Epithelial polarity: **The ins and outs of the fly epidermis**

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Epithelial cells must polarize and establish apical and basolateral membrane domains during development. Recent experiments have shed light on how apical–basal polarity is generated during cellularization in *Drosophila*, when around 6000 epithelial cells are created synchronously from a syncytium.

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The functions of an epithelium depend on the polarized organisation of the individual epithelial cells. The plasma membranes of epithelial cells are subdivided into apical and basolateral compartments, which differ in their protein and lipid composition [1]. The border between these compartments is established by the adherens junctions, which also act to maintain a tight connection between epithelial cells. How are these compartments and junctions established? Much of our knowledge about the establishment of epithelial cell polarity derives from the analysis of tissue culture cells which can be induced to polarize and form functional epithelia in a dish (see [2] for a detailed review).

The development of the *Drosophila* epidermis provides an ideal model system for a genetic analysis of the establishment of epithelial polarity *in vivo* (see [3] for an excellent review on epithelial polarity in *Drosophila*). Epidermal cells arise early during development of the fruitfly from a syncytium containing around 6000 nuclei. During embryonic development, a fully functional epithelium, containing clearly separated apical and basolateral membrane compartments, is gradually formed. Adherens junctions are first detected after cellularization; during development, these junctions progressively mature into zonula adherens, belt-like structures around the apex of epithelial cells.

A recent detailed study of cellularization has shed some new light on how polarity may be established in *Drosophila* epidermal cells. By carefully analyzing membrane flow during early embryogenesis, Lecuit and Wieschaus [4] found that individual membrane compartments are already established during the process of cellularization. This unusual compartmentalization of incompletely formed cells requires specialized junctions, and in another study the same group [5] has shown that a protein known as Nullo, which functions specifically during cellularization, is required for the formation of these distinct junctions.

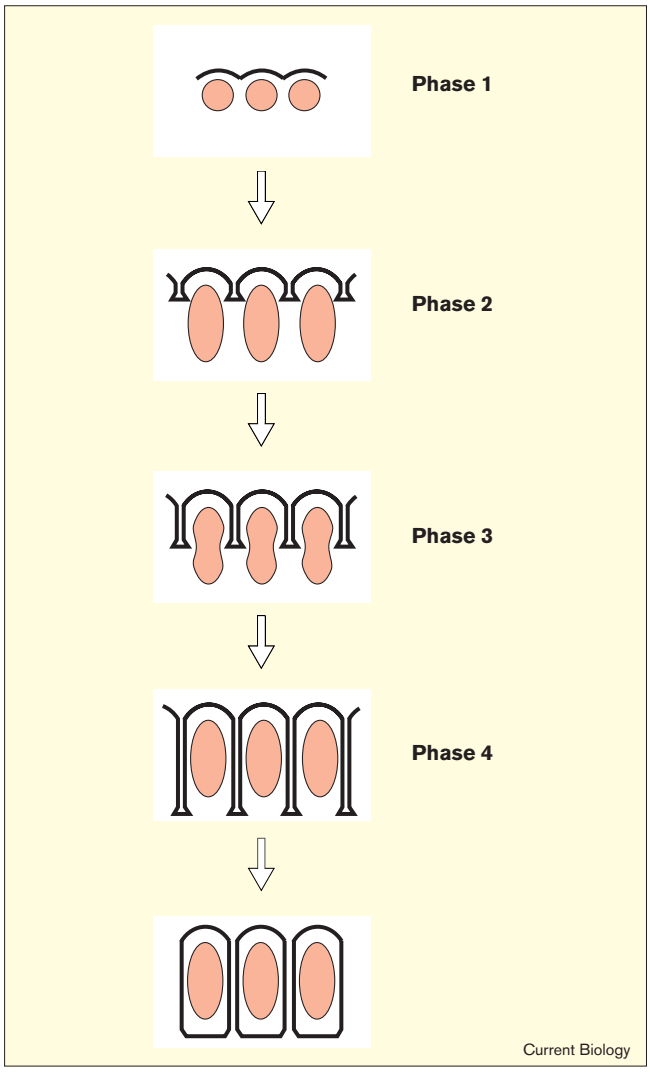
To understand these experiments, an overview of the morphological events during cellularization is essential. *Drosophila* development starts with a series of 13 rapid nuclear divisions. Towards the end of these syncytial cell cycles the nuclei move to the oocyte cell cortex where, after the last nuclear division, they are individually ensheathed by plasma membranes to form around 6000 individual epithelial cells. Morphologically, this cellularization process can be subdivided into four individual phases (Figure 1) [4,6]. During phase one, the first structurally distinct areas become visible at the cell cortex. While the cortical area overlying the nuclei is highly folded into microvilli, the region between the nuclei begins to extend basally to form a structure called the furrow canal, which is maintained throughout cellularization. Actin, myosin and the PDZ domain protein Discs-lost (Dlt) [7] concentrate in the furrow canal and serve as markers for this structure.

