would mimic Gβγ and prevent the activation of AC by Gpa2p, possibly in its GDP-bound form. This "nonproductive" interaction between Gpa2p and Gpb1/2p-Gpg1p is reminiscent of the mechanism of pseudosubstrate inhibitors of protein kinases, which mimic substrates to block the kinase from binding authentic substrates (Kemp and Pearson, 1991). As Gα subunits do not activate ACs via a catalytic activity, we propose the term "pseudostructural inhibitor" to describe the regulatory role of Gpb1/2p-Gpg1p. In addition, a second target for Gpb1/2p-Gpg1p must exist to effect its Gpa2p-independent regulation of AC activity. A primary candidate is AC itself, as some mammalian ACs are directly regulated by Gβγ (Simonds, 1999), although other targets, including Ras2p, which activates AC in response to internal acidification (Colombo et al., 1998), and phosphodiesterase, cannot be excluded. Kelch repeat proteins can bind separate proteins simultaneously on opposing surfaces of the β propeller domain (Adams et al., 2000); thus, one Gpb1/2p-Gpg1p dimer could negatively regulate both Gpa2p and AC. This placement within the signaling complex could also allow Gpb1/2p-Gpg1p to play a positive role by bringing these proteins into close proximity to facilitate signaling upon glucose detection.

The identification of redundant kelch repeat proteins and a small binding partner as a Gβγ structural mimic in *S. cerevisiae* is an exciting development in the areas of both glucose/cAMP signaling and pseudohyphal growth. As with many discoveries, it generates more questions than answers, at the moment, but will undoubtedly stimulate these fields into new insights and discoveries. In addition, the concept of structural mimics serves notice that clues to the function of seemingly novel proteins may be found by going beyond BLASTP and PSI-BLAST searches for sequence homologs.

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Selected Reading

Adams, J., Kelso, R., and Cooley, L. (2000). Trends Cell Biol. *10*, 17–24.

Blumer, K.J., and Thorner, J. (1990). Proc. Natl. Acad. Sci. USA *87*, 4363–4367.

Colombo, S., Ma, P., Cauwenberg, L., Winderickx, J., Crauwels, M., Teunissen, A., Nauwelaers, D., de Winde, J.H., Gorwa, M.F., Colavizza, D., and Thevelein, J.M. (1998). EMBO J. *17*, 3326–3341.

Harashima, T., and Heitman, J. (2002). Mol. Cell *10*, 163–173.

Kemp, B.E., and Pearson, R.B. (1991). Biochim. Biophys. Acta *1094*, 67–76.

Landry, S., and Hoffman, C.S. (2001). Genetics *157*, 1159–1168.

Lengeler, K.B., Davidson, R.C., D'Souza, C., Harashima, T., Shen, W.C., Wang, P., Pan, X., Waugh, M., and Heitman, J. (2000). Microbiol. Mol. Biol. Rev. *64*, 746–785.

Simonds, W.F. (1999). Trends Pharmacol. Sci. *20*, 66–73.

Welton, R.M., and Hoffman, C.S. (2000). Genetics *156*, 513–521.

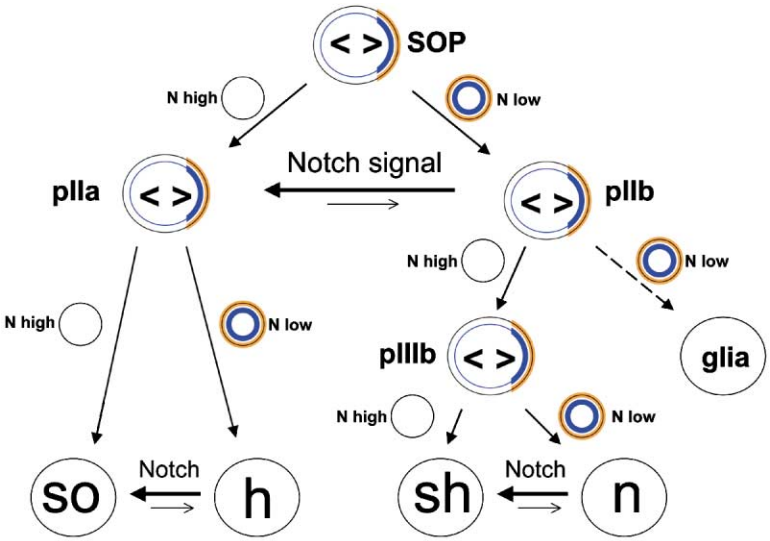
Numb: "Adapting" Notch for Endocytosis

During sensory organ precursor divisions in *Drosophila,* the *numb* gene product segregates asymmetrically into one of the two daughter cells, to which it confers a specific fate by inhibiting Notch signaling. In this issue of *Developmental Cell*, Berdnik et al. show that Numb recruits α-Adaptin and that this physical interaction plays a role in downregulating Notch, presumably by stimulating endocytosis of Notch.

Different mechanisms have evolved to ensure that diverse cellular populations can be generated during development. One of these mechanisms is intrinsic and employs asymmetric localization of cellular determinants during cell division. Numb is one of several gene products implicated in this process (reviewed in Posakony, 1994). Another mechanism is based on extrinsic intercellular signaling, whereby extracellular ligands activate transmembrane receptors. The Notch signaling pathway epitomizes this type of cell-cell communication. Both mechanisms play pivotal roles in *Drosophila* peripheral nervous system (PNS) development. As shown in the Figure, external sensory (ES) organs comprise five clonally related cells that are all derived from a single sensory organ precursor (SOP) through consecutive asymmetric divisions. Notch signaling is high in some cells and low in others, resulting in different cell fate specifications (reviewed in Posakony, 1994).

