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Dorsoventral Patterning: A Direct Route from Ovary to Embryo

Dispatch

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Recent data indicate that Gurken-mediated activation of the EGF receptor in the somatic follicle cells of the Drosophila ovary — required for dorsoventral patterning of the fly embryo — leads to cell-autonomous repression of pipe expression, suggesting that the EGF receptor signaling pathway acts directly to control pipe transcription.

Dorsoventral polarization of the *Drosophila* embryo depends on the prior establishment of dorsoventral polarity of the egg chamber within which the oocyte develops [1]. Midway through oogenesis, the germ-line derived oocyte and its 15 associated nutritive cells — the nurse cells — are ensheathed by an epithelium of somatically derived follicle cells (Figure 1). The asymmetric position of the oocyte nucleus along the dorsoventral axis of the oocyte determines the dorsal side of the egg chamber through the association of the nucleus with the RNA encoding Gurken, an activating ligand for the epidermal growth factor (EGF) receptor. The EGF receptor is expressed in all follicle cells surrounding the oocyte, but is activated at highest levels in follicle cells adjacent to the oocyte nucleus.

The activation of EGF receptor and the consequent establishment of dorsoventral polarity in the follicle cell layer has two distinct consequences: the determination of follicle cell subpopulations required for the production of appropriate structures along the dorsoventral axis of the eggshell; and the restriction of expression of the *pipe* gene to ventral follicle cells. Expression of *pipe*, in turn, is the cue that initiates the establishment of polarity in the resultant embryo. Two recent reports [2,3] indicate that Gurken-mediated activation of EGF receptor leads directly and cell-autonomously to repression of the *pipe* gene in the lateral and dorsal cells of the follicle layer. This is somewhat surprising, in part because Gurken protein has not been detected in much of the region within which *pipe* is repressed.

For the generation of eggshell polarity, EGF receptor activation in dorsal follicle cells induces the transcription of *rhomboid*, whose product augments signaling by the EGF receptor-activating ligand Spitz. Rhomboid, recently shown to be a novel transmembrane serine protease, cleaves Spitz within its transmembrane domain, resulting in the formation of active, diffusible ligand [4]. The production of active Spitz dorsally results in an autocrine enhancement of EGF receptor signalling in the follicle cells producing Rhomboid [5]. When EGF receptor activity reaches a certain threshold on the dorsal midline, the expression of Argos, a negatively regulating EGF receptor ligand, is induced. This results in downregulation of EGF receptor activity at the dorsal midline. However, two flanking dorsolateral strips of cells that have presumably encountered active Spitz still exhibit high levels of EGF receptor activity. As a result of these positive and negative feedbacks, the initial single peak of EGF receptor activity resolves into two lateral peaks. Later in oogenesis, these two dorsolateral groups of cells will produce the two dorsal respiratory appendages of the eggshell. Recently, by integrating genetic and biochemical information about the EGF receptor signalling network into a mechanistic model, Shvartsman *et al*. [6] have been able computationally to model the resolution of a single broad activation domain into a twin-peaked pattern, supporting the validity of the proposed mechanism.

In addition to patterning the egg chamber and the eggshell, activation of EGF receptor in the follicle cell layer during oogenesis establishes the dorsoventral axis of the embryo that will develop from the oocyte. EGF receptor signaling restricts the transcription of *pipe* to a zone comprising the ventral-most 30–40% cells along the dorsoventral circumference of the follicle layer [7]. The expression of *pipe* in the follicle cell layer leads, by a mechanism that is still not fully understood, to spatially specific ventral activation of a series of proteases acting in the perivitelline space between the embryonic membrane and the eggshell. This protease cascade ultimately processes Spätzle protein into an active ligand for Toll, a receptor distributed uniformly in the embryonic membrane, whose ventrally restricted activation defines the embryonic dorsoventral axis. The *pipe* gene encodes a putative glycosaminoglycan-modifying enzyme [7] which normally resides in the Golgi of follicle cells. A plausible model for Pipe action invokes the existence of an as yet uncharacterized glycoprotein target of Pipe, stably deposited in the egg, whose ventrally localized modification by Pipe leads to spatially restricted activation of the protease cascade.

