Alternating polarity in a linear array of *Scaptodrosophila* follicle cells

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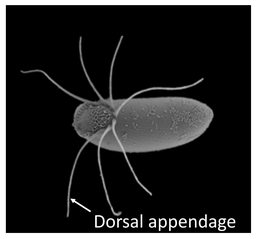
Abstract

Epithelial cells can exhibit multiple types of polarity. In addition to the apical-basal polarity generally found in epithelia, polarization within the plane of the epithelium can also be present. Examples include the planar cell polarity (PCP) pathway, in which cells are all oriented in a single direction within the plane of an epithelial sheet, or the planar polarity seen during Drosophila axis elongation, where Bazooka (Par-3) is localized to dorsal-ventral cell-cell interfaces and Myosin II is localized to anterior-posterior interfaces. Here we examine the in-plane polarity in the floor cells of the follicular epithelium of *Scaptodrosophila pattersoni* (which is conspecific with *Scaptodrosophila lebanonensis*). During stage 10 of oogenesis, these cells form a single curved line within the epithelium. In later stages, these cells deform to create the underside of the dorsal appendages, through a process that involves the substantial lengthening of alternating floor-floor interfaces. In other words, there is an alternating left/right pattern, so if a given floor cell specifically lengthens on its left edge, its neighboring floor cells lengthen on their right edges. The floor-floor interfaces that are fated to lengthen show a strong enrichment of Par-3, aPKC, and F-actin localization at the onset of lengthening. Pharmacological approaches suggest that alternating polarization of F-actin, or possibly the maintenance of this polarization, is not dependent on aPKC localization. We are continuing to examine the molecular basis underlying this unusual example of planar polarity.

Experimental system:

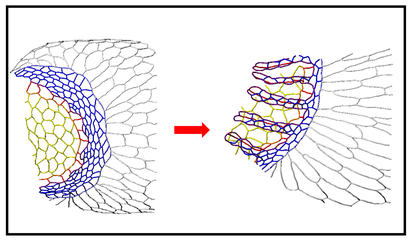
Dorsal appendages of *Scaptodrosophila* eggshells



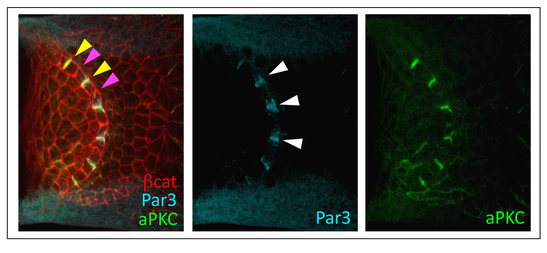
The eggshells of *Scaptodrosophila lebanonensis* (also known by the junior synonym *S. pattersoni* [1]) have a variable number of dorsal appendages [2-3], ranging from 4 to 8. The eggshell shown (left) has 7.

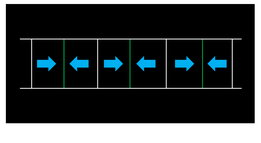
These dorsal appendages are formed from specific sub-types of follicle cells that rearrange and change shape during late oogenesis (see below).

In the 3D reconstruction of cell outlines shown to the right, the floor cells (red) begin as a single, arched row of cells at stage 10 (left). As the dorsal appendage tubes are formed, the floor cells change shape so that every second floor-floor edge elongates, forming the bottom of a single tube (right) [2].



Even before the floor cells change shape, the floor-floor edges fall into two classes: edges that are positive for Par3 and aPKC (yellow arrowheads, left), alternating with edges that are negative for these markers (magenta arrowheads) [2].





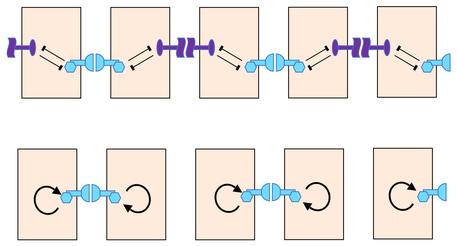
Since small Par3-positive puncta accumulate on both sides of positive edges (white arrowheads above), we think every floor cell expresses Par3 and aPKC, but is polarized so that these molecules accumulate on only one edge. Within the floor cell domain, this polarization alternates, i.e. left-right-left-right.

Hypotheses:

How is this pattern of alternating cell polarity established?

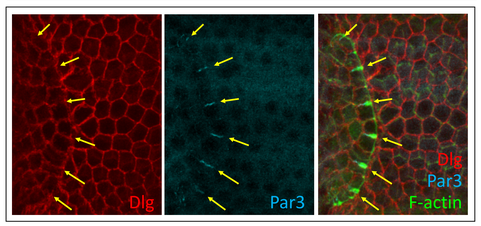
Other instances of cell polarity largely rely on some type of feedback. This can include negative interactions between two complexes of protein (X and Y) to prevent co-localization and/or positive feedback to recruit more complex components of the same type to regions with high concentrations [4].

