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Patterning: JAK–STAT Signalling in the *Drosophila* Follicular Epithelium

Dispatch

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During *Drosophila* oogenesis the follicular epithelium becomes subdivided into distinct cell populations. New reports have established that the Janus kinase (JAK) signalling pathway plays an important role in this process.

The proper functioning of a tissue or organ requires that each cell is specified correctly according to its position. This positional information is often provided by signalling molecules secreted from a local source which act in a concentration-dependent manner to specify distinct fates. New work by Xi *et al.* [1] has now shown that graded activation of the Janus kinase (JAK) pathway is involved in specifying cell fates along the anterior–posterior axis of the follicular epithelium in the *Drosophila* egg chamber. This report further extends other recent work which implicated JAK signalling in the induction of follicle cell fates [2–6].

A *Drosophila* egg chamber consists of fifteen nurse cells and one oocyte, surrounded by a monolayer of somatic follicle cells (reviewed in [7]). Accurate patterning of this follicle cell layer along the anterior–posterior axis is crucial for the establishment of polarity in the future embryo and for the deposition of a functional egg shell. The first differences between cells in the follicular epithelium are established very early in oogenesis, when a small number of cells stop dividing and give rise to two polar cells at each end of the egg chamber [8,9]. The remaining follicle cells undergo another four to five rounds of mitosis, generating an epithelium of about 1000 cells. During this period, the follicle cell layer gets further subdivided into two terminal regions and a central or mainbody region (Figure 1). The terminal domains are originally symmetrically patterned with cells adopting different fates depending on their distance from the poles [10].

This symmetry is broken when the TGFα-like ligand Gurken, produced in the oocyte, binds to the epidermal growth factor (EGF) receptor in the overlying terminal cells, instructing them to assume a posterior fate. Thus, a polarized anterior–posterior axis is established [10–12]. The differences between follicle cells along this axis become obvious when cell division ceases and cells start differentiating. Apart from the mainbody and posterior terminal follicle cells, three anterior terminal subpopulations can be distinguished: border, stretched and centripetal cells. The molecular and morphological changes each of these cell types undergoes are critical for the proper development of the egg.

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Several lines of evidence have pointed to a central role for the polar cells in organizing anterior–posterior polarity within the follicular epithelium. Ectopic polar cells can induce neighbouring cells to adopt a border cell fate or posterior terminal fate, depending on their position in the egg chamber [13–16]. Conversely, in the absence of polar cells, none of the three distinct anterior terminal cell fates gets specified [16]. These observations have led to a model in which a signal secreted by the polar cells acts as a morphogen to specify the terminal cell populations and to organize them into distinct domains, with border cells being induced at the highest level of signalling and centripetal cells at the lowest (Figure 1).

Until now, however, the molecular nature of this signal remained unknown. New evidence presented by Xi *et al*. [1] indicates that Upd, a ligand for the JAK pathway in *Drosophila*, may be that signal. JAK signalling is widely used throughout the animal kingdom to determine cell fates in response to extracellular signals (reviewed in [17]). Upd was a prime candidate to be the signal required for terminal follicle cell determination, because it is expressed specifically in the polar cells and recent work has shown that JAK signalling is required for the specification of at least one anterior fate, the border cells [2–5]. To investigate whether Upd acts as a morphogen to pattern the follicle cells, Xi *et al.* [1] analyzed egg chambers in which they genetically altered expression levels of several components of the JAK pathway and assessed cell fates by looking at the expression of different marker genes and by analyzing cellular morphology.

Several questions were addressed. First, is there a gradient of JAK activity in the follicular epithelium? The possibility of graded activation of the pathway had been previously suggested based on studies in the *Drosophila* eye and hindgut [18,19]. Xi *et al.* [1] observed graded expression of a number of JAK-responsive reporter genes, both in their endogenous domains and in response to ectopic Upd. A model in which Upd turns on a secondary signal which in turn patterns the rest of the epithelium is unlikely, as overexpression of intracellular JAK has a strictly cell-autonomous effect. Moreover, nuclear accumulation of STAT, the transcription factor downstream of JAK which may be a more direct read-out for pathway activation, is also clearly graded in the follicular epithelium, with maximum levels near the Upd-producing polar cells.

