**Katherine Burton (Supervisor: V. Tropepe).** Subpallial cell-cycling effects probed as social stimulatory-induced force underlying embryonic neuronal population dynamics

Socially-mediated changes in embryonic neural progenitor cell behaviour were characterized in 9-day post-fertilization *Danio rerio* larvae. Experiments sought to elucidate involvement of neurogenesis during establishment of neural plasticity. Olfactory bulb, optic tectum, pallial, and subpallial neurogenic niche populations underwent extensive quantification via use of endogenous Proliferating Cell Nuclear Antigen (PCNA) and exogenous 5-ethynyl-2´-deoxyuridine (EdU) marker characterization. Crucially, non-significant PCNA p-values highlighted need for subsequent testing via EdU-pulse trace methodology. EdU testing successfully yielded statistically significant results (p<0.01) between subpallial regions of control versus stimuli-deprived groups. Absence of a proliferative effect, in concomitant contrast with positive changes in EdU marker expression patterns, strongly implicated modified cell-cycling behaviour as the causative force driving observed cellular, and morphological differences.

**Yan Ling Iris Chiu (Supervisor: B. Chang).**  Phylogenetic reconstruction of teleost RH2 sequences reveals repeated amino acid substitutions at spectral tuning sites following gene duplications.

Cone opsins are light-sensitive proteins that mediate colour vision and are involved in the first step of the visual phototransduction process. Rhodopsin-like opsin (RH2), sensitive to green light, is found in many groups of vertebrates including teleost fish. The number of RH2 gene duplicates varies greatly among teleost fish, despite being a single-copy gene in many terrestrial vertebrate lineages. Gene duplication events increase the likelihood of acquiring functional mutations which may result in shifts in absorbance and kinetic properties. The absorbance shifts provide a wider range of spectral sensitivity and may have adaptive significance as aquatic environments tend to have more diverse spectral properties. A database of 356 RH2 gene sequences from teleost fish was created by manually collecting RH2 sequences from fish genomes that were not publicly accessible on Genbank and datamining existing sequence databases. These sequences were aligned using multiple different approaches. From these alignments, maximum likelihood and Bayesian phylogenetic gene trees were produced to explore the evolutionary history of this gene family in teleost fish. Our results show a pattern between RH2 duplication events and the amino acid residue at site 122, alternating between Glutamic acid and Glutamine, which is known to cause a blue-shift in peak absorbance in RH2. From the database created, the copy number of RH2 appears to be the greatest in shallow water inhabitants ranging from 2 to 4 copies, suggesting that variation in RH2 sequence and copy number is evidence of adaptation to environmental light conditions.

**Dorsa Derakhshan (Supervisor: J. Peever).** Identifying the cellular population in the Subcoeruleus region

Cataplexy, a pathognomonic sign of sleep disorder narcolepsy, is known as the sudden and inappropriate intrusion of REM sleep muscle paralysis during wakefulness. The neuronal circuitry underlying the regulation of REM sleep muscle paralysis remain to be fully characterized. Glutamatergic cells of subcoeruleus region (SubC) have been hypothesized to regulate REM sleep muscle paralysis. In this study, transgenic mouse model VGLUT2-Cre (SLC16A, Jackson Laboratory) was used to selectively express cre recombinase in glutamatergic cells to allow for targeted insertion of chemogenetic excitatory hM3Dq DREADD receptor into VGLUT2-SubC cells (n=2). The brains were frozen, sliced at 16 µm-thickness sections with an aseptic cryostat, and mounted onto RNAse-free slides. Fluorescence in-situ hybridization (FISH) was implemented to characterize SubC glutamatergic neurons through specific binding of vGLUT2 riboprobe to vGLUT2 mRNA. In combination with FISH, immunohistochemistry (IHC) was used to stain for chemogenetic receptors with primary antibody mCherry and secondary antibody Cy3. DAPI stain was also applied in order to characterize neuronal cell bodies. The VGLUT2-Cre brains were successfully stained for mCherry and vGLUT2 mRNA, and the co-expression of vGLUT2 mRNA and hM3DGq-mCherry was observed and quantified within the virally expressed hM3DGq SubC glutamatergic neurons by fluorescence microscopy. The efferent neuronal pathways from the SubC glutamatergic cells were also examined and strong projections in the ventral medulla and supramammillary gland was observed. This project aimed to validate the accuracy of viral DREADD delivery methodology for focally targeting SubC glutamatergic neurons, investigating efferent projections from SubC, and sets the stage for future investigations on the role of glutamatergic SubC cells in cataplexy and REM sleep.

**Madeleine Di Gregorio (Supervisor: S. Plotnikov).** Collective cell migration is regulated by microtubule dynamics in a PRICKLE1-dependent manner.

