

Documentation of the mechanistic, single-cell model

This supplementary material provides a summary of published evidence in support of the nodes and their relationships at the core of our model (Figure 3).

Input nodes

These nodes embody external influences and have no associated logical function.

Dpp

Decapentaplegic is a BMP ligand (a TGF- β superfamily member), secreted from the stretched FCs that overlay the nurse cells, and from stage 10 onwards in the centripetally migrating follicle cells [1]. It signals to the follicular epithelium through receptors Wit [30] and Tkv.

At stage 10, Dpp/BMP signaling activity can be detected in a narrow band along the anterior border of the follicular epithelium, slightly larger at the dorsal side [2,3].

Grk

Gurken is a TGF- α like protein secreted by the oocyte. It accumulates around the oocyte nucleus in the dorsal-anterior corner from stage 8 [4,5] and signals to the overlying follicular epithelium until the formation of the vitelline membrane at stage 10B [6].

Br_adj

This node denotes Br presence in neighboring cells (only those directly adjacent).

Aos_ext

This node denotes Aos presence in neighboring cells.

Rho_ext

This node denotes Rho presence in neighboring cells.

earlyBMP

dpp expression is first detectable at the end of stage 8 in approximately 20 to 30 somatic follicle cells at the anterior tip of the egg chamber [1]. However, there is evidence that the BMP pathway is active in early (<stage 6) egg chambers [7].

earlyEGF

Gurken is a TGF- α like protein secreted by the oocyte. In early stages, it is located in the posterior pole [4,5], where it activates EGF signaling in the overlying follicle cells.

Core nodes

The behavior of these “internal nodes” depends on other nodes (described in the bullet lists) following rules given in Figure 3.

Aos

argos expression begins around stage 10A in the dorsal centripetal follicle cells, before extending to the dorsal midline. The patch splits around stage 11-12 into a pattern reminiscent of the late pattern of *rho* [6,8–10]. It is targeted by

- Pnt, which induces *aos* in the neuroectoderm [11].
Overexpression of *pntP1* may reduce expression of EGF signaling targets *rhomboid* and *69D* (unpublished data mentioned by [12]).
Pnt may induce *aos* in the FCE (unpublished data mentioned by [6]).
- dpERK: in addition to the *argos* expression pattern, we know that in intervein cells in the wing imaginal disc, *aos* transcription is repressed by Cic in a cell autonomous way; Cic itself is targeted for degradation through the EGF signaling pathway [3,13–15].
- Mid: when EGFR is ectopically activated in the epithelium, *aos* transcription is restricted to the anterior side [8], which suggests repression by Mid.

Br

Broad-Complex (Br-C), is a transcription factor. It is a key marker of the roof region, expressed in two large patches of cells on each side of the dorsal midline [2,7,16–21]. *broad* mRNA is initially present at low levels everywhere, then repressed in an antero-dorsal T-shaped domain, before increasing in the roof domains and finally decreasing everywhere else.

br expression is controlled by two promoters [22]; only the BrL promoter is considered here.

- *Mirr* is required both to upregulate Br-C in the normal appendage primordium, and to downregulate it in the entire dorsal epithelium [20]. Fuchs et al. showed that *Mirr* activates the BrL promoter (late, Roof-specific Br pattern) and represses the BrE promoter (Early, ubiquitous Br pattern) [22].
- *Pnt* represses BrL (promoter controlling *broad* late expression) [22].

dpERK

Activation of the EGF pathway may be visualized directly through MAPK or ERK phosphorylation (indicated with dpERK), starting from a midline-centered pattern, which expands to encompass a larger part of the dorsal-anterior domain, before regressing to a spectacle-like pattern and finally to the L-shape pattern characteristic of the floor domain [6,9,10,21]. It is targeted by

- *Gkr* activates EGFR [4,8].
- *Argos* inhibits EGFR signaling by sequestering Spitz [23]. Secreted Spitz (after being cleaved by Rhomboid) activates EGFR [24–26].
- *X*, corresponding to a hypothetical enhancement of EGFR activity via a juxtacrine signal from the roof.

Mid

Midline expression marks the posterior domain of the follicular epithelium [27].