Second, does graded JAK activity specify all anterior and posterior terminal cells? Xi *et al.* [1] show conclusively that JAK signalling specifies posterior cell fate within the posterior terminal domain. The most anterior terminal cells, the border cells, also clearly require JAK signalling. Earlier work had already shown that high levels of JAK activity are necessary and sufficient for border cell identity, at least within the anterior terminal domain [2–4].

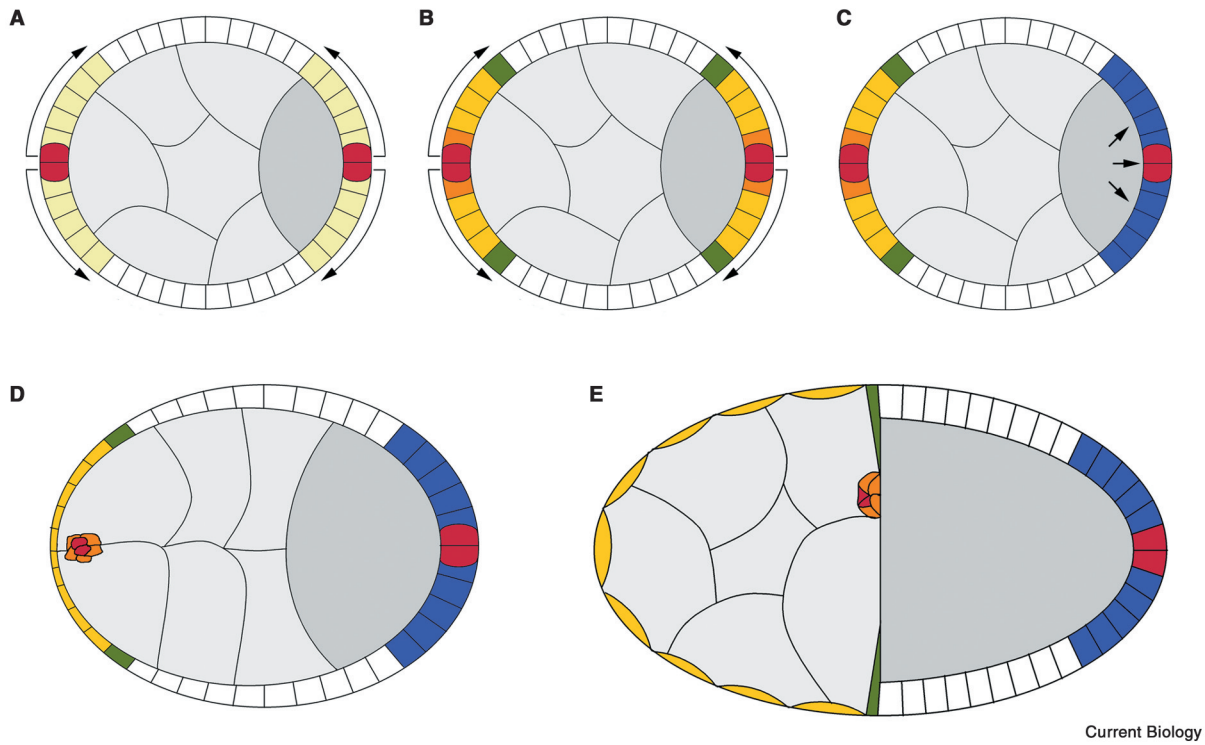

Figure 1. A model for anterior–posterior patterning of the follicle cell epithelium in *Drosophila* oogenesis.

