Cell Signalling:
Combining Pathways for Diversification and Reproducibility

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How a given signalling pathway can generate diverse outcomes is an open question. A new study shows that EGFR signalling in combination with JAK/STAT or BMP pathways induces different cell fates. Antagonistic interactions between downstream targets further stabilizes epithelial patterning.

The Drosophila ovary contains arrays of developing egg chambers formed by somatic follicle cells surrounding germline cells. Follicle cells form an epithelial monolayer essential for the establishment of the two main body axes of the future embryo, the anterior–posterior (A–P) and the dorsal–ventral (D–V). Intriguingly, the EGFR pathway is responsible for setting both the A–P and D–V axes. A new report, published recently in Current Biology shows that it is the combination of the EGFR pathway with either the JAK/STAT or the BMP pathways that ultimately determines different follicle cell fates. Oogenesis starts with the division of germline stem cells to produce 16 interconnected cells, one of which is selected as the future oocyte, while the remaining 15 take on a nurse-cell fate. Nurse cells produce RNAs and proteins that are transported into the growing oocyte, which comes to lie at the posterior of the egg chamber. Early in oogenesis, all the follicular cells look alike and form a morphologically homogeneous epithelium. As the egg chamber matures, the oocyte grows in size and the follicle cells proliferate until stage 6, when they cease division and start differentiating into specialized cell types. From this stage, different follicle cell fates are determined along the A–P and D–V axes of the egg chamber, an essential step for the future development of the embryo. Key to this fate determination process are two pairs of specialised follicle cells, called polar cells, found at opposite poles of the egg chamber (Figure 1). Polar cells stop division before the rest of the follicle cells and act as signalling centres during epithelial patterning. Two signalling pathways play a fundamental role in defining how the seemingly homogeneous follicular epithelium becomes asymmetric. While the JAK/STAT signalling cascade provides the follicle cells with positional information on their distance with respect to the polar cells, the EGFR pathway defines which of the two ends will become the posterior side and, later, where the dorsal side will form. Polar follicle cells express the unpaired (upd) gene, which encodes the main ligand of the JAK/STAT pathway (Figure 1A). Upd is secreted apically from the polar cells to the space contacting the germline cells, where it spreads to activate JAK/STAT signalling in the neighbouring follicle cells. Upd binding to its receptor Domeless (Dome) induces the activation of the JAK kinase bound to it. JAK phosphorylates Dome, allowing the docking of the transcription factor STAT, which also becomes phosphorylated by JAK. Phosphorylated STAT dimerises and moves to the nucleus activating its target genes. Analysis of STAT localisation in early egg chambers from stages 5–7 shows that follicle cells closer to the polar cells have higher levels of nuclear STAT, which decrease as cells are located further away from the Upd source. Thus, at the stages when the follicular epithelium is patterned, STAT nuclear accumulation serves as a read-out of JAK/STAT activation, the graded nuclear amounts suggesting differential Upd reception. This gradient correlates with the distance-dependent activation of JAK/STAT downstream targets, suggesting that Upd acts as a morphogen in the follicular epithelium. Most morphogens are secreted ligands whose concentration decreases with distance from the source.

