More sophisticated ecological approaches are also needed — the emphasis on changes in the leaf litter of individual plants has been too narrow. Shifts in plant community composition in response to high CO_2 can have dramatic effects on litter chemistry, and on carbon accumulation and nitrogen immobilization in soil (J. Dukes, Stanford Univ.). Changes induced by high CO_2 in the allocation of carbon to leaves, wood or roots will alter decomposition dynamics. Other environmental changes that will accompany the increasing concentration of CO_2 (warming and increased nitrogen deposition, for instance) will certainly influence decomposition rates, but their interactions with rising CO_2 are difficult to predict.

Another requirement is that experiments aiming to detect changes in the principal ecosystem properties that regulate nitrogen and carbon cycling must be guided by ecosystem models. The CENTURY model of decomposition[8] indicates that litter chemistry does influence decomposition, but the changes that have been observed in experiments are simply too small to have a detectable effect on decomposition. Total litter input is much more important.

Other old ideas also need to be re-examined. For example, slower decomposition that immobilizes nitrogen in microbial biomass has been considered a negative feedback on productivity. But immobilization is a transient phenomenon and can be a 'good' thing — an indication of a more fertile habitat resulting from increased root litter in CO_2-enriched grasslands[9]. And a surprising advance reported at the workshop is that slower initial decomposition rates can actually promote more complete long-term decomposition (B. Berg, Swedish Univ. Agric. Sci.); initial decomposition rate is promoted by high nitrogen concentration, but nitrogen retards long-term decomposition through inhibition of lignin-degrading enzymes and reactions that produce recalcitrant aromatic compounds[10]. Clearly, assessment of the carbon sequestration potential of soils in changing climates will require careful measurement of not just the litter inputs and decomposition, as well as other outputs from the ecosystem, but also analysis of the feedbacks between different processes and their temporal dynamics.

It is difficult to let favoured principles go. But the 15 years of work on the litter-quality hypothesis has led to a far richer understanding of how plants, decomposers and ecosystems will function as the atmosphere is progressively enriched with CO_2 and the climate changes. This understanding is especially needed as we struggle with the attempt to sequester the excess carbon we ourselves are emitting to the atmosphere[11]. □

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Developmental biology

Sending all the right signals

Norbert Perrimon and Joseph B. Duffy

During growth and development, cells signal to — and regulate the fates of — each other. Cells can initiate many developmental fates in response to a single signal and, throughout the years, two mechanisms have been identified[1]. In the first, receiving cells adopt different fates depending on the amount of signal or 'morphogen' that they receive. This implies that there is a concentration gradient of the signal, and that receiving cells can translate the quantitative amount of the signal they receive into different qualitative outcomes. The second mechanism is a 'relay mechanism', whereby receiving cells activate secondary signals that are subsequently responsible for the diversification of cell fates.

Reporting in *Cell*, Wasserman and Freeman[2] provide a striking example of how a simple instructive signal initiates the development of an elaborate pattern during oogenesis. The oocyte (derived from the germ line) is surrounded by an epithelial layer of somatically derived follicle cells, which produce the chorion (or eggshell) in the final stages of oogenesis. Among the most prominent features of the chorion are two dorsal filaments, or appendages, used for respiration. In the fruitfly *Drosophila*