Schematic representations of pre-stage 6 (A–C), stage 9 (D) or stage 10B (E) egg chambers. Anterior is to the left and posterior to the right. (A,B) Prior to stage 6 of oogenesis, the follicle cell layer gets subdivided into terminal (light yellow) and mainbody (white) domains (A). A symmetric prepattern is established within the terminal domains in which cells are committed to distinct fates depending on their proximity to the poles (B). According to the current model, a signal emanating from the two polar cells (red), located on each side of the egg chamber, specifies the terminal cell populations and organizes them into distinct domains. (C) The symmetry in this prepattern is broken when Gurken, produced by the oocyte (grey), binds to the EGF receptor in the overlying terminal cells and instructs them to adopt a posterior identity (blue). In the absence of Gurken–EGF receptor signalling these cells fail to become posterior and instead take up the three distinct anterior fates. (D,E) The differences between cells along the anterior–posterior axis become obvious after cells start differentiating. The three anterior cell types undergo a number of dramatic morphological changes. At the anterior tip, directly surrounding the polar cells, 6–8 border cells (orange) delaminate from the epithelium in the beginning of stage 9 and migrate in between the nurse cells to reach the anterior end of the oocyte by the beginning of stage 10. At the same time, a group of 40 stretched cells (dark yellow) adjacent to the border cells flatten to form a squamous epithelium over the nurse cells, while the remaining follicle cells move posteriorly to form a columnar epithelium covering the growing oocyte. During stage 10B (E), the columnar cells abutting the stretched cells migrate in between the nurse cells and the oocyte to cover the anterior of the oocyte. These cells are called centripetal cells (green).

But the conclusions are less straightforward in the case of the two other anterior cell types, stretched and centripetal cells. Beccari *et al.* [3] had previously reported that in *hop* or *stat92E* mutants — defective for the fly JAK and STAT homolog, respectively — stretched or centripetal cells are mostly specified correctly [3]. In contrast, Xi *et al.* [1] found that stretched cells fail to differentiate in *hop* loss-of-function clones, and that their number is slightly reduced in weak mutants. One possible explanation for the discrepancy between the two reports might be timing of clone induction: if mutant clones are generated after the onset of a certain 'specification program', cells might not be able to revert completely to a different fate. While the data presented by Xi *et al.* [1] are consistent with a gradient model, they could also be explained by a general requirement of JAK signalling to induce terminal fate, rather than a specific requirement for stretched cell identity. The observation that a centripetal marker can be turned on in mainbody follicle cells in response to ectopic Upd suggests but does not unambiguously prove a direct role for JAK signalling in determining centripetal identity. Again, JAK signalling might merely be required for the specification of terminal cells, and centripetal fate might be established by a secondary system, for instance, operating at the boundary between terminal and mainbody follicle cells.

Another conflicting result that needs to be resolved is the observation reported by Grammont and Irvine [16] that large *upd* mutant clones that include the anterior polar cells and thus the source of Upd production reduced the number of border cells but had no effects on stretched or centripetal cells. This seemingly contradictory result might be explained by redundancy among Upd ligands. Three other *upd*-like genes have been predicted from genome analysis, and deletion of all four genes causes embryonic phenotypes more severe than that of single *upd* mutants [20]. This suggests that, at least in the embryo, *upd* genes can act in a partially redundant way.

Finally, Xi *et al.* [1] acknowledge that other signals have to be invoked to fully understand anterior–posterior patterning in the follicular epithelium. One prediction from a simple gradient model would be that ectopic Upd production in the mainbody domain should be sufficient to turn on the most extreme terminal fates. This, however, is not the case. Whereas high-level pathway activation can occur in mainbody cells in response to ectopic JAK activation, as judged by STAT nuclear accumulation and induction of the high-threshold target *domeless–lacZ*, border cells can be induced only in the anterior terminal domain. This suggests that a secondary signal present at the termini is required for induction of at least border cell fate. Similarly, two signals are required to induce posterior fate. Xi *et al.* [1] show that the inability of mainbody cells to assume posterior identity in response to ectopic Upd can be overcome by coexpression of activated EGF receptor.

In conclusion, Xi *et al.* [1] show that graded JAK activity plays an important role in patterning the follicle cell layer. A simple model in which Upd secreted from the polar cells acts in a concentration-dependent manner to determine different cell fates along the anterior–posterior axis in the follicular epithelium, while consistent with many of the results, is not sufficient to explain all of the reported data. It is more likely that a gradient of JAK activity is superimposed on underlying differences within the epithelium. This might be a more typical situation in development than the assumption of a completely naïve sheet of cells being patterned by just one gradient. Overlaying a gradient on a field of cells with different responsiveness may lead to greater precision in patterning.

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