

# What goes up must come down

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**The activity of the epidermal-growth-factor receptor is highly regulated during *Drosophila* development. Two negative-feedback loops involving the proteins Kekk1 and Sprouty have been found to act by quite different inhibitory mechanisms.**

**T**he epidermal-growth-factor receptor (EGFR) activates a conserved signal-transduction pathway in mammals, flies and worms that regulates cell differentiation, proliferation and survival. Recent research from two laboratories now reveals new mechanisms through which EGFR signalling can switch itself off and highlights the impact that this negative feedback can have on development<sup>1,2</sup>.

During development of multicellular animals, cells communicate with each other by producing extracellular ligands that bind to transmembrane receptors and activate intracellular signalling pathways. For example, ligands that bind to the extracellular domains of receptor tyrosine kinases (RTKs) trigger activation of the Ras/MAP (mitogen-activated protein) kinase pathway, which in turn alters gene transcription and can ultimately modify cell behaviour. Genetic experiments in the fruitfly *Drosophila melanogaster* have been remarkably useful in establishing the molecular details and biological functions of this signalling pathway. In fact, MAP kinase is activated repeatedly by the EGFR tyrosine kinase during *Drosophila* development<sup>3</sup>, and by other RTKs that have more restricted functions.

*Drosophila* is also proving useful in the identification of molecules that are expressed as a result of RTK activation and which feed back to have a positive or negative effect on RTK signalling. Two papers in *Cell* have now revealed new and distinct ways in which signalling through the *Drosophila* EGFR can be switched off<sup>2</sup>. Ghiglione and colleagues<sup>2</sup> have studied the putative transmembrane protein Kekk1 and shown that it associates with the EGFR and is likely to interfere directly with EGFR

activation. Meanwhile, Casci and colleagues<sup>1</sup> have identified the protein Sprouty as an inhibitor of EGFR signalling and shown that Sprouty localizes to the inner surface of the plasma membrane and might antagonize signalling through its ability to bind to molecules further on in the signalling pathway<sup>1</sup>. Intriguingly, the expression of both Kekk1 and Sprouty is induced by EGFR activation, indicating that multiple negative-feedback loops may control EGFR signalling during development.

One function of the *Drosophila* EGFR is to induce a dorsal fate in the follicle cells that surround the oocyte and secrete the eggshell<sup>4</sup>. The dorsal–anterior surface of the mature eggshell is characterized by two filamentous structures called dorsal appendages, through which the embryo can breathe if the rest of the egg is buried. Dorsal-appendage development is initiated by Gurken, a homologue of the mammalian EGFR ligand, transforming growth factor- $\alpha$ . Gurken is expressed on the dorsal–anterior surface of the oocyte and activates EGFR signalling in the follicle cells that overlie the oocyte in that region. In a search for *in vivo* targets of EGFR signalling during dorsal-appendage formation, Ghiglione and colleagues found that the Kekk1 transcript accumulated in the dorsal–anterior follicle cells in a manner that was dependent on EGFR signalling<sup>2</sup>. When they overexpressed Kekk1 in the follicle cells they found that dorsal-appendage formation was inhibited, thus identifying Kekk1 as a negative regulator of EGFR signalling.

