Recent advances in understanding signal transduction pathways in worms and flies

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One major challenge in the fields of signal transduction and pattern formation is to understand how multiple signals are integrated to determine cell fates. Two developmental systems, vulval development in *Caenorhabditis elegans* and axis formation during *Drosophila melanogaster* oogenesis, require the epidermal growth factor receptor tyrosine kinase and the NOTCH signaling pathways to specify cell fates. Current work in both systems has provided new opportunities to investigate the potential for the cross-talk between these different signaling pathways.

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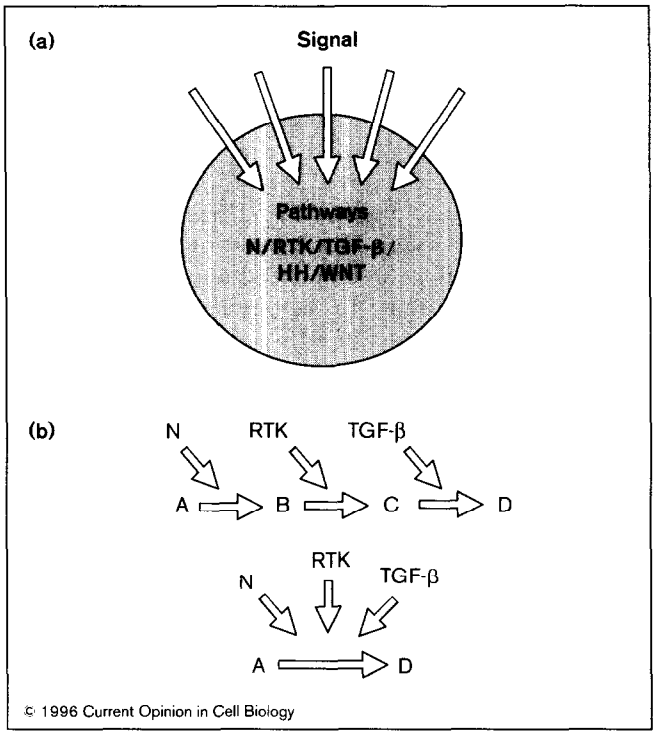
Current Opinion in Cell Biology 1996, 8:231–238

Abbreviations

A	anterior
AC	anchor cell
A/P	anterior/posterior
APc	anterior polar cell
DER	*Drosophila* epidermal growth factor receptor
D/V	dorsal/ventral
EGF	epidermal growth factor
MAPK	mitogen-activated protein kinase
P	posterior
PPc	posterior polar cell
RTK	receptor tyrosine kinase
SH	Src homology
TGF-β	transforming growth factor-β

Figure 1



Nature and temporal occurrence of the signaling pathways involved in patterning. **(a)** The types of signaling pathways involved in patterning. **(b)** Demonstrates two simple alternatives for the requirement of the multiple signaling pathways involved in cell-fate determination. The pathways may be required sequentially (top) or coincidentally (bottom), on the basis of a temporal distinction for the action of the pathways. A–D represent different cell fates. N, DELTA/NOTCH; HH, HEDGEHOG.

Introduction

Along the path to differentiation, cells often receive and respond to a multitude of signals in order to arrive at their final developmental fate. The choices that cells make along this path must occur relative to those of their neighbors to ensure proper patterning. Ultimately, however, the response of a specific cell relies upon its ability to transduce extracellular stimuli into the intracellular environment and establish a specific fate. Numerous pathways capable of such a signaling feat have been identified to date. These include the receptor tyrosine kinase (RTK), DELTA/NOTCH, transforming growth factor-β (TGF-β), WNT, and HEDGEHOG pathways (Fig. 1a). Extensive analyses of these pathways have identified a number of components involved in each pathway [1,2]. One of the critical issues remaining in cell signaling and patterning is to determine if the activities of these and other signaling pathways in cell specification events are temporally distinct or coincident (see Fig. 1b). If they are coincident, at what level does the interaction occur? Is it at the extracellular, cytoplasmic or transcriptional level? Crucial to this goal is the identification of developmental systems that involve multiple signaling events in cell fate specification. The identification of such systems has not come easily. However, the contributions of genetics to this issue have been highlighted in a series of recent papers. As we elaborate below, vulval development in *Caenorhabditis elegans* and the establishment of polarity in *Drosophila melanogaster* egg chambers have emerged as excellent paradigms to address the interaction between two signaling pathways, the epidermal growth factor receptor tyrosine kinase and NOTCH pathways.