The receiving cells are capable of reading the morphogen concentration and activate one target gene or another accordingly. In the egg chamber, the different levels of nuclear STAT result in the determination of different follicle cell types (Figure 1A). At the anterior, higher STAT-accumulating cells activate the slow border cells (slbo) gene and will later become the migratory border cells; lower STAT levels activate apontic (apt) as well as dpp, becoming stretched cells; further away, lower STAT levels can activate the BB127 marker in what will become centripetal cells. Finally, the main body follicle cells express the mirror (mirr) gene (Figure 1A). The early expression of mirr in the central part of the egg chamber requires neither JAK/STAT nor EGFR signalling activity. In fact, mirr expression is repressed by JAK/STAT signalling [8], suggesting efficient STAT activation does not reach the main body follicle cells. At the posterior pole of the egg chamber, the upd expressing polar cells also control follicle cell fate. However, posterior follicle cells do not activate the STAT anterior targets, but STAT posteriorspecific genes such as pnt, blot, Mid and H15. These posterior follicle cells have an important function in the organisation of the oocyte's cytoskeleton and, thus, in the A–P and D–V asymmetries of the egg. The differential transcriptional outcome of STAT in the posterior follicle cells is due to its interaction with the EGFR pathway. One of the EGFR ligands, the TGFa-like the cell. After translation and secretion, Gurken protein only reaches the neighbouring posterior follicle cells, modifying STAT's transcriptional outcome and leading to the activation of posterior markers instead of anterior ones (Figure 1A). In turn, posterior follicle cells with an activated STAT pathway signal back to the oocyte, reorganizing its cytoskeleton. The nature of this signal from the posterior follicle cells to the oocyte is unknown, but it causes the release of the nucleus from the posterior oocyte cortex, allowing the microtubules to push the nucleus, and the associated gurken mRNA, to the dorsal-anterior end of the oocyte. From this new position (Figure 1B), secreted Gurken protein now reaches the main body, mirr-expressing follicle cells that do not receive Upd. Besides Gurken the main body follicle cells also receive the Dpp/BMP ligand expressed from the neighbouring stretched and centripetal follicle cells. The combination of Gurken and Dpp signals reinforce mirr expression in the dorsal-anterior follicle cells, resulting in a completely different EGFR outcome from that in posterior cells. Furthermore, Fregoso Lomas et al. also demonstrate that the initial, non-overlapping expression of mirr in the dorsal follicle cells (induced by EGFR/ BMP) and Mid and H15 in posterior follicle cells (induced by EGFR/STAT) becomes reinforced through the cross-regulation of the downstream targets themselves. In the posterior follicle cells Mid and H15 repress mirr transcription, while in the dorsal main body follicle cells Mirr transcriptionally represses Mid and H15. Such negative cross-regulation stabilizes the formation of two different posterior follicle cell fates leading to the creation of sharp boundaries of gene expression. Interestingly, a negative interaction between STAT targets in the anterior follicle cells also leads to a similar binary decision. High STAT levels can activate slbo gene transcription, while lower levels induce apt. Slbo protein represses apt transcription and Apt represses slbo, generating a stable binary on/off switch that leads to the formation of migratory border cells (slbo positive) and stretched cells (slbo negative). In summary, the follicular epithelium represents a beautiful example of how the combination of diverse signalling pathways creates cell diversity by using morphogen gradients. These gradients initiate spatially restricted gene expression domains that become stabilized by negative cross-regulation of their downstream targets. In the follicle cells, JAK/STAT signalling outcome differs depending on whether it interacts with EGFR signalling or not. Similarly, the result of EGFR pathway activation is conditioned by its interaction with either the Dpp/BMP or the JAK/STAT pathways. Finding out how the temporal- and spatial-specific information provided by these signalling pathways converge on their downstream targets and particularly how their activating and repressing inputs are integrated on the target gene enhancers is a fundamental area of research.

