

Signaling during *Drosophila* development

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Research in the lab focuses on signaling processes operating during embryonic and post-embryonic development of *Drosophila*, to shape the pattern of the organism. We employ a combination of approaches, including Genetics, Cell biology and Biochemistry, and collaborate with Naama Barkai's group on computational analyses of developmental patterning.

The *Drosophila* EGF Receptor Pathway.

The spatial and temporal regulation of this pathway is achieved by tightly regulated intracellular trafficking of the ligand. The ligand is retained as a precursor in the ER, and is actively transported to the Golgi by a chaperon protein, where it undergoes cleavage by a seven-transmembrane domain protease. Our studies show that the same protease also induces cleavage of the chaperone, to assure that it will not undergo retrograde trafficking to carry more than one molecule of ligand. The level of the chaperone therefore determines the amount of active ligand that will be released. In the developing eye the ligand precursor is cleaved in the ER. We have identified a novel ER retention mechanism for the cleaved ligand, such that only restricted amounts of the cleaved ligand would be released from the ER and secreted. The machinery for retention and trafficking of the ligands is being explored genetically in cell culture, using dsRNA technology to knock down every gene in the genome and identify the genes regulating this process. Candidates identified in this manner will be studied further in flies.

One of the central issues in understanding patterning by morphogens is the conversion of a continuous activation profile to distinct borders of gene expression. We investigate this problem in the embryonic ventral ectoderm which is patterned by EGFR signaling. The key transcriptional responses in this tissue involve transcriptional activators and

repressors of the ETS family, that are modified by MAPK signaling. We demonstrate that sharp thresholds can be generated by a balance between phosphorylation and dephosphorylation reactions.

Establishment of Cell Polarity by the *Drosophila* VEGF receptor.

This receptor-tyrosine kinase is broadly expressed in polarized epithelial tissues. In the wing imaginal disc the activating ligands are specifically secreted on the apical side of cells, to induce polarized activation of the receptor. Consequently, polymerization of actin is restricted apically. Techniques to trace polarized receptor dimerization *in vivo* are being utilized, in order to elucidate the basis for polarized receptor activation.

Unidirectional Signaling by the Notch Pathway.

The Notch pathway is utilized during development to mediate cell-cell interactions by a membrane-bound ligand termed Delta. A sharp distinction between the cell sending the signal and the cell

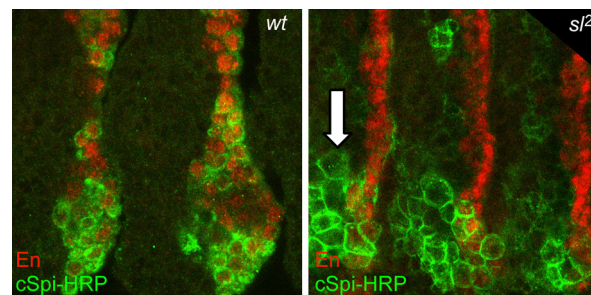


Fig. 1 Retention of cleaved EGFR ligand requires Phospholipase C (Sl). Expression of the cleaved EGFR ligand (cSpitz-green) in the embryo results in ER retention of the ligand in the expressing cells (red). In mutants for the PLC-gamma *sl* mutation, the ligand is readily secreted to the extracellular milieu (arrow).

receiving the signal is crucial for unidirectional signaling by this pathway. We identified a metalloprotease that is responsible for continuous cleavage of Delta, in order to eliminate it from the receiving cells and maintain the unidirectional flow of the signal. The role of this metalloprotease in different phases in which Notch signaling is employed is being investigated.

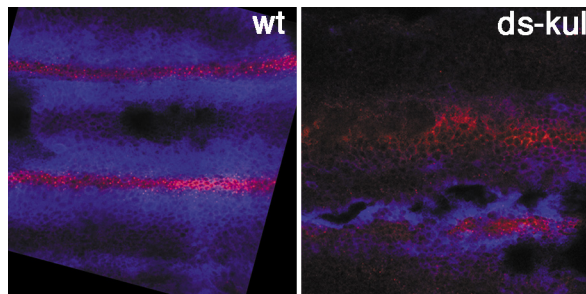


Fig. 2 The Kul metalloprotease maintains unidirectional Notch signaling.

In the pupal wing the ligand Delta (red) is expressed in the future veins, and activates the Notch pathway in the adjacent cells (blue). Reduction in the level of the Kul metalloprotease following expression of double-stranded RNA, leads to elevation of Delta levels in the juxta-vein cells, and disruption of the restricted spatial pattern of Notch activation.

Selected Publications

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