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Endocytosis, Endosome Trafficking, and the Regulation of *Drosophila* Development

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Key Words

morphogen gradient, cell signaling, patterning

Abstract

Endocytosis and endosome trafficking regulate cell signaling in unexpected ways. Here we review the contribution that *Drosophila* research has made to this exciting field. In addition to attenuating signaling, endocytosis shapes morphogen gradients, activates ligands, and regulates spatially receptor activation within a single cell. Moreover, some receptors signal from within endosomes, and the ability of a specific type of endosome to form controls the ability of cells to signal. Experiments in *Drosophila* reveal that through regulation of a variety of cell signaling pathways, endocytosis controls cell patterning and cell fate.

Contents

ENDOCYTOSIS AND MEMBRANES

Clathrin: a multimeric protein consisting of three heavy chains and two light chains that coat the endocytic vesicles

Endocytosis is the internalization of plasma membrane into the cytoplasm as vesicles or endosomes (**Figure 1**). Endocytosis in vertebrate cells, and probably also in *Drosophila*, occurs by one of two general mechanisms: clathrin-dependent and clathrin-independent internalization (Leroy & Wrana 2005). In clathrin-dependent endocytosis, with the help of the AP-2 adapter complex, clathrin triskelia form a cage structure around invaginated membrane, and the activity of the GTPase dynamin results in vesicle scission. Newly formed vesicles fuse with the early (sorting) endosome, and the internalized proteins either are shunted to the recycling endosome and sent back to the plasma membrane, or targeted to the multivesicular body/late endosome prior to secretion as an exosome or lysosomal degradation. Clathrin-independent internalization occurs at sites of lipid rafts, which are cholesterol-rich regions of the plasma membrane. The protein caveolin mediates some of the clathrin-independent events, forming membrane invaginations called caveolae. *Drosophila* have no caveolin, but other proteins associated with caveolae (i.e., flotillin and annexin) are present. Clathrin-independent endocytosis is not well characterized, but dynamin is usually involved, and the vesicles may be delivered to sorting endosomes.

Several different kinds of proteins and lipids regulate internalization and endosomal sorting. Rab proteins are membrane-associated, Ras-like GTPases that control membrane fusion (Zerial & McBride 2001). Different Rabs are associated with particular endosomes, and they regulate endosome/endosome and endosome/plasma membrane fusion. Inositol phospholipids, or phosphoinositides, constitute a small fraction of the phospholipids in the plasma membrane and endosome membranes. Distinct regions of the plasma membrane and different endosomes are enriched in specific varieties of phosphoinositides, which bind with differing affinities to proteins with lipid-binding domains (Lemmon 2003, LeRoy & Wrana 2005). For example, Hrs (hepatocyte growth factor–regulated tyrosine kinase substrate), a protein required for formation of the inner vesicles of multivesicular bodies (MVBs), is localized to early endosomes by binding PI3P (Komada & Kitamura 2005). Another

example is epsin, an endocytic adapter protein that in *Drosophila* is specifically required for internalization of the ligand Delta and Delta signaling (see below). Epsin is localized to the plasma membrane via its ENTH (epsin N-terminal homology) domain, which binds $PI(4,5)P_2$ (Legendre-Guillemin et al. 2004). Transmembrane proteins may have cytoplasmically located internalization signals that are part of their primary amino acid sequence (Bonifacino & Traub 2003). Alternatively, a Ub (ubiquitin) polypeptide that serves as an endocytosis signal may be added posttranslationally to the cytoplasmic domain (Hicke & Dunn 2003, Raiborg et al. 2003, Dupre et al. 2004).

Researchers in this field face a variety of challenges. For example, when one is imaging cells, it can be difficult to distinguish endocytic and secretory vesicles and to identify different endosomes along the endocytic pathway. Many new markers have been developed for the endosomes already identified, but there may well be other kinds of endosomes awaiting discovery. Experiments with mutants are also problematic in that the same mutation can often affect both secretion and endocytosis or several different steps in endosomal sorting. New techniques that allow targeting of mutations to specific cells are helping to overcome this obstacle. There are several recent general reviews of the role of endocytosis in cell signaling and development in *Drosophila* (Seto et al. 2002, Piddini & Vincent 2003, Dudu et al. 2004).

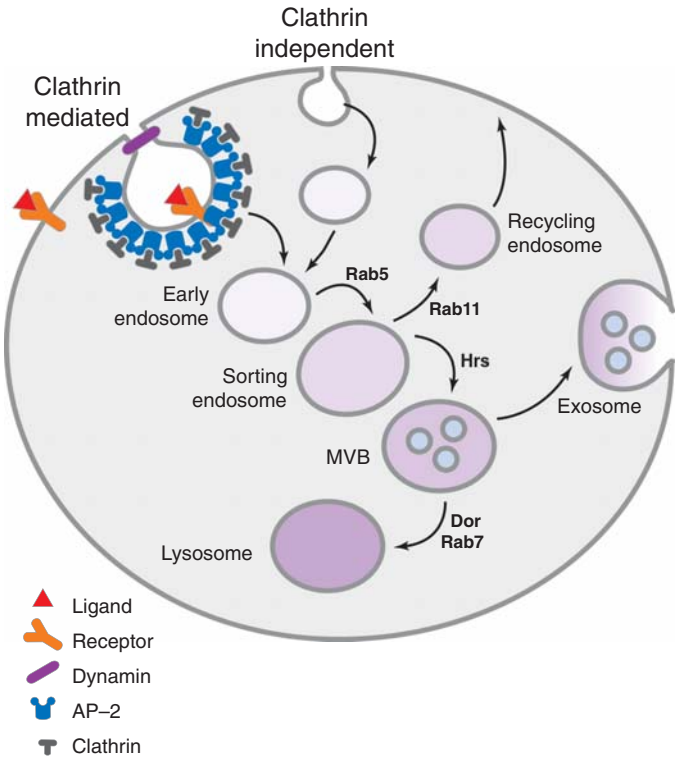


Figure 1.

A simple view of the endocytic pathway. Clathrin-mediated and clathrin-independent vesicle formation is shown. Internalized membrane proteins either are sent back to the membrane by the recycling pathway or as exosomes, or else degraded in the lysosome. See text for details.

MORPHOGENS: GRADIENT FORMATION AND SIGNALING

Morphogens are factors that specify cellular identity in a concentration-dependent manner. Three protein morphogens, Hedgehog (Hh), Wingless (Wg; a Wnt), and Decapentaplegic (Dpp; a TGF-β), are diffusible ligands that form gradients in the embryo and the wing disc. Most evidence suggests that all three protein gradients are formed by extracellular diffusion, which is regulated by HSPG (heparan sulfate proteoglycan) proteins on the outer plasma membrane. Endocytosis regulates the shapes of the gradients as well as signaling by the respective morphogen receptors. There have been several interesting reviews of this topic, including Cadigan (2002), Vincent & Dubois (2002), Entchev & Gonzalez-Gaitan (2002), Gonzalez-Gaitan (2003a,b), Raftery & Sutherland (2003), Seto & Bellen (2004), Tabata & Takei (2004), and Zhu & Scott (2004).

Dynamin: a GTPase known as Shibire in *Drosophila* and a core component of the endocytic machinery required for the pinching off of vesicles

Endosome: a membrane-bound organelle carrying endocytosed materials

Roles of Morphogens in Embryo and Wing Disc Patterning

Each segment in the embryo is 12 cells wide along the anterior-posterior (AP) axis (**Figure 2**). *hh* is expressed by the most

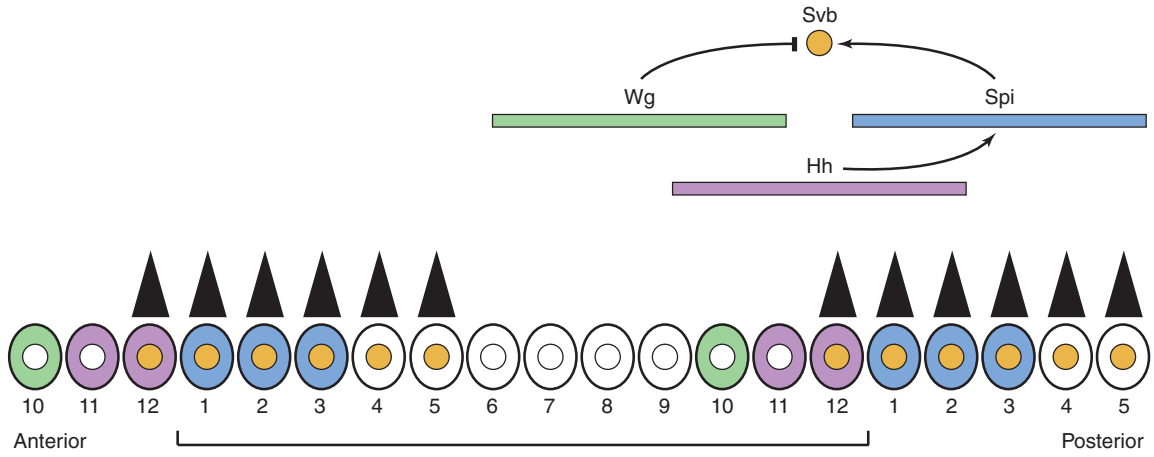


Figure 2.

Morphogens that pattern the Drosophila embryo along the AP axis. Cells 1–12 constitute a segment (*black line*), where cell 1 is anterior and cell 12 is posterior. The black triangles are denticles, and cell nuclei are small circles. Wg secretion by cell 10 and Hh secretion by cells 11 and 12 result in protein gradients spanning the regions shown. Hh pathway activation results in expression and secretion of the Egfr ligand Spitz (Spi) by cells 1–3, which activates expression of the transcription factor Shavenbaby (Svb). Conversely, Wg signaling represses Svb expression, resulting in Svb expression in cells 12 and 1–5. Cells that express Svb form denticles.

posterior two cells of the segment. Hh signaling activates *wg* expression in cell 10 and *rhomboid* (*rho*) in cells 1–3. Wg controls the fates of cells 6–11 by repressing denticle formation in those cells that thus give rise to "naked cuticle." By contrast, *rho* expression results in denticle formation on the cuticles of all the other cells. *dpp* patterns the embryo along the dorsal-ventral (DV) axis. Finally, *dpp* is expressed uniformly in dorsal-lateral cells, and a Dpp activity gradient is formed through a complex process that involves endocytosis.

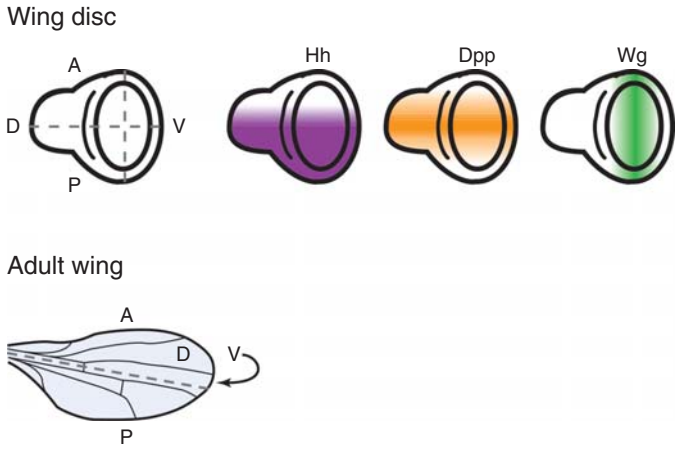


Figure 3

Morphogens that pattern the Drosophila wing disc. The protein gradients formed by expression of three different secreted morphogens are shown. The colored circles indicate those cells that secrete the morphogen. See text for details.

The wing disc is divided into A and P compartments; cells in different compartments do not intermix (**Figure 3**). *hh* is expressed in the P compartment and is secreted into the A compartment, where it induces expression of several target genes, including *dpp*, and patterns the central region of the wing. *dpp* is expressed in a stripe of A cells at the AP compartment border and specifies cell fate along the AP axis of the wing, beyond the central region. Dpp forms a long-range gradient that activates target genes at different distances from the source. *wg* is expressed at the DV boundary, which will become the wing edge, and the Wg gradient activates expression of target genes at different distances from the DV boundary.

Dpp

The Dpp gradient in the wing disc is shaped by endocytosis. Dpp gradient formation in the wing is a consequence of controlled protein dispersal from the cells that secrete it and of protein removal from the extracellular space. There has been controversy in the literature regarding whether Dpp is dispersed by rounds of endocytosis and secretion (planar transcytosis) or by extracellular diffusion (Entchev et al. 2000; Teleman & Cohen 2000; Teleman et al. 2001; Entchev & Gonzalez-Gaitan 2002; Gonzalez-Gaitan 2003a,b; Belenkaya et al. 2004; Zhu & Scott 2004). Different mathematical models support either side of the argument (Lander et al. 2002; Eldar et al. 2003, 2004; Kruse et al. 2004). Recent evidence indicates that, at least in the wing, Dpp, like Hh and Wg (see below), is most likely dispersed by extracellular diffusion, which is regulated by HSPG proteins. Endocytosis, however, does play a pivotal role in shaping the gradient (**Figure 4**).