- earlyEGF: early, posterior Grk signalling activates *Mid* expression [27].
- earlyBMP: there is indirect evidence that BMP signaling affects the posterior boundary of the appendage primordia [8,18,28]. Considering the contrasting results of BMP pathway disruption on *br* expression [3,28–30], we postulate that this effect has to occur early, and propose it functions through repression of *mid*.

Mirr

The transcription factor *Mirror* is activated in a large dorsal-anterior patch of the epithelium by low levels of EGFR activity [28,31,32].

- dpERK; *mirr* is induced in a large dorsal-anterior patch of the epithelium by low levels of EGF activity through *Cic* [14,28,31,32].
- *Mid* represses *mirr* and its target *br* [27].

Pnt

The gene encoding the transcription factor *Pointed* is expressed along the dorsal midline at stage 10A, and in the roof region at stages 10B-11 [3,12].

- dpERK: *pnt* is induced by high levels of EGF signaling activity [2,11,12].

Rho

rho expression pattern seems to correlate with EGF signaling activity as it evolves from the whole dorsal-anterior region in early stages to the characteristic one-cell wide floor region, adjacent to the roof, in late stages [4,8,33,34]. Rhomboid, a protease, cleaves Spitz, which is then secreted [25,26].

- *Mirr*: ectopic expression of *mirr* induces *rho* ectopic expression [31,35]. There is evidence that some anterior clue is required to induce *rhomboid* [8,18,29], and *mirror* could relay that signal to Rho.
- *Br*, which is required cell-autonomously to repress *rho* expression. Broad binding sites are predicted in the promoter region of *rho* [36].
- dpERK: *rho* transcription is repressed by the transcription factor CF2 [34]; CF2 is downregulated by the EGFR pathway [37].
- *Dpp* signaling represses *br* in the anterior follicle cells, via pMAD; strong *dpp* overexpression represses *Br* entirely [2,3].

Integration nodes

A

Combines the values of Aos and Aos_ext.

- Aos and Aos_ext: Argos is secreted [38]. The *argos* gene encodes a diffusible factor that regulates cell fate decisions in the Drosophila eye. Rule for the epithelial model:
level 1: at least 5 cells among the proper cell and 126 neighbors (direct and or at a distance up to 6 cells) express Aos.

S

Combines the values of Rho and Rho_ext, to account for Spitz signaling.

- Rho and Rho_ext: Rho activates Spitz, which is then secreted [25,26]. Rules for the epithelial model:
level 2: autocrine (Rho at level 2) or at least 6 cells over six (direct) neighbors
level 1: autocrine (Rho at level 1) or between 2 and 5 cells among the six (direct) neighbors.

X

Hypothetical mechanism. EGFR activity seems to be enhanced in cells in contact with the roof region ("spectacle-shaped" pattern in [9,10,21]). Moreover, Rho is expressed along the high-Notch side of a high/low Notch border; ectopic *rho* expression may be induced by ectopic high/low Notch barriers generated by Notch-clones [36].

- Br_adj and Br. Br is taken as a marker of the roof. As such, it activates X in the neighboring cells, while inhibiting it in the roof itself.