During the next three phases of cellularization, the furrow canal is pulled inwards by an actin-myosin-dependent mechanism, which operates at progressively increasing speed. In phase two, this movement is still very slow and in fact barely detectable, but the nuclei extend in an apical-basal direction. Phase three is characterized by a slow inward movement of the furrow canals, until they have reached the basal part of the nuclei. During phase four, the speed of furrow canal movement suddenly doubles. The movement stops when the furrows are around 35 μm long and a contractile ring at their basal end pinches off the newly formed blastoderm cells.

From the time of their birth, blastoderm cells contain apical and basolateral membrane compartments which are marked by distinct localization of certain transmembrane or membrane-associated proteins [3]. What is the origin of these compartments? Are different compartments formed from different sources of membrane material during cellularization? Earlier models of cellularization assumed that microvilli, which are present above each nucleus before cellularization but are gone after cellularization, are the source of the membrane material required during cellularization. Such a model is incompatible with membrane compartmentalization during cellularization, unless one assumes that microvilli are already subdivided into compartments according to their future position along the apical-basal axis.

Using a newly developed method for membrane tracking, Lecuit and Wieschaus [4] found that most of the blastoderm cell membrane is actually recruited from intracellular sources and inserted during cellularization at

Figure 1



During early *Drosophila* development, 6000 individual cells are formed from a syncytium in a process called cellularization. This figure illustrates the four distinct phases which can be distinguished during this process (see text for details).

distinct spots. They injected limiting amounts of the fluorescently labelled lectin wheat germ agglutinin (WGA) close to the apical surface of cellularizing *Drosophila* embryos (Figure 2). WGA binds rapidly and permanently to sugar residues present on membrane proteins, and thus creates a mark for this membrane area which then can be followed during cellularization by video time-lapse microscopy.

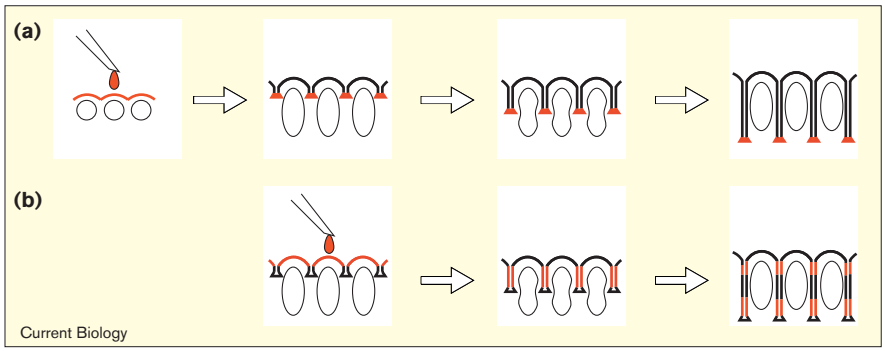
Early injection of fluorescently labelled WGA marked the entire membrane surface overlying the nuclei, which contains the microvilli (Figure 2a). Surprisingly, however, the early-injected label was found to stay confined to the furrow canal throughout cellularization, and to move inward with the cellularization front. This suggests that the furrow canal is an isolated membrane domain, and shows that at least two distinct membrane domains are already formed at the onset of cellularization. Label injected during phase 2 or 3 of cellularization was found to be displaced from the apical surface and move inward at the speed of the cellularization front, even though it never entered the furrow canal (Figure 2b). During phase 4, however, the pattern of membrane movement changes. When labeled WGA was injected during this fast phase of cellularization, the apical membrane maintained its label while the lateral fraction moved inward with the cellularization front, leaving a gap of unlabelled membrane between these two areas.

These observations show that the plasma membrane is already subdivided into individual compartments during cellularization, and that new membrane is inserted at distinct sites — apically first, and then at a more lateral position during phase 4. What is the source of the newly inserted plasma membrane? Earlier electron microscopy experiments had shown the presence of cytoplasmic membrane stacks, called lamellar bodies, which were apparently connected to the cleavage furrows and progressively disappeared during cellularization [8], suggesting that membrane insertion occurs from cytoplasmic sources. The transmembrane protein Neurotactin is incorporated into plasma membranes during cellularization and can serve as a marker for the membrane synthesis pathway [4]. Neurotactin is first detected in close proximity to cis-Golgi membranes and later in exactly the area where membrane insertion occurs during phase 4. After disruption of microtubules, the protein fails to incorporate into the plasma membrane and accumulates in cis-Golgi associated membrane structures instead. These results suggest that new membrane is inserted from the Golgi/endoplasmic reticulum compartment via the secretory pathway in a microtubule-dependent process.