When Dpp secretion sequences are fused to GFP, sGFP does not form an extracellular gradient but merely fills the extracellular space. Thus, GFP cannot form a gradient by simple diffusion alone. However, most Dpp is extracellular, and an extracellular Dpp-GFP gradient that coincides with Dpp activity (activation of an effector protein) has been observed (Belenkaya et al. 2004). Although Dpp-GFP is also seen in *shi*$^{+}$-dependent vesicles, endocytosis appears not to be required for movement of Dpp between cells. *shi*$^{-}$ clones of cells located between the Dpp source and wild-type cells do not block Dpp movement, as they have no effect on expression of Dpp effector proteins in the wild-type cells. Also, when the Dpp-GFP source cells are *shi*$^{+}$ and all other cells in the disc are *shi*$^{-}$, the extracellular Dpp-GFP gradient forms (Belenkaya et al. 2004; see also Entchev et al. 2000).

The difference in behavior between sGFP and Dpp-GFP is likely due to regulation of diffusion by HSPG proteins as well as to endocytosis mediated by the Dpp receptor Thickvein (Tkv), both of which shape the Dpp gradient. Extracellular Dpp-GFP appears to accumulate around the *shi*$^{-}$ clones, suggesting that endocytosis normally downregulates Dpp levels. *tkv*$^{-}$ clones accumulate Dpp-GFP around them, facing the source. In addition, Tkv accumulates on *shi*^{ts}-mutant cells after a brief temperature shift, suggesting that Tkv is normally endocytosed (Belenkaya et al. 2004; see also Entchev et al. 2000).

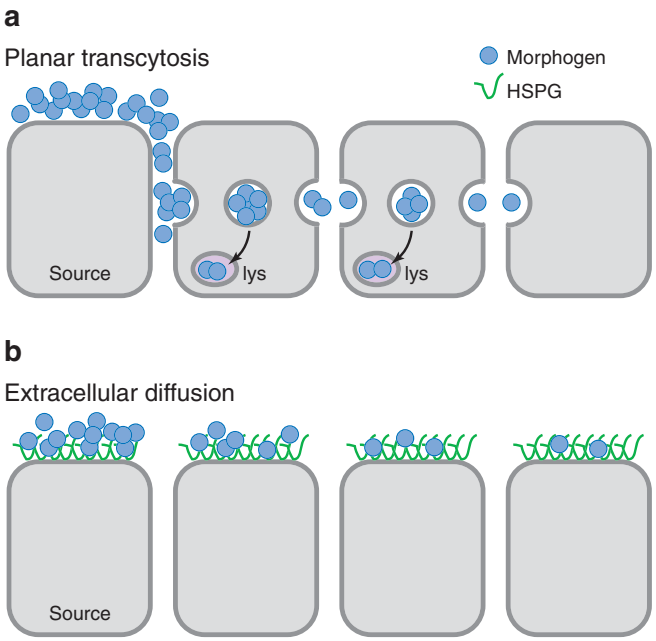


Figure 4

Models for morphogen gradient formation mechanisms. Two extreme models for the mechanism by which morphogen gradients are formed are depicted. In the planar transcytosis model, after secretion from the source cell, the morphogen proteins are dispersed through cell distances by rounds of receptor-mediated endocytosis and resecretion. At each step, some of the internalized morphogen is degraded. In the extracellular diffusion model, morphogen proteins secreted from the source cell are dispersed through cell distances with the guidance of HSPG proteins. Although the morphogen is dispersed extracellularly, this model does not exclude a role for receptor-mediated endocytosis in removing morphogen from the extracellular space and thereby helping to form the gradient. See text for details.

The Dpp gradient in embryos is generated by an opposing Sog gradient whose formation requires endocytosis. In embryos, a dorsally high gradient of Dpp protein is

Rab: small Ras-like GTPases that control membrane fusion and endosome trafficking

Hrs: Hepatocyte growth factor–regulated tyrosine kinase substrate

MVB: multivesicular body

formed by interaction with a ventrally high gradient of a secreted protein called Shortened gastrulation (Sog). Endocytosis is an important mechanism for generating the Sog gradient. Sog antagonizes Dpp activity as well as the activity of a ubiquitous TGF-β called Screw (Scw). Sog, together with another protein called Twisted gastrulation (Tsg), forms complexes with Dpp and Scw, preventing them from binding receptors. Sog and Tsg also facilitate dorsal movement of Dpp. The result is that, although *dpp* is transcribed dorsally and *scw* mRNA is ubiquitous, Dpp and Scw proteins form a DV gradient (Srinivasan et al. 2002). The Sog gradient is formed by specific proteolysis by the metalloprotease Tolloid (Tld) and by dynamin-mediated endocytosis of Sog in dorsal cells. In shi^{ts} embryos shifted briefly to nonpermissive temperature, there is an increase in active extracellular Sog in dorsal cells. As shi^{ts}; tld^{-} double mutants show an additive increase in dorsal Sog levels, endocytosis is independent of the Tld mechanism (Marques et al. 1997, Srinivasan et al. 2002, Mizutani et al. 2005).

Dpp signaling from endosomes. In vertebrate cells, TGF-β signaling occurs from endosomes (Seto et al. 2002; Gonzalez-Gaitan 2003a,b; LeRoy & Wrana 2005). TGF-β ligand binding induces assembly of heteromeric type I/type II ligand complexes. The type II receptor transphosphorylates the type I receptor, which then phosphorylates a transcription factor called an R-Smad, resulting in its nuclear translocation. A key regulator of signaling is SARA (Smad anchor for receptor activation), an adaptor between the type I receptor and R-Smads (Tsukazaki et al. 1998). Sara is a FYVE protein (i.e., it contains an F-Y-V-E amino acid motif), and it binds PI3P and associates with endosomes. Internalization of the receptor complex may be essential for bringing the type I receptor to Sara and R-Smads at the endosome and/or for generating an environment that facilitates phosphorylation of R-Smads (**Figure 5*a***).

Dpp signaling in *Drosophila* may also occur from an endosome. A *Drosophila* Sara homolog has been identified and shown to be involved in Dpp signaling (Bennett & Alphey 2002), but nothing is known yet about its subcellular localization. However, target protein expression is reduced cell autonomously in shi^{-}-mutant clones in the wing disc, indicating that endocytosis is required for Dpp signaling (Belenkaya et al. 2004). Also, Rab5 overexpression stimulates Dpp signaling, whereas blocking Rab5 function has the opposite effect; conversely, Rab7 gain of function attenuates Dpp (Entchev et al. 2000). Finally, studies of the *spinster* (*spin*) gene, which encodes a late endosomal protein, suggest that Dpp signaling in *Drosophila* is endosomal (Sanyal & Ramaswami 2002, Sweeney & Davis 2002). *spin* mutants have overgrown synapses owing to an increase in TGF-β (probably Dpp) signaling caused by a failure to degrade signaling complexes in the lysosome. These mutants also have an increased number of endosomes at the synapse and interact genetically with Dpp receptor mutants in a manner consistent with *spin*, resulting in increased Dpp signaling. Thus, Spin may attenuate Dpp signaling by directing signaling endosomes down a degradative pathway.

Hedgehog

The Hh gradient in the wing disc and embryo is shaped by endocytosis. Like Dpp, Hh protein in the wing disc is dispersed by extracellular diffusion facilitated by HSPGs, and the Hh gradient is shaped by receptor-mediated endocytosis (Han et al. 2004, Torroja et al. 2004). Unlike Dpp, Hh is lipid modified (as is Wg; see below), an observation that has raised the question of how proteins with such strong membrane affinity move extracellularly. Hh (and Wg) travel from cell to cell in the wing by reversible association with lipophorin particles that form on the exoplasmic face of the plasma membrane (Panakova et al. 2005). Lipophorin RNAi disrupts lipid transport and decreases the range

Epsin: eps15-interacting protein, known as Liquid facets in *Drosophila*, is a modular endocytic adapter with a C-terminal ENTH domain and motifs that bind ubiquitin, clathrin, and other proteins

Ub (ubiquitin): a 76-amino-acid polypeptide that is covalently linked via an isopeptide bond to an internal lysine residue of a substrate protein or another ubiquitin moiety

Morphogen: a molecule that determines cell fate in a concentration-dependent manner

Wing disc: *Drosophila* imaginal discs are folded sacs of epithelial monolayers. The imaginal disc cells proliferate toward the end of larval life and evert during metamorphosis to become external structures of the adult fly such as the eyes, antennae, wings, and legs

Compartment: a cell lineage within a tissue that does not intermix with other cell lineages. The wing imaginal disc is divided into anterior and posterior compartments

of Hh signaling and Hh protein dispersal from 11 cells to 6 cells from the source. Hh accumulates at abnormally high levels in the first five rows near the source; most of the accumulation is in endosomes with the Hh receptor Patched (Ptc).

Endocytosis is not required for Hh protein dispersal in the wing. Hh does not accumulate between the source cells and *shi*$^{-}$ clones. In addition, wild-type cells posterior to the *shi*$^{-}$ clones show normal expression of Hh target genes (Han et al. 2004). However, Ptc-mediated endocytosis appears to shape the Hh gradient. Punctate Hh^{+}Ptc^{+}Rab7^{+} structures are usually observed in three to four rows of A compartment cells abutting the AP boundary. These are absent in the *shi* clones, and Hh or Hh-GFP accumulates on the cell membranes of the clones. Also, in *deep orange* (*dor*$^{-}$) clones, Hh^{+}Ptc^{+} vesicles accumulate. These results suggest that Hh bound to Ptc normally is internalized and degraded (Chen & Struhl 1996, Torroja et al. 2004).

Similarly, in the embryo, endocytosis shapes the Hh gradient. Cytoplasmic Hh-containing particles are Ptc dependent, suggesting that Hh bound to Ptc is internalized. Also, when the Hh signal receiving cells express *shi*^{DN} (*shi* dominant negative), the spreading of Hh is too broad. Moreover, there is much less Hh observed in cells that overexpress Ptc. Finally, most Hh^{+}Rab7^{+} vesicles are also Ptc^{+}, and in *ptc*$^{-}$ cells there is no Hh/Rab7 colocalization. Taken together,

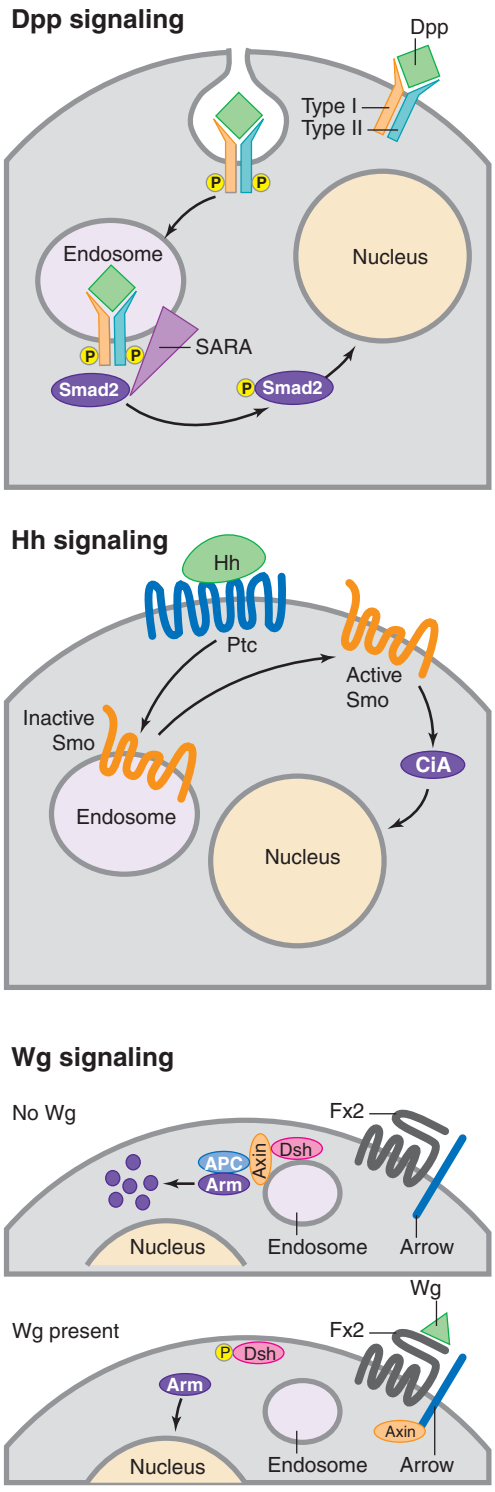


Figure 5

Models for roles of endocytosis in morphogen signaling pathways. Possible roles for endocytosis in three morphogen signaling pathways are depicted. (*a*) Dpp signaling may occur from Sara-containing endosomes. (*b*) Hh signaling results in Smo activation, which may require Smo relocalization from an endosome to the plasma membrane. (*c*) Activation of Wg signaling results in movement of Dsh and Axin to the plasma membrane, which results in stabilization of Arm. See text for details.

these results suggest that Ptc targets Hh to the lysosome (Gallet & Therond 2005).