References

1. Twombly V, Blackman RK, Jin H, Graff JM, Padgett RW, et al. (1996) The TGF-beta signaling pathway is essential for Drosophila oogenesis. *Development* 122: 1555–1565.
2. Deng WM, Bownes M (1997) Two signalling pathways specify localised expression of the Broad-Complex in Drosophila eggshell patterning and morphogenesis. *Development* 124: 4639–4647.
3. Yakoby N, Lembong J, Schüpbach T, Shvartsman SY (2008) Drosophila eggshell is patterned by sequential action of feedforward and feedback loops. *Development* 135: 343–351. doi:10.1242/dev.008920.
4. Neuman-Silberberg FS, Schupbach T (1994) Dorsoventral axis formation in Drosophila depends on the correct dosage of the gene *gurken*. *Development* 120: 2457–2463.
5. Neuman-Silberberg FS, Schüpbach T (1996) The Drosophila TGF-alpha-like protein *Gurken*: expression and cellular localization during Drosophila oogenesis. *Mech Dev* 59: 105–113.
6. Wasserman JD, Freeman M (1998) An autoregulatory cascade of EGF receptor signaling patterns the Drosophila egg. *Cell* 95: 355–364.
7. Chen Y, Schüpbach T (2006) The role of *brinker* in eggshell patterning. *Mech Dev* 123: 395–406. doi:10.1016/j.mod.2006.03.007.
8. Queenan AM, Ghabrial A, Schüpbach T (1997) Ectopic activation of *torpedo/Egfr*, a Drosophila receptor tyrosine kinase, dorsalizes both the eggshell and the embryo. *Development* 124: 3871–3880.
9. Peri F, Bökel C, Roth S (1999) Local *Gurken* signaling and dynamic MAPK activation during Drosophila oogenesis. *Mech Dev* 81: 75–88.
10. Nakamura Y, Matsuno K (2003) Species-specific activation of EGF receptor signaling underlies evolutionary diversity in the dorsal appendage number of the genus *Drosophila* eggshells. *Mech Dev* 120: 897–907. doi:10.1016/S0925-4773(03)00164-3.
11. Chang D, Xu N, Luo K (2003) Degradation of cyclin B is required for the onset of anaphase in Mammalian cells. *J Biol Chem* 278: 37865–37873. doi:10.1074/jbc.M306376200.

