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SPOTLIGHT

Modulating apical–basal polarity by building and deconstructing a Yurt

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Cell polarity is regulated by protein networks in the apical and basolateral domains that repress one another by mutually antagonistic interactions. Gamblin et al. (2018. *J. Cell Biol.* https://doi.org/10.1083/jcb.201803099) reveal that apical Crumbs is antagonized by oligomerization of basolateral Yurt, while Yurt oligomerization is in turn negatively regulated by the apical kinase aPKC.

You may not realize it, but building and maintaining epithelia was and remains one of your most important tasks. You started early, with the trophectoderm, your first tissue, and the neural tube, precursor of your nervous system. Building and maintaining your gut, kidney tubules, and blood vessels made and keep you functional. One key feature of epithelial sheets is their polarization along the apical–basal axis, allowing, for example, intestinal cells to move glucose from the lumen to the bloodstream by correctly positioning glucose symporters and antiporters apically versus basally. Epithelia also create barriers between body compartments, preventing free movement of molecules across the sheet. The minimal machinery to build epithelia includes proteins of cadherin-based cell–cell adherens junctions, which divide the apical and basolateral domains, and basal integrin-based cell–matrix junctions. Barrier function requires assembling claudin-based occluding junctions such as vertebrate tight junctions, which are apical to adherens junctions, or insect septate junctions, which are just basal to them. Positioning adherens and tight junctions correctly is critical to apical–basal polarity. In this issue, Gamblin et al. provide new insight into this process.

Genetic analysis in *Drosophila melanogaster* and *Caenorhabditis elegans* identified proteins with essential roles in polarity establishment and maintenance. Cell biological and biochemical followup work revealed that these encode a set of multiprotein complexes that localize either apically or basolaterally (1, 2): the apical Par complex, which includes atypical protein kinase C (aPKC), the apical Crumbs complex, and the basolateral Scribble/Dlg/Lgl module and Par1 kinase. Mutually antagonistic relationships between apical and basolateral complexes (Fig. 1 A) mediated in part by antagonistic cross-phosphorylation keep the apical and basolateral domains segregated. This also positions the occluding junctions, though the circuitry involved differs between vertebrates and insects.

In 2006, 22 years after its identification in the Nobel Prize–winning screens for genes required for the embryonic body plan, another player called Yurt entered the field (3). Yurt encodes a protein in the FourPointOne-Ezrin-Radixin-Moesin (FERM) domain superfamily (4). Yurt initially localizes along the lateral domain, but during terminal differentiation, it is recruited to the apical domain (Fig. 1 A). Clues as to its mechanism of action came from the realization that Yurt's FERM domain binds the Crumbs C terminus, antagonizing Crumbs function. This suggested Yurt acts early to prevent Crumbs localization basally and later restrains Crumbs's function apically, preventing overexpansion of the apical domain. A role in restricting Crumbs function continues in retinal development in both flies and zebrafish (5). Yurt and its mouse homologue EPB41L5 also regulate barrier function (6). Finally, data from mouse mutants and cultured cells also support roles for the Yurt homologue in regulating the junctional actomyosin cytoskeleton (7, 8) at both apical junctions and basal focal adhesions.

Scientists then placed Yurt into the broader network of antagonistic interactions that maintain the apical and basolateral domains. Both domains have resident kinases that play important roles—for example, the apical kinase aPKC phosphorylates Bazooka (fly Par3) to exclude it from the apical domain and thus position adherens junctions, while phosphorylation of apical proteins by the basolateral kinase Par1 plays a complementary role (2). aPKC plays a key role in regulating Yurt localization (9). aPKC binds Yurt via its FERM-associated (FA) domain and phosphorylates Yurt on a series of conserved serine/threonine residues (Fig. 1, A and B, top), inactivating it. Powerful genetic tools confirmed the importance of this. A nonphosphorylatable Yurt mutant could more strongly antagonize Crumbs activity, leading to virtual loss of the apical domain, and Yurt is thought to act in turn to prevent aPKC activity from expanding basolaterally.

Gamblin et al. now extend this work, exploring mechanisms by which aPKC phosphorylation regulates Yurt activity and thus epithelial polarization (10). By coimmunoprecipitation and proximity ligation assays, they observed Yurt–Yurt interactions in cultured cells and fly embryos. Yurt's mammalian orthologue EPB41L5 also oligomerizes, indicating an evolutionarily conserved function. The FERM and FA domains are required for robust Yurt oligomerization, and scanning mutagenesis was used to alter hydrophobic amino acids on the FERM domain's F3 lobe. Mutating F281R + W283R abolished oligomerization but did not affect membrane localization, suggesting Yurt was still correctly folded. Thus, the F3 lobe of the FERM domain is part of the oligomerization interface.