Planar transcytosis: a model for dispersal of protein morphogens in which the protein, once secreted from the source cell, spreads through cell distances by repeated rounds of endocytosis and resecretion

Shi: Shibire

Endocytosis and endosome trafficking may be integral elements of Hh signaling in the wing disc. Hh/Ptc binding activates signaling by relieving inhibition of the activity of another membrane protein, Smoothened (Smo). Ptc is a 12-pass membrane protein, and Smo is a 7-pass membrane protein. The mechanism of the Ptc/Smo interaction is unclear (see Hooper & Scott 2005 for discussion). There are two general kinds of models: those in which Ptc and Smo interact directly, and those in which they interact indirectly. In both models, relocalization of Smo from endosomes to the plasma membrane is required for signaling. However, there are also contradictory data suggesting that endocytosis is dispensible for Hh signaling.

In the absence of Hh, Smo is observed mainly in endosomes, and Ptc is both in endosomes (most Ptc does not colocalize with Smo) and at the cell surface. In the presence of Hh, Ptc goes to lysosomes, and Smo goes to the plasma membrane (Denef et al. 2000, Incardona et al. 2002, Evans et al. 2003, Zhu et al. 2003, Stegman et al. 2004). The activity of Smo coincides with its localization at the plasma membrane: GFP-Smo relocates to the cell surface when Hh is added to cells, Ptc inactivation results in Smo at the plasma membrane in the absence of Hh, Smo with activating mutations is at the plasma membrane constitutively, Smo modified so that it is always at the cell surface is active constitutively, and Smo at the plasma membrane is inactive only when Ptc is also retained there (Zhu et al. 2003, Nakano et al. 2004).

If Hh and Smo interact indirectly, a likely model is that Hh/Ptc binding at the plasma membrane results in relocalization of Smo-containing endosomes to the plasma membrane and thus in Smo activation (**Figure 5*b***). In the direct interaction model, Ptc and Smo are initially together at the membrane, and Hh/Ptc binding separates Ptc from Smo. This may occur by internalization of Hh/Ptc or by internalization of Hh/Ptc/Smo and then trafficking of Hh/Ptc to the lysosome and Smo back to the membrane.

In *Drosophila* there may be more evidence in favor of the indirect model. In the absence of Hh, Smo is not observed at the membrane with Ptc (see above). Also, a mutant Ptc protein that cannot be internalized is able to activate Hh signaling (Torroja et al. 2004). Most of the evidence for direct interaction between Ptc and Smo comes from experiments with Shh in vertebrate cells (Incardona et al. 2002, Stegman et al. 2004). Ptc and Smo colocalize extensively before Shh treatment. In the presence of Shh, they enter endosomes together. Subsequently, Smo endosomes segregate away from Shh/Ptc endosomes, which go to the lysosome. The SSD (sterol-sensing domain) of Ptc, which may target Ptc to a specific membrane compartment, is not required for Hh/Ptc binding but is required for Ptc to inhibit Smo. This suggests that Hh binding must inactivate the SSD and thereby divert Ptc into a distinct trafficking route, possibly to get it away from Smo (Chen & Struhl 1996, Martin et al. 2001, Strutt et al. 2001, Kuwabara & Labouesse 2002).

One major caveat to all these models is that they all require endocytosis of Smo, and Hh signaling can be activated in *shi*$^{-}$ wing disc cells (Han et al. 2004, Torroja et al. 2004) and in embryos (Gallet & Therond 2005). One way to resolve this apparent paradox is if the *shi* alleles do not abolish endocytosis completely.

If Ptc does regulate Smo subcellular localization indirectly, what might be the mechanism? Smo is phosphorylated as a result of Hh/Ptc binding, but it is unclear whether that is a cause or an effect of plasma membrane localization (Denef et al. 2000). It may be relevant that Ptc has an RND (resistance-nodulation-cell division) permease domain that is required for it to inhibit Smo. One idea is that Ptc at the membrane may inhibit endosomal Smo relocalization to the plasma membrane through regulation of the entrance into the cell of a small molecule, for example, an

activator of a Smo phosphatase. Hh/Ptc binding may block the permease activity, resulting in Smo phosphorylation (Martin et al. 2001, Strutt et al. 2001, Taipale et al. 2002, Johnson et al. 2002).

Targeting release of Hh-containing endosomes to specific membrane compartments in the embryo correlates with target gene activation. In embryos, the region of the cell from which Hh is secreted governs the response of the receiving cells (Gallet et al. 2003). For *wg* expression to be activated, secretion of Hh from the source cell in an anterior direction must be apical. The formation of the apical endosomes requires cholesterol modification of Hh; an SSD-containing protein called Dispatched; and HSPG proteins, which mediate Hh spreading. By contrast, posterior signaling (*rho* activation) does not require cholesterol or HSPGs, and basolateral Hh secretion is sufficient. These curious observations may suggest that receptors and/or signaling complexes are localized differently in anterior versus posterior cells.

Wingless

Wg gradient formation in embryos requires endocytosis and asymmetric trafficking to lysosomes, and perhaps transcytosis. Most evidence suggests that in the embryo, the role of endocytosis is to shape the Wg gradient. The Wg gradient emanates from a single row of cells in each segment and is initially symmetrical. The gradient becomes asymmetrical [one cell posterior and four cells anterior (**Figure 2**)] owing to increased Wg degradation in cells posterior to the source (Dubois et al. 2001). The main evidence for this model comes from experiments in which an HRP-Wg fusion protein is expressed as the sole source of Wg protein. In contrast to the Wg portion of the fusion protein, HRP (horse radish peroxidase) is stable in late endosomes and thus serves as a marker for endosomes in which HRP-Wg was present. In cells anterior to the HRP-Wg source, vesicles (presumed to be early endosomes) contain both HRP and Wg, whereas vesicles (presumably late endosomes) in posterior cells contain HRP only. Also, posterior cells have four times more HRP-containing MVBs than do anterior cells. Moreover, in *dor* mutants, in which endosomes cannot fuse with the lysosome, Wg accumulates in giant MVBs. Finally, in *chc* (*clathrin heavy chain*) mutants, in which no clathrin-dependent endocytosis can take place, Wg disperses further from the source and accumulates at the plasma membranes of posterior cells. Genetic experiments that implicate HSPG proteins in Wg gradient formation support the hypotheses that Wg is dispersed through extracellular diffusion and that its dispersal limited by endocytosis (Desbordes et al. 2005).

chc: clathrin heavy chain

There is, however, contradictory evidence in favor of a role for endocytosis (planar transcytosis) in Wg dispersal in embryos. In *shi*- mutant embryos, extracellular Wg accumulates around the source cell and does not spread (Bejsovec & Wieschaus 1995, Moline et al. 1999). The apparent contradiction between these results and the *chc* experiments described above (Dubois et al. 2001) remains unresolved.

The Wg gradient in the wing disc is shaped by endocytosis. Like Dpp and Hh, Wg protein in the wing disc is dispersed by extracellular diffusion facilitated by HSPGs, and the Wg gradient is shaped by receptor-mediated endocytosis. The main evidence that endocytosis is dispensible for Wg dispersal is that Wg spreads extracellularly through *shi*$^{-}$ cell clones (Strigini & Cohen 1999). Recent experiments indicate that like Hh, Wg moves extracellularly in lipophorin particles (Panakova et al. 2005). These particles, also called argosomes, were thought originally to be plasma membrane–containing vesicles that move Wg from cell to cell by planar transcytosis (Greco et al. 2001). In cells that express lipophorin RNAi, Wg spreads over shorter distances, and long-range signaling is disrupted.

The Wg gradient is shaped by two receptors: Frizzled 2 (Fz2), a seven-pass transmembrane receptor, captures Wg and provides an internalization signal, whereas Arrow, a single-pass transmembrane receptor related to the LDL receptor, provides a lysosomal trafficking signal (Piddini et al. 2005). Wg and Fz2 are observed together in endocytic vesicles, and Wg accumulates intracellularly in *dor* or *hrs*-mutant cells, suggesting that Wg is normally degraded in lysosomes. Overexpression of Fz2-gpi, a Fz2 protein that can bind extracellular Wg but cannot be internalized, results in less Wg endocytosis and a more extensive Wg gradient. This result suggests that Wg/Fz2 endocytosis restricts the range of Wg signaling. However, overexpression of wild-type Fz2 results in Wg stabilization (Cadigan et al. 1998). This paradox was resolved by the observation that co-overexpression of Arrow suppresses the stabilization effect on Wg of Fz2 (Piddini et al. 2005). How Arrow targets Wg to the lysosome is unknown.

Wg signaling requires relocalization of Dishevelled and Axin. The activation of Wg signaling is independent of endocytosis; overexpression of Wg in *chc*$^{-}$ embryos results in naked cuticle, indicative of Wg signaling (Dubois et al. 2001). However, intracellular trafficking of vesicles containing Dishevelled (Dsh) protein determines the specificity of the signal.

Fz and Dsh mediate Wg signaling through the Armadillo (Arm) protein and also are part of an otherwise distinct pathway for determining planar cell polarity (PCP) (Wharton 2003, Seto & Bellen 2004). When at the plasma membrane, Dsh functions in the PCP pathway, and when in endosomes, in Wg signaling (**Figure 5*c***). Dsh mutants with impaired plasma membrane association affect PCP but not Wg signaling, and Dsh mutants with impaired vesicular localization disrupt Wg signaling only (Yanagawa et al. 1995, Rothbacher et al. 2000, Axelrod et al. 1998, Boutros et al. 1998, Axelrod 2001, Penton et al. 2002).

Another protein in the Wg signaling pathway, Axin, is also regulated by endosomal trafficking (**Figure 5*c***). Axin is a component of a complex that phosphorylates Arm, leading to Arm degradation (Hamada et al. 1999, Willert et al. 1999, Cliffe et al. 2003). In response to Wg signaling, and dependent on Dsh, Axin relocates from endosomes to the plasma membrane (Cliffe et al. 2003). Axin relocalization correlates with Arm stabilization, suggesting that Axin at the plasma membrane cannot phosphorylate Arm.

NOTCH SIGNALING

Notch Pathway

Notch signaling regulates numerous developmental decisions in probably all *Drosophila* tissues. The Notch receptor is a single-pass transmembrane protein that in *Drosophila* has two different transmembrane ligands, Delta (Delta) and Serrate (Ser). Ligand binding activates cleavage of the Notch extracellular domain (S2 cleavage) by an ADAM protease, which is then followed by cleavage of the intracellular domain (S3 cleavage) by the γ-secretase complex. The released intracellular domain enters the nucleus, where, along with Suppressor of Hairless [Su(H)], it regulates target gene expression (**Figure 6**). (See LeBorgne et al. 2005a for a recent review of Notch signaling and endocytosis.)

The role of endocytosis in *Drosophila* Notch signaling has been studied in cell culture (S2 cells) and in vivo, mainly in wing and eye imaginal discs and in developing bristles (**Figure 7**). In the wing disc, signaling by Delta and Ser that specifies the wing margin at the DV compartment boundary has been analyzed. The role of endocytosis in Notch signaling in the eye disc has been best defined in the ommatidial preclusters that emerge just posterior to the morphogenetic furrow. Delta signaling from photoreceptor R2/5 and R3/4 precursors prevents adjacent precluster cells from becoming ectopic R-cells. Bristles originate from a sensory organ precursor (SOP)

cell called pI that divides to give rise to a neural precursor (pIIb) and a nonneural cell (pIIa) whose fates are determined by Notch/Delta signaling. Asymmetric distribution within pI of cytoplasmic determinants that regulate endocytosis results, after mitosis, in pIIb sending a Delta signal that activates Notch in pIIa.

Endocytosis of Notch Ligands by Signaling Cells Is Required for Notch Activation in Receiving Cells

A surprising observation is that Notch activation in the receiving cell requires internalization of the ligand by the signaling cell (Seugnet et al. 1997; Parks et al. 2000; Pavlopoulos et al. 2001; Lai et al. 2001; Itoh et al. 2003; Le Borgne & Schweisguth 2003; Li & Baker 2004; Overstreet et al. 2004; Wang and Struhl 2004, 2005). In the wing and eye discs, three key players in this process are known: two RING (Really Interesting New Gene) finger E3 proteins—Neuralized (Neur) and *Drosophila* Mind bomb (D-mib)—and Liquid facets (Lqf), the *Drosophila* homolog of endocytic epsin. In the simplest model for the mechanism of ligand internalization, the intracellular domain of Delta or Ser is monoubiquitinated by either Neur or D-mib, and Ub serves as a signal for endocytosis (**Figure 8**). Neur and D-mib are functionally interchangeable but expressed in complementary cells (Lai et al. 2005, Le Borgne et al. 2005b, Pitsouli & Delidakis 2005, Wang & Struhl 2005). Lqf, an endocytic adaptor protein, is essential specifically for clathrin-mediated endocytosis of Delta (Cadavid et al. 2000; Overstreet et al. 2003, 2004; Wang & Struhl 2004).

