V0 components were reported to associate with target membrane SNAREs and to play a role in the fusion of yeast vacuoles (Peters et al., 2001). Despite these observations, the conclusion that the +H/ATPase might also function as a fusion pore has remained difficult for many cell biologists to accept.

This issue of Cell reports the latest “sighting” of the +H/ATPase. Hiesinger et al. (2005) performed a forward genetic screen for proteins required for neurotransmission in Drosophila. They identified the gene vha100, the 100 kDa component of V0 that is generally referred to as Voa. There are four isoforms of vha100 in Drosophila. The screen identified vha100-1, which is expressed specifically in the nervous system. Mutations that resulted in truncated vha100-1 led to a significant decrease in transmission. Even spontaneous, calcium-independent neurotransmission was severely impaired, consistent with the interpretation that Voa plays a role independent of V1.

One of the difficulties in assigning a role in fusion to V0, however, is its already established role in acidifying vesicles, which drives the uptake of neurotransmitter. Decreased neurotransmission could therefore be due to reduced neurotransmitter in vesicles. To distinguish between these actions, Hiesinger et al. examined vesicle acidification using the fluorescent, lipophilic dye FM1-43. Cycling vesicles can be labeled with FM1-43 by exposing nerve terminals during stimulation then washing away extracellular dye. The amount of dye taken up reflects the number of vesicles that fused with the membrane. This measure is relatively insensitive to vesicle acidification, as Hiesinger et al. show by demonstrating that the drug bafilomycin, which disrupts proton gradients, has little effect on FM dye labeling. Loss of full-length vha100-1 produced a significant decrease in FM labeling, consistent with a decrease in vesicle fusion.

The authors took this analysis one step further by examining the ability of Drosophila vha100 to rescue loss of homologous proteins in yeast. Loss of the yeast genes VPH1 and STV1 renders cells unable to grow on neutral media and disrupts trafficking in endocytic compartments. Expression of vha100 specifically rescued trafficking deficits in vph1/stv1 double mutants. It did not restore vacuolar acidification, suggesting that Voa performs a conserved function specific to trafficking.

As with its yeast analog (Peters et al., 2001), Hiesinger et al. find that vha100-1 binds to t-SNAREs, suggesting a model in which SNARE proteins guide V0 pores in vesicle and acceptor membranes into apposition, thus forming a pore spanning both membranes (Almers, 2001). However, the data are also consistent with a pore comprised of V0 in the vesicle and t-SNAREs in the acceptor membrane. This latter model is consistent with recent reports suggesting that the t-SNARE syntaxin is a component of the fusion pore (Han and Jackson, 2005; Han et al., 2004). A third possibility suggested by the fact that the screen identified Voa and not the pore forming proteolipid Voc, is that Voa acts as an accessory to both the +H/ATPase pore and a fusion pore comprised of SNAREs, though studies of yeast vacuole fusion suggest that all of V0 is involved (Peters et al., 2001). In any case, if V0 is involved in fusion, it is likely to act independently of V1 since the bulky, cytoplasmic V1 would be expected to hinder membrane apposition. This suggests that one should find V0 in isolation and perhaps in greater number per vesicle than V1.

The fact that Voa was found to be necessary for neurotransmission in a forward genetic screen, coupled with compelling data indicating that it does more than acidify vesicles, makes the strongest case to date that the +H/ATPase plays a role in vesicle fusion. Still the crucial piece of evidence—that loss of V0 halts all fusion—is lacking. Hiesinger et al. found that fusion induced by hypertonic sucrose, which is lost in SNARE mutants, was still present in vha100-1 mutants. While this may mean that other V0 components can function in the absence of Voa, it may also mean that SNAREs are the primary component of the fusion pore and that V0 acts either in addition or as an accessory. V0’s double duty may preclude the definitive test of its role in fusion, however, since its role in vesicle acidification is likely to be necessary for cell survival.

Sandra Bajjalieh
Department of Pharmacology
University of Washington
D429 HSB
Box 357280
Seattle, Washington 98195

Selected Reading

DOI 10.1016/j.cell.2005.05.002

Temporal Regulation of Planar Cell Polarity: Insights from the Drosophila Eye

In this issue of Cell, Djiane et al. (2005) identify a first regulatory link between planar cell polarity (PCP) sig-
naling and apical-basal polarity. The authors propose that a component of the apical Crumbs complex regulates the phosphorylation of the Frizzled (Fz) PCP receptor, thus modulating PCP in the Drosophila eye.