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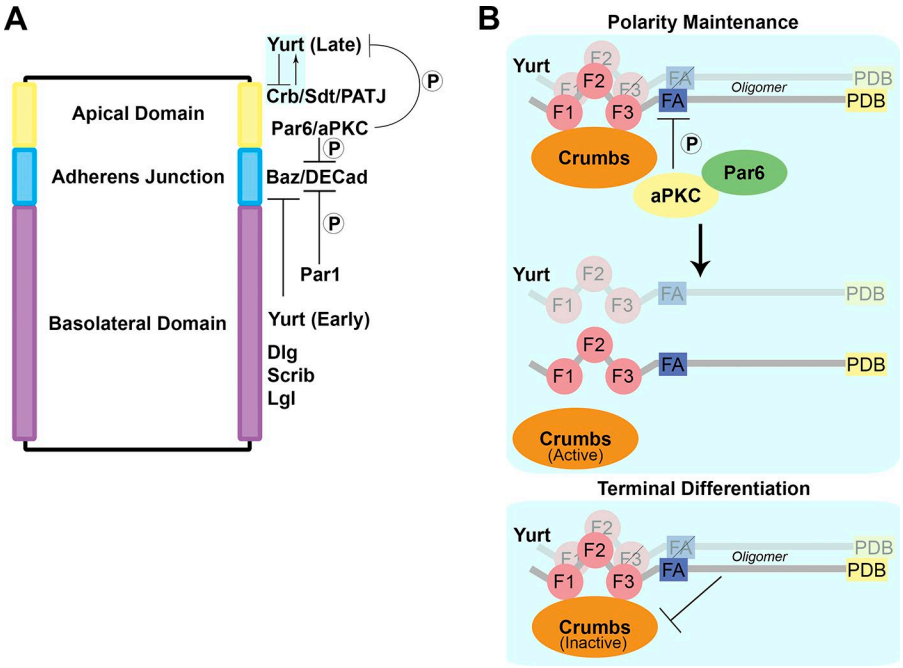

Figure 1. **Models for the role of Yurt in epithelial polarity and terminal differentiation during *Drosophila* embryonic development. (A)** Model illustrating regulatory interactions between Yurt and epithelial polarity proteins. **(B)** Top: Early during the polarity maintenance phase, apical aPKC prevents premature apical localization of Yurt by phosphorylating Yurt's FA domain, disrupting Yurt oligomerization, preventing binding to Crumbs, and keeping Crumbs in an active state. Bottom: Later, during terminal differentiation, Yurt is recruited by Crumbs to the apical membrane, where it forms part of the Crumbs complex and negatively regulates Crumbs function. PDB, PDZ domain binding site.

The FERM domain mutant thus provided Gamblin et al. with a tool to assess the functional significance of Yurt oligomerization in epithelial polarization (10). They used CRISPR/Cas9 to introduce the F281R and W283R mutants into the *yurt* locus and generate embryos where YurtF281R + W283R was the only Yurt protein present. Although YurtF281R + W283R mutants could still access the plasma membrane, they no longer restricted Crumbs apically; instead Crumbs expanded throughout the basolateral domain. YurtF281R + W283R cannot coimmunoprecipitate with Crumbs; therefore, oligomerization of Yurt appears necessary for its physical and functional interaction with Crumbs. The oligomerization-defective mutant had defects in morphogenesis including head involution and dorsal failure, and its epidermal epithelial architecture mimicked full loss of Yurt, demonstrating the importance of Yurt oligomerization in Crumbs inactivation (Fig. 1 B, bottom). Consistent with conservation of mechanisms, a mutation in the FERM domain identified in the zebrafish Yurt homologue Mosaic Eyes blocked oligomerization and Crumbs binding.

Gamblin et al. then explored how Yurt self-assembly is controlled (10), following up their earlier observation that aPKC phosphorylates Yurt to restrain its apical localization (9). aPKC knockdown increased WT Yurt oligomerization, while aPKC activation reduced it. Mutation of five aPKC phosphorylation sites in Yurt's FA domain previously identified by mass spectroscopy revealed that a phosphomimetic mutant had reduced oligomerization. Together, these data suggest that aPKC phosphorylation of Yurt destabilizes its oligomerization state (Fig. 1 B, top). It remains unknown, however, if this function is exclusive to aPKC or if the kinase Pak1 acts redundantly in this process.

These data place Yurt firmly in the center of the network that maintains robust apical–basal polarity and open up many exciting questions. Is the Yurt oligomer a dimer or a much larger multimer? Do all of Yurt's functions, including its roles in fly septate junctions and in regulating myosin contractility, also require oligomerization? By what mechanism(s) does Yurt limit basolateral aPKC activity? More broadly, it is becoming ever clearer that different tissues use different subsets of the polarity maintenance network. Is Yurt a universal polarity modulator, and if not, what other mechanisms take its place? By working together, we can all contribute to this developing story.

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