Delta and Ser appear to be internalized by the signaling cell because they are observed in vesicles that are *shi*$^{+}$ dependent (Kooh et al. 1993, Parks et al. 1995, Kramer & Phistry 1996). The results of many different experiments connect Delta and Ser endocytosis with signaling. First, mutant Delta proteins that cannot be internalized cannot signal (Parks et al. 2000). Second, in *neur*- and *D-mib*-mutant cells, Delta and Ser accumulate on the plasma membrane owing to a failure to be internalized, and genetic analysis indicates that *neur* and *D-mib* are required in the signaling cells. In SOPs, Neur is asymmetrically localized within pI and is inherited exclusively by the neural precursor cell pIIb. When pI is *neur*$^{-}$, two pIIb cells are produced because pIIb cannot activate Notch in its neighbor, and Delta accumulates on the membranes of both cells. Antibody uptake experiments show that Delta fails to be internalized in the absence of Neur. Mosaic analysis indicates that the requirement for *neur*$^{+}$ is in pIIb (LeBorgne & Schweisguth 2003). Similar conclusions were reached in wing and eye discs for *neur* and *D-mib* function in internalization of both Delta and Ser (Pavlopoulos et al. 2001, Lai et al. 2001, Li & Baker 2004, Overstreet et al. 2004, Le Borgne et al. 2005b, Pitsouli & Delidakis 2005, Wang & Struhl 2005). Finally, clonal analysis in the eye and wing discs show that *lqf*$^{+}$ is required in the signaling cells to activate Notch in the receiving cells, and Delta or Ser accumulates on the plasma membranes of *lqf*-mutant cells (Overstreet et al. 2004; Wang & Struhl 2004, 2005). *lqf*$^{+}$ function requires *neur*$^{+}$ or *D-mib*$^{+}$

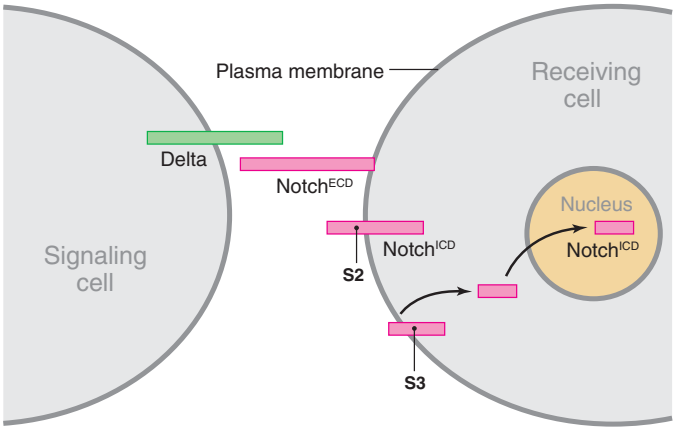


Figure 6

Notch signaling pathway. Upon ligand binding, Notch is cleaved once extracellularly (S2) and once intracellularly (S3). Intracellular cleavage allows a fragment of Notch to enter the nucleus, where it regulates transcription of target genes. Endocytosis is important in both the signaling and receiving cells. See text for details.

Lqf: Liquid facets

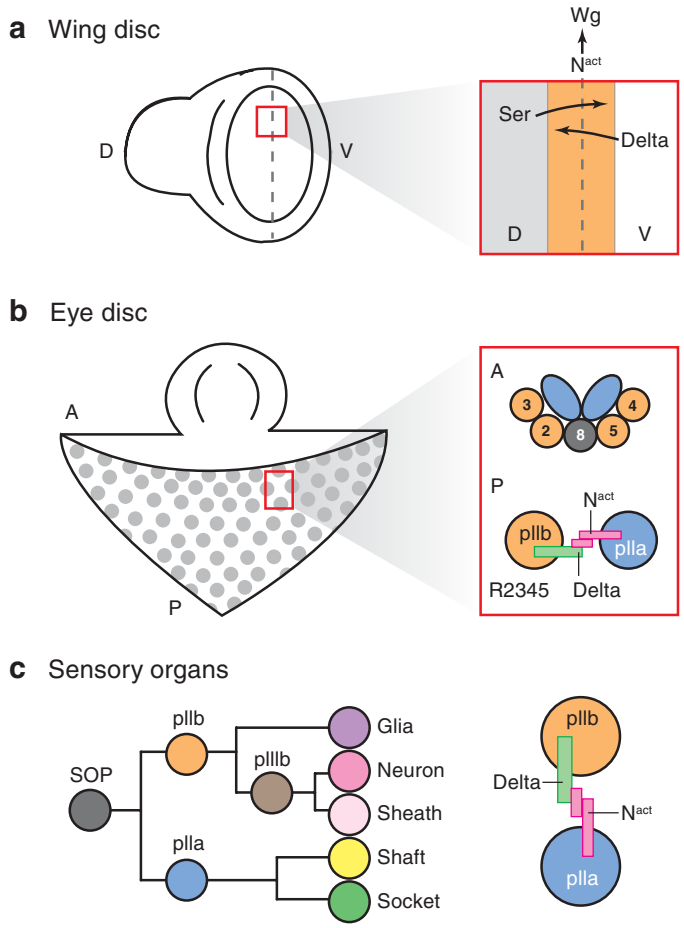


Figure 7

Notch signaling events during Drosophila development, for which Notch ligand endocytosis by the signaling cell has been shown to be required for signaling. (*a*) The DV boundary of the wing disc is defined by Notch activation (N^{act}), which turns on Wg expression. Notch activation is confined to a stripe of cells through differential expression and activation of two different Notch ligands, Serrate (Ser) and Delta (Dl). Ser is expressed by dorsal cells but is an active ligand only for Notch expressed by ventral cells. Conversely, Dl is expressed by ventral cells but is an active Notch ligand only in dorsal cells. Signaling by either Dl or Ser requires Lqf-mediated endocytosis of ligand by the signaling cells. (*b*) Precluster cells not destined to become photoreceptors (*blue*) are excluded from developing ommatidia by activation of Notch. Photoreceptor cell precursors R2/5 and R3/4 send a Dl signal to the blue cells that depends on Lqf-mediated endocytosis. (*c*) In the peripheral nervous sytem, an SOP divides, and one daughter cell (pIIb) is specified as a neural precursor and prevents neural development of the other daughter cell (pIIa) through Notch/Delta signaling. Delta signaling by pIIb and Notch activation in pIIa require Delta internalization by pIIb. See text for details.

activity, which suggests that Lqf, which has Ub interaction motifs (UIMs), may recognize mono-Ub on the ligand intracellular domain (Wang & Struhl 2004).

Why is ligand internalization by the signaling cell required for signaling? Many models have been proposed; they fall into two classes (see Le Borgne et al. 2005a for a review) (**Figure 9**). In the first class, one supposes that ligand is internalized after binding to the Notch receptor. In this case, ligand endocytosis may facilitate S2 cleavage, perhaps by exerting force on the cleavage site. Alternatively, ligand may be internalized prior to receptor binding. In this case, ligand would be processed endosomally into the active form and either secreted as exosomes or recycled back to the plasma membrane.

The observation that Delta and the Notch extracellular domain (N^{ecd}) sometimes can be observed to colocalize in endosomes supports the idea that Delta internalization facilitates S2 cleavage (Parks et al. 2000). The exosome model may explain the long-range activity of Delta that is sometimes observed (De Joussineau et al. 2003, Le Borgne & Schweisguth 2003). Evidence is accumulating in favor of the recycling model, which was first proposed as a result of an observation on the function of Lqf. In the wing and eye discs, most of the endosomal Delta observed is internalized in a *lqf*$^+$-independent manner. This led to the proposal that Lqf-dependent internalization of Delta routes it to a specific endosomal pathway for activation and recycling. The idea is supported by an experiment in which replacement of the Delta intracellular domain with an LDL receptor fragment that mediates recycling results in *lqf*$^+$-independent Delta signaling (Wang & Struhl 2004).

Experiments in SOPs with *rab11* and *sec15* mutants lend more support to the recycling model (**Figure 10**). pIIb is ensured to become the Delta signaling cell by its exclusive inheritance of Neur (see above) and Numb (see below). Similarly, there is a mechanism to ensure that pIIa becomes the receiving

cell: It involves regulation of recycling endosome formation (Emery et al. 2005, Jafar-Nejad et al. 2005). Formation of Rab11-containing endosomes is blocked in pIIa by inhibition of recruitment of necessary factors, one of which is a protein called Nuclear fallout (Nuf), to the centrosome. Rab11-containing endosomes, some of which contain Delta, form near the centrosome only in pIIb. Expression of a dominant-negative Rab11 in SOPs causes Delta to accumulate in large Hrs-containing vesicles. The asymmetry of Rab11 endosome formation was connected to Delta signaling and cell fate specification with the following experiment: pIIa may be transformed into a Delta signaling cell (pIIb) by co-overexpressing Nuf and constitutively active Rab11, which induces recycling endosome formation (Emery et al. 2005). Sec15, a homolog of a yeast protein required for secretory vesicle transport, colocalizes with Rab11 in pI and both progeny cells. Sec15 is required for Delta trafficking in pIIb and, presumably through an effect on Delta signaling, Notch activation in pIIa. *sec15* mutants undergo pIIa-to-pIIb transformations because Notch is not activated. In *sec15* mutants, Rab11 is upregulated and distributed abnormally, and Delta$^+$/Rab5$^+$/Hrs$^+$ vesicles accumulate aberrantly. These results suggest that although Sec15 is distributed symmetrically in pIIa and pIIb, it is required in pIIb for Delta endosome recycling (Jafar-Nejad et al. 2005, Wu et al. 2005).

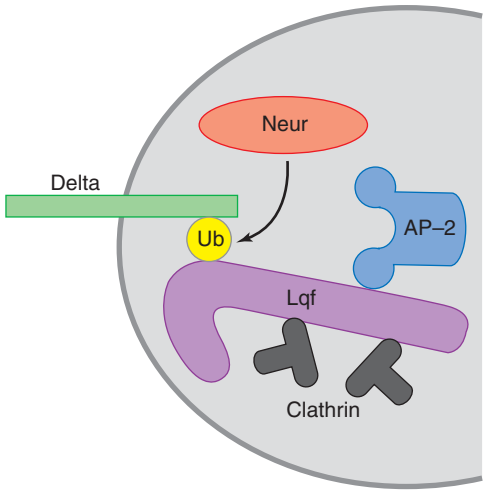


Figure 8

Mechanism of Delta internalization. A simple model showing the proteins involved in Delta (or Ser) internalization that leads to signaling are shown. In complementary cell types, either Neur or D-mib ubiquitinate Delta. Lqf (epsin) is essential for endocytosis of Delta that leads to signaling. Lqf binds the plasma membrane with its N terminus, and it also binds Ub, clathrin, and AP-2. It is not clear why Lqf is required specifically for Delta signaling.

Inhibition of Notch Activation in Signaling Cells by Endocytosis

In SOPs, there are two different pathways in pIIb for ensuring that it becomes the signaling cell; both involve endocytosis. Neur, an essential activator of Delta, is inherited by pIIb only (see above). Numb, a negative regulator of Notch, and α-adaptin, an AP-2 subunit, are also segregated to pIIb (Rhyu et al. 1994, Berdnik et al. 2002, Le Borgne & Schweisguth 2003). The idea is that Numb and α-adaptin internalize Notch and/or a four-pass transmembrane protein called Sanpodo (Spdo), which results in Notch and/or Spdo degradation and the prevention of Notch activation in pIIb (**Figure 10**) (Dye et al. 1998, Skeath & Doe 1998, O'Connor-Giles & Skeath 2003, Hutterer & Knoblich 2005).

In *numb* mutants, Notch is activated in the presumptive pIIb, transforming it into pIIa (Guo et al. 1996). Numb binds to the intracellular domain of Notch and α-adaptin, suggesting that the function of Numb is to endocytose Notch in pIIb (Guo et al. 1996, Berdnik et al. 2002). However, as Notch levels at the plasma membrane appear the same in pIIa and pIIb, the target of Numb may be Spdo.