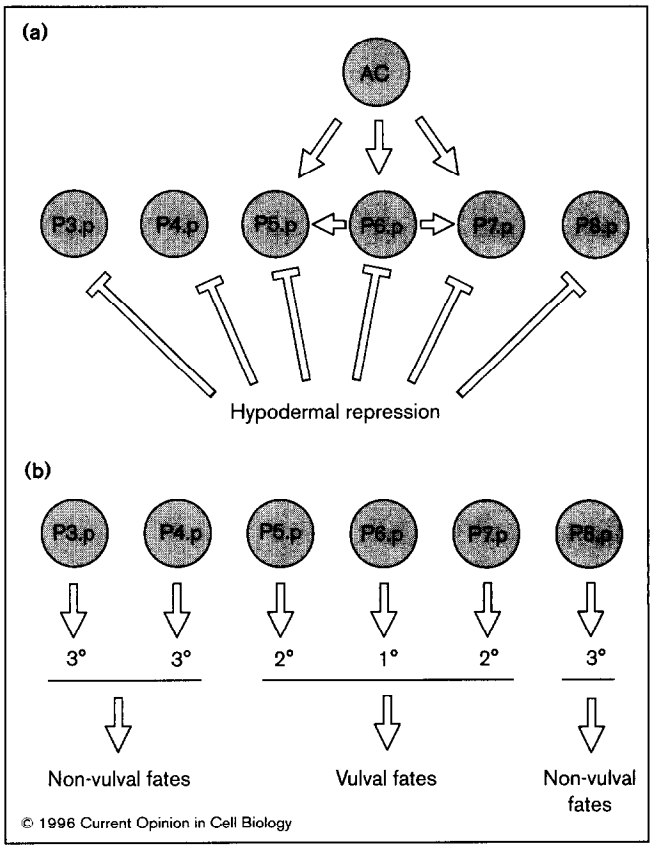
Formation of the vulva involves both the LET-23 RTK and LIN-12 pathways

During the third larval stage of *C. elegans*, the precursors of the adult vulva are specified from an equivalence group of 'Pn.p' cells. These six ectodermal blast cells, termed P3.p–P8.p, are capable of vulval specification in response to an inductive signal (Fig. 2; reviewed in [3]). This signal, encoded by the epidermal growth factor (EGF) family member LIN-3, is derived from the adjacent gonadal anchor cell (AC) and triggers activation of the LET-23 EGF receptor tyrosine kinase (RTK) signaling pathway [4] (see Fig. 3). In normal development the cell closest to the AC signal (P6.p) responds to the LIN-3 signal by adopting the primary (1°), fate, while its neighbors (P5.p and P7.p) adopt a secondary (2°) fate (Fig. 2). Both of these Pn.p fates give rise to progeny that differentiate into vulval tissue. In contrast, those cells farthest from the AC (P3.p, P4.p, and P8.p) adopt an uninduced tertiary (3°) fate, and their progeny contribute to the surrounding hypodermal syncytium. As such, loss of LIN-3 activity results in the loss of 1° and 2° fates and a vulvaless phenotype. However, LIN-3 is not the sole source of patterning information during vulval development. Proper vulval patterning also requires two other signaling activities. One of these signals is produced by the 1° Pn.p, P6.p, to commit neighboring Pn.p cells to a 2° fate [5]. This lateral signal appears to be received by LIN-12, a member of the NOTCH family of transmembrane receptors [6,7]. The other signaling activity involves the inhibitory LIN-15 signal, which acts in the surrounding hypodermis to limit the specification of vulval fates in Pn.p cells [8–10].

