Biol 463 Final Project First Draft

Background information

In animals, permeability barriers are created to control movement of compounds across various tissues such as the brain, intestine and epidermis. Since permeability barriers protects animals against pathogens, their establishment during development is deemed necessary. In vertebrates, the blood-brain barrier isolates and protects the brain from potential damage from blood born growth factors, neural active compounds and ions (Schulte et al.,2003). In Drosophila melanogaster, disruption of the blood-brain barrier may cause paralysis as the permeated potassium from the hemolymph can block action potentials in neurons (Auld et al., 1995). Failure in junction and barrier formation usually lead to detrimental disease or fatality. Since permeability barriers are primarily formed from junctions, the study of junction assembly during development provide vital insight on barrier formation.

Tight junctions(TJs) are present in vertebrate epithelia to form permeability barrier. In insects, the blood-brain barrier serves a similar function as in vertebrates. In the epithelia of insects, septate junctions(SJs) establish the permeability barrier. While septate junctions and tight junctions perform analogous function, they differ in their ultrastructure (Figure 1A). Interestingly, the core complex SJ proteins are functionally and molecularly conserved to form paranodal myelinated axon's node of Ranvier in vertebrates, while the septate junction in the Drosophila nervous system is found to subperineurial glia cells (Figure 1C) (Bhat et al., 2001). Hence, understanding the formation of septate junction would provide insight to the formation of paranodal junction in vertebrates.

At the point of contact of three neighbouring cells, a specialized type of junction, Tricellular junction (TCJ), is formed. At TCJ,TJs or SJs of three neighbouring cells converges in vertebrate and insects respectively (Figure 1B,1D).In fruitfly Drosophila, Gliotactin(Gli) is found to be necessary to the SJ localization at TCJ, and the formation of glial-based blood-nerve barrier. Gli contains PDZ binding motif and an SH3 domain. Disc large(Dlg) association with Gli is necessary for formation of TCJ and localization of Gli. Previous Dlg knockdown experiments suggest that interaction between Glio and Dlg stabilizes Gliotactin (Sharif Khodaei, 2017). Although Gli contains a PDZ-binding motif on Gli's C-terminal and Dlg contains a PDZ domain, their interaction is likely through other intermediary proteins since Gli localization is independent of it's PDZ binding motif (Schulte et al, 2006). However, it was observed that extensive endocytosis occurs to form large intracellular vesicle when PDZ binding motif of Gli is defective(Sharif Khodaei, 2017). This suggests that PDZ binding motif has other functional means. Dlg is an evolutionarily conserved tumour suppressor. Dlg-mutants exhibit overgrowth in larval brain and imagainal discs. While the protein interactions with Dlg in septate junction formation is not known, Dlg is known to be necessary in establishment of cell polarity (Sharif Khodaei, 2017). Cell-polarity and tissue polarity provides structural integrity and suppresses malignant phenotype of mutant cells. Loss of cell-polarity is known to cause tumour initiation (Lee and Vasioukhin, 2008).

In vertebrate studies, the conservation of Gli and Dlg interaction is observed in their respective vertebrate homologs, Neuroligin and PSD-95 (Schulte et al. 2006). Meanwhile human Disc large(hDlg) is observed to function similarly to Drosophila Dlg. Laprise et al. (2003) demonstrated hDlg is required for the assembly of adherens junctions in the epithelia while hDlg1 was shown to reduce expression of invasion human cervical carcinomas (Frese et al, 2006). Since hDlg1 has been studied extensively for its oncogenic function, more is known about its interactome. As conservation of protein interaction is

observed, the hDlg interactome can be utilized to extend the interactome of Dlg to investigate which proteins are involved in the formation of septate junction and possibly the paranodal junctions.

Because manipulation of the peripheral nervous system is more difficult than manipulation of imaginal discs, the investigation of septate junction and tricelluar junction formation can be done at the wing imaginal disc where cells are larger. A list of proteins which have been shown to interact with hDlg1 was acquire from UniProt. A NCBI protein blast search was performed to identify potential Drosophila homologs. Drosophila proteins that exhibit more than 50% identity to hDlg interactome protein were selected to compile a list of Drosophila protein that may interact with Dlg. To choose candidate proteins that may interact with Dlg, proteins that have contain conserved PDZ domains and SH3 domain.

Research Question:

What are the proteins necessary for the formation of the SJ at the TCJ at the wing imaginal disc?

Hypothesis:

Veli, RE30311p, RE51991p, X11L, Jagar, alpha-actinin and moesin proteins are necessary in the for proper formation of septate junctions at tricellular junction by interacting with Dlg and or Gli.

Predication:

Because veli proteins, RE30311p, RE51991p have highly conserved PDZ domains, they are likely candidates for Gli interaction mediated by PDZ binding motif. Jagar contains SH3 domain which is likely to interact with proline rich motif of Gli. Alpha-actinin and moesin have high percent identity and alignment score conservation. Both proteins interact with the cytoskeleton and may indirectly interact with Gliotactin by positioning it at the TCJ with microtubules or actin filaments interactions.