The role of Spdo in Notch/Delta signaling is controversial. In *spdo* mutants, Notch is not activated in the presumptive pIIa, so it is transformed into pIIb (Hutterer & Knoblich 2005, Roegiers et al. 2005). Several studies indicate that Spdo is at the plasma membrane in pIIa but is exclusively endosomal in pIIb, which led to a model in which Spdo at the membrane is required for Notch activation in pIIa. It follows, then, that Numb may

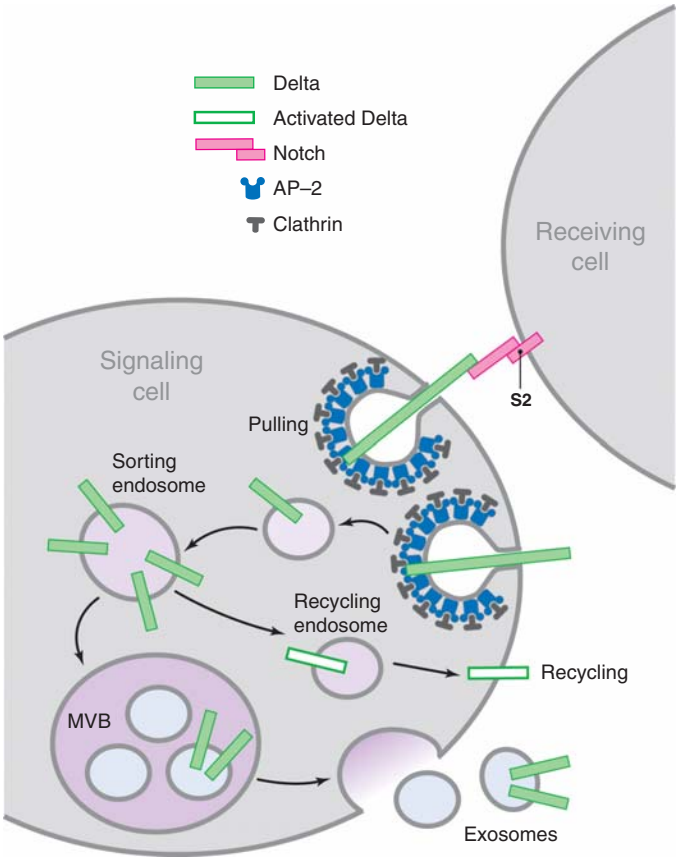


Figure 9

Models for why Delta endocytosis by the signaling cells is required for signaling. Delta internalization by the signaling cell may facilitate S2 cleavage (pulling). Alternatively, Delta endocytosis may enable the activation of Delta in an endosome as well as its return to the plasma membrane in active form (recycling). In a similar model, Delta internalization allows formation of Delta-containing exosomes. See text for details.

prevent Notch activation in pIIb by promoting Spdo internalization. Numb/Spdo complexes have been identified, and in *numb* or α-*adaptin* mutants, Spdo is not internalized and instead accumulates at the plasma membrane (Hutterer & Knoblich 2005, Langevin et al. 2005, Roegiers et al. 2005). A different study argues that Spdo is required for Notch activation not in pIIa but in pIIb for Delta recycling. Jafar-Nejad et al. (2005) observe Spdo at the membrane in both pIIa and pIIb, but only Spdo$^+$ Delta$^+$ Rab11$^+$ vesicles in pIIb. Moreover, in *sec15* mutants, in which Delta cannot be activated because of a defect in recycling endosome trafficking (see above), trafficking of Spdo$^+$ endosomes is aberrant, and Spdo no longer colocalizes with Rab11. These authors suggest that Spdo functions in Delta recycling and that a defect in Spdo localization in *sec15* mutants may contribute to the *sec15*-mutant phenotype.

Endocytosis and Endosomal Trafficking of Notch in Signal-Receiving Cells Regulates Ligand-Independent Notch Activation

Notch internalization may also lead to signaling in a ligand and Su(H)-independent manner from endosomes (**Figure 11**). Biochemical experiments, genetic interactions, and imaging in the wing disc show that this event is regulated by three different E3 ubiquitin-ligase proteins. Two HECT (homologous to E6-AP C terminus)-domain E3s, Nedd4 (neural precursor cell–expressed developmentally downregulated 4) and Su(dx) (Suppressor of deltex), target Notch for lysosomal degradation, and Dx (Deltex), a RING E3, antagonizes their activities, shunting Notch into an endosomal signaling pathway. All three E3s are present in endosomes, but they may also be present at the plasma membrane. Thus, it is not clear where the ubiquitination events occur.

Notch appears to be internalized and sent through the degradative pathway. Notch^{icd} and Notch^{ecd} are found together in Hrs$^+$ endosomes, indicating that nonactivated (noncleaved) Notch is internalized (Fehon et al. 1990, Wilkin et al. 2004). Also, in *hrs*$^-$ cells in which lysosomal degradation is blocked, Notch accumulates in large vesicles (Jekely & Rorth 2003).

Notch is targeted for lysosomal degradation by Nedd4 and Su(dx), which prevent Notch from entering the recycling pathway. Nedd4 ubiquitinates Notch^{icd}. Loss-of-function *Nedd4* or *Su(dx)* mutants have little effect on Notch localization or signaling,

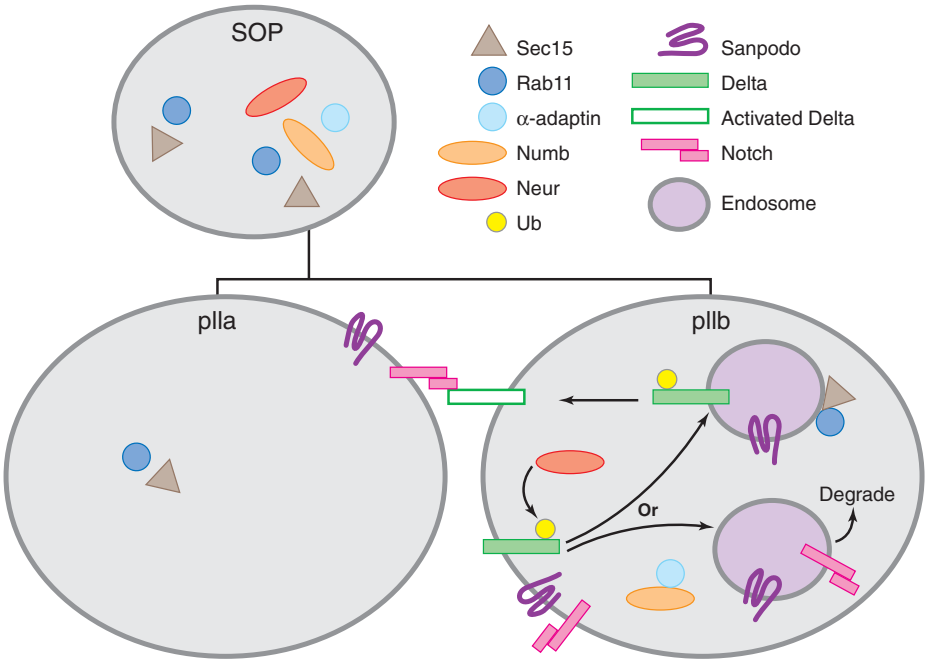


Figure 10

How the SOP daughter cell pIIb becomes the Delta signaling cell. Delta signaling by pIIb activates Notch in pIIa, which prevents neural determination of pIIa. Several different mechanisms ensure that pIIb becomes the signaling cell and that pIIa becomes the receiving cell. First, Numb, Neur, and α-adaptin segregate asymmetrically into pIIb and internalize Notch and Sanpodo, leading to their degradation. Second, Rab11-containing recycling endosomes are able to form only in pIIb. Third, Sanpodo may be present at the plasma membrane only in pIIa, in which it is required for Notch activation. Alternatively or additionally, Sanpodo may stimulate Delta internalization and activation in pIIb. See text for details.

perhaps because of redundancy with a third Nedd4 family member, Dsmurf. However, overexpression of Nedd4 or Su(dx) phenocopies partial loss of Notch activity and results in Notch accumulation in $Rab7^+$ vesicles. Moreover, overexpression of a dominant-negative Su(dx) protein leads to missorting of Notch into $Rab11^+$ vesicles (Fostier et al. 1998, Cornell et al. 1999, Mazaleyrat et al. 2003, Sakata et al. 2004, Wilkin et al. 2004).

Dx promotes ligand-independent and Su(H)-independent Notch signaling from endosomes. Dx binds Notch and promotes Notch relocation from the plasma membrane to vesicles. Notch target gene expression is reduced in *dx* mutants, whereas *dx* overexpression leads to cell-autonomous, Notch-dependent activation of Notch target genes independent of Delta, Ser, and Su(H) (Hori et al. 2004). Experiments with Notch alleles that produce truncated receptors also support the existence of a Dx-dependent, Su(H)-independent signal (Ramain et al. 2001). In addition, expression of dominant-negative Rab5 inhibits Dx-mediated Notch activation, suggesting that ligand-independent Notch signaling requires that Notch accumulate in late endosomes (Hori et al. 2004).

Dx activity antagonizes that of Nedd4. Dx overexpression leads to increased Notch accumulation in vesicles in vivo, suggesting that Dx antagonizes Notch degradation (Hori et al. 2004). Overexpression of dominant-negative Nedd4 in S2 cells promotes ligand-independent activation of Notch target genes, and this effect is enhanced by simultaneous

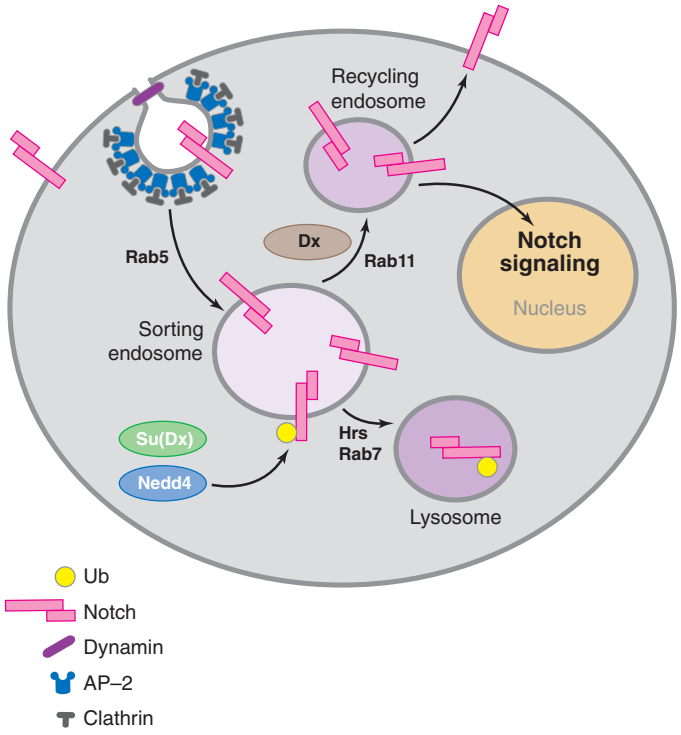


Figure 11

Ligand-independent Notch signaling from endosomes. Internalization and sorting of Notch is regulated by three ubiquitin ligases [Nedd4, Su(Dx), and Dx]. Notch sorted to recycling endosomes may signal from the endosomes in a ligand-independent manner. See text for details.

overexpression of Dx. Thus, dominant-negative Nedd4 prevents Notch-containing endosomes from entering the degradative pathway, and Dx targets them to an endosomal environment in which Notch can signal (Sakata et al. 2004).

PROTEIN RECEPTOR TYROSINE KINASE SIGNALING

The role of endocytosis in receptor tyrosine kinase (RTK) signaling has been studied mainly in vertebrate cells, and there are many reviews of this work (Carpenter 2000, Wiley & Burke 2001, Sorkin & von Zastrow 2002, Dikic 2003, Dikic & Giordano 2003, Miaczynska et al. 2004). Endocytosis attenuates signaling as well as bringing active signaling complexes in endosomes to intracellular locations of effector proteins. In addition, signaling can regulate the endocytic machinery itself. Most likely these mechanisms are conserved in *Drosophila*. Analysis of RTK signaling in a few developmental contexts in *Drosophila* has revealed some novel mechanisms for regulation by endocytosis.