One of the perplexing aspects of EGF receptor regulation of embryonic polarity is that, while *pipe* is expressed in only the ventral most-third of the follicle cell layer, the genes activated by EGF receptor signalling are expressed in the dorsal-most third. Gurken protein is only detectable dorsally [8–10]; there is, however, a domain — the lateral third of the dorsoventral circumference — in which the region where *pipe* is repressed and the Gurken domain do not appear to overlap. Jordan *et al*. [11] proposed the existence of a second, long-range signal, the expression of which depends on EGF receptor activation, and which in turn regulates *pipe* expression.

This proposal was based on the observation that Mirror, a homeobox-containing transcription factor, is

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produced dorsally in a Gurken-dependent manner. Experimental perturbations of Mirror production led to changes in the expression of *fringe*, a known target of Mirror, which are consistent with Mirror being a repressor of *fringe* expression in follicle cells. Misexpression of Mirror in follicle cells also resulted in alterations in the pattern of *pipe* expression, in this case at a distance. Because *fringe* encodes a glycosyltransferase known to modulate activation of the Notch receptor by its ligands [12,13], these observations led to the proposal that Gurken-induced Mirror leads to formation of a Notch–Fringe expression boundary in follicle cells in which Notch is activated. This activation of Notch signaling was presumed to activate expression of secreted molecules which diffuse away from the expressing cells, acting over a distance to negatively regulate *pipe* expression. Consistent with this model, expression of an activated form of Notch in a restricted domain of follicle cells led to repression of *pipe* beyond the region expressing Notch.

The notion of a secondary signal mediating *pipe* repression at a distance was complicated by studies of the influence of the *d-cbl* gene on patterning. The Cbl family proteins are Ring-finger domain E3 ubiquitin ligases, which are thought to downregulate EGF receptor activity by targeting active receptor molecules for degradation [14]. By generating clones of homozygous mutant cells in heterozygous females, Pai *et al*. [15] identified a mutation in *d-cbl*, the wild-type product of which is required for patterning the eggshell as well as the embryonic dorsoventral axis. Only *d-cbl* mutant clones at ventral positions within the follicle cell epithelium led to dorsalized embryonic progeny. Ventral mutant *d-cbl* clones also exhibited a strictly cell-autonomous loss of *pipe* expression. Surprisingly, the mutant clones caused inappropriate activation of EGF receptor signalling which was dependent on Gurken, despite the fact that the Gurken distribution has not been observed to extend to the ventral side of the follicle.

Taken together, these data strongly suggest that EGF receptor-dependent signalling is directly responsible for repression of *pipe* activity along the dorsal two-thirds of the egg chamber circumference. One possible explanation is that Gurken protein might diffuse from its dorsal source, producing a graded distribution broader than has been detected with antibody, extending even to the ventral side of the egg chamber where it activates EGF receptor to repress *pipe* (Figure 1). Alternatively, the regulation of *pipe* expression might be analogous to eggshell patterning, with dorsally localized Gurken leading to the formation of Spitz, or another EGF receptor ligand, which diffuses from its dorsal source to form an EGF receptor activation gradient that represses *pipe* over the dorsal two-thirds of the follicle circumference.

Two recent studies [2,3] support the view that EGF receptor signalling acts directly to repress *pipe*. James *et al*. [2] generated mutant follicle cell clones using null alleles of *Ras* and found that *pipe* transcription was derepressed cell-autonomously in dorsal or lateral regions of the follicle; Peri *et al*.[3] observed a similar derepression of *pipe* expression in *Raf* mutant