Directional cell migration is fundamental during physiological processes such as tissue development, wound healing and immune response as well as pathological processes such as cancer metastasis. To migrate directionally cells must exhibit polarity with regards to the distribution of signalling molecules as well as organization of the cytoskeleton including actin microfilaments and microtubules. Over the last two decades, immense progress has been made in understanding how organization of actin cytoskeleton is regulated in migrating cells. However, much less is known about molecular mechanism that control polarity and dynamics of the microtubules. Recently, PRICKLE1, a member of the planar cell polarity complex, has been shown to regulate polarity in non-epithelial cells. Here I hypothesized a role for PRICKLE1 in the regulation of cancer cell migration. By using human breast adenocarcinoma cells (MDA-MB-231) in combination with siRNA–mediated gene silencing and live-cell imaging I demonstrated that PRICKLE1 is essential to target microtubules to the focal adhesion. I showed that knock down of PRICKLE1 in MDA-MB-231 cells did not affect cellular response to extracellular matrix molecules. Microtubule plus-tip tracking revealed PRICKLE1 knock down cells displayed significantly faster microtubule filament growth than wild type cells. Microtubule plus end filament growth was also observed to be faster in the absence of focal adhesions. Since focal adhesions stabilize microtubules via the talin-KANK1 interaction, PRICKLE1 expression was subsequently depleted in talin homozygous knock out murine embryonic fibroblasts (MEFs) transfected with either wild type talin or talin G1404L, a single point mutation which disrupts KANK1 binding. While talin expression completely rescued the fibroblast phenotype lost in homozygous knock out MEFs, microtubule dynamics remain dependent on PRICKLE-1 stabilizing function. Collectively, these results show that PRICKLE-1 stabilizes microtubules at focal adhesion complexes and consequently regulates cell migration.

**Eduardo Garcia (Supervisor: M. Ringuette).** Investigating the role of SPARC in the retina repair mechanism of *Drosophila Melanogaster,* and the efficiency of GFP tagged SPARC.

PDF-1, a homolog for vascular endothelial growth factor (VGEF) and platelet derived growth factor (PDGF), has been found to play a pivotal role in retina repair in the fruit fly. It is understood that it’s secreted as a response to damage, and it activates downstream effectors to induce tissue repair. However, the mechanism by which PDF-1 acts on these downstream players is still unknown. In mammalian models, SPARC is known to bind VGEF and PDGF respectively, thereby making it a suitable candidate in the PDF-1 induced pathway. We used the UAS-GAL4 system with an eye specific promoter (GMR), to drive the knock down of SPARC in the eye of the fly. We also utilized confocal microscopy to evaluate the efficiency of the GPF tag in localizing SPARC in both the fat body, and embryo stage of *Drosophila M*. Future directions include administrating UV damage at the late third instar larval stage, to observe if retinal repair is at all hindered by the absence of SPARC.

**Eleni Giannopoulos (Supervisor: D. Desveaux).** Proteomic Characterization of an *Arabidopsis* Immune Complex Using Proximity-Based BioID

Plants are able to suppress infection by pathogenic bacteria via a two-layer immune system. In the first layer of immunity, known as PAMP-triggered immunity (PTI), pathogen-associated molecular patterns (PAMPs) activate the extracellular pattern recognition receptor (PRR). In the second layer of immunity, known as effector-triggered immunity (ETI), type III secretion effectors are recognized by the intracellular resistance (R) protein. This detection can either occur directly, or indirectly. During indirect recognition by the R protein, these type III effectors modify a “sensor” protein. Although it has been shown that these recognition complexes are involved in immunity, very little is known about the involvement of other proteins in this system. Proximity-dependent biotin identification (BioID) is a method that has been used to label proximate proteins. We identified stable, homozygous transgenic *Arabidopsis thaliana* lines expressing the ZAR1 R protein, the ZED1 pseudokinase sensor protein and HopZ1a effector all fused to promiscuous biotinylating protein (BirA\*) under a DEX-inducible promoter. Once we identified these homozygous lines that had strong expression, BioID was used in order to isolate the proximate biotinylated protein targets via streptavidin-based affinity purification. Mass spectrometry was performed to identify these candidate proteins, specific to our baits. In order to test their involvement in immunity, we ordered knockout Salk lines of these proteins and performed DNA extraction in order to identify homozygous lines containing the T-DNA insert. We hypothesize that these proteins have a critical role in plant immunity.

**Mohamad Hamieh (Supervisor: J. Peever).** REM sleep circuit: functional connection of the SubC glutamatergic neurons.

Glutamatergic neurons of the Sub-Coeruleus region (SubC) have been hypothesized to regulate REM sleep and its features such as muscle paralysis. Recently, we have demonstrated that optogenetic stimulation of the region increases the duration of REM sleep. This study aims to quantify the number of active neurons during optogenetic activation. This was determined by immunohistochemistry labelling of c-fos, a marker of neural activity, in conjunction with the labelling of a fluorescent tag, enhanced yellow fluorescent proteins (eYFP), which indicates the expression of opsins. In addition, we confirm that these SubC activated neurons are glutamatergic by either using a reporter mouse line which expresses a red fluorescent protein (i.e., tdTomato) in glutamatergic neurons (i.e., neurons expressing vesicular glutamate transporter (VGLUT2)); or by staining for the mRNA of VGLUT2 using fluorescent in situ hybridization (FISH). We found that 93.75% of neurons expressing eYFP were VGLUT2+, and a majority of these neurons expressed c-fos when activated by light (10 Hz, 10-s ON/10-s OFF) compared to control (i.e., no light). We also observed the activation of neurons in the ventral medulla (vM), a downstream target of SubC neurons, indicating a functional connection between these two neuronal structures. These results support the hypothesis that neurons in the SubC-vM circuit control REM sleep muscle paralysis.

