However, there still remains a great deal to be learned about the molecules and mechanisms underlying apical-basal polarity in epithelial cells. The precise mechanisms by which the known players interact remains largely unknown. Moreover, it is likely that not all proteins that contribute to polarity regulation have yet been identified. A recent study has alluded to the existence of another unknown basolateral polarity complex that acts to maintain polarity at late stages of *Drosophila* embryogenesis. This is supported by the finding that polarity is recovered at later stages of embryogenesis in both Yrt/Cora double mutants and Scrib mutants (Laprise et al., 2009).

The goal of my research is to identify novel genes/proteins that play key roles in apical- basal polarity.I used an interdisciplinary approach combining genetics, confocal microscopy and molecular biology to identify novel regulators of epithelial polarity. I have conducted a deficiency screen for defects in epithelial polarity. Defects in polarity were scored using antibody staining with Crb and Dlg as markers of membrane polarity. I have completed the screen of the second chromosome and have identified several candidate genes. I have chosen to further investigate 2 candidate lines, Df(2R)BSC346 and Df(2R)ED1791 to narrow down the genes of interest through complementation crosses.

**Kiat Yi Tan (Supervisor: D. Guttman)**. The role of HopZ1a autoacetylation in plant immunity.

The type III secretion system plays an important role in bacterial pathogenesis in plants. *Pseudomonas* *syringae* is a plant pathogen that uses this system to inject virulence proteins termed type III effectors into plant hosts to cause disease. HopZ is a well-studied family of effectors. Two alleles of this family, HopZ1a and HopZ1b, elicit an immune response in the model plant host *Arabidopsis thaliana*. Despite sharing a 72% amino acid similarity, HopZ1b induces a much weaker response compared to HopZ1a. HopZ1a has been shown to be an acetyltransferase where autoacetylation of a conserved lysine residue is necessary for eliciting an immune response in *A. thaliana*. Recently, additional serine autoacetylation sites have been found for HopZ1a but their significance is unknown. HopZ1b, despite high sequence similarity to HopZ1a, lack the same residues. To investigate whether these autoacetylated serines are necessary for induction of the plant immune response, we mutated the HopZ1a serine residues to the corresponding residues on HopZ1b and *vice versa*. The mutants were assayed *in planta* for the ability to induce an immune response. Surprisingly, these results suggest the autoacetylation sites do not play a role in subverting plant immune response as initially thought.

**Matthew Volpini (Supervisor: V. Tropepe)**. Characterization of retinal regeneration in zebrafish (*Danio rerio*) in response to a focal UV lesion protocol.

Zebrafish exhibit the ability to regenerate retinal tissue in response to acute injury by specifically replacing damaged retinal cell sub-types. The regenerative response is largely mediated by the activation of Muller glia cells, which undergo a specific series of steps in order to replace the lost cell types, namely: dedifferentiation, proliferation, migration, and re-differentiation. Ultimately, the daughter cells that are derived from the activated Muller glia migrate to the correct retinal position and differentiate into the lost retinal cell types, restoring vision to the animal. In my project, a UV lesion protocol was developed to specifically ablate photoreceptors and induce the regenerative response. The proliferative component of the regenerative response was visualized by exposing the zebrafish to an EdU pulse at appropriate time-points post-lesion, followed by tissue immunohistochemistry (IHC) analysis. IHC imaging analysis revealed that the UV retinal lesion resulted in EdU+ cells localized to both the inner nuclear layer (INL) and outer nuclear layer (ONL) of the retina. Interestingly, the intensity of the lesion protocol seemed to be correlated with the kind of proliferative response observed. Sufficiently high-intensity lesions resulted in EdU+ cells in both the INL and ONL, and included cells that exhibited cell morphologies consistent with both Muller glia, as well as rod precursor cells (RPCs; another progenitor cell type). Conversely, low-intensity lesions resulted in EdU+ cells that were localized exclusively to the ONL, and exhibited a morphology consistent only with RPCs.

**Nina Wang (Supervisor: N. Provart)**. Cis-elements regulating subcategories of plant immune response: analyses and visualization tools.

The plant immune system can be divided into two classes, PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI). The first class is a basal immune response activated by pattern recognition receptors in response to microbial- or pathogen-associated molecular patterns. The second class is an amplified immune response, often associated with a type of programmed cell death, termed hypersensitive response, at the site of infection. The Provart and Desveaux labs have identified subsets of genes specific for early and late phases of PTI and ETI; however, the regulatory networks of these genes are not yet fully understood. Here we extend a Provart lab data visualization tool, GeneSlider, to permit the exploration of cis-regulatory elements in the promoter subsets and conserved regions of Brassicaceae. Conserved non-coding regions identified by Haurdy *et al*. will be displayed along with transcription factor binding sites determined by Weirauch *et al*. and filtered with a functional depth cut-off to reduce false positive identification as determined in this work. Functional depth is the value necessary for functional binding to occur between a transcription factor and its binding site. Depending on the genome-wide distribution of functional depth values for each motif, a functional depth cut-off of 0.5 or mean plus 2 or 3 standard deviations (SD) is assigned. Significant matches will be converted to a GFF file and loaded into the GeneSlider database. The extension of GeneSlider to include identification of cis-regulatory elements offers a new tool for generating hypotheses to explore the regulatory networks governing plant-pathogen interactions.

**Yuanchao Xue (Supervisor: K. Yoshioka)**. Is the suppressor of *dnd1*, *rdd1*, at a crossroads of immunity and development?

The *Arabidopsis* mutant *rdd1* (*repressor of dnd1*) was identified as a novel suppressor of *defense no death 1* (*dnd1*) mutant. *dnd1* is an autoimmune mutant caused by a loss of function of *AtCNGC2*, a member of the cyclic nucleotide-gated ion channel (CNGC) family. It has been suggested that CNGCs are involved in various physiological processes such as defence and development. A combination of map-based cloning and whole genome sequencing revealed that *RDD1* is likely *YUCCA6*, an auxin biosynthesis gene. Furthermore, the results of double mutant analysis indicated that *rdd1* is likely a knockout allele of *YUCCA6*. Auxin is an important plant hormone that can affect cellular division, expansion, and organization. In this study, the physiological responses of a *YUCCA6* knockout mutant and *rdd1* were compared to confirm the aforementioned result. Response to exogenous treatment of auxin was analysed and compared to determine the sensitivity of auxin in both mutants. Based on the assumption that *rdd1* is a knockout allele of *YUCCA6* – an auxin biosynthesis gene, a reduced auxin levels in both *rdd1* and *YUCCA6* knockout mutant should correspondingly alter their sensitivities to exogenous treatment of auxin in a similar manner. As expected, the result did show a similar auxin sensitivities in both mutants. Furthermore, the aforementioned finding proposes that in addition to auxin biosynthesis, *YUCCA6*/*RDD1* may have a role in pathogen defence.

**Mitsue Zaman (Supervisor: T. Harris)**. The role of Arf small G proteins and candidate interacting proteins in the maintenance of epithelial structures.