The *numb* gene was isolated in the Jans' lab more than a decade ago (Uemura et al., 1989). Numb determines the fate of the SOP progeny: loss of *numb* leads to transformation of the majority of sensory neurons of the PNS into support cells (see Figure). The *numb* loss-of-function phenotype is, in many respects, opposite to that associated with the loss of Notch. Interaction of Notch, a transmembrane receptor, with its ligand, Delta, another transmembrane protein, is necessary for the SOP progeny to acquire proper identity. At each asymmetric division, a differential level of Notch signaling between the two daughter cells causes them to adopt different fates. Epistasis experiments in which double mutants of *Notch* and *numb* were generated place *numb* genetically upstream of *Notch*. The contrasting phenotypes of *Notch* and *numb*, together with the discovery that the proteins can bind to each other directly, implied that Numb dictates the fate of SOP progeny by negatively regulating Notch (Guo et al., 1996). Although *tramtrack*, a downstream target of *Notch*, was proposed to act as a "readout" for Notch-Numb activity, there was, so far, no mechanistic insight as to how cells can integrate cell-intrinsic signals, like Numb, with extrinsic cues, such as Notch activation.

The study by Berdnik et al. (2002) sheds light onto the mechanism of action of *numb* in the fruit fly. Using



Asymmetric Segregation of α-Adaptin Endows the SOP Daughter Cells with Distinct Fates

Numb (orange) is expressed in the SOP and before cell division localizes to the plasma membrane overlying one of the centrosomes. Numb recruits α-Adaptin (blue) to one side of the dividing progenitor cells. Because of its asymmetric localization, this complex is predominantly inherited by one of the two daughter cells, the pIIb. This leads to downregulation of Notch receptor activity (N) in this cell. The Numb/α-Adaptin complex acts similarly on all of the binary decisions in the course of sensory organ development. As a result, five different cells are generated (so, socket cell; h, hair cell; sh, sheath cell; n, neuron; glia).

a powerful genetic strategy developed by Dickson and colleagues (Newsome et al., 2000), the authors found that mutations that affect the ear domain of α-Adaptin mimic the loss-of-function phenotype of *numb* in adult sensory organ development. α-Adaptin consists of a head domain, an ear domain (also called the appendage domain), and a hinge domain that links the two. It is part of the adaptor protein-2 (AP-2) complex, which plays a pivotal role in clathrin-mediated endocytosis at the plasma membrane: it recruits the endocytic machinery to specific target sites by interacting with both clathrin and endocytic substrates (Gonzalez-Gaitan and Jackle, 1997; reviewed in Seto et al., 2002). Hence, mutations that cause a loss of α-Adaptin are embryonic lethal. However, mutations in the ear domain are much less severe and cause defects mainly in sensory organ development (Berdnik et al., 2002). The ear domain can bind various accessory proteins, like AP-180, Epsin, Eps-15, Dynamin, and Amphiphysin. It is therefore thought to have a regulatory role in this process. While it has been shown previously that a vertebrate homolog of Numb can also bind the ear domain of α-Adaptin, the in vivo relevance of this interaction was unclear (Santolini et al., 2000).

Berdnik et al. show that, during metaphase, the α-Adaptin protein is concentrated to one side of the cell cortex, along with Numb. In *numb* mutant tissue α-Adaptin fails to localize asymmetrically. Furthermore, epistasis analysis places *α-adaptin* genetically upstream of *Notch* and downstream of *numb*. Since Numb can directly interact with the Notch intracellular domain, Berdnik et al. propose a model in which Numb antagonizes Notch activity by recruiting α-Adaptin. This probably stimulates endocytosis of Notch and serves as a mechanism to downregulate signaling. It is therefore tempting to speculate that other proteins involved in endocytosis and in early and late endosome formation will contribute to Notch signaling. It is already known that loss of Dynamin, which is involved in pinching off vesicles from the membrane, mimics the loss of function of *Notch*. In addition, Notch has recently been shown to be ubiquitinated, and this modification is thought to promote internalization and/or lysosomal degradation. Suppressor of deltex/Itch is an excellent candidate E3 ligase that may ubiquitinate Notch (reviewed in Lai, 2002). In addition, the vertebrate "ligand of Numb protein X" (LNX) has been shown to mediate ubiquitination of Numb. Finally, Eps-15, which binds the aspartate-proline-phenylalanine (DPF) motif found in Numb, is thought to bring different proteins involved in endocytosis together via protein-protein interactions (reviewed in Seto et al., 2002). Hence, Berdnik et al. not only provide a key link between intrinsic and extrinsic signaling, but they also open a window at the interface of developmental signaling pathways and cell biological processes.

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Selected Reading

Berdnik, D., Torok, T., Gonzalez-Gaitan, M., and Knoblich, J.A. (2002). Dev. Cell *3*, 221–231.

Gonzalez-Gaitan, M., and Jackle, H. (1997). Cell *88*, 767–776.

Guo, M., Jan, L.Y., and Jan, Y.N. (1996). Neuron *17*, 27–41.

Lai, E.C. (2002). Curr. Biol. *12*, R74–R78.

Newsome, T.P., Asling, B., and Dickson, B.J. (2000). Development *127*, 851–860.

Posakony, J.W. (1994). Cell *76*, 415–418.

Santolini, E., Puri, C., Salcini, A.E., Gagliani, M.C., Pelicci, P.G., Tacchetti, C., and Di Fiore, P.P. (2000). J. Cell Biol. *151*, 1345–1352.

Seto, E.S., Bellen, H.J., and Lloyd, T.E. (2002). Genes Dev. *16*, 1314–1336.

Uemura, T., Shepherd, S., Ackerman, L., Jan, L.Y., and Jan, Y.N. (1989). Cell *58*, 349–360.