Three recent papers on *C. elegans* have attempted to directly address the role of LIN-3/LET-23 signaling in the specification of 1° and 2° fates [11••–13••]. Katz *et al.* [11••] expressed varying concentrations of a transgene encoding a secreted form of the EGF domain of LIN-3. By following Pn.p fates both in response to these different levels of LIN-3 activity and in different Pn.p contexts, the following conclusions were made. First, the secreted EGF domain is capable of inducing vulval fates. Second, in the absence of an AC and in an isolated Pn.p (P7.p), LIN-3 could induce 1° or 2° fates in a dose-dependent manner. Consistent with this, in an intact Pn.p field, 2° fates could be found bordered on both sides by 3° Pn.ps. Finally, in an intact Pn.p field, high levels of unlocalized LIN-3 were capable of overriding the lateral signal and inducing fates intermediate between 1° and 2° fates in adjacent cells. Similarly, high levels of localized LIN-3 were capable of completely overriding the lateral signal and inducing 1° fates in neighboring cells. All of these results point towards the ability of the secreted form of LIN-3 to specify 1° and 2° fates in a concentration-dependent manner.

With similar intentions, but a different approach to that outlined above, Simske and Kim [13••], and Koga and Ohshima [12••], used a mosaic technique to address the role of LIN-3 signaling in the specification of 1° and

Figure 2

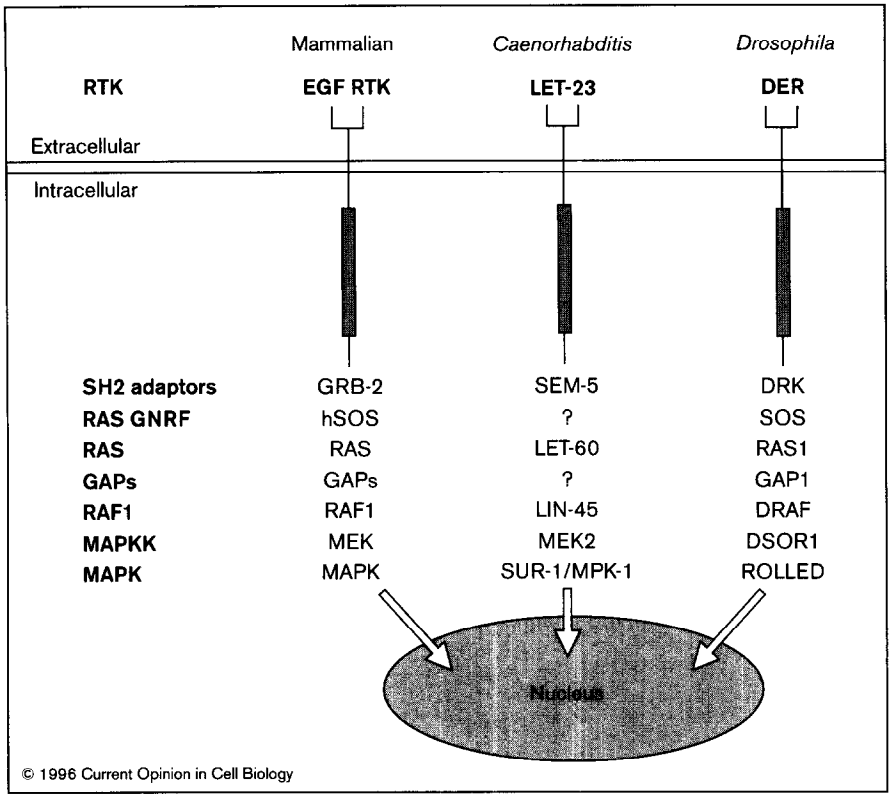


C. elegans vulval development. **(a)** The anchor cell inductive signal (arrows from AC), the P6.p/1° lateral signal (arrows from P6.p) and the hypodermal repressive signal (which acts on the surrounding hypodermis to limit the specification of vulval cell fates) have an effect upon the vulval precursors (Pn.p). **(b)** The combined action of these signals results in the specification of 1°, 2°, and 3° cell fates. Each Pn.p then follows the appropriate developmental pathway and assumes a vulval or non-vulval fate.