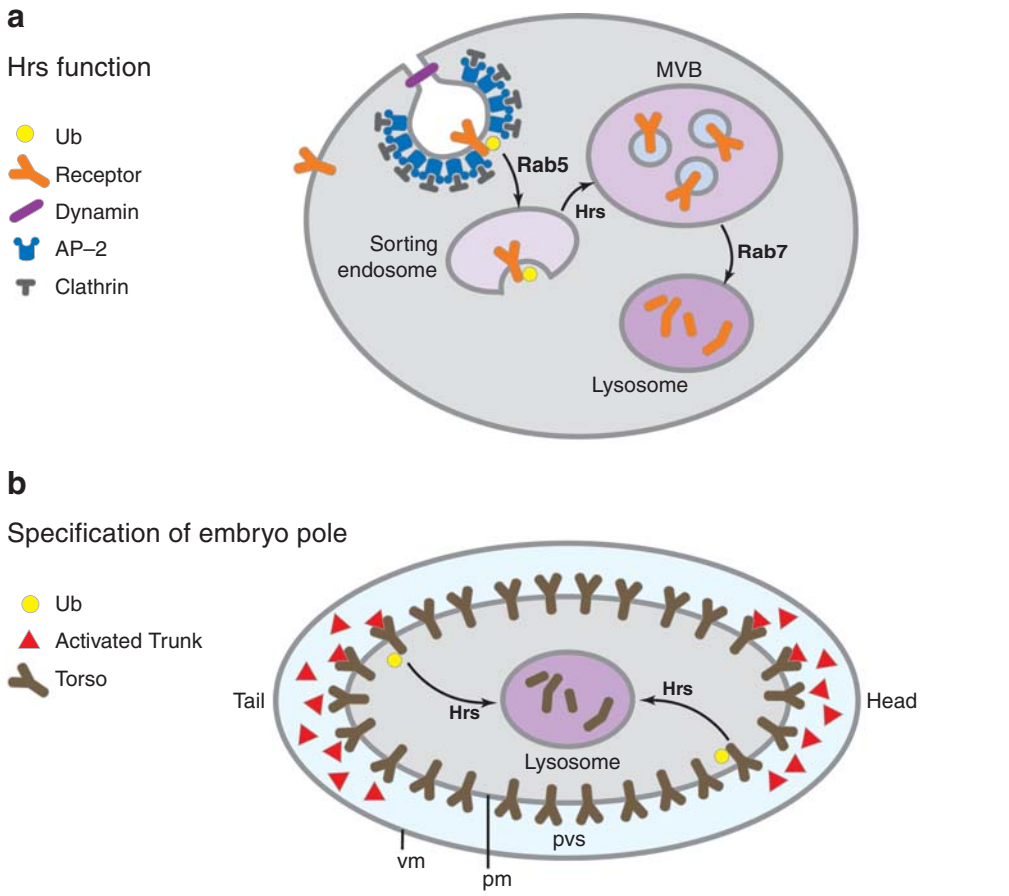
Hrs Is Required for Attenuation of RTK Signaling in Embryos

Analysis of *hrs* mutants has shown that signaling by *Drosophila* RTKs is attenuated by endocytosis. Hrs is homologous to yeast Vps27p (vacuolar protein sorting 27p), which regulates trafficking of endosomes to the vacuole, the yeast equivalent of the lysosome (reviewed in Raiborg et al. 2003). As mentioned above, Hrs has a FYVE domain that binds endosomal membranes, and it also has a UIM. Electron microscopy of *hrs*-mutant larval garland cells (large cells with a high rate of endocytosis) shows that hrs^+ is required for membrane invagination in MVB formation, a prerequisite for lysosomal targeting (Lloyd et al. 2002). This activity of Hrs is needed to downregulate signaling from two RTKs, Torso and Egfr (epidermal growth factor receptor) (**Figure 12**).

Torso signaling is required for specification of the head and tail (the anterior-most and posterior-most regions, respectively) of the embryo (Li 2005). The Torso receptor is expressed ubiquitously but binds a diffusible ligand secreted at the poles. Ligand binding activates the Ras/MAPK (mitogen activated protein kinase) pathway. In *hrs* mutants,

Figure 12

Regulation of RTK signaling by Hrs. (*a*) Hrs is required for formation of internal vesicles in MVBs, which fuse with the lysosome. Hrs-dependent degradation of activated RTKs is a mechanism to attenuate signaling. (*b*) The termini of the embryo are specified by activation of the RTK Torso by its ligand Trunk. Hrs-dependent degradation of activated Torso receptors away from the termini controls patterning. vm, vitelline membrane; pm, plasma membrane; pvs, perivitelline space. (*c*) The embryonic ventral ectoderm is specified by Egfr activation by the ligand Spitz. Hrs-dependent degradation of activated Egfr prevents broadening of the ventral region of the embryo. See text for details.



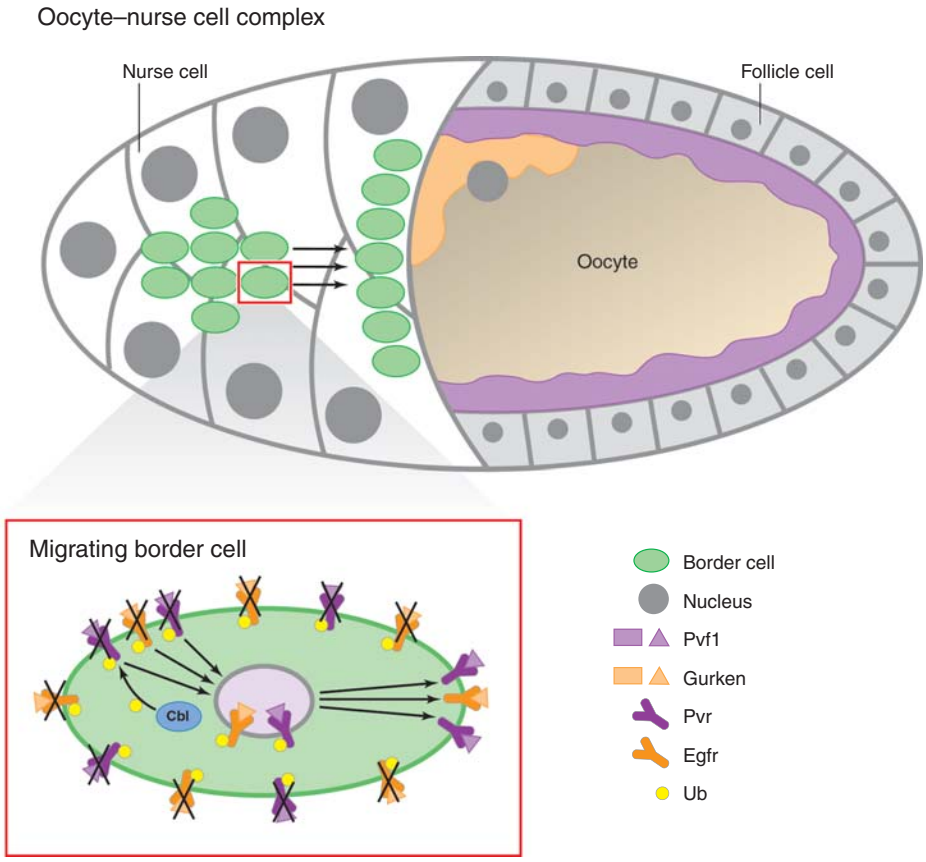


Figure 13

Control of cell migration by Cbl-dependent RTK endocytosis. Drosophila egg chambers consist of somatic cells (follicle cells) and 16 germ line cells (15 nurse cells and 1 oocyte). Particular follicle cells called border cells (*green*) migrate to the oocyte, guided by the RTKs Egfr and Pvr, whose ligands are expressed in the oocyte. The direction of border cell migration is controlled by concentrating the RTKS to the cell cortex at the leading edge of movement. This is achieved through endocytosis of receptors not at the leading edge and through their recycling to cortex at the edge of movement. See text for details.

signal activation is broadened spatially and prolonged temporally owing to a failure of Torso degradation (Lloyd et al. 2002).

Egfr signaling in the embryos is required for ventral ectoderm determination (reviewed in Shilo 1992, Schweitzer & Shilo 1997, Casci & Freeman 1999). The active form of ligand Spitz is secreted by cells along the ventral midline, which leads to activation of Egfr and the Ras/MAPK pathway in the ventral ectoderm. Embryos lacking *hrs* die with numerous morphological defects, suggesting that many different RTK signaling pathways are disrupted. In these embryos, Egfr is overactivated (levels of active Egfr are increased), resulting in a broadened area of receptor activation and an expansion of the region of ventral fate (Lloyd et al. 2002).

Signaling by other RTKs and also other receptors is attenuated by Hrs-dependent degradation. *hrs*-mutant epithelial cells accumulate multiple signaling receptors in endosomes. Most of the receptors are not bound to ligand, indicating that turnover of both active and inactive receptors attenuates signaling (Jekely & Rorth 2003).

Cbl-Mediated Endocytosis Restricts RTK Signaling Spatially in Border Cells During Oogenesis

Cbl (Casitas B-cell lymphoma) is a RING E3 ubiquitin ligase that in vertebrate cells has been shown to target RTKs for lysosomal degradation (reviewed in Thien & Langdon 2001, Rubin et al. 2005). Analysis of the *D-cbl*-mutant phenotype in oogenesis reveals that endocytosis of activated RTKs is a mechanism for restricting signaling spatially within single follicle cells called border cells.

Border cells, which form an eight-cell cluster, migrate to the oocyte in a stereotyped manner during oogenesis (Starz-Gaiano & Montell 2004) (**Figure 13**). Signaling by Pvr and Egfr guides the migration (Duchek & Rorth 2001, Duchek et al. 2001). Border cells express the two RTKs, and oocytes express the ligands Pvf1 and Gurken, respectively. Many *D-cbl*$^{-}$ border cells fail to migrate, and this phenotype is suppressed in heterozygotes for the Egfr ligand (*gurken*$^{-}$/*gurken*$^{+}$) and enhanced by Pvr or Egfr overexpression. This suggests that in *D-cbl* mutants, RTK signaling is in some sense overactive. However, it is not an increased level of RTK signaling but loss of localization that causes the *D-cbl*-mutant phenotype. In wild-type border cells, active RTK, monitored by antiphospho-tyrosine, is localized at the cell cortex, at the leading edge of cell movement. In *D-cbl* mutants, activated RTK is mislocalized all around the cell cortex. Moreover, expression of dominant-negative Shi in border cells results in the same effect. Taken together, these results suggest a model in which endocytosis of active RTKs leads to their recycling to regions of higher signaling and concentrates the signaling activity to one edge of the cell (Jekely et al. 2005).

CONCLUDING REMARKS

Who could have imagined that cell signaling, and thereby cell fate, would be regulated through baroque mechanisms involving endocytosis and endosomal trafficking? *Drosophila* has been a premier model system for developmental biologists interested in cell patterning. It is becoming increasingly apparent that to understand the complexities of the proteins and signaling pathways that pattern cells, we have to look further than the cell membrane and nucleus. For years, endosomes have been lackluster organelles to most *Drosophila* researchers. Endosomes are now having their day, but with the large number of laboratories hot on their trails, probably not the last laugh!

SUMMARY POINTS

1. Endocytosis is a mechanism for downregulating signaling by internalizing ligand-bound as well as inactive receptors and targeting them for the lysosome.
2. Endocytosis upregulates signaling as some receptors signal from endosomes.
3. Receptor-mediated endocytosis contributes to the shaping of morphogen gradients by removing morphogen from the extracellular space.
4. Ligand activation in endosomes and subsequent recycling may regulate signaling.
5. Regulation of endosome formation is a means to control signaling.
6. Regulation of endosomal routing is a means to control signaling outcomes.
7. Spatially restricted endocytosis of receptors used as guidance cues for migrating cells can control the direction of cell movement.

UNRESOLVED ISSUES AND FUTURE DIRECTIONS

1. Does planar transcytosis contribute at all to morphogen gradient formation?
2. How does Hh/Ptc binding relieve Smo inhibition by Ptc, and does the mechanism involve endosomal trafficking?
3. Why must Notch ligands be internalized in order to signal, and why is epsin (Lqf) essential for this?
4. What are all the mechanisms by which endocytosis regulates RTK signaling?

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LITERATURE CITED

Axelrod JD, Miller JR, Shulman JM, Moon RT, Perrimon N. 1998. Differential recruitment of Dishevelled provides signaling specificity in the planar cell polarity and Wingless signaling pathways. *Genes Dev.* 12:2610–22

Axelrod JD. 2001. Unipolar membrane association of Dishevelled mediates Frizzled planar cell polarity signaling. *Genes Dev.* 15:1182–87

Bejsovec A,Wieschaus E. 1995. Signaling activites of the *Drosophila wingless* gene are separately mutable and appear to be transduced at the cell surface. *Genetics* 139:309–20

Belenkaya TY, Han C, Yan D, Opoka RJ, Khodoun M, et al. 2004. *Drosophila* Dpp morphogen movement is independent of dynamin-mediated endocytosis but regulated by the glypican members of heparan sulfate proteoglycans. *Cell* 119:231–44

This paper provides strong evidence that Dpp dispersal from the source in the wing occurs not by planar transcytosis but by extracellular movement aided by HSPG proteins.