**Natalie Hoffmann (Supervisor: D. Desveaux).** Natural variation of disease resistance to the *Pseudomonas syringae* effector HopX1 in *Arabidopsis thaliana.*

*Pseudomonas syringae* is a Gram-negative bacterial pathogen that causes disease in many plant species, including the model plant *Arabidopsis thaliana*. *P. syringae* is able to directly inject virulence proteins called effectors into plant cells through a type III secretion system to suppress plant immunity and cause disease. Plant species have evolved to recognize effectors using a diverse array of *Resistance* (*R-*) genes, which activate a robust immune response. HopX1, a member of the HopX family of effectors, is a cysteine protease that targets the jasmonic acid plant hormone pathway and suppresses immunity in susceptible plants. However, mechanisms of HopX1 detection in resistant plantsremain largely uncharacterized. In order to better define the genetic and molecular basis of HopX1 recognition, we cataloged the natural variation of HopX1 resistance in an infection assayofglobal *A. thaliana* accessions collected by the 1001 Genomes Project. We hypothesized *R*-gene diversity between accessions would lead to altered disease susceptibility to infection with *P. syringae* pv*. tomato* DC3000 expressing HopX1 (*Pto*DC3000HopX1). In an infection assay of 86 accessions, we identified 17 accessions (20%) with enhanced resistance to *Pto*DC3000, and two accessions, Stepn-2 and Vie-0, with increased specific resistance to *Pto*DC3000HopX1. Resistant accessions showed significantly lower leaf chlorosis following infection compared to the Columbia-0 control. Future studies with Stepn-2 and Vie-0 could identify genetic polymorphisms underlying HopX1 recognition and give insight into disease resistance in *A. thaliana.*

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

During zebrafish embryonic development, epiboly, an essential morphogenetic event, occurs. Zebrafish epiboly is characterized as the spreading and thinning of a multilayered cell mass to engulf the large yolk cell during gastrulation. 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* and *rab25a* are similar in terms of spatial and temporal expression patterns in the embryo. Additionally, to further understand the exact function of Rab25b, loss of function experiments utilizing morpholinos and CRISPR-Cas9, were performed. The results of the morpholino knockdown study showed that *rab25b* did not cause an epiboly delay and the delay was evident only when a combination of *rab11a*, *rab25a*, and *rab25b* morpholinos was used. Further research must be done with the CRISPR-Cas9 generated rab25b mutants, to elucidate the exact function of Rab25b.

**Abiramy Jeyagaran (Supervisor: S. Plotnikov).** Characterization of talin E1770A

mutation in mouse embryonic fibroblasts.

Cell migration is a crucial part of many developmental processes, and as such, needs to be tightly regulated. Protrusions at the leading edge of the cell, formation of nascent focal adhesions (FAs), and release of rear adhesions allow cells to sense and move around its’ surroundings. In migrating cells, many FA molecules such as integrins and talins exist in either a closed (inactive), or open (ECM-binding competent) conformation. The transition of integrins to the open state is induced when its cytoplasmic tail interacts with talin. The binding of talins to the intracellular domain of integrins needs to be highly regulated to ensure effective cell migration. Mouse embryonic fibroblasts (MEFs) heterozygous and homozygous for talin mutation E1770A that results in a constitutively active talin were studied for its effects on FA turnover and cell migration. The talin mutants had larger, more stable FAs; however, analysis of traction force microscopy (TFM) data showed that despite the larger FA size, the mutants exerted less force on the substrate. Finally, I assessed the physiological consequence of talin E1770A mutation. I found that the cells heterozygous and homozygous for the talin E1770A mutation migrate more directionally with greater speed compared to the controls. Together, these data demonstrate that tight regulation of talin activation is dispensable for efficient cell migration, but is critical for force transmission by the FAs.

**Yong Won Jin (Supervisor: U. Tepass).** Role of apical endocytosis and membrane trafficking during neuroblast ingression in *Drosophila melanogaster*.

Epithelial‑to‑mesenchymal transitions (EMT) entail the loss of apical‑basal polarity and intercellular adhesion by epithelial cells. EMT are prevalent during carcinogenesis, yet, knowledge about the cellular mechanisms driving these processes is limited. To gain insight into how the apical cell domain and cellular junctions are lost, we employed an imaging friendly model system ‑ the ingressing neuroblasts (NB) of the Drosophila embryo. Photoconversion of a myristoilated membrane marker, GAP43, fused to mEOSFP revealed differential membrane dynamics between ingressing NB and non‑ingressing cells. FRAP experiments using endogenous E‑Cadherin‑GFP, a core component of intercellular junctions, allowed comparisons of E‑cadherin turnover between ingressing and non‑ingressing cells. Blocking apical endocytosis via inhibition of Clathrin and Dynamin substantially reduced E‑cadherin turnover and stalled ingression. Similarly, inhibition of myosin II significantly reduced E‑cadherin dynamics, suggesting that actomyosin promotes E‑cadherin internalization during cell ingression. In agreement, pulses of E‑cadherin endocytosis were detected that correlated with ratcheted, myosin II driven contractions of the apical cortex driving NB ingression. Results collectively point to significant differences in membrane dynamics and E‑Cadherin turnover between ingressing and non-ingressing cells.