2° fates. They engineered animals lacking endogenous LET-23 RTK or LIN-7 (LIN-7 is also required in Pn.ps to respond to the AC signal) activity, but having either of the encoding genes present on extrachromosomal arrays. Loss of the extrachromosomal array would result in the production of different Pn.ps lacking either LET-23 RTK or LIN-7 activity. This mosaic loss allowed them to test the requirement for the reception and transduction of the LIN-3 inductive signal in cells that normally adopt the 1° (P6.p) or the 2° (P5.p and P7.p) fate. Both groups report similar results. First, the presence of LET-23 RTK and LIN-7 activity is required for a Pn.p to adopt the 1° fate. Second, the 2° fate could be established in Pn.ps lacking LET-23 RTK and LIN-7 activity if they were adjacent to a 1°-fated Pn.p. Finally, in the absence of the 1° fate in P6.p (due to lack of LET-23 or LIN-7 activity), P7.p adopted a tertiary (3°) fate regardless of LET-23 or LIN-7 activity. These results indicate that LIN-3 activity induces, via the LET-23 RTK signaling pathway, the 1° fate in P6.p. P6.p can subsequently induce, independently of the

Figure 3

Common elements of a signaling transduction pathway. Cytoplasmic elements of RTK signaling that have been identified and conserved in mammals, worms and flies are listed. GNRF, guanine nucleotide release factor; GAP, GTPase-activating protein; MAPKK, MAPK kinase.



LET-23 RTK pathway, the 2° fate in P5.p and P7.p. This indicates that activation and reception of the lateral signal, via the NOTCH-related LIN-12 receptor, is sufficient to induce the 2° fate in P5.p and P7.p. This is consistent with the previous demonstration that expression of an activated form of LIN-12 in all Pn.ps can induce 2° fates [14]. Interestingly, in some mosaic animals, when P6.p lacked LET-23 activity P5.p assumed a 1° fate, indicating that P5.p is capable of receiving and responding to the LIN-3 signal, consistent with the results of Katz *et al.* [11••].

The results of [12••,13••], coupled with the results of [11••] and with previous knowledge, indicate that LIN-3 activity is also important in specifying a 2° fate in P5.p and P7.p (reviewed in [15]). A reconciliation of all these results, as suggested in these papers, invokes both the concentration of LIN-3 and the reception of the lateral/LIN-12 signal as contributing to the invariant pattern of 2° fate in P5.p and P7.p cells in wild-type animals. Yet many intriguing questions still remain. For example, in mosaic animals lacking LET-23 activity in P6.p, what are the levels of LIN-3 activity that lead to a a 1° fate in P5.p? Does P5.p receive higher than normal levels of LIN-3 in the absence of LET-23 activity in P6.p, or is it the failure to receive a secondary signal that results in a 1° fate? Along the same lines, it will be informative to determine if secreted LIN-3 can induce the 2° fate in P5.p and P7.p in a *lin-12* mutant background, although it should be noted that previous results indicate that LIN-12 activity is necessary and sufficient for the specification of 2° fates (reviewed in [15]). Finally, what is the molecular relationship of the repressive LIN-15 signal to the LIN-3 and LIN-12 signals?

Oocyte patterning and axis determination involves both DER and NOTCH activities

Interestingly, work on *Drosophila* oogenesis has revealed the involvement of two signaling pathways in axis determination that are similar to the pathways found in *C. elegans* vulval development [16–20,21••,22••]. During oogenesis, a germline stem cell gives rise, through incomplete cytokinesis, to a cyst of sixteen interconnected cells (reviewed in [23]). One of these cells will form the oocyte while the other fifteen will become nurse cells. At this point, the entire cyst is surrounded by an epithelial sheet of somatically derived follicle cells. At these stages of oogenesis, two unique populations of follicle cells, the anterior (A) and posterior (P) polar cells (hereafter referred to as APcs and PPcs), become distinct at the poles of the egg chamber ([24]; see Fig. 4). Decreasing the activity of the NOTCH transmembrane receptor disrupts PPc fate and leads to an alteration in the anterior/posterior (A/P) axis of the oocyte [19]. In what was previously thought to be independent of A/P axis formation, the movement of the oocyte nucleus from the posterior to the anterior