Benmerah A. 2004. Endocytosis: signaling from endocytic membranes to the nucleus. *Curr. Biol.* 14:R314–16

Bennett D, Alphey L. 2002. PP1 binds SARA and negatively regulates DPP signaling in *Drosophila melanogaster*. *Nat. Genet.* 31:419–23

Berdnik D, Torok T, Gonzalez-Gaitan M, Knoblich JA. 2002. The endocytic protein α-adaptin is required for Numb-mediated asymmetric cell division in *Drosophila*. *Dev. Cell* 3:221–31

Bonifacino JS, Traub LM. 2003. Signals for sorting of transmembrane proteins to endosomes and lysosomes. *Annu. Rev. Biochem.* 72:395–447

Boutros M, Paricio N, Strutt DI, Mlodzik M. 1998. Dishevelled activates JNK and discriminates between JNK pathways in planar polarity and Wingless signaling. *Cell* 94:109–18

Cadavid AL, Ginzel A, Fischer JA. 2000. The function of the *Drosophila* Fat facets deubiquitinating enzyme in limiting photoreceptor cell number is intimately associated with endocytosis. *Development* 127:1727–36

Cadigan KM, Fish MP, Rulifson EJ, Nusse R. 1998. Wingless repression of *Drosophila frizzled 2* expression shapes the Wingless morphogen gradient in the wing. *Cell* 93:767–77

Cadigan KM. 2002. Regulating morphogen gradients in the *Drosophila* wing. *Semin. Cell Dev. Biol.* 13:83–90

Carpenter G. 2000. The EGF receptor: a nexus for trafficking and signaling. *Bioessays* 22:697–07

Casci T, Freeman M. 1999. Control of EGF receptor signaling: lessons from fruitflies. *Cancer Metastasis Rev.* 18:181–01

Chen Y, Struhl G. 1996. Dual roles for Patched in sequestering and transducing Hedgehog. *Cell* 87:553–63

Cliffe A, Hamada F, Bienz M. 2003. A role of Dishevelled in relocating Axin to the plasma membrane during Wingless signaling. *Curr. Biol.* 27:960–66

Cornell M, Evans DA, Mann R, Fostier M, Flasza M, et al. 1999. The *Drosophila melanogaster Suppressor of deltex* gene,a regulator of the Notch receptor signaling pathways, is an E3 class ubiquitin ligase. *Genetics* 152:567–76

De Joussineau C, Soule J, Martin M, Anguille C, Montcourrier P, Alexandre D. 2003. Delta-promoted filopodia mediate long-range lateral inhibition in *Drosophila*. *Nature* 426:555–59

Denef N, Neubuser D, Perez L, Cohen SM. 2000. Hedgehog induces opposite changes in turnover and subcellular localization of Patched and Smoothened. *Cell* 102:521–31

Desbordes SC, Chandraratna D, Sanson B. 2005. A screen for genes regulating the Wingless gradient in *Drosophila* embryos. *Genetics* 170:749–66

Dikic I. 2003. Mechanisms controlling EGF receptor endocytosis and degradation. *Biochem. Soc. Trans.* 31:1178–81

Dikic I, Giordano S. 2003. Negative receptor signaling. *Curr. Opin. Cell Biol.* 15:128–35

Dubois L, Lecourtois M, Alexandre C, Hirst E, Vincent JP. 2001. Regulated endocytic routing modulates Wingless signaling in *Drosophila* embryos. *Cell* 105:613–24

Duchek P, Rorth P. 2001. Guidance of cell migration by EGF receptor signaling during *Drosophila* oogenesis. *Science* 291:131–33

Duchek P, Somogyl K, Jekely G, Beccari S, Rorth P. 2001. Guidance of cell migration by the *Drosophila* PDGF/VEGF receptor. *Cell* 107:17–26

Dudu V, Pantazis P, Gonzalez-Gaitan M 2004. Membrane traffic during embryonic development: epithelial formation, cell fate decisions and differentiation. *Curr. Opin. Cell Biol.* 16:407–14

Dupre S, Urban-Grimal D, Haguenauer-Tsapis R. 2004. Ubiquitin and endocytic internalization in yeast and animal cells. *Biochim. Biophys. Acta* 1695:89–111

Dye CA, Lee JK, Atkinson RC, Brewster R, Han PL, Bellen HJ. 1998. The *Drosophila sanpodo* gene controls sibling cell fate and encodes a tropomodulin homolog, and actin/tropomyosin-associated protein. *Development* 125:1845–56

Eldar A, Rosin D, Shilo BZ, Barkai N. 2003. Self-enhanced ligand degradation underlies robustness of morphogen gradients. *Dev. Cell* 5:635–46

Eldar A, Shilo BZ, Barkai N. 2004. Elucidating mechanisms underlying robustness of morphogen gradients. *Curr. Opin. Genet. Dev.* 14:435–39

Emery G, Hutterer A, Berdnik D, Mayer B, Wirtz-Peitz F, et al. 2005. Asymmetric Rab11 endosomes regulate Delta recycling and specify cell fate in the *Drosophila* nervous system. *Cell* 122:763–73

Entchev EV, Schwabedissen A, Gonzalez-Gaitan M. 2000. Gradient formation of the TGF-beta homolog Dpp. *Cell* 103:981–91

Entchev EV, Gonzalez-Gaitan MA. 2002. Morphogen gradient formation and vesicular trafficking. *Traffic* 3:98–109

Evans TM, Ferguson C, Wainwright BJ, Parton RG, Wicking C. 2003. Rab23, a negative regulator of Hedgehog signaling, localizes to the plasma membrane and the endocytic pathway. *Traffic* 4:869–84

This paper shows that inhibition of recycling endosomal formation in the pIIa SOP cell may be a mechanism for specifying pIIa as the Notch signaling-receiving cell.

Fehon RG, Kooh PJ, Rebay I, Regan CL, Xu T, et al. 1990. Molecular interactions between the protein products of the neurogenic loci *Notch* and *Delta*, two EGF-homologous genes in *Drosophila Cell* 61:523–34

Fostier M, Evans DA, Artavanis-Tsakonas S, Baron M. 1998. Genetic characterization of the *Drosophila melanogaster Suppressor of deltex* gene: a regulator of Notch signaling. *Genetics* 150:1477–85

Gallet A, Rodriguez R, Ruel L, Therond PP. 2003. Cholesterol modification of Hedgehog is required for trafficking and movement. *Dev. Cell* 4:191–204

Gallet A, Therond PP. 2005. Temporal modulation of the Hedgehog morphogen gradient by a Patched-dependent targeting to lysosomal compartment. *Dev. Biol.* 277:51–62

Gonzalez-Gaitan M. 2003a. Endocytic trafficking during *Drosophila* development. *Mech. Dev.* 120:1265–82

This paper shows that Hh dispersal from the source in embryos is independent of endocytosis.

Gonzalez-Gaitan M. 2003b. Signal dispersal and transduction through the endocytic pathway. *Nat. Rev. Mol. Cell Biol.* 4:213–24

Greco V, Hannus M, Eaton S. 2001. Argosomes: a potential vehicle for the spread of morphogens through epithelia. *Cell* 106:633–45

Guo M, Jan LY, Jan YN. 1996. Control of daughter cell fates during asymmetric division: interaction of Numb and Notch. *Neuron* 17:27–41

This paper shows that unlike canonical Notch signaling, which occurs at the plasma membrane, ligand- and Su(H)-independent Notch signaling occurs in late endosomes.

Haglund K, Sigismund S, Polo S, Szymkiewicz I, Di Fiore PP, Dikic I. 2003. Multiple monoubiquitination of RTKs is sufficient for their endocytosis and degradation. *Nat. Cell Biol.* 5:461–66

Hamada F, Tomoyasu Y, Takatsu Y, Nakamura M, Nagai S, et al. 1999. Negative regulation of Wingless signaling by D-axin, a *Drosophila* homolog of axin. *Science* 283:1739–42

Han C, Belenkaya TY, Wang B, Lin X. 2004. *Drosophila* glypicans control the cell-to-cell movement of Hedgehog by a dynamin-independent process. *Development* 131:601–11

Hicke L, Dunn R. 2003. Regulation of membrane protein transport by ubiquitin and ubiquitin-binding proteins. *Annu. Rev. Cell Dev. Bio.* 19:141–72

Hooper JE, Scott MP. 2005. Communicating with hedgehogs. *Nat. Rev. Mol. Cell Biol.* 6:306–17

Hori K, Fostier M, Ito M, Fuwa TJ, Go MJ, et al. 2004. *Drosophila* Deltex mediates Suppressor-of-Hairless-independent and late-endosomal activation of Notch signaling. *Development* 131:5527–37

Hutterer A, Knoblich JA. 2005. Numb and α-adaptin regulate Sanpodo endocytosis to specify cell fate in *Drosophila* external sensory organs. *EMBO Rep.* 6:836–42

Incardona JP, Gruenberg J, Roelink H. 2002. Sonic hedgehog induces the segregation of Patched and Smoothened in endosomes. *Curr. Biol.* 12:983–95

Itoh M, Kim CH, Palardy G, Oda T, Jiang YJ, et al. 2003. Mind bomb is a ubiquitin ligase that is essential for efficient activation of Notch signaling by Delta. *Dev. Cell* 4:67–82

Jafar-Nejad H, Andrews HK, Acar M, Bayat V, Wirtz-Peitz F, et al. 2005. Sec15, a component of the exocyst, promotes Notch signaling during the asymmetric division of *Drosophila* sensory organ precursors. *Dev. Cell* 9:351–63

Jekely G, Rorth P. 2003. Hrs mediates downregulation of multiple signaling receptors in *Drosophila*. *EMBO Rep.* 4:1163–68

Jekely G, Sung HH, Luque CM, Rorth P. 2005. Regulators of endocytosis maintain localized receptor tyrosine kinase signaling in guided migration. *Dev. Cell* 9:197–207

Johnson RL, Zhou L, Bailey EC. 2002. Distinct consequences of sterol sensor mutation in *Drosophila* and mouse Patched homologs. *Dev. Biol.* 242:224–35

This work shows that in SOPs, Sec15 is required in pIIb for Notch activation in pIIa. The *sec15*-mutant phenotype suggests that Sec15, with Spdo, is required for Delta activation by recycling.

This work shows that Cbl-mediated internalization of EGFR regulates the spatial distribution of activated EGFR within individual cells.

Komada M, Kitamura N. 2005. The Hrs/STAM complex in the downregulation of receptor tyrosine kinases. *J. Biochem.* (Tokyo) 137:1–8

Kooh PJ, Fehon RG,Muskavitch MA. 1993. Implications of dynamic patterns of Delta and Notch expression for cellular interactions during *Drosophila* development. *Development* 117:493–507

Kramer H, Phistry M. 1996. Mutations in the *Drosophila hook* gene inhibit endocytosis of the Boss transmembrane ligand into multivesicular bodies. *J. Cell Biol.* 133:1205–15

Kruse K, Pantazis P, Bollenbach T, Julicher F, Gonzalez-Gaitan M. 2004. Dpp gradient formation by dynamin-dependent endocytosis: receptor trafficking and the diffusion model. *Development* 131:4843–56

Kuwabara PE, Labouesse M. 2002. The sterol-sensing domain: multiple families, a unique role? *Trends Genet.* 18:193–201

Lai EC, Deblandre GA, Kintner C, Rubin GM. 2001. *Drosophila* Neuralized is a ubiquitin ligase that promotes the internalization and degradation of Delta. *Dev. Cell* 1:783–94

Lai EC, Roegiers F, Qin X, Jan YN, Rubin GM. 2005. The ubiquitin ligase *Drosophila* Mind bomb promotes Notch signaling by regulating the localization and activity of Serrate and Delta. *Development* 132:2319–32

Lander AD, Nie Q, Wan FY. 2002. Do morphogen gradients arise by diffusion? *Dev. Cell* 2:785–96

Langevin J, Le Borgne R, Rosenfeld F, Gho M, Schweisguth F, Bellaiche Y. 2005. Lethal giant larvae controls the localization of Notch-signaling regulators Numb, Neuralized, and Sanpodo in *Drosophila* sensory-organ precursor cells. *Curr. Biol.* 15:955–62

Le Borgne R, Schweisguth F. 2003. Unequal segregation of Neuralized biases Notch activation during asymmetric cell division. *Dev. Cell* 5:139–48

Le Borgne R, Bardin A, Schweisguth F. 2005a. The roles of receptor and ligand endocytosis in regulating Notch signaling. *Development* 132:1751–62

Le Borgne R, Remaud S, Hamel S, Schweisguth F. 2005b. Two distinct E3 ubiquitin ligases have complementary function in the regulation of Delta and Serrate signaling in *Drosophila*. *PLoS Biol.* 3:e96

Le Roy C, Wrana JL. 2005. Clathrin- and nonclathrin-mediated endocytic regulation of cell signaling. *Nat. Rev. Mol. Cell Biol.* 6:112–26

Legendre-Guillemin V, Wasiak S, Hussain NK, Angers A, McPherson PS. 2004. ENTH/ANTH proteins and clathrin-mediated membrane budding. *J. Cell Sci.* 117:9–18

Lemmon MA. 2003. Phosphoinositide recognition domains. *Traffic* 4:201–13

Levkowitz G, Waterman H, Ettenberg SA, Katz M, Tsygankov AY, et al. 1999. Ubiquitin ligase activity and tyrosine phosphorylation underlie suppression of growth factor signaling by c-Cbl/Sli-1. *Mol. Cell.* 4:1029–40

Li WX. 2005. Functions and mechanisms of receptor tyrosine kinase Torso signaling: lessons from *Drosophila* embryonic terminal development. *Dev. Dyn.* 232:656–72

Li Y, Baker NE. 2004. The roles of *cis*-inactivation by Notch ligands and of *neuralized* during eye and bristle patterning in *Drosophila*. *BMC Dev. Biol.* 27:5

Lloyd TE, Atkinson R, Wu MN, Zhou Y, Pennetta G, Bellen HJ. 2002. Hrs regulates endosome membrane invagination and tyrosine kinase receptor signaling in *Drosophila*. *Cell.* 108:261–69

Marques G, Musacchio M, Shimell MJ, Wunnenberg-Stapleton K, Cho KW, O'Connor MB. 1997. Production of a DPP activity gradient in the early *Drosophila* embryo through the opposing actions of the SOG and TLD proteins. *Cell* 91:417–26

Analysis of the *hrs*-mutant phenotype shows that the protein is required for MVB formation. Moreover, RTK signaling is attenuated by endocytosis and Hrs-mediated endosomal trafficking to the lysosome.