12. Morimoto AM, Jordan KC, Tietze K, Britton JS, O'Neill EM, et al. (1996) Pointed, an ETS domain transcription factor, negatively regulates the EGF receptor pathway in *Drosophila* oogenesis. *Development* 122: 3745–3754.
13. Roch F, Jiménez G, Casanova J (2002) EGFR signalling inhibits Capicua-dependent repression during specification of *Drosophila* wing veins. *Development* 129: 993–1002.
14. Atkey MR, Lachance J-FB, Walczak M, Rebello T, Nilson LA (2006) Capicua regulates follicle cell fate in the *Drosophila* ovary through repression of mirror. *Development* 133: 2115–2123. doi:10.1242/dev.02369.
15. Astigarraga S, Grossman R, Díaz-Delfin J, Caelles C, Paroush Z, et al. (2007) A MAPK docking site is critical for downregulation of Capicua by Torso and EGFR RTK signaling. *EMBO J* 26: 668–677. doi:10.1038/sj.emboj.7601532.
16. Tzolovsky G, Deng WM, Schlitt T, Bownes M (1999) The function of the broad-complex during *Drosophila* melanogaster oogenesis. *Genetics* 153: 1371–1383.
17. Dequier E, Souid S, Pál M, Maróy P, Lepesant JA, et al. (2001) Top-DER- and Dpp-dependent requirements for the *Drosophila* *fos/kayak* gene in follicular epithelium morphogenesis. *Mech Dev* 106: 47–60.
18. Ward EJ, Berg CA (2005) Juxtaposition between two cell types is necessary for dorsal appendage tube formation. *Mech Dev* 122: 241–255. doi:10.1016/j.mod.2004.10.006.
19. Yakoby N, Bristow CA, Gong D, Schafer X, Lembong J, et al. (2008) A combinatorial code for pattern formation in *Drosophila* oogenesis. *Dev Cell* 15: 725–737. doi:10.1016/j.devcel.2008.09.008.
20. Boisclair Lachance J-F, Fregoso Lomas M, Eleiche A, Bouchard Kerr P, Nilson LA (2009) Graded *Egfr* activity patterns the *Drosophila* eggshell independently of autocrine feedback. *Development* 136: 2893–2902. doi:10.1242/dev.036103.
21. Zartman JJ, Kanodia JS, Cheung LS, Shvartsman SY (2009) Feedback control of the EGFR signaling gradient: superposition of domain-splitting events in *Drosophila* oogenesis. *Development* 136: 2903–2911. doi:10.1242/dev.039545.
22. Fuchs A, Cheung LS, Charbonnier E, Shvartsman SY, Pyrowolakis G (2012) Transcriptional interpretation of the EGF receptor signaling gradient. *Proc Natl Acad Sci USA* 109: 1572–1577. doi:10.1073/pnas.1115190109.
23. Klein DE, Nappi VM, Reeves GT, Shvartsman SY, Lemmon MA (2004) Argos inhibits epidermal growth factor receptor signalling by ligand sequestration. *Nature* 430: 1040–1044. doi:10.1038/nature02840.
24. Rutledge BJ, Zhang K, Bier E, Jan YN, Perrimon N (1992) The *Drosophila* *spitz* gene encodes a putative EGF-like growth factor involved in dorsal-ventral axis formation and neurogenesis. *Genes Dev* 6: 1503–1517.
25. Lee LA, Elfring LK, Bosco G, Orr-Weaver TL (2001) A genetic screen for suppressors and enhancers of the *Drosophila* PAN GU cell cycle kinase identifies cyclin B as a target. *Genetics* 158: 1545–1556.
26. Urban S, Lee JR, Freeman M (2001) *Drosophila* rhomboid-1 defines a family of putative intramembrane serine proteases. *Cell* 107: 173–182.
27. Fregoso Lomas M, Hails F, Lachance J-FB, Nilson LA (2013) Response to the dorsal anterior gradient of EGFR signaling in *Drosophila* oogenesis is prepatterned by earlier posterior EGFR activation. *Cell Reports* 4: 791–802. doi:10.1016/j.celrep.2013.07.038.
28. Shrivage BV, Altmann G, Technau M, Roth S (2007) The role of Dpp and its inhibitors during eggshell patterning in *Drosophila*. *Development* 134: 2261–2271. doi:10.1242/dev.02856.
29. Peri F, Roth S (2000) Combined activities of Gurken and decapentaplegic specify dorsal chorion structures of the *Drosophila* egg. *Development* 127: 841–850.
30. Marmion RA, Jevtic M, Springhorn A, Pyrowolakis G, Yakoby N (2013) The *Drosophila* BMPRII, wishful thinking, is required for eggshell patterning. *Dev Biol* 375: 45–53. doi:10.1016/j.ydbio.2012.12.011.
31. Jordan KC, Clegg NJ, Blasi JA, Morimoto AM, Sen J, et al. (2000) The homeobox gene mirror links EGF signalling to embryonic dorso-ventral axis formation through notch activation. *Nat Genet* 24: 429–433. doi:10.1038/74294.
32. Zhao D, Woolner S, Bownes M (2000) The Mirror transcription factor links signalling pathways in *Drosophila* oogenesis. *Dev Genes Evol* 210: 449–457. doi:10.1007/s004270050332.
33. Ruohola-Baker H, Grell E, Chou TB, Baker D, Jan LY, et al. (1993) Spatially localized rhomboid is required for establishment of the dorsal-ventral axis in *Drosophila* oogenesis. *Cell* 73: 953–965.
34. Hsu T, Bagni C, Sutherland JD, Kafatos FC (1996) The transcriptional factor CF2 is a mediator of EGF-R-activated dorsoventral patterning in *Drosophila* oogenesis. *Genes Dev* 10: 1411–1421.

doi:10.1101/gad.10.11.1411.

35. Jordan KC, Hatfield SD, Tworoger M, Ward EJ, Fischer KA, et al. (2005) Genome wide analysis of transcript levels after perturbation of the EGFR pathway in the *Drosophila* ovary. *Dev Dyn* 232: 709–724.
doi:10.1002/dvdy.20318.
36. Ward EJ, Zhou X, Riddiford LM, Berg CA, Ruohola-Baker H (2006) Border of Notch activity establishes a boundary between the two dorsal appendage tube cell types. *Dev Biol* 297: 461–470.
doi:10.1016/j.ydbio.2006.05.021.
37. Mastrova EY, Hsu T (1998) Down-regulation of transcription factor CF2 by *Drosophila* Ras/MAP kinase signaling in oogenesis: cytoplasmic retention and degradation. *Genes Dev* 12: 1166–1175.
38. Freeman M, Klämbt C, Goodman CS, Rubin GM (1992) The *argos* gene encodes a diffusible factor that regulates cell fate decisions in the *Drosophila* eye. *Cell* 69: 963–975.