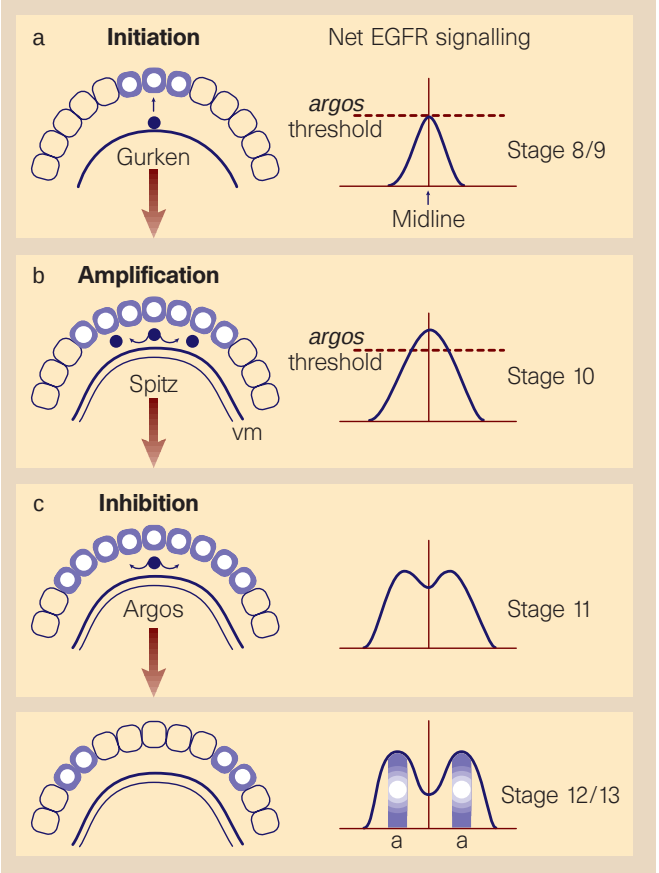

Figure 1 **Cell-fate specification during dorsal patterning of the *Drosophila* egg. Wasserman and Freeman[2] have shown that patterning involves three stages of cell-fate specification.** a, **Activity of the epidermal growth factor receptor (EGFR) in the epithelial layer of follicle cells is initiated by a signal from the oocyte called Gurken.** b, **Activity of the EGFR is amplified through autocrine signalling — EGFR-mediated transcription of the *vein* gene, and of the *rho* gene, which increases the activity of another EGFR ligand, Spitz.** c, **EGFR also stimulates the transcription of *argos*, which inhibits the EGFR in the cells where it is most highly expressed. (Adapted from ref. 2.)**

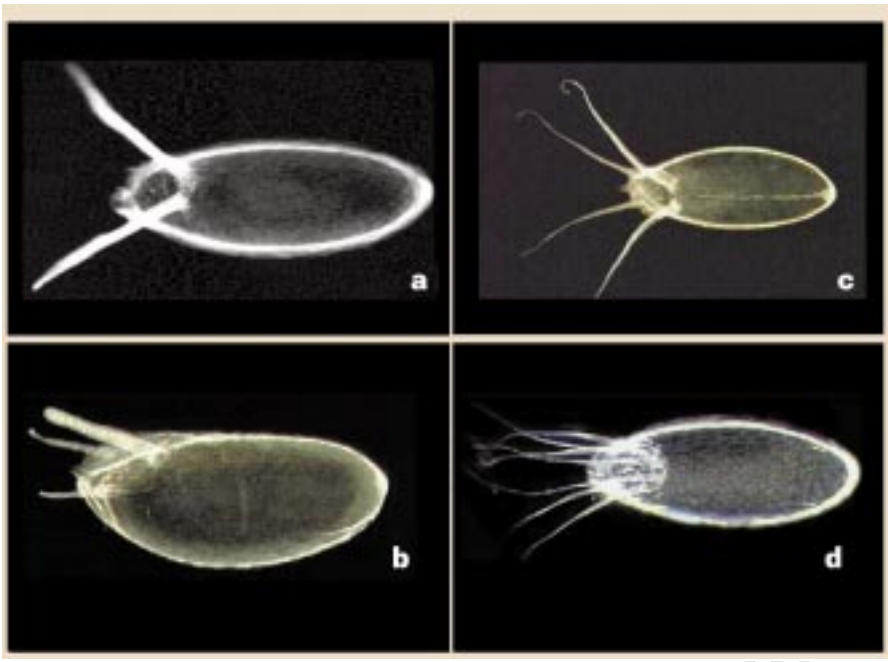

Figure 2 **Diversity of dorsal appendages in *Drosophila* species. Chorions from**: a, ***D. melanogaster*** **(2 appendages)**; b, ***D. phalerata phalerata*** **(3)**; c, ***D. virilis*** **(4)**; d, ***D. latifasciaeformis*** **(>4).**

melanogaster, the fate of dorsal cells is specified by activation of the epithelial growth factor receptor (EGFR) in the follicle cells (see ref. 3 for review). Activation depends on an asymmetric signal encoded by Gurken (Grk), a transforming growth factor (TGF)-α-like molecule that originates from the oocyte. The Grk pathway initiates development of the respiratory appendages in the dorsal/anterior region of the chorion. But what is the nature of its contribution?

