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The Molecular Mechanisms of Fringe Modification on Drosophila Notch: Examining the Structure and Function of Notch EGF Repeats

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The Notch signaling pathway is an evolutionarily conserved signaling pathway that plays critical roles in both development and disease. The Notch receptor, a large cell-surface single-pass transmembrane protein, is canonically activated upon binding to ligand expressed on an adjacent cell. In Drosophila, Notch can bind to one of two ligands, Delta or Serrate, to activate downstream signaling events. A marked characteristic of the Notch protein is that there are thirty-six tandem epidermal growth factor-like (EGF) repeats in the extracellular domain (ECD). EGF repeats 11-12 are designated the ligand binding domain, and are necessary and sufficient for ligand binding, although recently other EGF repeats have also been implicated in ligand binding. Interestingly, glycosylation of EGF repeats is not only essential for Notch activity, but also modulates Notch activity. Extension of the O-fucose by Fringe with a β3-linked GlcNAc affects how Notch responds to ligands expressed on adjacent cells, specifically increasing Notch-Delta binding while decreasing Notch-Serrate binding. However, the exact mechanism by which Fringe modification alters ligand binding is unknown. We predict that Fringe modification changes the conformation of the EGF repeats in the ECD, therefore changing the ability of the EGF repeats to interact with either ligand. First, we performed mass spectral studies on EGF repeats 1-36 from Drosophila Notch and found that Fringe modifies some EGF repeats more efficiently than others. We will be using site-directed mutagenesis and cell-based binding assays to study which O-fucose sites are important for mediating the ligand binding effect by Fringe. In addition, using electron microscopy we have seen that Fringe modification causes slight conformational changes in the Notch ECD. Additional studies are in progress to better understand how Fringe modifications regulate Notch-ligand binding. Supported by NIH grant R01GM061126 and T32GM008468.