**Priya Kaur (Supervisor: M. Ringuette).** The effect of VEGF and Domain III SPARC peptide interaction on angiogenesis.

The process of angiogenesis is tightly regulated by cooperative interactions among endothelial cells and components of the extracellular matrix (ECM). Vascular Endothelial Growth Factor (VEGF) is a potent angiogenic factor that induces vascularization and is known to be sequestered by the ECM component SPARC (Secreted Protein, Acidic, Rich in Cysteine). This sequestering confers anti-angiogenic effects due to SPARC binding VEGF. Highly vascularized *ex ovo* chicken chorioallantoic membrane (CAM) assays were used to study the interactions of a mimetic domain III peptide of the SPARC protein (SF3) with VEGF to elucidate the specific domain interactions affecting anti-angiogenesis. Matrigel plugs with VEGF, SF3, and Heparin were incubated at vascular branch points and the change in length, branching and number of vascular tubules was visualised using light microscopy and quantified. My data suggests a decrease in angiogenesis and branching of vascular tubules in the presence of SF3, confirming an anti-angiogenic effect due to SF3-VEGF interaction.

**Kaiyan Kou (Supervisor: E. Nambara).** Functional analysis of cis-elements in the promoters of the osmotic stress-responsive genes in Arabidopsis

The regulation of transcription plays an important role in plant growth and physiology. The interaction between the transcription factors (TFs) and corresponding cis elements (cis regulatory elements/motifs) in the promoter is the critical process for the transcription to be regulated. ABA Responsive Element (ABRE) is a cis-element required for ABA-induced transcription and is enriched in the promoter of osmotic stress-responsive genes. It was previously shown that three copies of ABREs in the promoter (3ABREpro) gave the weak induction of osmotic stress-responsive gene expression. In this project, I made the synthetic promoters composed of 3ABREpro with various fourth cis-element (DRE, MYC, MYB, CE3, ABRE), known to be involved in osmotic stress-responsive gene expression. The synthetic promoters were fused to GUS reporter gene and transformed into *Arabidopsis thaliana*. I currently obtained transgenic plants containing the reporter gene driven by a synthetic promoter with 4 copies of ABREs (4ABREpro). I will present the reporter expression patterns of the 4ABREpro lines in the poster day.

**Lewis Kurschner (Supervisor: D. Guttman).** Host immunity elevates the mutation rate of infecting *Pseudomonas syringae*

The bacterium Pseudomonas syringae is able to cause disease on a wide variety of agriculturally important crop plants. When P. syringae causes disease, it first colonizes the intercellular leaf space known as the apoplast. It then uses a modified pilus to inject a multitude of virulent factors known as effectors directly into the host cell, with the collective function of successfully colonizing the host environment. However, plants have evolved intracellular receptors which detect these effectors and trigger an immune response termed Effector Triggered Immunity (ETI). When ETI is triggered it generates a hostile environment characterized by localized cell death that reduces the spread of the pathogen into surrounding tissue. Currently, it is unknown what effect this hostile environment has on the mutation rate of infecting P. syringae. When the bean pathogen, P. syringae pv. phaseolicola (Pph) 1302A, infects the bean cultivar Tendergreen, ETI is triggered by recognition of a Pph 1302A effector. In our experiment, we developed a method of extracting large quantities of ETI-induced and untreated apoplast from Tendergreen beans. We then used this apoplastic fluid as a media to perform an in vitro mutation rate quantifying assay known as a fluctuation assay. We found that exposure to the ETI-induced apoplast results in a significantly higher mutation rate in Pph 1302A compared to growth in the untreated apoplast. This could be caused by the induction of the stress-induced mutagenesis response which has been shown to elevate the mutation rates of bacteria in stressful environments.

**Pik Kwan Lau (Supervisor: K. Yoshioka).** Measurement of ROS and Hypersensitive Response in *Arabidopsis thaliana*Cyclic Nucleotide Gated Ion Channel Knock-out Mutants.

Cyclic nucleotide gated ion channels (CNGCs) have been identified in both animals and plants; however, the role of CNGCs in plants is less extensively studied in comparison to its role animals. Some of the CNGC members have been found to mediate important physiological processes such as development, light signalling, salt stress and plant-pathogen interaction. Twenty members of CNGCs have been identified in *Arabidopsis thaliana*. Homozygous knockouts of various members of CNGCs in *A. thaliana* were determined. Out of these, homozygous knockouts of CNGC1, 3, 5, 16 were utilized in the examination of their response to pathogen-associated molecular pattern (PAMP) by using flg22, a fragment of the bacteria flagellin consisting of 22 amino acids. Through its binding to the FLS2 receptor in the plants, pathogen-induced immunity is triggered and result in the induction of oxidative burst. Hypersensitive response or programmed cell death can also be triggered by pathogen infiltration to isolate the infected area in an attempt to prevent the spread of the infection. The ability of the CNGC knockouts in *A. thaliana* to induce hypersensitive response and oxidative burst was examined to investigate the role of these members of the CNGC family in *A. thaliana* in mediating pathways leading to pathogen resistance.