cortex of the oocyte leads to the establishment of dorsal follicular fates (Fig. 4). Accompanying this migration is the localization of the mRNA and protein of another EGF family member, GURKEN [20,22••]. *gurken* mRNA, and subsequently protein, becomes apically localized with respect to the dorsal-anterior oocyte nucleus. This asymmetric GURKEN localization induces dorsal fates by activating the TORPEDO/*Drosophila* EGF receptor (DER) RTK signaling pathway in the overlying follicular epithelium (reviewed in [25]). Ventrally, the absence of DER activity results in the production of a signal that establishes the embryonic dorsal/ventral (D/V) axis.

Some time ago, a link between the organization of the A/P and D/V axes was suggested by the observation of a duplicated anterior micropyle at the posterior of the embryo in the ventralizing mutant *gurken* [16]. In an elegant display of the power of genetics and phenotypic analysis, two current papers have firmly linked the GURKEN/DER RTK and NOTCH signaling pathways with the establishment of both the A/P and the D/V axes [21••,22••]. The results and conclusions of these papers are discussed below.

During the early stages of oogenesis, the oocyte nucleus and its associated *gurken* mRNA are located at the posterior of the oocyte [20]. At mid-oogenesis, the oocyte nucleus and *gurken* mRNA relocalize to the anterior cortical region, where GURKEN/DER signaling specifies dorsal follicle cell fates (reviewed in [25]). Work by Gonzalez-Reyes *et al.* [21••] and Roth *et al.* [22••] has now demonstrated that the early posterior localization of GURKEN specifies posterior follicle cell fates through the activation of the DER RTK pathway. First, both reports demonstrated that a reduction in the activity of GURKEN, DER or the novel protein CORNICHON results in a transformation of posterior follicle cell fates to anterior follicle cell fates (Fig. 5). Second, they showed that, in response to this transformation, the polarity of the oocyte is disrupted (Fig. 5) Using the oocyte nucleus with a plus end directed kinesin motor/β-galactosidase fusion protein (kinesin/LacZ) and *bicoid* (*bcd*) and *oskar* (*osk*) mRNAs as markers they demonstrated that oocyte polarity becomes A/P/A, instead of A/P. The normally anteriorly localized *bcd* mRNA becomes localized to both poles of the oocyte, while the normally posteriorly localized kinesin/LacZ and *osk* mRNA are now positioned at the center of the oocyte.

Figure 4

Drosophila axis organization in egg chambers. A simplified view of oogenesis. In response to GURKEN signaling from the oocyte to what will become the posterior follicle cells (top), the A/P axis is organized (bottom). This results in a reorganization of the cytoskeletal network and subsequent specification of D/V fates (bottom). In addition, the NOTCH pathway interacts in some fashion with this signaling to also regulate the proper specification of posterior follicle cells. Because the nature of this interaction is still unclear, we have not depicted it here.