In this knockdown screen:

If protein knock down results in Gliotactin delocalization, septate junction would not be formed properly. Protein would be deemed necessary in septate junction formation.

If protein knock down causes overgrowth in wing imaginal discs, dlg recruitment is likely disrupted. Protein is likely necessary for cell polarity establishment and may also play a role in septate junction formation.

Relevance

Permeability barriers have important physiological roles. When permeability barriers break down dramatic consequences occur in both vertebrates and invertebrates. Protection from toxic metabolites, regulation of nutrients, oxygen, ions exchange, and cell homeostasis are disrupted and may results in lethality and pathogenic invasion when barriers are defective. In identifying which proteins are necessary in forming septate junction during development, one can propose a comprehensive mechanistic model of septate junction and paranodal junction formation. In determining the mechanisms underlying septate junction and paranodal junction formation, one can better understand how the blood brain barrier(bbb) is established and may contribute to devising ways to bypass it to deliver compounds to the brain. Furthermore, results from this project may allow one devise a method to disrupt permeability barrier to develop a pesticide.

Approach

1) Using the GAL4-UAS to drive RNAi to knock down candidate proteins in the wing disc to screen which proteins are necessary for Gli and Dlg localization.

GAL4/UAS is a yeast drive system that can be used to express RNAi at specific sites within a tissue type. To drive RNAi at the posterior compartment of the wing imaginal disc only while the anterior compartment of the wing imaginal disc would serve as an internal control, the promoter of *Engrailed(eng)* may be utilized. *Eng* a protein expressed in the posterior compartment of the wing imaginal disc during Drosophila development(Figure 2). GAL4 is a yeast transcription factor while binds to UAS and drives UAS expression. To create knock down mutants, females flies homozygous for engrail promoter-GAL4(*eng-gal4*), Gli tagged with YFP and Dlg tagged with RFP can be crossed with male flies homozygous for UAS-RNAi. The F1 progeny would express RNAi at the posterior compartment of the wing imaginal disc as F1 would inherit both the *eng-GAL4* maternally and UAS-RNAi paternally (Figure 3). UAS-RNAi fly lines specific to each candidate protein is commercially available. As a control for RNAi expression, a crossed would be performed with males homozygous for UAS-mCD8::GFP (mouse CD8 protein tagged with GFP). Using Fluorescence microscopy on dissected wing imaginal discs from third instar larvae, the expression of Gli and Dlg can be visualized as they are endogenous tagged with YFP and RFP respectively.

2) Using Gliotactin promoter to drive GAL4 expression to induce expression of proteins that delocalize Gli and Dlg. Observe if Blood Brain Barrier is disrupted.

After identifying which proteins are necessary in septate junction formation in the wing imaginal disc tricellular junction, a secondary screen can be performed to investigate if those proteins are necessary in establishing the Blood Brain Barrier. The GAL4/UAS system can be implemented. In this experiment, instead of using the promoter of *eng,* the promotor for *Gliotactin* is used to to drive expression of GAL4 in glia. Similarly, the F1 of the cross between *Gli-GAL4* females and UAS-RNAi males would express RNAi to knock down protein expression in the glia. To observe whether the BBB has been disrupted by the knock down, instar larvaes can be exposed to a hemolymph dye. Observation of dye in the brain lobes would indicate disruption in the BBB which resulted in the permeability of the dye in the brain lobes.

3) Determining if protein function is domain dependent

Future Direction

Determining hierarchy of proteins.

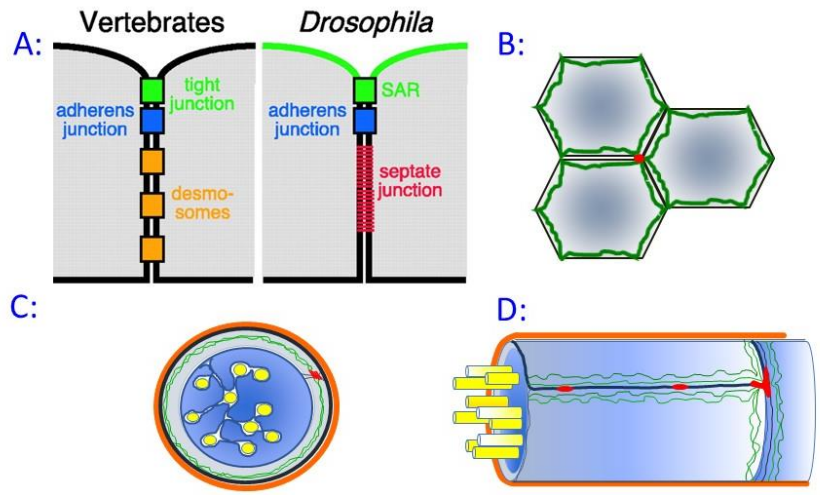

Figure 1. A) In Drosophila, ladder-like septate junction adheres adjacent membranes basal to adherens junctions. In vertebrates, tight junctions apical to adherens junctions forms kissing points between adjacent plasma membranes(Schulte et al., 2003). Image modified from Cox and Hardin (2004). B) Tricellur junction. C) Septate junction in the subperineurial glia D) Trimembrane junction in Drosophila glia.