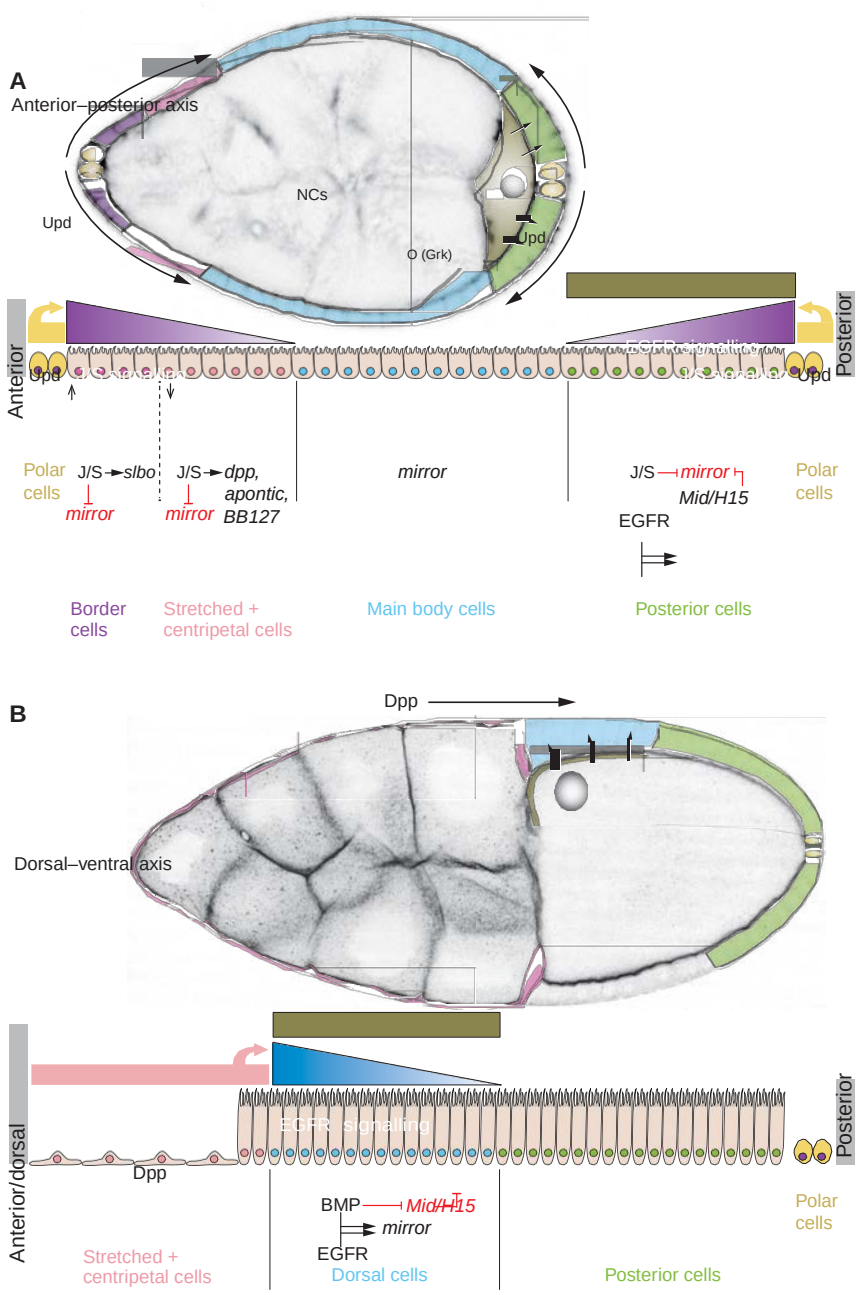

Figure 1. Schematic representation of the signalling pathways patterning the Drosophila follicular epithelium. Sagittal images of egg chambers at stage 6 (A) and stage 10 (B) are coloured to show the various follicle cell types. The fifteen nurse cells (NCs) and the posteriorly placed oocyte (Oo) are surrounded by a monolayered follicular epithelium. A linear representation of the follicular epithelium is depicted under each image, together with the signalling pathways controlling its patterning. (A) Early in oogenesis, all follicle cells have a similar shape. Gurken (Grk) secretion from the oocyte specifies posterior follicle cells. Upd ligand secretion from the polar cells generates a gradient of nuclear STAT that activates different targets depending on the levels of JAK/STAT (J/S) signalling and the presence or not of EGFR activation. Early mirror expression in the main body follicle cells is JAK/STAT- and EGFR-independent. (B) At later stages, anterior follicle cells differentiate into border cells, which delaminate from the epithelium and migrate between the nurse cells to reach the anterior of the oocyte (not shown in B for simplicity), while stretched and centripetal cells acquire their particular shape and position (pink). As a result of the nucleus re-localisation (grey sphere) to the anterior of the oocyte the Gurken ligand now reaches the in main body follicle cells, specifying the dorsal cells and up-regulating mirror expression collaboration with BMP signalling. A mutual negative interaction between the EGFR downstream targets mirr and Mid/H15 stabilizes follicle cell regionalization.
In this figure anterior is left and dorsal up.