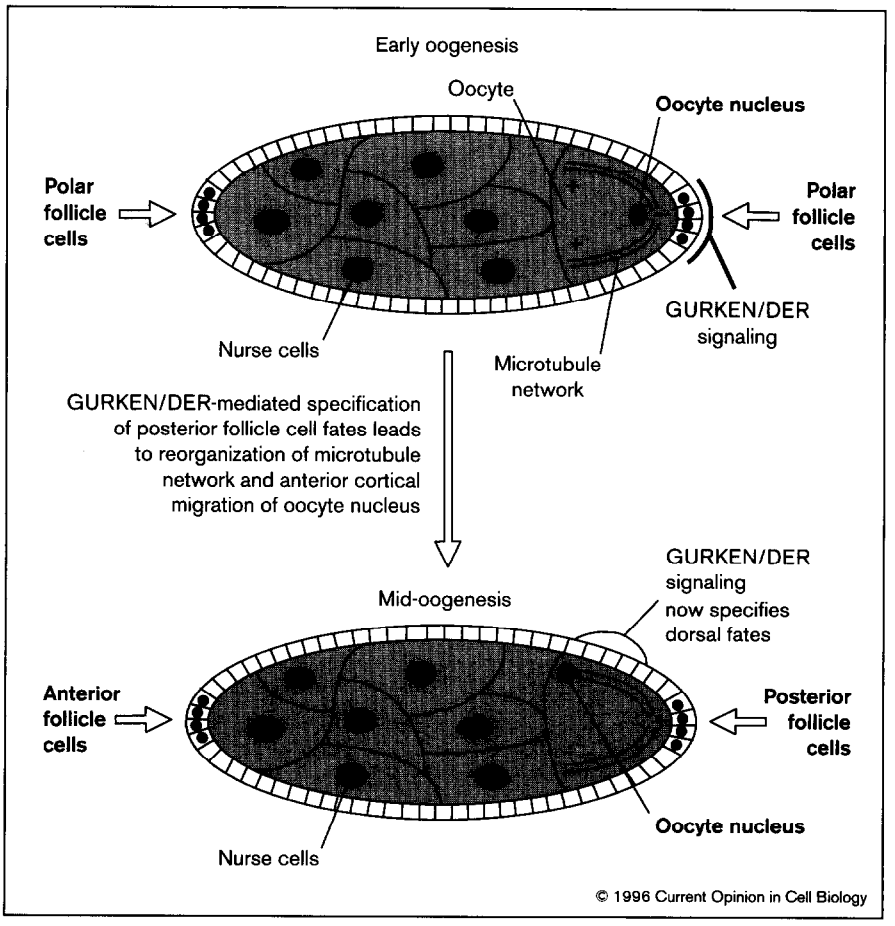
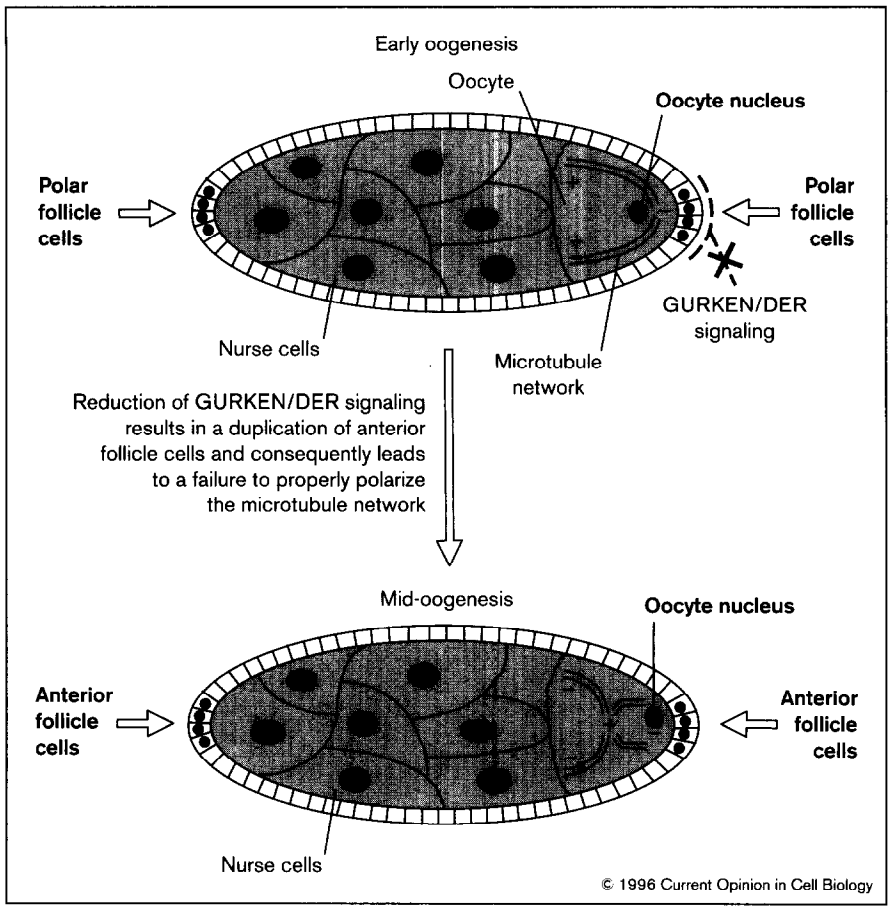


Figure 5

Drosophila axis organization in egg chambers with disrupted GURKEN/NOTCH signaling (NOTCH signaling is not shown but the effects of disruption of NOTCH are similar to the effects of disruption of GURKEN). In the absence of normal levels of GURKEN or NOTCH signaling, posterior follicle cell fates are improperly specified. This results in a duplication of anterior fates and the formation of an A/P/A axis. As a result of the abnormal organization of the cytoskeletal network, the D/V axis is also disrupted.