**Helen Liu (Supervisor: D. Christendat).** Identification of DNA sequence of QuiR1 protein and Qui1 operon promoter Binding sequence and affinity for transcriptional activation of Quinate and Shikimate Metabolism genes by QuiR1, a LysR Type Transcriptional regulator in Listeria Monocytogenes.

This project centres around the molecular mechanism of transcriptional regulation of genes involved utilization of plant derived compound, quinate, in food borne pathogen *Listeria monocytogenes*. Previous researches have identified two operons, operon qui1 (lmo0489-0491) and qui2 (lmo2234-2237), encoding for the quinate utilization enzymes including quinate/shikimate dehydrogenase (YdiB), shikimate dehydrogenase (Ndsdh), dehydroquinate dehydratase (DHQD), and dehydroshikimate dehydratase (DSD). Upstream to the qui1 operon is a LysR-Type Transcriptional Regulator (LTTR), QuiR1, which has demonstrated different mechanism of regulation. In attempt to understand the transcriptional regulatory model, the sequences and position of promoter binding sites need to be identified. This project aims to identify the regulatory binding sites and promoter recognition sequence and also establish promoter affinity through DNase foot-printing assay with the QuiR1 protein. From which determined the binding site to be within the intergenic regions and established a consensus motif for LTTR regulation.

**Sehrish Mahmood (Supervisor: T. Harris).** Investigating the role of Hippo pathway in cytoskeleton regulation in early *Drosophila* embryo.

Hippo pathway is involved in *Drosophila* organ growth. However, it is unclear whether Hippo pathway affects organ growth through cell structural components, such as cytoskeleton. To dissect the role of Hippo pathway in structural development, the effect of perturbation of individual pathway components on actin regulation was investigated at early embryo stage. It was hypothesized that if Hippo pathway plays a role in regulating cytoskeleton during early embryogenesis, then defects in morphology of early embryos will be seen. In this study, specific Hippo pathway genes, namely, Hippo, Warts, Merlin, Salvador, Yorkie and Ajuba were perturbed in *Drosophila* embryos using RNAi approach. Each gene was perturbed by maternally expressing at least 2 shRNA constructs targeting different nucleotide sequences in the gene. The embryos were screened in 2 steps. First, embryos were screened for disruption of cleavage furrow or abnormality of cell arrangement at cellularization stage to identify the specific Hippo pathway genes that affect the embryo development. In step 2, the specific constructs showing defects on initial screen were stained for specific staging markers such as phosphohistone (PH3), in order to further investigate the role of the genes at earlier stage. On initial screen, knocking down Hippo, Merlin, Salvador, or Ajuba did not affect embryo development at cellularization stage. However, when knocking down Warts, embryos showed non-synchronized cell division and disruption of cellularization structure. Further staining of Warts constructs for PH3 showed non-synchronized cell division at syncytial stage. Meanwhile, one of the Yorkie shRNA construct did not lay eggs when crossed with the strong driver (MTD), probably due to the strong effects on oogenesis. Further study of this construct is needed to confirm the role of Yorkie. Overall, the results suggest that Hippo pathway may affect embryo development through actin regulation by Warts. This study gave insight into the role of the Hippo pathway in regulation of cytoskeleton and tissue structure in *Drosophila*.

**Faiza A. Mahmud (Supervisor: J. Peever).** Characterization of novel hypothalamic murine adult-derived cell lines, mHypoA-ORX/GFP2-4: expression of orexin and its implications in narcolepsy.

The lateral hypothalamus of the brain is dominated by neurons secreting orexin, a neuropeptide heavily involved in regulating sleep and wakefulness states. Narcolepsy is a severe neurological sleep disorder characterized by the loss of orexin neurons in the lateral hypothalamus which produces dysfunction in regulating wakefulness and rapid eye movement (REM) sleep. A novel secondary cell line had been extracted from a presumptive orexinergic population of the lateral hypothalamus. However, due to the heterogeneity of the hypothalamus, it is necessary to define the morphology and chemical phenotype of this novel cell line. To do this, we performed immunocytochemistry against orexin. The findings of the current *in vitro* study suggest that these novel hypothalamic cell lines, mHypoA-ORX/GFP2-4, express orexin. This suggests that these cells present a morphological and chemical phenotype identical to that of orexin neurons found within the murine lateral hypothalamus *in vivo*. Current treatment options for narcolepsy only temporarily relieve its notable symptoms; however, none have been considered long-term viable cures for the disorder. Characterization of mHypoA-ORX/GFP allows consideration of cell replacement therapy as a prospective therapeutic strategy for narcolepsy by recovering lost orexin neurons within the lateral hypothalamus, thus curing this sleep disorder.

**Kathleen Miao (Supervisor: K. Yoshioka)** The *Arabidopsis thaliana* cyclic nucleotide-gated ion channels AtCNGC 3, 5, and 14 are involved in root gravitropism.

