PROSPECTUS: THE ROLE OF EHBP-1 IN THE NOTCH SIGNALING PATHWAY

Notch signaling is an evolutionarily conserved pathway by which juxtaposed cells communicate with one another. The downstream effects of this communication vary widely to include differential cell-fate determination, stem cell maintenance, boundary formation, cell proliferation and apoptosis (High and Epstein, 2008). Notably, the pathway functions throughout an organism's lifespan, and is highly dependent on the cellular context (High and Epstein, 2008).

This pathway has been implicated in a range of diseases, including cancer (Roy et al., 2007) stroke (Nichols et al., 2007), cardiac development (High and Epstein, 2008), and cardiac disease (High and Epstein, 2008). In order to launch effective preventative measures against these illnesses, it is important to gain an in depth understanding of not just what role the Notch pathway plays in each disease, but also how the Notch pathway functions.

In mammals, there are four different kinds of Notch receptors (Notch 1-4), and five ligands (Delta-like-1, -3, and -4, Jagged 1 and Jagged 2) (Radtke and Raj, 2003). Comparatively, the pathway is more simplified in Drosophila melanogasler, in which there is a single Notch receptor and two ligands, called Delta and Serrate (Radtke and Raj, 2003). Activation of the Notch signaling pathway begins with the binding of receptor and ligand of two adjacent cells (Figure 1). At this point, the cytoplasmic portion of the Notch receptor (called the Notch Intracellular Domain, or NICD is cleaved and released from the membrane into the cytoplasm. The NJCD then enters the nucleus to bind to and inactivate a suppressor complex. In this way, the NICD activates the transcription of target genes (Radtke and Raj, 2003). It is important to note that multiple levels of regulation can modify the activation of the Notch receptor and Delta/Serrate ligand. Although this activation process has been extensively researched, it is not yet fully understood. It is widely
believed that endocytosis, membrane trafficking and signaling modulators all strictly regulated the activation of both Notch and Delta/Serrate (Nichols et al., 2007). For example, although one would expect the signal-sending cells to show high amounts of Delta/Serrate ligands on their surface, the ligands are actually internalized in intracellular vesicles (Nichols et al., 2007). Furthermore, cells defective in endocytosis accumulate Delta/Serrate ligands on their surface, yet are unable to activate a Notch receptor (Nichols et al., 2007). Thus, the processes of trafficking, endocytosis, and exocytosis are crucial for the activation of the Delta/Serrate ligand.

Members of the lab conducted a forward genetic screen in order to search for Notch signaling mutants. Because a loss of Notch signaling leads to lethality, mosaic flies were created such that the loss of Notch signaling only affected specific tissues. Notch signaling is known to direct the development of External Sensory Organs (ESOs) in Drosophila, thus, flies with abnormal bristle formation were determined to be an earmark of Notch loss-of-function.

Mutants isolated from this screen were categorized by lab members into complementation groups, and mapped by meiotic recombination. One group of mutant was found to map to CG15609, the Drosophila ortholog of EH-domain Binding Protein 1 (dEHBP-1). Little is known about the function of EHBP-1 in either mammals or Drosophila, but the structure has been analyzed by homology alignment to reveal three domains. The first is a highly conserved N-terminal domain of unknown function that is conserved from C. elegans to humans. The second domain is a CH domain, "which is thought to be responsible for binding to actin polymers (Guilhenne et al., 2004). The third region is a CAAX motif at the C-terminus of the protein. In other proteins with this motif, the CAAX domain is responsible for membrane tethering. The mutations which mapped to CG15609 were found to have splicing errors that caused premature
stop codons along the length of the gene, thus preventing the production of the full length protein.

One approach to understanding the Notch signaling pathway is to understand the components that allow it to function properly. With this methodology in mind, a number of experiments have been designed in order to better understand the function of EHBP-1 in the Notch pathway. This major theme is approached in two different contexts.

The first project of my thesis will have two aims: first, to determine if EHBP-1 functions in tissues other than the ESO where Notch signaling is known to play a role, and second, to determine whether EHBP-1 functions in the cells that send the signal or in the cells that receive the signal. Both of these aims may be addressed by using the *Drosophila* ovaries as a model tissue. *Drosophila* ovaries are an appropriate system to address these concerns because of their unique developmental attributes. Fly egg chambers develop in one of two ovaries, each of which consists of parallel ovarioles (Matthies). Egg development begins at the anterior portion the ovariole, called germarium, and proceeds linearly along the ovariole towards the posterior end of the ovary. As it proceeds developmentally, the egg chamber is referred to as a cyst (Matthies). The cyst is surrounded by somatically derived epithelial cells, referred to as "follicle cells" (Matthies). In the early, anterior stages of cyst development, the follicle cells undergo regular mitosis. At stage seven of the cyst's development, the germ cells send a signal to the follicle cells via the Notch signaling pathway that allows the follicle cells to enter endocycle. In this cycle, the follicle cells replicate their DNA, but do not undergo cytokinesis. Before the follicle cells undergo mitosis again in stage ten of their development, each cell will have sixteen copies of DNA.

The switch from mitosis to endocycle in the follicle cells can be monitored by the presence of certain proteins. Specifically, the follicle cell's mitotic cycle is marked by the presence of the protein Cut. Alternatively, when the cell moves into endocycle, the protein Hindsight (Hnt)
represses Cut. Thus, mitosis is marked by an absence of Hnt and presence of Cut, and endocycle is marked by an absence of Cut and presence of Hnt.

In addition to having a Notch signaling occurrence that can be easily monitored, ovaries are also a unique and advantageous tissue because of their structure. As stated previously, each cyst contains sixteen germ-line cells surrounded by a thin layer of follicle cells. Through genetic crossing and induced recombination, the germ cells and follicle cells can be mutated independently of one another so that they are homozygous for a premature stop codon in CG15609 (specifically, CG1560~8). By creating a mutant signal-sending cell or signal-receiving cell (while keeping its counterpart wild-type), and then monitoring the Notch signaling, one should be able to determine whether EHBP-l plays a role in the sending of the Notch signal or the receiving of the Notch signal.

The second project of my thesis will examine the role of EHBP-l both ex vivo and in vivo. Its aim will be to analyze the function of EHBP-l as a whole through understanding the function of its parts. Specifically, we will examine where and how the protein localizes within a cell. This question will be addressed using two successive strategies. First, ten alternate forms of EHBP-l have been created, all of which are FLAG-tagged. Nine of the forms are truncations that express some, but not all of the three critical domains. The tenth form contains a point mutation in the CAAX motif so that it expresses an alanine instead of a cysteine. These alternate EHBP-l forms will be transfected into S2 cells and differentially analyzed. By examining which forms are able to localize in the wild-type manner, we will determine which domains are critical for correct localization within the cell. The second approach to the EHBP-l analysis will be to determine with which structures EHBP-l co-localizes in vivo. In this plan, EHBP-l is over-expressed in the salivary glands of Drosophila larvae. The salivary glands of third-instar Drosophila larvae have
extremely large cells that can be stained and examined easily for co-localization studies.

In summary, through each of these multi-faceted projects, I will try to define the role EHBPI in the Notch pathway, as well as the molecular mechanisms that underlie its intracellular localization.
BIBLIOGRAPHY


