

The Ups and Downs of Life in an Epithelium

Helmut Krämer

Center for Basic Neuroscience and Department of Cell Biology, University of Texas Southwestern Medical Center, Dallas, Texas 75390-9111

Polarized epithelial layers protect tissues from the harsh conditions of the outside world, such as the outside of an embryo or the lumen of a gut. This “outside” is faced by an epithelial cell’s apical domain, which contains a set of membrane proteins and cytoskeletal elements that is distinct from the proteins found on the cell’s basolateral domain (Yeaman et al., 1999). Establishing the differences that define these domains is one of the fundamental challenges confronting an epithelial cell. Several recent papers in the *Journal of Cell Biology* (Hunter and Wieschaus, 2000; Lecuit and Wieschaus, 2000; Sisson et al., 2000; Tanentzapf et al., 2000; Wodarz et al., 2000) use *Drosophila* to explore this process, and they showed surprising parallels among epithelia in *Drosophila*, vertebrates, and *Caenorhabditis elegans*.

In the early *Drosophila* embryo, the synchronized emergence of epithelial polarity provides a unique tool for studying the determinants of apical–basal polarity. The embryo’s first 13 nuclear divisions occur in a syncytium; subsequently, about 6,000 nuclei move just below the cell surface. In synchronized motion, the membranes between the nuclei invaginate and, just one hour later, each nucleus is contained within a polarized cell exhibiting well defined apical, basal, and lateral domains. Although these domains share some similarities in *Drosophila* and vertebrate epithelia, morphological differences have hindered attempts to draw parallels across species.

One feature of apical–basal polarity that is conserved is the adherens junction, which is the major site of epithelial cell–cell contact (Yeaman et al., 1999; Gumbiner, 2000). The adherens junction is one component of the junctional complex that divides apical and basolateral membrane domains. In *Drosophila*, *C. elegans*, and vertebrate epithelia, the core elements of adherens junctions are cadherin adhesion proteins whose intracellular domains interact with α -Catenin and β -Catenin/Armadillo (ARM)¹ proteins (Fig. 1).

Address correspondence to Helmut Krämer, Center for Basic Neuroscience and Department of Cell Biology, University of Texas Southwestern Medical Center, Dallas, TX 75390-9111. Tel.: (214) 648-1860. Fax: (214) 648-1801. E-mail: kramer@utswmed.edu

¹Abbreviations used in this paper: aPKC, atypical protein kinase C; ARM, armadillo; DaPKC, PDZ-domain protein Bazooka and the atypical protein kinase C; LAP, leucine-rich repeat and PDZ domain.

Unlike the adherens junction, other cell–cell contact sites exhibit pronounced differences between vertebrate and insect epithelia. In vertebrates, tight junctions located just apical to the adherens junctions seal the epithelium against diffusion between the cells (“gate function”) and also provide a barrier for lipids and proteins within the membrane (“fence function”) (Yeaman et al., 1999). The structure of tight junctions is not well understood, though several constituents are known (Furuse et al., 1998; Izumi et al., 1998; Tsukita and Furuse, 1999; Yeaman et al., 1999). Unlike apically located tight junctions, insect septate junctions are found just basal to the adherens junction. Septate-junction ultrastructure, which is characterized by a striated pattern between cells, also differs from that of tight junctions (Tepass and Hartenstein, 1994). Because tight junctions are not apparent in insect tissues, septate junctions have been considered their functional equivalent (Bryant, 1997). However, this supposition is questionable given recent discoveries of molecules necessary for the formation of different junctions (Bilder and Perrimon, 2000; Tanentzapf et al., 2000; Wodarz et al., 2000).

Scribble and Disc-large are two components of septate junctions in *Drosophila* (Woods and Bryant, 1991; Bilder and Perrimon, 2000). Together with Lethal-giant-larvae, they block the invasion of apical and adherens-junctions proteins into basolateral domains. For example, in embryos mutant for any of these three genes, the apical marker Crumbs and the adherens-junction marker ARM are no longer restricted to their proper domains (Bilder et al., 2000). The *scribble* gene encodes one of the leucine-rich repeat and PDZ domain (LAP) linker proteins, which are so named because they contain both of these protein–protein interaction domains (Bilder and Perrimon, 2000; Borg et al., 2000; Legouis et al., 2000).

Like Scribble, other basolaterally located members of the LAP family are involved in maintaining the basolateral domain. For example, the *scribble* homolog ERBIN is necessary for the basolateral location of the mammalian epidermal growth factor receptor homolog Her-2/ErbB2 (Legouis et al., 2000). Furthermore, the *LET-413* gene encodes a LAP protein that is required for siting adherens junctions and apical markers in *C. elegans* (Legouis et al., 2000), which is in striking similarity to the *scribble* phenotype in *Drosophila* (Bilder and Perrimon, 2000). There-

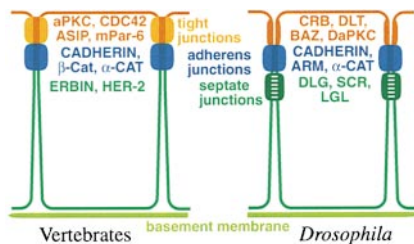


Figure 1. Proteins that compose different junctional complexes in *Drosophila* and vertebrate cells are conserved and localize to equivalent membrane domains. See text for further details.

fore, LAP proteins provide an example of a familiar theme found in many specialized membrane domains, including epithelial junctions, synapses, and *Drosophila* rhabdomeres. The functional assembly of each of these membrane domains depends on PDZ domain-containing proteins clustering distinct transmembrane and cytoskeletal proteins and excluding others (for reviews see Fanning and Anderson, 1999; Garner et al., 2000).