Martin V, Carrillo G, Torroja C, Guerrero I. 2001. The sterol-sensing domain of Patched protein seems to control Smoothened activity through Patched vesicular trafficking. *Curr. Biol.* 11:601–7

Mazaleyrat SL, Fostier M, Wilkin MB, Aslam H, Evans DA, et al. 2003. Down-regulation of Notch target gene expression by Suppressor of deltex. *Dev. Biol.* 255:363–72

Miaczynska M, Pelkmans L, Zerial M. 2004. Not just a sink: endosomes in control of signal transduction. *Curr. Opin. Cell Biol.* 16:400–6

Mizutani CM, Nie Q, Wan FY, Zhang YT, Vilmos P, et al. 2005. Formation of the BMP activity gradient in the *Drosophila* embryo. *Dev. Cell* 8:915–24

Moline MM, Southern C, Bejsovec A. 1999. Directionality of Wingless protein transport influences epidermal patterning in the *Drosophila* embryo. *Development* 126:4375–84

Nakano Y, Nystedt S, Shivdasani AA, Strutt H, Thomas C, Ingham PW. 2004. Functional domains and subcellular distribution of the Hedgehog transducing protein Smoothened in *Drosophila*. *Mech. Dev.* 121:507–18

O'Connor-Giles KM, Skeath JB. 2003. Numb inhibits membrane localization of Sanpodo, a four-pass transmembrane protein, to promote asymmetric divisions in *Drosophila*. *Dev. Cell* 5:231–43

Overstreet E, Chen X, Wendland B, Fischer JA. 2003. Either part of a Droosphila epsin protein, divided after the ENTH domain, functions in endocytosis of Delta in the developing eye. *Curr. Biol.* 13:854–60

Overstreet E, Fitch E. Fischer JA. 2004. Fat facets and Liquid facets promote Delta endocytosis and Delta signaling in the signaling cells. *Development* 131:5355–66

This paper shows that in the eye, Lqf (epsin) is required specifically for Delta endocytosis and Delta signaling. (See also Wang & Struhl 2004.)

Panakova D, Sprong H, Marois E, Thiele C, Eaton S. 2005. Lipoprotein particles are required for Hedgehog and Wingless signaling. *Nature* 435:58–65

Parks AL, Turner FR, Muskavitch MA. 1995. Relationships between complex Delta expression and the specification of retinal cell fates during *Drosophila* eye development. *Mech. Dev.* 50:201–16

Parks AL, Klueg KM, Stout JR, Muskavitch MA. 2000. Ligand endocytosis drives receptor dissociation and activation in the Notch pathway. *Development* 127:1373–85

Pavlopoulos E, Pitsouli C, Klueg KM, Muskavitch MA, Moschonas NK, Delidakis C. 2001. Neuralized encodes a peripheral membrane protein involved in Delta signaling and endocytosis. *Dev. Cell* 1:807–16

Penton A,Wodarz A, Nusse R. 2002. A mutational analysis of Dishevelled in *Drosophila* defines novel domains in the Dishevelled protein as well as novel suppressing alleles of *axin*. *Genetics* 161:747–62

Piddini E, Vincent JP. 2003. Modulation of developmental signals by endocytosis: different means and many ends. *Curr. Opin. Cell Biol.* 15:474–81

Piddini E, Marshall F, Dubois L, Hirst E, Vincent JP. 2005. Arrow (LRP6) and Frizzled2 cooperate to degrade Wingless in *Drosophila* imaginal discs. *Development* 132:5479–89

Pitsouli C, Delidakis C. 2005. The interplay between DSL proteins and ubiquitin ligases in Notch signaling. *Development* 132:4041–50

Raftery LA, Sutherland DJ. 2003. Gradients and thresholds: BMP response gradients unveiled in *Drosophila* embryos. *Trends Genet.* 19:701–8

Raiborg C, Rusten TE, Stenmark H. 2003. Protein sorting into multivesiclar endosomes. *Curr. Opin. Cell Biol.* 15:446–55

Ramain P, Khechumian K, Seugnet L, Arbogast N, Ackermann C, Heitzler P. 2001. Novel Notch alleles reveal a Deltex-dependent pathway repressing neural fate. *Curr. Biol.* 11:1729–38

Rhyu MS, Jan LY, Jan YN. 1994. Asymmetric distribution of Numb protein during division of the sensory organ precursor cell confers distinct fates to daughter cells. *Cell* 76:477–91

Roegiers F, Jan LY, Jan YN. 2005. Regulation of membrane localization of Sanpodo by Lethal giant larvae and Neuralized in asymmetrically dividing cells of *Drosophila* sensory organs. *Mol. Biol. Cell* 16:3480–87

Rothbacher U, Laurent MN, Deardorff MA, Klein PS, Cho KW, Fraser SE. 2000. Dishevelled phosphorylation, subcellular localization and multimerization regulate its role in early embryogenesis. *EMBO J.* 19:1010–22

Rubin C, Gur G, Yarden Y. 2005. Negative regulation of receptor tyrosine kinases: unexpected links to c-Cbl and receptor ubiquitylation. *Cell Res.* 15:66–71

Sachse M, Urbe S, Oorschot V, Strous GJ, Klumperman J. 2002. Bilayered clathrin coats on endosomal vacuoles are involved in protein sorting toward lysosomes. *Mol. Biol. Cell.* 13:1313–28

Sakata T, Sakaguchi H, Tsuda L, Higashitani A, Aigaki T, et al. 2004. *Drosophila* Nedd4 regulates endocytosis of Notch and suppresses its ligand-independent activation. *Curr. Biol.* 11:1729–38

Sanyal S, Ramaswami M. 2002. Spinsters, synaptic defects, and amaurotic idiocy. *Neuron* 36:335–38

Schupbach T. 1987. Germ line and soma cooperate during oogenesis to establish the dorsoventral pattern of egg shell and embryo in *Drosophila melanogaster*. *Cell* 49:699–707

Schweitzer R, Shilo BZ. 1997. A thousand and one roles for the *Drosophila* EGF receptor. *Trends Genet.* 13:191–96

Seto ES, Bellen HJ, Lloyd TE. 2002. When cell biology meets development: endocytic regulation of signaling pathways. *Genes Dev.* 16:1314–36

Seto ES, Bellen HJ. 2004. The ins and outs of Wingless signaling. *Trends Cell Biol.* 14:45–53

Seugnet L, Simpson P, Haenlin M. 1997. Reqeuirement for dynamin during Notch signaling in *Drosophila* neurogenesis. *Dev. Biol.* 192:585–98

Shilo BZ. 1992. Roles of receptor tyrosine kinases in *Drosophila* development. *FASEB J.* 6:2915–22

Skeath JB, Doe CQ. 1998. Sanpodo and Notch act in opposition to Numb to distinguish sibling neuron fates in the *Drosophila* CNS. *Development* 125:1857–65

Sorkin A, von Zastrow M. 2002. Signal transduction and endocytosis: close encounters of many kinds. *Nat. Rev. Mol. Cell Biol.* 3:600–14

Srinivasan S, Rashka KE, Bier E. 2002. Creation of a Sog morphogen gradient in the *Drosophila* embryo. *Dev. Cell* 2:91–101

Starz-Gaiano M, Montell DJ. 2004. Genes that drive invasion and migration in Drososphila. *Curr. Opin. Genet. Dev.* 14:86–91

Stegman MA, Goetz JA, Ascano MJ, Ogden SK, Nybakken KE, Robbins DJ. 2004. The Kinesin-related protein Costal2 associates with membranes in a Hedgehog-sensitive, Smoothened-independent manner. *J. Biol. Chem.* 279:7064–71

Strigini M,Cohen SM. 1999. Formation of morphogen gradients in the *Drosophila* wing. *Semin. Cell Dev. Biol.* 10:335–44

Strutt H, Thomas C, Nakano Y, Stark D, Neave B, et al. 2001. Mutations in the sterol-sensing domain of Patched suggest a role for vesicular trafficking in Smoothened regulation. *Curr. Biol.* 11:608–13

Sweeney ST, Davis GW. 2002. Unrestricted synaptic growth in spinster: a late endosomal protein implicated in TGF-β-mediated synaptic growth regulation. *Neuron* 36:403–16

Tabata T, Takei Y. 2004. Morphogens, their identification and regulation. *Development* 131:703–12

Taipale J, Cooper MK, Maiti T, Beachy PA. 2002. Patched acts catalytically to suppress the activity of Smoothened. *Nature* 418:892–97

Teleman AA, Cohen SM. 2000. Dpp gradient formation in the *Drosophila* wing imaginal disc. *Cell* 103:971–80

Teleman AA, Stringini M, Cohen SM. 2001. Shaping morphogen gradients. *Cell* 105:559–62

Thien CB, Langdon WY. 2001. Cbl: many adaptations to regulate protein tyrosine kinases. *Nat. Rev. Mol. Cell Biol.* 2:294–307

Torroja C, Gorfinkiel N, Guerrero I. 2004. Patched controls the Hedgehog gradient by endocytosis in a dynamin-dependent manner, but this internalization does not play a major role in signal transduction. *Development* 131:2395–408

Tsukazaki T, Chiang TA, Davison AF, Attisano L, Wrana JL. 1998. SARA, a FYVE domain protein that recruits Smad2 to the TGFβ receptor. *Cell* 95:779–91

Vincent JP, Dubois L. 2002. Morphogen transport along epithelia, an integrated trafficking problem. *Dev. Cell* 3:615–23

Wang W, Struhl G. 2004. *Drosophila* epsin mediates a select endocytic pathway that DSL ligands must enter to activate Notch. *Development* 131:5367–80

Wang W, Struhl G. 2005. Distinct roles for Mind bomb, Neuralized and epsin in mediating DSL endocytosis and signaling in *Drosophila*. *Development* 132:2883–94

Wharton KAJ. 2003. Runnin' with the Dvl: proteins that associate with Dsh/Dvl and their significance to Wnt signal transduction. *Dev. Biol.* 253:1–17

Wiley HS, Burke PM. 2001. Regulation of receptor tyrosine kinase signaling by endocytic trafficking. *Traffic* 2:12–18

Wilkin MB, Carbery AM, Fostier M, Aslam H, Mazaleyrat SL, et al. 2004. Regulation of Notch endosomal sorting and signaling by *Drosophila* Nedd4 family proteins. *Curr. Biol.* 14:2237–44

Willert K, Logan CY, Arora A, Fish M, Nusse R. 1999. A *Drosophila Axin* homolog, D-axin, inhibits Wnt signaling. *Development* 126:4165–73

Wu S, Mehta SQ, Pichaud F, Bellen HJ, Quiocho FA. 2005. Sec15 interacts with Rab11 via a novel domain and affects Rab11 localization in vivo. *Nat. Struct. Mol. Biol.* 12:879–85

Yanagawa S, van Leeuwen F, Wodarz A, Klingensmith J, Nusse R. 1995. The Dishevelled protein is modified by Wingless signaling in *Drosophila*. *Genes Dev.* 9:1087–97

Zerial M, McBride H. 2001. Rab proteins as membrane organizers. *Nat. Rev. Mol. Cell Bio.* 2:107–19

Zhu AJ, Zheng L, Suyama K, Scott MP. 2003. Altered localization of *Drosophila* Smoothened protein activates Hedgehog signal transduction. *Genes Dev.* 17:1240–52

Zhu AJ, Scott MP. 2004. Incredible journey: How do developmental signals travel through tissue? *Genes Dev*. 18:2985–97

This paper uses an endocytosis-defective Ptc allele to show that Hh gradient formation in the wing does not require endocytosis and that Hh signaling does not require Ptc internalization.

This work shows that in the wing, Lqf (epsin) is required within the signaling cells for Delta endocytosis and signaling. Moreover, the results of gain-of-function experiments suggest that Lqf-dependent Delta endocytosis leads to its activation through recycling.

This work shows that the E3 Ub ligase Su(dx) regulates postendocytic sorting of Notch to the late endosome where it can signal, as opposed to the lysosome where Notch would be degraded.