Most eukaryotic cells are polarized along a single axis. This polarity axis may be either static, as in epithelial cells, or dynamic, as in migratory cells that rapidly re-orient their polarity axis when they turn around. Some cells, however, display two polarity axes: a main axis acquired first and a secondary axis established later. For instance, epithelial cells, which are polarized along an apical-basal axis, may secondarily become polarized within the plane of the epithelium, perpendicular to their initial apical-basal axis (Eaton, 1997; Strutt, 2003). This polarity is referred to as planar cell polarity (PCP).

A good system to study PCP is the Drosophila eye. In this tissue, PCP is reflected by the mirror image orientation of ommatidia of opposite chiral forms across the dorsoventral midline, also known as the equator. All dorsal ommatidia point up, whereas all ventral ommatidia point down. This planar arrangement is generated in the larva within the eye primordium. It depends on the relative position within each ommatidium of the R3 and R4 photoreceptor cells along the equator-polar axis. In wild-type ommatidia, R3 cells are closer to the equator, whereas R4 cells are closer to the poles. Mutations in the fz gene randomize the R3/R4 decision, hence the loss of PCP in the adult eye. Two equipotential photoreceptor cells share the potential to become R3 or R4. The binary R3/R4 fate decision is regulated by Notch and Fz. Notch activation promotes adoption of the R4 fate, whereas Fz inhibits Notch. Current models propose that the equatorial cell (i.e., close to the equator) has high Fz activity, hence low Notch activity, and becomes R3. Conversely, the polar cell (i.e., away from the equator) has low Fz activity, hence high Notch activity, and therefore adopts the R4 fate. This cell fate choice occurs within a single layered epithelium that has inherited its apical-basal polarity from the early embryo. Formation of apical-basal polarity in the Drosophila embryo involves the apical Crumbs and Par complexes (Knust and Bossinger, 2002), which have recently been shown to physically and functionally interact (Hurd et al., 2003; Wang et al., 2004). Thus, epithelial polarity and PCP appear to be temporally and mechanistically distinct processes. In this issue of Cell, Djiane et al. (2005) uncover the first molecular links between Fz and two components of the Crumbs and Par complexes.

Previous studies have indicated that apical localization of Fz is a prerequisite for PCP signaling (Wu et al., 2004), suggesting that apical complexes may positively regulate PCP signaling by promoting apical localization of Fz. Consistent with this possibility, Djiane et al. (2005) now find that the C-terminal PDZ binding motif (PBM) of Fz directly binds a component of the apical Crumbs complex known as dPatj (Drosophila Pals1-associated tight junction protein). Whether dPatj is required to localize Fz at the apical cortex, however, remains to be determined. Additionally, the C-terminal tail of Fz contains two serine residues that can be phosphorylated in vitro by aPKC (atypical protein kinase C), a component of the apical Par complex. While it remains to be investigated whether endogenous Fz is phosphorylated in vivo, this suggests that Fz may be a phosphorylation target of aPKC. Thus, binding of dPatj to Fz may help to recruit aPKC to its phosphorylation target. Importantly, a phosphomimetic mutant form of Fz, Fz-EE, appears to be inactive in a gain-of-function assay, while a nonphosphorylatable form of Fz, Fz-AA, behaves similarly to wild-type Fz. Thus, phosphorylation of Fz by aPKC appears to inhibit Fz activity. This result is, at first glance, surprising, as it suggests that apical aPKC-containing complexes may inhibit rather than promote Fz signaling.