Since the polarity of these cell alternates, there must be some way for each cell to "read" the cell polarity of its neighbors. This could occur if one or more polarity complex member is a homophilic adhesion molecule, but could also occur through other means, including secreted ligands or mechanical signals. Just two combinations of these possible mechanisms are shown to the right.



Results

To look for other determinants of alternating cell polarity, we performed immunostaining to screen for candidate molecules that localize specifically to either Par/aPKC-positive or Par/aPKC-negative floor cell edges.



Since Par3 and aPKC are involved in establishing apical-basal polarity, the first set of candidates screened were other proteins involved in apical-basal polarity.

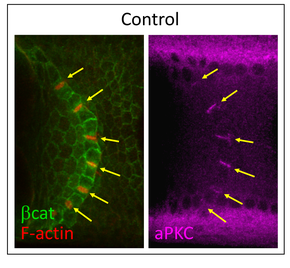
PatJ (a member of the Crumbs complex) showed slight enrichment on the Par3-positive edges.

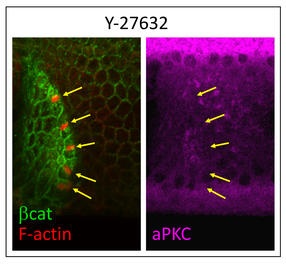
Dlg (a member of the Scribble polarity complex) showed no obvious polarity.

Unexpectedly, F-actin, which was used to visualize cell boundaries, showed dramatic enrichment on the Par3-positive edges.

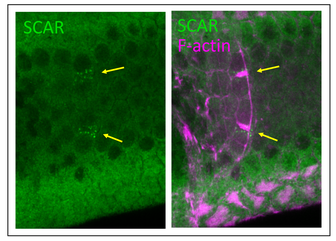
To determine the epistatic interactions among the polarity components identified so far, egg chambers were cultured *in vitro* in a variety of pharmacological inhibitors, then immunostained. Culturing the egg chambers in the presence of Y-27632 (a Rho Kinase inhibitor with known off-target effects on aPKC) reproducibly blocked the alternating pattern of aPKC localization, but not the alternating pattern of F-actin localization.

Further experiments are needed to determine whether these results reflect establishment or maintenance of polarity. However, they suggest that polarized F-actin accumulation may be upstream of aPKC.



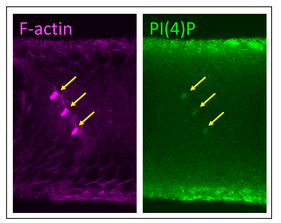


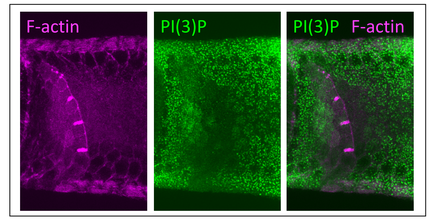
Therefore, the next set of candidates examined by immunostaining were actin regulators.

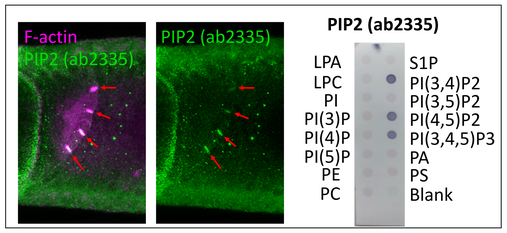


Of the small number of actin-binding or actin-regulating proteins found to be polarized in this system, one of the most intriguing was SCAR, which promotes actin nucleation through Arp2/3.

SCAR and several other actin regulators have domains thought to mediate binding to phosphoinositides, so this family of molecules was examined next.







Antibodies against PI(4)P and PIP2 localize clearly to the actin-rich floor-floor edges.

At the same developmental time, PI(3)P positive vesicles are largely depleted from floor cells.

The PIP2 antibody used (ab2335) was raised against PI(4,5)P2, a known regulator of actin dynamics, but also binds to PI(3,4)P2 and PIP3 (see above and [5]). The identity of the molecule recognized in this tissue is crucial question to answer in future experiments.

Summary

Several molecules exhibit alternating polarity in *Scaptodrosophila* floor cells; these include Par3, aPKC, F-actin, SCAR, and PI(4)P.

Further studies examining the epistatic relationships among these molecules are essential to begin determining the mechanisms underlying alternating polarity in this system.

References

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Acknowledgements

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