Integration of posterior positional cues patterning the follicular epithelium of the Drosophila ovary

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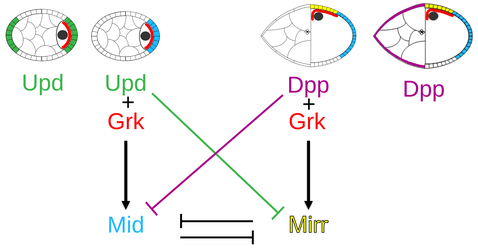
Mutually exclusive EGFR signaling outcomes in epithelial patterning



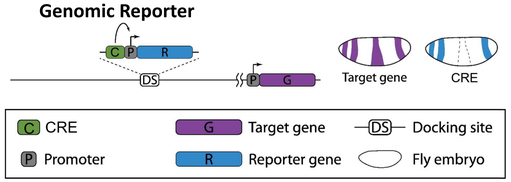
EGFR signaling establishes both Anterior-Posterior (AP) and Dorsal-Ventral (DV) axes of the follicular epithelium of the Drosophila egg chamber. The ligand Grk, secreted by the underlying oocyte, activates EGFR in the surrounding follicle cells. Grk/EGFR signaling induces Midline (Mid) in posterior follicle cells in early stages of oogenesis and Mirror (Mirr) in dorsal-anterior follicle cells in later stages.

Which EGFR target is expressed is determined by antiparallel gradients of the JAK/STAT ligand Upd and the BMP ligand Dpp. Each signal activates one target and represses the other. This outcome is stabilized by the mutual repression between Mid and Mirr.

Here, we are asking whether Mid and Mirr are direct targets of JAK/STAT signaling and how inputs from Upd and Grk are integrated to define posterior fate.

Methodology

Genomic Reporter

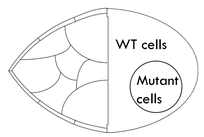


To understand how inputs from signaling pathways are captured, we are using genomic reporter constructs to study cis-regulatory elements (CRE).

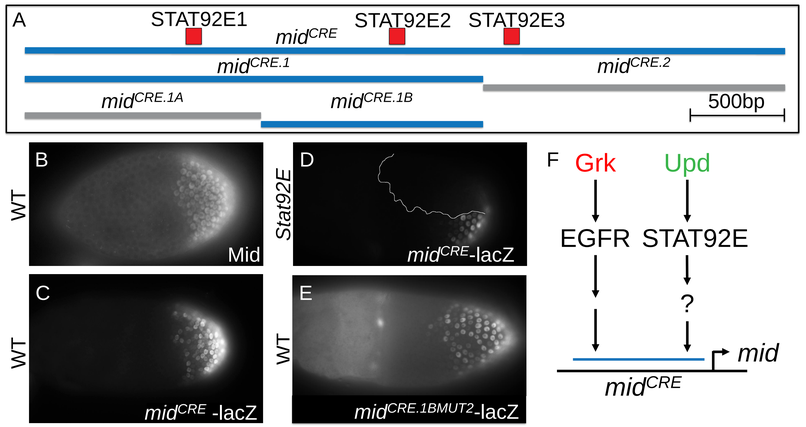
(Adapted from Kvon, Genomics, 2015)

Mosaic Analysis

We assess reporter expression in clones unable to respond to signaling or experiencing ectopic signaling.

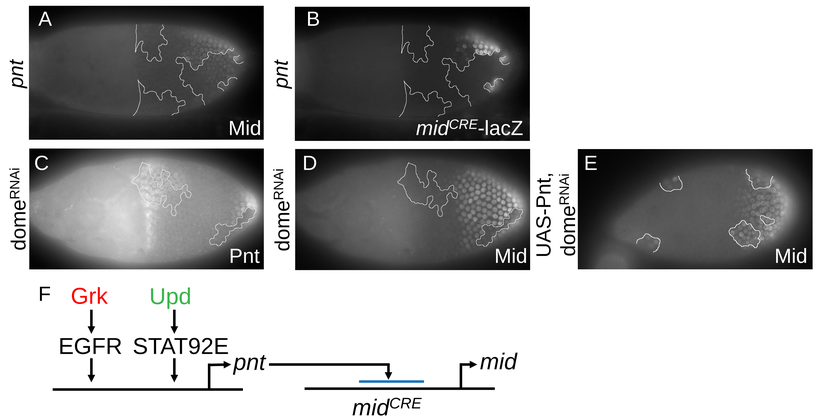


Is *mid* directly activated by JAK/STAT signaling ?