Wasserman and Freeman now document formation of the respiratory appendages by two distinct signalling mechanisms (Fig. 1). Initially, Grk activates EGFR in a paracrine fashion, through signalling between the oocyte and follicle cells. This establishes an initial profile of EGFR activity in a single, central domain within the dorsal anterior follicle cells. Paracrine signalling then activates a second phase of signalling during which the activity of EGFR is amplified in an autocrine fashion — the follicle cells signal to themselves.

The molecular details are as follows. In response to the initial phase of EGFR activity, transcription of the *rhomboid* (*rho*) and *vein* genes is induced in the follicle cells. The Vein protein, like Grk, encodes a putative ligand that can stimulate EGFR. The Rho protein, on the other hand, is not a ligand for EGFR, but it stimulates the activity of a third TGF-α-like ligand for EGFR, which is encoded by the *spitz* (*spi*) gene (see refs 4 and 5 for reviews). Thus, in response to the primary induction, a second phase of autocrine signalling involving two additional EGFR ligands leads to amplification of EGFR activity. But why? Wasserman and Freeman have an intriguing idea. During these final stages of oogenesis, the follicle cells synthesize the vitelline membrane, which covers the oocyte and separates off the epithelial cells from the oocyte. This would block the activation of EGFR by the Grk signal from the oocyte. By maintaining the activation of EGFR in an autocrine fashion, however, this barrier is effectively circumvented.

How does this molecular activity result in the elaboration of two appendages? A third response to activity of the EGFR is transcription of the *argos* (*aos*) gene. In contrast to Grk, Vein and Spi, Aos encodes a secreted inhibitor of the EGFR. Wasserman and Freeman's findings indicate that Aos alters the initial profile of EGFR activity in the follicle cells. Aos acts within the peak of EGFR activity at the dorsal anterior, leading to repression along the centre of this profile. Effectively, repression splits the single peak into two (Fig. 1).

The authors confirmed this hypothesis by examining receptor-signalling activity in the following manner. Binding of ligand to the EGFR activates a cascade, involving the kinases Raf, mitogen-activated protein kinase kinase (MEK) and mitogen-activated protein kinase (MAPK), that has been conserved from invertebrates to vertebrates. MAPK is activated through phosphorylation by MEK at two sites. Antibodies that recognize this diphosphorylated form of MAPK can be used to examine the readout of signalling activity and, consistent with Wasserman and Freeman's theory, these antibodies indicated a single domain of activated MAPK in the dorsal anterior. This domain then resolves into two bilateral domains, marking the future position of the respiratory appendages.

Wasserman and Freeman propose that there are three stages of cell-fate specification: initiation of EGFR activity, its amplification, and subsequent repositioning. Furthermore, they document a hitherto unknown regulatory strategy — a transition from a paracrine signal to its subsequent autocrine amplification and inhibition. These findings have both evolutionary and ecological consequences. There are enormous diversities in the morphology, pattern and number of respiratory appendages in drosophilid chorions, ranging from no appendages to more than four (Fig. 2). These patterns seem to have evolved independently on numerous occasions[6], presumably as a result of selective forces imposed by ecological constraints. With the molecular insight gained from *D. melanogaster*, we can now investigate the selective mechanisms that operate on a signal-transduction and pattern-forming pathway from molecular, evolutionary and ecological standpoints.

How has signalling been modified, and patterning differences achieved, over the course of evolution? One intriguing model is that diversity in the patterning of respiratory appendages has been achieved by increasing the complexity of EGFR regulation. Perhaps other positive and negative regulators of the EGFR remain to be identified. If so, such studies may have clinical implications. For example, misregulation of members of the EGFR/ErbB family of receptor tyrosine kinases has been implicated in more than 30% of human breast cancers, so by identifying new ways to inhibit these enzymes we may be able to develop cancer therapeutics.

Another model is that the diversity in appendage patterning has come about through cross-talk with other signalling pathways. Interestingly, the Decapentaplegic/TGF-β pathway is also involved in patterning of the appendages[7] so, possibly, cross-talk between the EGFR and TGF-β pathway has been exploited to diversify patterning of the dorsal appendages. Regardless of how diversity in patterning is achieved, work in the fruitfly has once again proved to be ripe for the picking. □

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