*Arabidopsis thaliana* has 20 members of cyclic nucleotide-gated ion channels (AtCNGCs) that are non-selective and involved in regulating signal transductions important to plant development and defense. For example, AtCNGC14 is known to regulate root gravitropism and knockout mutants exhibit delayed root bending, while AtCNGC2 is involved in Ca2+ signaling with knockout mutants displaying Ca2+ hypersensitive and dwarf phenotypes. However, other AtCNGC members may potentially be involved in these physiological processes. Hence, current research focuses on deciphering a variety of biological functions of each AtCNGC. To achieve this goal, homozygous Transfer-DNA (T-DNA) insertion knockout lines were generated for various AtCNGCs to be used in phenotypic analysis. Then, root gravitropism and calcium sensitivity on homozygous T-DNA knockout lines of *cngc3, cngc5,* and cngc*14* (positive control)were tested. Root tip bending angles of 5 days old mutant seedlings grown on 1% sucrose MS media plates were measured 7 and 24 hours after tilting plates sideways by 90º. Mutant lines *cngc3-6634*, *cngc5-9893*, *cngc5-3762* and *cngc14-5823* were significantly delayed in root bending compared to wildtype; average root bending angles of *cngc5-9893* and *cngc14-5823* were comparable but both were significantly different from other lines (Student’s t test, *P* < 0.05). These findings suggest that CNGC3, 5, and 14 are likely involved in regulating root gravitropism, and CNGC5 may play a more essential role similar to CNGC14 as mutants exhibited stronger phenotypes. Since defects in Ca2+ signaling can lead to delayed root gravitropism, Ca2+ sensitivity experiments were performed on mutant lines grown in 1% sucrose MS media containing 0, 10, and 20 mM Ca2+ levels. Currently, seedlings are in growth phase and at 3 weeks of age, their fresh weights will be measured. If mutants are not impaired in Ca2+ signaling, then the delay in root gravitropism is more likely due to unresponsiveness to auxin, since auxin is an upstream regulator of Ca2+ signaling. Future studies can examine the effect of auxin treatment on *cngc3*, *5*, and *14* to further reveal the signaling pathways and biological functions of AtCNGCs.

**Hyun-Kee (Nicolaes) Min** **(Supervisor: D. Christendat).** Kinetics and crystallization of *Brassica rapa* and *Solanum lycopersicum* quinate dehydrogenase.

Quinate dehydrogenase (QDH) catalyzes the conversion of 3-dehydroquinate to quinate, as a branching point in the shikimate pathway. Quinate is a feeding deterrent in plants, and is the precursor of chlorogenic acid, which is an important intermediate in lignin biosynthesis. In order to determine the mechanism behind substrate specificity and catalysis of *B. rapa* and *S. lycopersicum* QDH, kinetics with both quinate and shikimate as substrates were assayed, and crystal screenings were done using Hampton Research Crystal Screen solutions. Kinetics assay showed that overall, *B. rapa* QDH reported lower activities compared to *S. lycopersicum* QDH. *B. rapa* QDH showed significantly higher activity with shikimate compared to quinate, whereas *S. lycipersicum* QDH showed higher activity with quinate than with shikimate.

**Angelica Miraples (Supervisor: K. Yoshioka).** Establishing the CRISPR/Cas9 system in tomato and testing system efficiency in *Arabidopsis thaliana*.

The bacterial CRISPR/ Cas9 system was originally discovered as a bacterial defense against bacteriophages, which was quickly adapted to create a new powerful technology to edit genomes including plants. So far the basic research to understand gene function in plants has been done mainly using the model plant, *Arabidopsis*. Now we can utilize the knowledge gained from *Arabidopsis* for modifying crop plants. However, to transform CRISPR constructs into many crop plants, tissue culture regeneration methods have to be established in our laboratory. Thus, in this project a range of *Agrobacterium*-mediated transformation and tissue culture protocols were tested to determine an effective technique to produce transgenic tomato plants for CRISPR/Cas9 gene editing. Several key components identified to influence explant survival during transformation and callus formation include tomato cultivars, antibiotics for *Agrobacterium* elimination, hormones for cell differentiation and temperature of explant incubation. In addition, two CRISPR/Cas9 vector constructs targeting the cyclic nucleotide-gated ion channel 2 (*CNGC2*)gene were generated and transformed into *Arabidopsis thaliana* to test prior to tomato plant transformation. T₁ plants were analysed for dwarfed morphology as a read out for *CNGC2* mutagenesis and genotyped to confirm CRISPR/Cas9 directed deletion. .

**Alborz Noorani (Supervisor: D. Guttman).** Comparative genomics of 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 (CF) 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 87 strains of this genus from public databases, including 11 strains isolated from CF patients. 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. Additionally, we performed selection analysis to identify genes that are under positive selection. Among positively selected genes from CF isolated strains, we found genetic factors that are associated with virulence.

**Hilary Pang (Supervisor: K. Yip).** Therapeutically targeting the lysosomal degradation of collagen for the reversal of fibrosis.

