these mutants include muscle fibers that extend past their appropriate attachment sites, as well as muscles with multiple, persistent filopodia. Here we present characterization of the isolated mutant lines and preliminary results implicating a gene known to function in cytoskeletal organization. Further exploration into these muscle mutants will contribute crucial data for understanding muscle guidance and development.

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Program/Abstract # 219
The planar cell polarity pathway regulates parietal endoderm outgrowth
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Parietal extraembryonic endoderm (PE) contributes to the yolk sac and is the first migratory cell type in the developing mammalian embryo. We study this migratory event using the F9 teratocarcinoma cell in vitro model system. In suspension culture, F9 cells form embryoid bodies (EBs) consisting of an inner core of undifferentiated stem cells, surrounded by an outer layer of visceral endoderm. When EBs are plated on ECM substrates, PE migrates away from the EB as a sheet of cells that are enriched in migratory and adhesion structures. To determine if PE migration is regulated by the PCP pathway, we first determined if migratory cells were polarized. Based on Golgi localization, more than 70% of the outgrowth cells are polarized in the direction of migration. In the planar cell polarity pathway (PCP), which mediated directed cell migration events, Wnt ligand binds the Frizzled receptor activating Disheveled, which activates Daam1, leading to activation of the Rho/ROCK pathway. Perturbation of the Wnt pathway using the soluble Frizzled Receptor (sFRP) increases outgrowth migration distance and inhibits cell polarity. Our previous data showed that Rho/ROCK inhibited outgrowth migrates further and faster, as observed with Wnt inhibition and we now show that under this condition cells are not polarized. We hypothesize that a Wnt source in the EB repels emerging PE, promoting outgrowth of polarized cells. Candidate Wnts implicated in the PCP pathway are also expressed in F9 EBs. Currently we are investigating the location of Wnts and Frizzled receptors in the outgrowth system and using genetic approaches to perturb the PCP pathway.

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Program/Abstract # 220
Turtle (tutl) is required for photoreceptor axon targeting in Drosophila
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In the *Drosophila* visual system, photoreceptor (R cell) axons innervate the optic ganglia such that R1–R6 project to the superficial lamina layer and R7–R8 target the deeper medulla. The mechanisms regulating this specific innervation pattern remain largely unknown. In this study, we show that the neural-specific immunoglobulin family member, *turtle* (*tutl*), is required for normal lamina targeting. Examination of R2–R5 axons labeled with rough-tau-lacZ revealed a failure of these axons to properly terminate at the lamina layer in *tutl* loss-of-function mutants. This mistargeting phenotype is also observed in flies in which *tutl* has been knocked down by RNA interference. Further, this defect appears to be intrinsic to the photoreceptor cells. R2–R5 misprojections persisted in mosaic flies in which the *tutl* gene was specifically deleted in the eye. The lamina glial layers, which are known to have an important role in terminating R1–R6 axons, were properly generated and organized. Examination of plastic sections of adult eyes and immunohistochemistry with various R-cell-specific markers did not reveal any defects in photoreceptor cell specification or differentiation or indicate morphological abnormalities. Cell labeling indicates that *tutl* is expressed in at least a subset of R cells. We are presently using a combination of molecular and genetic approaches to identify proteins (i.e. its ligand and downstream effectors) that interact with *tutl* to regulate R-cell axon targeting in the visual system. This work was funded by CIHR.

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Program/Abstract # 221
Nonstop and Rap/Fzr/Cdh1 interact to regulate cell cycle progression and retinal axon targeting
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The *nonstop* (not) gene encodes a ubiquitin-specific protease (UBP) which is required for proper glia migration and axon targeting in the developing nervous system. The molecular mechanisms by which Nonstop, a de-ubiquitinating enzyme, regulates glia migration and axon targeting are not well understood. UBPs function to disassemble multi-ubiquitin chains from proteins destined to the 26S proteasome. UBPs have bidirectional control in the rate of protein degradation, accelerating degradation by allowing the recycling of free ubiquitin or inhibiting proteolysis by removing ubiquitin tags from proteins and therefore preventing further degradation. Rap/Fzr is the *Drosophila* homolog of the mammalian *Cdhl*, an activator of the anaphase promoting complex (APC), a ubiquitin ligase complex. We previously showed that Rap/Fzr regulates mitotic progression by targeting cyclins and promoting cell cycle exit in the developing eye and the embryo. In the following study, we present data that suggest a novel functional role for Rap/Fzr and Nonstop. Nonstop acts as a dominant suppressor of the *rap/fzr* loss-of-function phenotype. Our data
also show that Nonstop regulates APC activity and plays a novel role in cell cycle progression. Co-immunoprecipitation studies show that Nonstop physically interacts with Rap/Fzr. In addition, in the developing optic lobe, we show that loss-of-function \textit{rap/fzr} mutants lead to mis-targeting of R1–R6 axons similar to \textit{nonstop} null mutants. These results suggest that Rap/Fzr and Nonstop interact and regulate both axon targeting and cell cycle progression.

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Program/Abstract # 222

**The role of roundabouts in commissure formation in the zebrafish forebrain**

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In order to integrate information from both sides of a bilaterally symmetric organism, axons must cross the midline between two hemispheres. Such wiring of the nervous system utilizes an elaborate communication system between an array of guidance receptors on the growth cones of pathfinding commissural axons and surrounding environmental cues. How do all these cues simultaneously function to point axons or migrating cells in the right direction? To answer this, we have characterized a simple system in the zebrafish forebrain to assay commissural axons, their astroglial growth substrate and the Slit–Robo guidance system. We show that commissural axons grow along a bridge-like structure made of astroglial cells. In addition, Slit functions not only to guide axons across the forebrain, but also to position the astroglial bridge. Interestingly, \textit{roundabouts} (\textit{robo1–4}) are differentially expressed in commissural neurons and in the astroglial bridge. We use a loss of function approach to determine which Robo receptors are necessary in commissural axons and/or glial cells for proper midline positioning. Our results suggest that Robo1 may function directly in glial cell repulsion, setting up a permissive bridge for the crossing of POC axons, while Robo2 may function directly in POC axon guidance. We are currently taking a combinatorial \textit{robo} loss of function and \textit{slit} gain of function approach to test this, and to determine which Robos are required to mediate Slit signaling. Characterizing this system will allow us to examine how glial cells and axons interact using the Slit-Robo guidance system during commissure formation.

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