While molecular interaction and overexpression studies support the hypothesis that aPKC inhibits Fz PCP signaling, a more stringent test is, of course, loss-of-function analysis in flies. However, complete loss of aPKC function in clones results in cell lethality, thus preventing analysis of its role in PCP. Nevertheless, expression of a constitutively active form of aPKC leads to a mild PCP phenotype, consistent with aPKC inhibition of Fz. Unlike aPKC, the possible role of dPatj in PCP can be

---

**Figure 1. A Model for the Temporal Regulation of PCP Establishment**

Diagram illustrating the temporal correlation between the downregulation of dPatj (blue line) and Fz PCP signaling activity (yellow line) in the developing eye (top). Fz activity is proposed to be kept low by inhibitory phosphorylation of its intracellular tail by aPKC. Developmentally programmed down-regulation of dPatj would relieve Fz from this aPKC-mediated inhibition. The relevant polarization and fate determination events are shown below in pairs of epithelial cells. Apical (blue) and basal (green) membrane domains are indicated in blue and green, respectively. Asymmetric distribution of PCP proteins at the levels of cell-cell junctions is shown in yellow (Fz at the R4 side of the R3/R4 interface) and red (Strabismus at the R3/R4 interface) (Strutt, 2003). Nondifferentiated cells (left) are first recruited to become R3/R4 precursor cells (center). PCP signaling reproducibly biases the R3/R4 fate decision (see text).
easily studied, since dPatj mutant flies are viable and show no detectable apical-basal polarity defects (Pielage et al., 2003). Loss of dPatj activity does not randomize the R3/R4 decision, but, interestingly, symmetrical R3/R3 ommatidia are seen in dPatj mutant flies. R3/R3 ommatidia are associated with high Fz activity. Furthermore, a 2-fold reduction of dPatj activity enhances the gain-of-function phenotypes induced by Fz overexpression. Thus, dPatj appears to antagonize Fz PCP signaling. Whether this effect of dPatj on Fz activity is mediated via its direct interaction with Fz, however, is not entirely clear since a Fz-GFP C-terminal fusion protein lacking the C-terminal PBM involved in dPatj binding localizes apically and rescues a complete loss of fz activity (Strutt, 2001).

What could be the functional significance of the aPKC- and dPatj-mediated inhibition of apical Fz? It is important to note that Fz localizes at the apical cortex of eye epithelial cells long before they differentiate (i.e., anterior to the eye morphogenetic furrow) and acquire their second polarity axis. Although it is not entirely clear when Fz signals to establish PCP (Strutt and Strutt, 2002), Fz appears to signal to establish PCP only during a brief period of time preceding the R3/R4 decision. Thus, one hypothesis is that aPKC-mediated phosphorylation of Fz defines this temporal window of Fz signaling by inhibiting Fz prior to and after this period. Consistent with this hypothesis, the level of dPatj accumulation is specifically downregulated in the R3/R4 precursor cells when PCP signaling is thought to occur. Moreover, this downregulation of dPatj does not depend on Fz signaling, as it is still observed in PCP mutant flies. Additionally, the level of Bazooka (Baz; the Drosophila Par3 homologue) is upregulated in the R3/R4 precursor cells, and this upregulation also does not depend on PCP signaling. Loss of baz activity in clones results in symmetrical R4/R4 ommatidia (associated with low Fz signaling), and a 2-fold reduction of baz activity suppresses Fz overexpression phenotypes. These data, therefore, suggest that Baz positively regulates Fz signaling. Baz does not appear to act by regulating the levels of dPatj. Whether Baz acts by antagonizing aPKC activity or by yet another mechanism remains to be determined. Together, these observations suggest a model whereby the downregulation of dPatj and upregulation of Baz release Fz from aPKC-mediated inhibition and thus define when Fz signaling is active and PCP is established (Figure 1). One prediction of this model is that PCP, as reflected by the asymmetric distribution of Fz at the apical cortex of R3/R4 cell pairs, may be established earlier in developing dPatj mutant eyes.

The notions that PCP signaling is inhibited by components of apical polarity complexes and that this inhibition is important to define when PCP is established are novel. Moreover, inhibition of Fz PCP signaling by apical-basal polarity complexes may reflect a more general property of cell polarity regulation, which is that cells may more easily interpret a single polarity cue at one time. Accordingly, one first response of polarized cells to a novel polarity information such as PCP may be to downregulate preexisting polarity cues. Future studies will no doubt test whether PCP formation in the eye actually requires a transient downregulation of apical-basal polarity in R3/R4 cells.

François Schweisguth
Ecole Normale Supérieure
CNRS UMR8542
46 rue d’Ulm
75230 Paris Cedex 05
France

Selected Reading
DOI 10.1016/j.cell.2005.05.006