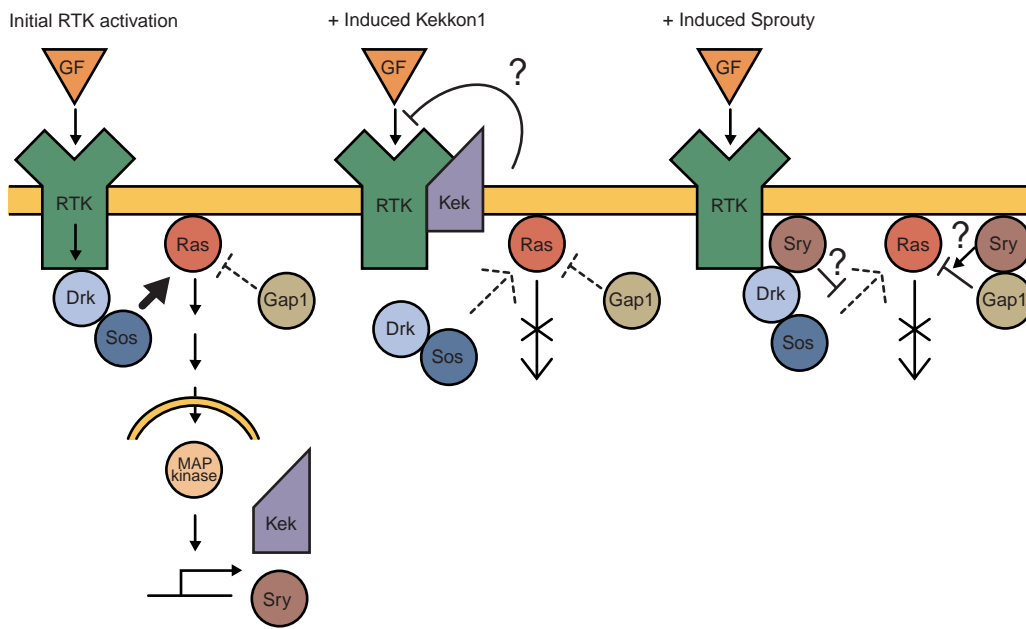
Kekk1 is a putative transmembrane protein that has features in common with proteins involved in cell adhesion and signalling<sup>5</sup>. It contains leucine-rich repeats, an immunoglobulin-like motif and glycosylation motifs in the probable extracellular domain, and has a large intracellular domain of unknown function. Both genetic and biochemical experiments indicate that Kekk1 is likely to inhibit EGFR signalling at the level of the receptor<sup>2</sup>. The inhibition of dorsal-appendage formation by Kekk1 could be overcome by co-expressing activated versions of downstream targets or the EGFR itself, but not by overexpressing

Rhomboid, a seven-transmembrane-domain protein that enhances ligand-induced activation of the EGFR. Consistent with the idea that Kekk1 inhibits EGFR activation, Kekk1 can associate with the EGFR. Moreover, both the inhibition of dorsal-appendage formation and the association of Kekk1 with the EGFR depend on the extracellular and transmembrane regions of Kekk1 but not its intracellular domain.

Together, these observations indicate that activation of the EGFR in follicle cells induces the production of an EGFR antagonist, Kekk1, that can inhibit EGFR signalling by interfering with EGFR activation. It is worth noting, however, that flies and eggs completely lacking Kekk1 are viable and fertile. Although eggs produced by flies without Kekk1 have more widely spaced dorsal appendages (similar to those induced by mild activation of the Gurken/EGFR pathway), the effects are subtle, and neither the patterning of the embryo nor the hatching rate are affected under laboratory conditions. Therefore, the Kekk1 negative-feedback loop does not seem to be necessary for normal egg development, and may instead provide a negative-feedback mechanism that can maintain patterning if signalling through the EGFR is disrupted by other means. Alternatively, redundancy might be at work: in animals lacking Kekk1, the related proteins Kekk2 and Kekk3 (refs 2,5) might be able to replace its function.

Another function of EGFR signalling in *Drosophila* development is in the recruitment of cells into ommatidial preclusters in the developing eye<sup>6</sup>. One previously identified inhibitor of the EGFR during this process is the secreted protein Argos. Overexpression of Argos during eye development results in adult eyes that are rough in appearance, so Casci and co-workers<sup>1</sup> used this as a readout to search for new molecules involved in EGFR signalling. They screened for mutations that could modify Argos-induced rough eyes and isolated several mutations in a previously identified gene called *Sprouty*. Reducing signalling through the EGFR pathway in the eye, either by overexpressing Argos or by subtle inactivating mutations in the endogenous EGFR gene, generates rough eyes with fewer ommatidial cells than normal. When one copy of the *Sprouty* gene is removed from those flies, the rough eyes revert to their normal smooth appearance. This suggests that *Sprouty* may normally inhibit EGFR signalling and that its removal can compensate for the inhibition of EGFR signalling by other means. Consistent with the idea that *Sprouty* inhibits EGFR signalling, patches of eye cells lacking *Sprouty* have defects typical of overactivation of the EGFR pathway — extra ommatidial cells — whereas the opposite effect is seen when *Sprouty* is overexpressed<sup>1</sup>.