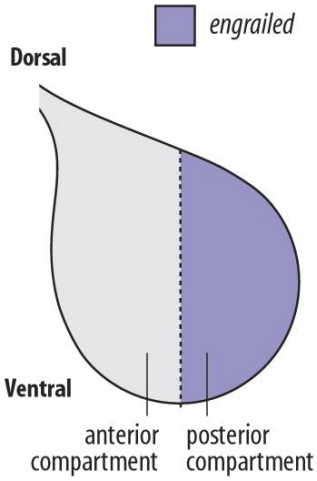

Figure 2. Expression pattern of *engrailed* in the wing imaginal disc.

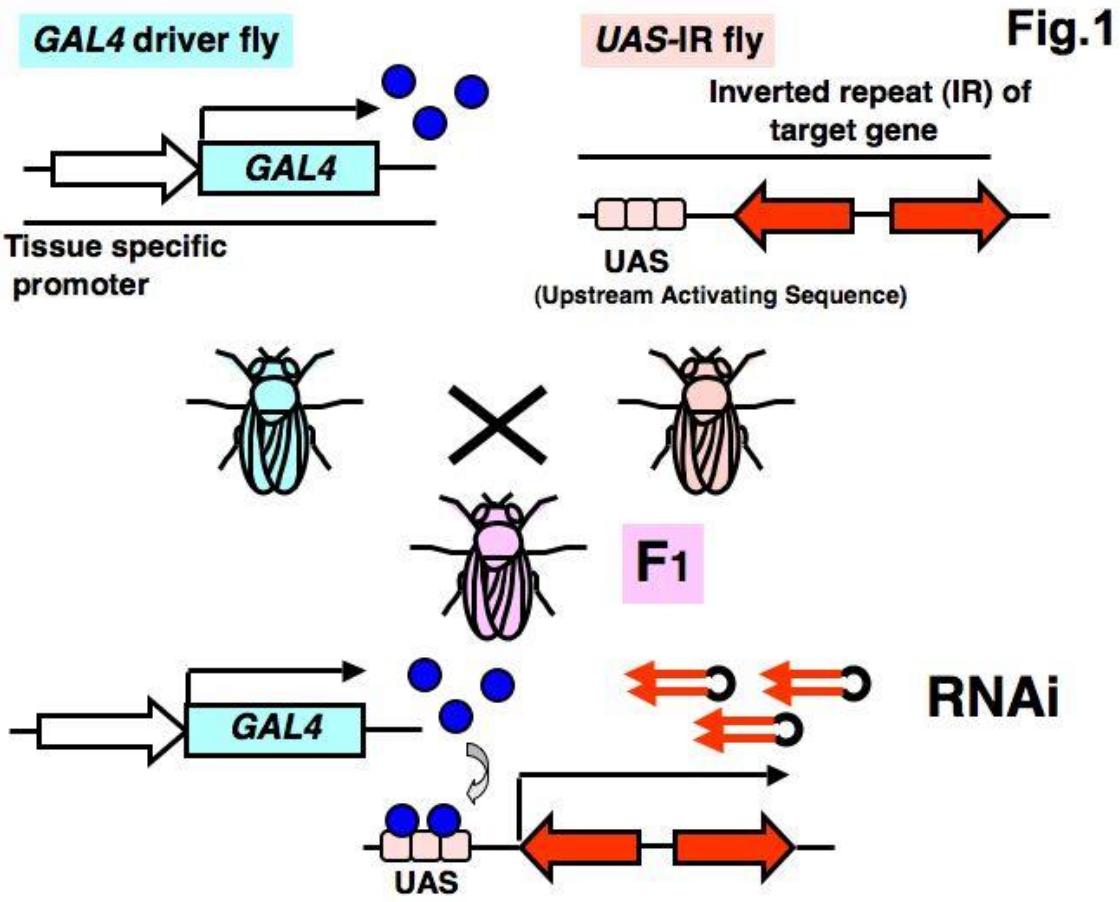

Figure 3. Schematic depicting the Gal/UAS-RNAi driver system.

Links to references (references to be organized later)

http://www.jbc.org/content/279/11/10157.long

http://jcs.biologists.org/content/121/8/1141

http://jcggdb.jp/GlycoPOD/protocolShow.action?nodeId=t32