**Background:** Fibrosis, the excess scarring of normal tissue, is one of the leading causes of organ dysfunction. Specifically, radiation-induced fibrosis affects up to 70% of cancer patients after radiotherapy. Hallmarks of this disease include the TGF-β-mediated increase in collagen deposition and impaired tissue elasticity. This study uncovers the potential of Drug C as a therapy for the reversal of fibrosis.
**Methods:** Fibrosis was modelled by leg contraction of the murine hind limb. Hind limb tissue sections were stained with Masson’s trichrome and analyzed by ImageJ to measure collagen expression. The therapeutic role of Drug C in collagen degradation and lysosome expression was assessed *in vitro* using live cell imaging of dermal primary human fibroblasts (dPHFs) exposed to DQ-collagen-1 (exhibits fluorescence when degraded) and LysoTracker (stains lysosomes). Images were acquired by confocal microscopy, fluorescence intensity was quantified by Volocity, and cell area was measured by ImageJ. **Results:** Treatment of Drug C improved leg contraction and decreased collagen expression *in vivo*. Live cell imaging of dPHFs showed that Drug C significantly increased collagen degradation and lysosome expression.
**Conclusions:** Drug C promotes collagen degradation, reducing the fibrotic phenotype *in vivo* and *in vitro.* This intervention may function through increased expression of lysosomes. Further understanding the pathogenesis of fibrosis may reveal Drug C as a potential therapy for the reversal of this disease.

**Mahira Safaralizade (Supervisor: D. Godt).** Terminal Filament specific Gal4 driver.

**Alexander Sullivan (Supervisor: N. Provart).** User-submission extension for the eFP-Seq Browser

In 2016, Priyank Purohit and Dr. Nicholas Provart developed a web-application called the multi-track RNA-seq browser (renamed to eFP-Seq Browser) which allowed for a user to visualize *Arabidopsis thaliana* RNA-seq mapping coverage to see expression levels of exons while utilizing electronic fluorescent pictographic (eFP) images to visually represent tissue localization under different growth conditions and developmental stages. Though this web-application was successful in representing a fixed 113 RNA-Seq datasets, an extension was developed to allow an individual to submit user-generated dataset that they may have collected themselves or through the NCBI/EBI databases to analyze using the eFP-Seq Browser. This extension allows for the eFP-Seq Browser to be more viable for the average user as it allows anyone that has a BAM and BAM index file of their data utilizing Amazon AWS or Google Drive cloud services to visualize and analyze using the provided tools given within the eFP-Seq Browser.

**Trinh Vo (Supervisor: D. Desveaux).** Elucidating the genetic components of the Arabidopsis RLCK VII kinase family in HopF2-Triggered Immunity.

*Pseudomonas syringae* is a gram-negative bacterial phytopathogen that possesses a type III secretion system to inject effector proteins into host cells. These virulent factors act to suppress plant immune responses, enabling unhindered pathogen growth. In turn, plants have evolved resistance (R) proteins that can recognize certain effectors to activate a secondary immune response known as effector-triggered immunity (ETI). It has been shown that effector recognition by R proteins can be direct or indirect. For instance, the Arabidopsis host kinase PBL2 mimics a virulence target of the effector AvrAC from *Xanthomonas campestris*. Subsequently, AvrAC interacts with PBL2 and modifications are detected by the R protein ZAR1 to activate an ETI response. Currently, we have found that the R protein ZAR1 also recognizes the effector HopF2a from *P. syringae*; however, the mechanism of recognition has not been determined. We hypothesize that ZAR1 may be monitoring another member within the PBL kinase family for HopF2a-induced modifications. Using reverse genetic screening, we aim to determine if other members of the PBL kinase family are required for HopF2a recognition. This may show that ZAR1 can expand the range of effectors it can recognize by associating with multiple proteins within the kinase family, thereby broadening the scope of pathogens it can protect the plant against.

**Calvin Won (Supervisor: T. Harris).** Conserved Hippo-signaling pathway effects on cytoskeletal formation and overall growth in early cleavage stage *Drosophila* embryos.

Recently the Hippo-signaling pathway has been found to have a key role in controlling tissue growth and development, from managing individual cell shape to proliferation. While the exact mechanisms of associated upstream pathways of Hippo-signaling are unclear, there are several known downstream steps through which this pathway enacts transcriptional change, including multiple kinases, growth suppressors and transcription factors, most having homologues across species. Though this pathway has been researched both in drosophila as well as in mammals, these studies have primarily dealt with later stage development models. This project will study the effects of the Hippo-signaling pathway in early cleavage stage drosophila embryos via multiple shRNAi knockdown screens for individual components, including RassF, Expanded and Crumbs. The main purpose of this screen, and project, is to determine whether the Hippo-signaling pathway has a direct regulatory role on drosophila actin cytoskeleton. By using the early cleavage stage embryos, it is possible to isolate the direct effect Hippo-signaling components have on development due to the well documentation of early drosophila growth via different pathways, where Hippo is not expected to play a part. Our hypothesis is that the Hippo-signaling pathway will have little to no effect on the actual growth of early cleavage stage drosophila embryos, but will have a role in cytoskeleton formation. A genetic screen knocking down six Hippo-signaling components: *RassF*, *Crumbs*, *Kibra*, *Fat*, *Expanded* and *Warts* was completed, with multiple lines per gene. Male *Drosophila* carrying the shRNAi construct for the respective gene were selected and crossed with females from a maternal triple driver (MTD). F2-generation embryos were then fixed and analyzed via immunostaining, with ms-Pnut and rb-PH3, and microscopy to assess any potential defects. Of the six genes studied, five resulted in WT phenotype, with normal honey-comb patterning during cellularization. Defects were observed in several embryos within one *Warts* line with patterning partially disrupted, indicating potential for *Warts* being an essential cytoskeletal regulator in early cleavage stage embryos. Further studies utilizing other antibodies as well as various embryonic stages will be required to confirm.