Interestingly, similar effects on oocyte polarity had been described previously [19]. Ruohola *et al.* [19] showed that reducing the activity of the NOTCH signaling pathway in the follicular epithelium also resulted in a failure to repolarize the oocyte during mid-oogenesis and a subsequent abnormal A/P/A axis. Thus, in response to both GURKEN and NOTCH signaling, posterior follicle cells produce a secondary signal during mid-oogenesis that initiates a reorganization of the cytoskeletal network (reviewed in [26]). This establishes proper A/P polarization and results in the anterior cortical migration of the oocyte nucleus and its associated *gurken* mRNA. As described earlier, this initiates the specification of D/V polarity via GURKEN/DER RTK signaling to the overlying follicular epithelium (reviewed in [25]). Therefore, induction of DER RTK activity in the posterior follicle cells, in cooperation with NOTCH signaling, controls the organization of both the A/P and D/V axes. Yet, even with this knowledge, we are confronted with numerous other issues to be resolved. For example, what is the nature of the secondary signal sent to the oocyte in response to GURKEN/NOTCH signaling? How does this signal direct the reorganization of microtubule polarity? The above and previous results indicate that the polarity of the microtubule cytoskeleton at this stage is oriented with minus ends at the anterior end of the oocyte and plus ends at the posterior end (reviewed in [23,26]). However, the colocalization of a plus and a minus end directed motor (kinesin/LacZ and dynein, respectively) within the oocyte in response to this signaling provides an intriguing twist to this problem [27•,28•]. Initial work has suggested that protein kinase A may be involved in this cytoskeletal reorganization [29].

Integration of the EGF RTK and NOTCH/LIN-12 pathways

The two examples described above stress that in *C. elegans*, as in *Drosophila*, the combined induction of the EGF RTK and NOTCH/LIN-12 pathways appears to be required for establishing the pattern of a particular group of cells. In both cases, a single cell (the AC in worm vulval development and the oocyte in fly axis formation) is signaling through an EGF RTK pathway to an adjacent set of cells, which are communicating amongst themselves via the NOTCH/LIN-12 pathway. Knowing this, it is tempting to speculate that molecular insight into either pathway may identify mechanisms common to both. The availability of these two paradigms should help identify the level at which these two signaling pathways intersect.

With respect to activation of the RTK signaling cassette by an EGF family ligand, work in both vertebrates and invertebrates has identified and ordered many of the components of this intracellular signaling cassette. Components of this cassette that have been identified and characterized in *C. elegans* and *Drosophila* are shown in Figure 3 (reviewed in [1,30]). Functional conservation has been highlighted in four cases in which it has been demonstrated that the Src homology (SH)3–SH2–SH3 adaptor protein DRK, in addition to the kinases Raf, MEK and mitogen-activated protein kinase (MAPK), is capable of providing cross-species activity [31–35].

Similarly, numerous components of the NOTCH signaling pathway have been identified in both vertebrates and invertebrates. Due to space constraints, readers are directed to [2] for an excellent review of this pathway.

In establishing cell fate, the EGF RTK and NOTCH pathways may be temporally distinct or coincident in their activities. In the latter case, they may independently converge on the transcriptional regulation of the same downstream targets. Alternatively, convergence may happen at the level of a particular transducer of one pathway. For example, the activation of the LET-23/DER RTK pathway could modify the activity of one of the integral components of LIN-12/NOTCH signaling. The ability to genetically isolate suppressors and enhancers of mutants in these specific paradigms should be informative with regard to these issues. If the integration of RTK and NOTCH/LIN-12 pathways occurs directly, it will be interesting to compare whether the same molecular mechanisms have been conserved among different RTKs.