Even though the furrow canal is set aside as independent membrane domain at the beginning of cellularization, no junctions have been described that form so early during development that they could separate the furrow canal from the rest of the plasma membrane. A careful analysis of the distribution during cellularization of the *Drosophila* β-catenin homolog Armadillo and *D*E-cadherin, both markers for adherens junctions, showed that both proteins concentrate during phase 1 of cellularization in a region just apical to the furrow canal [5]. Indeed, electron microscopy revealed a region of tight membrane association at the same position, suggesting the existence of a second, basal adherens junction. The basal junction moves inward with the cleavage furrow, suggesting that it continues to separate the furrow canal as an independent membrane domain. By the end of cellularization, however, most Armadillo protein was seen to relocalize to the apical junction.

Figure 2

Membrane labelling using fluorophore-coupled lectins has distinguished separate membrane domains which form sequentially during *Drosophila* embryo cellularization. **(a)** Labelling during phase 1; **(b)** labelling during phase 2 or 3. (See text for details.)



This relocalization of Armadillo coincides with the degradation of the protein Nullo, which had been identified as the product of one of the few zygotic loci required for the process of cellularization. In *nullo* mutants, defects occur in the characteristic reorganisation of the actin–myosin network at the onset of cellularization and many of the cleavage furrows are not formed [9]. In the furrows that do form, Armadillo does not accumulate in the basal junction, even though the later apical accumulation is unaffected. Conversely, prolonged expression of Nullo causes a defect in apical junction formation. Thus, Nullo is required for the formation of basal junctions, while it inhibits apical junction formation. How might Nullo perform this function? Nullo protein can be detected in both the basal junction and the furrow canal, suggesting that it does not just determine the position of Armadillo accumulation. Nullo might facilitate Armadillo accumulation to a point of close membrane contact. The fact that prolonged *nullo* expression recruits Armadillo to the whole lateral membrane and prevents its apical concentration is consistent with this hypothesis.

While Nullo is required for basal junction assembly, no single mutant has been described up to now that causes a similar defect of the apical junction. In *stardust*, *bazooka* double mutant embryos, however, the apical junction fails to assemble and Armadillo protein accumulates in a punctate pattern along the apicolateral cortex [10]. This strong genetic interaction may actually reflect a requirement for these two proteins in adherens junction establishment which is masked by the strong maternal contribution that has been described at least for *bazooka*. Analysis of embryos that are both maternally and zygotically null-mutant for these genes may reveal whether they play a similar role in formation of the apical junction as Nullo does in formation of the basal junction.

The mechanisms that establish epithelial polarity during *Drosophila* cellularization and in cultured epithelial cells are characteristically different, even though they use overlapping mechanisms. In cultured cells, sites of cell–cell contact, mediated by cadherins, or of cell–extracellular matrix interaction, mediated by integrins, serve as points of reference [11]. Both forms of cell adhesion induce localized assembly of cytoskeletal structures, as well as signalling networks at the contact site. Cadherin binds to β-catenin and recruits α-catenin, an actin-bundling protein. Integrins directly bind to the cytoskeletal proteins α-actinin and vinculin. These cytoskeletal changes in turn lead to assembly of a specialized membrane skeleton on the cytoplasmic face of the membrane at the contact site, which in turn may alter its protein content by modulating membrane endocytosis or generating targeting patches for transport vesicles.

In *Drosophila*, the future axis of polarity is predetermined by the anatomy of the embryo. Here, the basal junction seems to be the first point of reference during cellularization. The fact that Armadillo and *D*E-Cadherin localize to this junction make it functionally equivalent to the cell–cell contact points in tissue culture cells. The requirement for Nullo, which does not have obvious homologs outside *Drosophila*, may reflect the fact that junctions do not form at cell-contact points, but at a precise position next to the actin–myosin contractile ring in the furrow canal. Even though the early syncytial development is a speciality of *Drosophila*, it will be interesting to determine whether similar mechanisms couple junction formation to embryonic anatomy in other organisms.

The establishment of separate membrane domains during very early embryonic development has actually also been observed in other organisms. In *Xenopus*, early development does not occur in a syncytium, but the cytokinesis of the first cell division — and actually cytokinesis in general — may be mechanistically quite similar to cellularization in flies. During both events, the plasma membrane is pulled inwards in an actin–myosin-dependent mechanism and new plasma membrane is inserted during this process [12]. New membrane insertion occurs exclusively into the furrow and not into the exterior plasma membrane [13]. Furthermore, proteins containing a basolateral sorting signal

accumulate in the furrow and are excluded from the outer surface [13]. It will be interesting to determine whether this sorting mechanism is actually the basis for establishing the apical domain of the embryonic epithelium, which will eventually form from the outer surface. No homolog of Nullo has been found in *Xenopus*, but the requirement for such a basal junction as reference point for the early establishment of membrane domains may actually extend well beyond *Drosophila*.

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