A similar mechanism may also operate in defining the apical surface. Crumbs is an apical transmembrane protein whose interaction with the PDZ-protein Disc lost is critical to establishing epithelial polarity in the fly embryo (Tepass et al., 1990; Bhat et al., 1999; Klebes and Knust, 2000; Tanentzapf et al., 2000). The importance of this interaction is stressed by the finding that most of Crumbs' function can be provided by a truncated form of Crumbs that retains only the transmembrane domain and the short cytoplasmic domain that interacts with Disc lost (Wodarz et al., 1995; Klebes and Knust, 2000). Crumbs overexpression results in a relative expansion of apical domains, whereas loss of Crumbs disrupts the structure of the embryonic epithelium (Tepass et al., 1990; Wodarz et al., 1995). Because *Crumbs* null mutations have such severe phenotypes, the primary consequences of its loss are not easily characterized.

Tepass and co-workers circumvented this difficulty by examining the signals that specify apical and lateral surfaces in the follicle-cell epithelium (Tanentzapf et al., 2000). In this epithelium, which surrounds each cluster of germline cells from which *Drosophila* oocytes develop, tissue integrity was maintained despite the loss of apical polarity. In ovaries that lacked germ cells, basal and lateral markers remained separate in follicle cells. In contrast, apical and lateral markers were no longer clearly segregated and Crumbs expression was lost from the apical surface. These specific mislocalization results indicate that the apical signal is provided by the germ cells.

The authors went on to induce mosaic follicle epithelia in which some cells had lost the *crumbs* or *disc lost* genes (Tanentzapf et al., 2000). Both mutations caused a partial loss of apical markers, consistent with the hypothesis that Crumbs acts through Disc lost (Bhat et al., 1999; Klebes and Knust, 2000; Tanentzapf et al., 2000). This idea was further strengthened by the colocalization of Disc lost and Crumbs to apical membranes. Both were especially enriched in the lateral domains just apical to the adherens junctions, a site which corresponds to that of tight junctions in vertebrates (Tanentzapf et al., 2000). Although no morphological specialization in this region is distinguishable, it will be interesting to see whether some of the cell

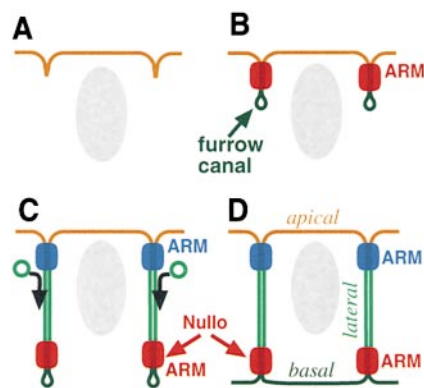


Figure 2. Establishment of polarity is tightly coupled to cellularization in *Drosophila* embryos. The temporal order of membrane insertion is translated into spatial differences by the isolation of different membrane domains from the main site of insertion (Hunter and Wieschaus, 2000; Lecuit and Wieschaus, 2000). (A) Shortly after the initial stage of cellularization, (B) a first set of junctional complexes, marked by ARM, is formed just below the embryonic cell surface. These complexes seal off the most basal membrane domain that forms the furrow canals. (C) As cellularization proceeds, the initial set (red) of junctions move basally together with the furrow canals. A second set of junctions (blue) is formed just below the cell surface and separates the apical and lateral domains. Membrane insertion basal to these junctions continues to extend the lateral domain. (D) The furrow canals will form the basal membrane domains and shortly thereafter, as the embryo initiates gastrulation, the unusual basal junctions disappear.

signaling, "gate" or "fence," functions associated with vertebrate tight junctions (Yeaman et al., 1999) are also found in this domain of invertebrate epithelia.

Because some apical markers are retained in *crumbs* and *disc lost* mutant clones, additional determinants for apical domains must exist. Knust and co-workers now show that the PDZ-domain protein Bazooka and the atypical protein kinase C (DaPKC) are two key components of such an apical determinant in the *Drosophila* embryo (Wodarz et al., 2000). The *bazooka* gene was implicated in establishing cell polarity by its mutant phenotype and its similarity to the *Par-3* gene, which is necessary for orienting mitotic spindles in early cell divisions of *C. elegans* embryos (Etemad-Moghadam et al., 1995; Kuchinke et al., 1998). Because in *C. elegans* and vertebrates aPKCs bind to Bazooka homologs (Izumi et al., 1998; Tabuse et al., 1998), Knust and co-workers explored the role of the single *DaPKC* gene found in the *Drosophila* genome. Analysis of *DaPKC* and *bazooka* mutants revealed that the apical localization of each protein depended on the other (Wodarz et al., 2000). This indicates that Bazooka is not only an anchor for DaPKC, but also depends on DaPKC function for its localization, consistent with the phosphorylation of its murine homolog m-PAR-3 by the aPKC ζ isoform (Lin et al., 2000).