**Alexander Wong (Supervisor: J. Peever)** Histological verification of optogenetically hyperpolarized noradrenergic locus coeruleus cells in the context of apnea-induced long-term facilitation.

The respiratory system is highly adaptive and can undergo plasticity. Long-term facilitation (LTF) is a form of respiratory plasticity where respiratory motor neuron output progressively increases under normoxic conditions in response to a repetitive stimulus (e.g. apneas), strengthening contraction of breathing muscles for up to 60 minutes post stimulus. Previously it has been shown that apnea-induced LTF requires noradrenaline and activates the locus coeruleus (LC). Optogenetic inactivation of LC neurons completely abolished LTF and should therefore be histologically verifiable by comparing neuronal activity with unmodified control neurons. Using a series of immunohistological approaches and microscopy, I aim to verify whether optically abolished LTF was truly a result of inactivated LC neuron firing. These results would determine if apnea-induced LTF is dependent on the activation or recruitment of noradrenergic LC neurons.

**Anthony Wong (Supervisor: D. Godt).** Regulation of collective cell migration in *Drosophila* ovariesby the transcription factor Traffic jam.

**Dorsa Zabihipour (Supervisor: M. Ringuette).** Extracellular matrix encapsulation of ovarian cancer tissues.

According to the American Cancer Society, ovarian cancer is the 5th most common cause of cancer deaths in women. Ovarian cancer metastasizes extensively and can spread vastly through seeding on the peritoneum wall, further enabling it to invade the body. Previous research has demonstrated the presence of a matrix encapsulation of non-metastatic melanoma tumours, suggesting that encapsulation could be a metastatic inhibitor. In this project I investigated the presence of matrix encapsulations in various human patient tissue samples invaded by high-grade ovarian cancer, through histological stainings, and SEM and fluorescent imaging. We found that only a subset of these tissues exhibited an encapsulation of rigid and aligned organization of ECM, highly dense in collagen I. This data suggests a potential host mechanism used to suppress further metastasis of developing tumours, similar to that seen in non-metastatic melanoma. Furthermore, Semaphorin 3F, a chemo-repulsant for endothelial cells, is down regulated in non-metastatic melanoma in nude mice models. My next step is to investigate the role of semaphorin 3F regulation in the encapsulation process in ovarian cancers.

**Yiwen Zhang (Supervisor: R. Fernandez-Gonzalez). Title:** Cell Size Regulation during *Drosophila* Embryonic Wound Repair.

Embryos repair epidermal wounds significantly faster than adults. Embryonic wounds heal with little-to-no inflammation or scarring, in a process that is conserved across species. Thus, understanding the molecular mechanisms of embryonic wound repair might provide strategies for improved treatments of adult wounds. It is thought that cytoskeletal rearrangements drive embryonic wound repair. However, our lab found that p38 kinase, a component of the MAPK pathway which is important for regulating cell size, is critical for wound closure in *Drosophila* embryos. Inhibiting p38 did not disrupt the cytoskeletal rearrangements associated with wound repair, but completely blocked wound healing. I investigated whether changes in the size of the cells around the wound were associated to tissue repair. To this end, I measured the apical area of the cells immediately adjacent to the wound. Cells around the wound shrank by 48% initially, as the wound expanded, and then regained some area during the healing process, up to 78% of their initial size. The cells position dorsal and ventral to the wound experienced significantly greater area losses (54%) than the anterior-posterior cells (38%), consistent with the pattern of tension in the epidermis. My data indicate that other cells beyond those at the wound edge must change their size during wound repair. I am currently quantifying area changes in embryos in which p38 is inhibited as well as cells further away from the wound. My experiments will reveal whether cell size changes contribute to wound repair, and what the role of p38 is in controlling cell size during rapid wound healing.

**Joanna Zhu (Supervisor: J. Kim).** Modulation of cholecystokinin-containing GABAergic interneurons in ventral hippocampus to nucleus accumbens pathway during contextual reward conditioning.

In the ventral hippocampus, pyramidal cells that project to the nucleus accumbens (vHPC-NAc) have been found to be recruited during contextual reward processing (Ciocchi et al., 2015, Lee et al., 2014). We hypothesize that vHPC-NAc cells may be modulated by inhibitory cholecystokinin-containing (CCK) GABAergic interneurons. Reduction of CCK interneuron activity may lead to disinhibition of pyramidal cells in the vHPC-NAc pathway and enhancement of contextual reward memory. To examine the functional connectivity between CCK interneurons and vHPC-NAc cells, we expressed ArChT, a light sensitive inhibitory opsin, in CCK interneurons (ArChT-GABA-CCK). Retrograde tracing was performed by injection of cholera toxin subunit B (CTB) into the NAc, followed by measuring levels of c-fos, an immediate early gene, in pyramidal cells of the vHPC after exposure to a context containing sucrose. In ArChT-GABA-CCK mice who received light illumination, increased co-labeling of CTB and c-fos was found. CCK interneurons play a major role in regulating pyramidal cell activity in the vHPC-NAc pathway during learning of context reward associations.