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**Figure 1** Two different ways of achieving negative-feedback inhibition of receptor tyrosine kinase signalling pathways. Activation of a receptor tyrosine kinase (RTK) by its growth factor (GF) ligand results in the activation of Ras and MAP kinase (left), and leads to expression of the proteins Kekkton1 (Kek) and Sprouty (Sry). Kekkton1 directly inhibits further activity of the RTK by associating with it in the membrane (centre). Sprouty, on the other hand, inhibits signalling from the RTK indirectly, by associating with downstream components of the pathway and inhibiting Ras activation (right).

Interestingly, the Sprouty gene was first identified because it could inhibit signalling through the fibroblast-growth-factor receptor during development of the *Drosophila* airways, the trachea<sup>7</sup>. In addition, Casci and colleagues<sup>1</sup> have found that Sprouty inhibits signalling through the receptor tyrosine kinase Torso in the early *Drosophila* embryo. These observations immediately suggest that Sprouty might be a general inhibitor of RTK signalling that acts in a less specific manner than Kekkton1.

Hacohen and colleagues<sup>7</sup> found previously that removing Sprouty function from one cell type during tracheal development could cause tracheal branching defects in other nearby cells. This led them to postulate that Sprouty was an extracellular-membrane-associated or secreted protein. In contrast, Casci and colleagues<sup>1</sup> found that the differentiation of specific cells during eye development was affected only when Sprouty was removed from those cells themselves. Together these results indicate that Sprouty can inhibit signalling by several RTKs, and can have non-cell-autonomous effects (in the trachea) and cell-autonomous effects (in the eye).

These observations prompted Casci and colleagues to investigate more closely where Sprouty is found and how it might work. They found that Sprouty is an intracellular protein that associates with the inner surface of the plasma membrane through its cysteine-rich carboxy terminus and binds to the signalling molecules Drk and Gap1 through its amino terminus. Drk and Gap1 regulate Ras activity, indicating that Sprouty might function as a general inhibitor of RTK signalling by localizing to the inner surface of the plasma membrane and modulating Ras activity.

So, although EGFR-induced expression of either Kekkton1 or Sprouty has a similar inhibitory effect on EGFR signalling, the negative-feedback mechanisms involved differ considerably (Fig. 1). The molecular details still have to be determined, but it looks as though Kekkton1 interferes directly with the extracellular activation of the EGFR whereas Sprouty binds to intracellular downstream proteins and can inhibit signalling by other RTKs as well. As well as Kekkton1 and Sprouty, other inhibitors of EGFR signalling have been identified, such as Argos, whose production is again induced by EGFR activation<sup>8,9</sup>. EGFR signalling also triggers the expression of molecules that provide positive-feedback loops and can amplify the original EGFR signal. For example, activation of the EGFR by its ligand Gurken in the follicle cells induces the expression of Vein, another EGFR ligand, and of Rho, which enhances the activation of EGFR by a third EGFR ligand, Spitz<sup>9</sup>.

Why do these complex positive and negative feedback mechanisms for signalling through the EGFR exist? Several explanations are possible. Signalling through the Ras/MAP kinase pathway is used repeatedly during *Drosophila* development and has different effects depending on the state of the target cell and the level of Ras/MAP kinase activation. This means that the spatial and temporal regulation of EGFR signalling is likely to be critical. The various regulatory feedback mechanisms may exist to prevent regions of Ras/MAP kinase activation from overlapping and to enable signalling through the pathway to respond to and resist perturbation. The combined production of inhibitors and activators that act in different ways and penetrate different dis-

tances through the developing tissue could generate spatial and temporal regions of differing levels of Ras/MAP kinase signalling. Evidence is accumulating to suggest that this is one of the ways in which patterned structures are generated during development<sup>6,9</sup>.

Another question raised by this work is whether analogous molecules feed back on activated RTKs in other organisms. Given the evolutionary conservation of the RTK/Ras/MAP kinase signalling module, it seems likely that its refinement by positive and negative feedback will also have been conserved. Certainly there are Kekkton-like molecules with a similar arrangement of leucine-rich repeats and immunoglobulin motifs in vertebrates and worms. Human homologues of Sprouty have also been identified, although this homology is largely restricted to Sprouty's membrane-targeting domain. Much research at present is focused on characterizing feedback during signalling, and our understanding of this complex and intriguing process is likely to increase in the near future. □

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