Along with aPKCs, Par-3 homologs are constituents of large complexes that include Par-6 and CDC42 (Joberty et al., 2000; Lin et al., 2000; Qiu et al., 2000). CDC42 is a small GTPase that regulates multiple elements of the actin cytoskeleton (Kaibuchi et al., 1999) and selective membrane transport to the basolateral membrane (Kro-

schewski et al., 1999). How this well-conserved complex cooperates in integrating the polarization of membrane domains and cytoskeletal elements is unknown. Interestingly, Bazooka is not only necessary for polarity in epithelial cells, but it also orients asymmetric cell divisions in the nervous system (Schaefer et al., 2000; Wodarz et al., 2000; Yu et al., 2000), which is similar to the role of the Par-3 complex in the *C. elegans* embryo (Etemad-Moghadam et al., 1995).

The extent to which the determinants of apical-basal polarity and the components of epithelial junctions are conserved suggests that the basic mechanism by which apical-basal polarity is established is also universal. It is therefore intriguing to study cellularization, the one hour during *Drosophila* embryogenesis in which 6,000 polarized cells are formed. Two recent papers from the Wieschaus laboratory closely examine this time window (Hunter and Wieschaus, 2000; Lecuit and Wieschaus, 2000) and present two striking findings. First, the emergence of polarity in the epithelium is tightly integrated with the process of cellularization. Second, the *Drosophila* embryo not only establishes cellular junctions in a synchronized manner, it actually does so twice in each cell (Fig. 2).

Lecuit and Wieschaus (2000) probed the growth of the invaginating membranes to address a long-standing question: what is the source of the membranes that fuel the dramatic extension of the plasmalemma during cellularization? By following the fate of the newly synthesized Neu transmembrane protein, they established that secretion from the Golgi complex is the predominant source of new membranes. This result is further supported by the study of Sullivan and co-workers in this issue of the *Journal of Cell Biology* (Sisson et al., 2000). These authors made use of an antibody against the Lava lamp protein, a novel peripheral Golgi protein, which was isolated as a result of its association with microtubules and actin filaments. Disruption of the Golgi complex organization by the injection of anti-Lava lamp antibodies or Brefeldin A into early embryos dramatically inhibited cellularization (Sisson et al., 2000).

By labeling emerging or preexisting surface membranes using the Neu protein or lectin-coated beads, Lecuit and Wieschaus also demonstrated that insertion of these Golgi-derived membranes preferentially occurs at a defined site: the apical most region of the lateral domain (Lecuit and Wieschaus, 2000). To their surprise, after an initial phase of unrestricted diffusion in the membrane, the newly added membrane was excluded from the leading edge of the invaginating membrane, the furrow canal. This result is inconsistent with the prevailing model, which proposes that the bulk of membrane is inserted at the leading edge (Lencar and Singer, 1995). The functional isolation of the furrow canal membranes could be important for the specific assembly of the actin cytoskeleton at the leading edge of the invaginating membranes. Contraction of this localized actomyosin network at the leading edge is one of the elements thought to provide the force for membrane invagination (Schejter and Wieschaus, 1993; Sisson et al., 2000).

The fence that separates the furrow canal membranes appears to be formed by a first junctional complex just apical to the furrow canal (Fig. 2). As the membranes invaginate, these initial junctions, which eventually separate the basal and lateral domains, stay close to the furrow canal as

it is moved basally. These initial junctions contain the junctional proteins ARM, Cadherin, and Disc-lost (Bhat et al., 1999; Hunter and Wieschaus, 2000; Lecuit and Wieschaus, 2000), a composition very similar to the second junctional complex formed later during cellularization in a more conventional position, separating the apical and lateral membrane domains. To form the unusual set of basal junctions, cells require the Nullo protein; in its absence elements of basal junctions are scattered along the lateral domains (Hunter and Wieschaus, 2000). Whereas homologs of other junctional-complex components are found in *C. elegans* and vertebrates, Nullo is a novel protein, consistent with its role in a process unique to *Drosophila*.

The finding that membranes insert in a restricted site during cellularization (Lecuit and Wieschaus, 2000) adds strong support to a model previously proposed for polarized cells in vertebrate epithelia. In this model the Sec6/8 multiprotein complex acts to restrict delivery of secretory vesicles to the basolateral membrane (Grindstaff et al., 1998; Yeaman et al., 1999). The direct observation of such restricted insertion in *Drosophila* is a testimony to the advantages that this system offers for deciphering the signals that tell a cell what is up and what is down.

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