Sending signals: an update

To fully understand signal integration and how it relates to patterning will ultimately require the identification of all the components involved in each of the above pathways. Already, the recent characterization of a number of particular loci has proven to be revealing. As we will see below, in *Drosophila* and in *C. elegans* an ongoing molecular analysis of mutants has led to some appealing models for the regulation of EGF RTK signaling activity. It is possible that the activators and repressors of EGF RTK signaling are controlled by other pathways, thus providing molecular mechanisms for cross-talk between signaling pathways.

In *Drosophila*, both positive and negative regulators of the DER RTK have been characterized. On the positive side, the transmembrane proteins RHOMBOID and STAR have been postulated to generate a secreted and therefore activated form of SPITZ, a second EGF-like ligand for the DER RTK [36•]. Supporting this notion, overexpression of the transmembrane form of SPITZ had no phenotypic effects, whereas overexpression of a secreted form resulted in alterations of cell fates [36•]. Thus, in activating the DER RTK via SPITZ, processing seems to be the limiting event. One wonders if a similar event is also relevant to the activity of LIN-3 in *C. elegans* vulval development and GURKEN in *Drosophila* oogenesis. Conversely, genetic, molecular and biochemical data have provided the first *in vivo* evidence for the negative regulation of an RTK via a feedback loop [37•,38•]. Activation of DER signaling apparently leads to the transcriptional activation of a secreted transforming growth factor-α-like factor, termed ARGOS, that acts as an extracellular inhibitor of DER activity [37•,38•].

Similarly, in *C. elegans*, both positive and negative regulators have been identified. LIN-2A, which acts in Pn.ps to promote vulval development, has been identified as a membrane-associated guanylate kinase (MAGUK) [39•]. Interestingly, a kinase-inactive form of LIN-2A is functional in vulval patterning [39•]. These data, together with other genetic and biochemical evidence, have led to a model in which the LIN-2A MAGUK functions in a structural manner, positioning the LET-23 RTK at cell junctions for reception of the LIN-3 inductive signal [40]. Similarly, UNC-101 might also be involved in regulating LET-23 receptor localization [30,41•]. Molecular analysis has indicated that UNC-101 is homologous to the medium chain of the mouse clathrin-associated protein adaptin AP47 [41•]. Adaptins are capable of binding EGF RTKs and acting as tumor suppressors, roles consistent with UNC-101's negative regulation of the LET-23 pathway [42,43]. Finally, the inhibitory LIN-15 signal, which acts in the surrounding hypodermis to limit the specification of vulval fates in Pn.p cells, encodes two novel proteins [8–10]. Loss of LIN-15 activity results in excess vulval fates and a multivulval phenotype [44].

Conclusions and future perspectives

Studies in the field of signal transduction and pattern formation can be subdivided into those that aim to identify components of specific signaling pathways and those that address the integration of multiple signals. In this review, we have focused our discussion on recent papers which illustrate two systems in *C. elegans* and *Drosophila* that should help to elucidate how apparently distinct signaling pathways cooperate in cell-fate establishment and pattern formation. Current findings concerning extracellular and transmembrane proteins that regulate the activation of the EGF RTK have provided some initial suggestions as to how other signaling pathways may modify the activation of the EGF RTK pathway. It is apparent that one of the most difficult issues to be resolved in this field is to determine if two signaling pathways operate at the same time within a single cell. The identification of target promoters will become critical in the characterization of these interactions, as ultimately the effect of these signaling pathways is to establish cell fate through transcriptional regulation. Thus, another fruitful approach to revealing the molecular basis of cross-talk will be to identify target genes that respond to multiple signaling activities and to characterize their regulatory regions. If a response element is shared between two pathways, characterization of the

corresponding binding proteins will constitute a logical approach to identifying the integration points between two pathways. In this fashion, classical and reverse genetics may arrive at common ground.

Acknowledgements

We appreciate helpful comments and criticisms on the manuscript from Bob Boswell, Min Han, Zak Wills, and Petra Belady.

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