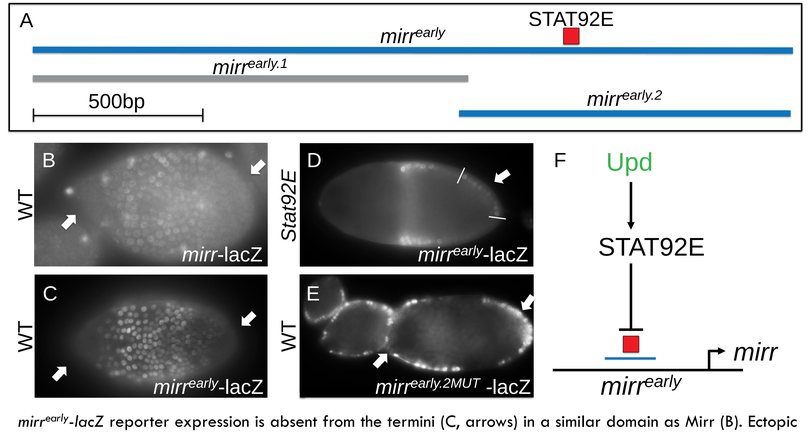
The *mid*^{CRE}-lacZ reporter is active in posterior follicle cells (C) in a similar domain as endogenous Mid (B), and is activated by both Grk and Upd (D) similar to what is observed with Mid suggesting that this reporter contains a *mid* CRE. Derivative constructs were generated to further define the CRE and narrow down the search for putative STAT92E binding sites (A). Disruption of a putative STAT92E binding site (STAT92E2) in the *mid*$^{CRE.1B}$ derivative (*mid*$^{CRE.1BMUT2}$-lacZ) did not affect expression of the lacZ reporter gene (E) suggesting that this CRE is not activated directly through binding of Stat92E (F).

Is JAK/STAT signaling regulating *mid* through Pnt ?



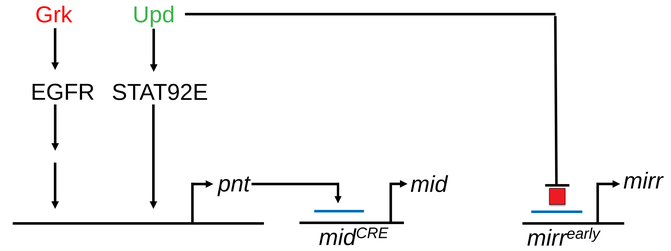
Loss of the EGFR effector Pointed (Pnt) leads to loss of Mid expression (A) and of the *mid*^{CRE}-lacZ reporter (B) indicating that Pnt regulates both *mid* and the *mid*^{CRE}-lacZ reporter. Pnt is also lost in clones lacking the Upd receptor Dome (C) indicating that *pnt* is regulated by JAK/STAT signaling in posterior follicle cells. Loss of Pnt due to lack of JAK/STAT signaling correlates with loss of Mid observed in clones unable to respond to Upd (D). Reintroduction of Pnt in clones lacking *dome* rescues loss of *mid* (E) suggesting that the role of Upd in *mid* regulation is to provide sufficient levels of Pnt (F).

Is *mirr* directly repressed by JAK/STAT signaling ?



mirr^{early}-lacZ reporter expression is absent from the termini (C, arrows) in a similar domain as Mirr (B). Ectopic expression in *Stat92E* mutant clones shows that this absence is due to repression by Upd (D, arrow). Derivative constructs were generated to further define the CRE and narrow down the search for putative STAT92E binding sites (A). Disruption of a putative binding site in the *mirr*$^{early.2}$ derivative (*mirr*$^{early.2MUT}$-lacZ) relieves the repression (E, arrows) at the termini and mimics loss of *Stat92E* suggesting that this CRE is directly repressed by binding of STAT92E. This is suggesting that *mirr* is directly repressed by JAK/STAT signaling through binding of STAT92E to the *mirr*^{early} CRE (F).

Upd determines posterior fate by influencing the outcome of EGFR signaling



Upd regulates alternative EGFR targets in two different ways:

DIRECT repression of *mirr* through binding of STAT92E to the *mirr*^{early} CRE.

INDIRECT activation of *mid* through the regulation of levels of the EGFR effect Pnt.

Whether Upd directly regulates *pnt* through STAT92E, remains unclear.