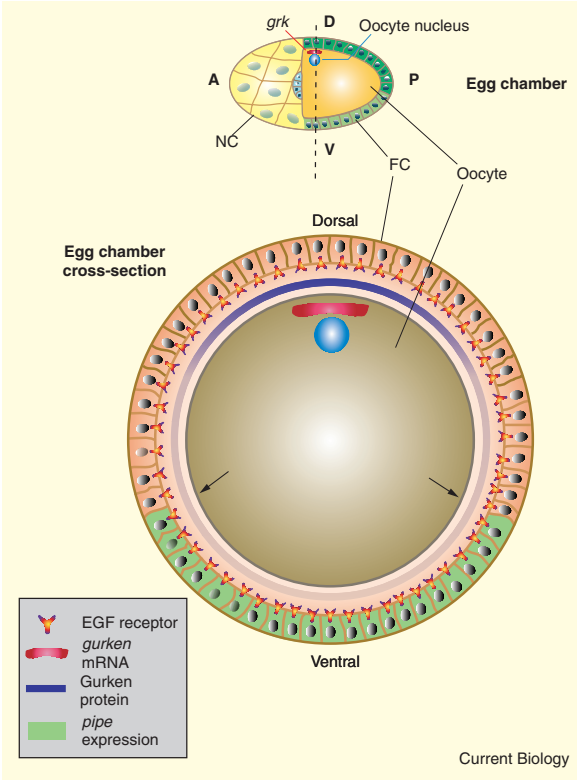

Figure 1. A model for direct regulation of *pipe* expression by Gurken-mediated activation of the EGF receptor.

At the top is a diagram showing the arrangement of the egg chamber at mid-oogenesis, indicating the positions of the nurse cells (NC) and oocyte, the follicle cells (FC) and the dorsally positioned oocyte nucleus. The *grk* RNA (red) is closely associated with the nucleus. At the bottom is shown a cross-section through the egg chamber, at a position along the anterior–posterior axis corresponding to the location of the oocyte nucleus (see dotted line at top). Gurken protein (purple) is translated at highest levels dorsally and secreted into the space between the oocyte and the follicle layer (shown disproportionately expanded here), forming a concentration gradient from dorsal to ventral. At a critical location along the dorsoventral axis (arrows), the concentration of Gurken protein falls to levels too low to mediate EGF receptor-dependent repression of *pipe*, and ventral expression of *pipe* ensues.

clones. These observations indicate that EGF receptor signaling — which leads to downstream activation of Raf and Ras — is required in all follicle cells in which *pipe* is normally repressed, and that cells in which EGF receptor-mediated signalling is blocked do not receive other secondary signals that lead to *pipe* repression. Peri *et al.* [3] also found that *pipe* expression is cell autonomously repressed in follicle cell clones producing a ligand-independent activated form of EGF receptor. Expression of *pipe* was not affected outside of the clone, showing that cells receiving the EGF receptor signal do not initiate a secondary signaling cascade involved in *pipe* repression at a distance.

Peri *et al*. [3] also revisited the model that EGF receptor signalling operates indirectly via interactions with Mirror, Fringe and Notch. After generating follicle cell clones mutant for *mirror* or *fringe*, they failed to

observe any alterations in *pipe* expression, contradicting the model in which EGF receptor-activated Mirror expression defines an organizing center important for *pipe* repression. Peri *et al*. [3] provide a potential explanation for the discrepancy, noting that while their experiments assessed the effects of *fringe* and *mirror* loss-of-function mutations, Jordan *et al*. [10] relied mainly on misexpression studies. Ectopic expression of *mirror* can induce Rhomboid expression which, in turn, generates activated Spitz. Activated Spitz generated in this way is capable of causing *pipe* repression, although this seems not to be what is occurring normally *in vivo*.

Both James *et al*. [2] and Peri *et al*. [3] observed that *Ras* and *Raf* clones causing ectopic *pipe* expression in the follicle cell layer did not invariably lead to the production of embryos with aberrant dorsoventral polarity. Both groups conclude that robust mechanisms are in place downstream of *pipe* activation to ensure appropriate dorsoventral patterning of the embryo. Based on studies of Morisato [16], this mechanism may involve an inhibitory fragment of the Spätzle protein formed during proteolytic generation of the Toll ligand, which acts to autoregulate the perivitelline protease cascade and regulate its spatial limits. While this is a plausible explanation, further experiments will be necessary to explain the robust nature of the relationship between *pipe* expression and the embryonic dorsoventral pattern. Moreover, now that it seems quite clear that the regulation of *pipe* expression by EGF receptor signaling is direct, it will be interesting to learn the identities of the transcriptional regulatory proteins through which EGF receptor controls *pipe* expression and the mechanism through which EGF receptor signalling exerts that control.

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