Wingless signalling: **More about the Wingless morphogen**

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Recent work on pattern formation in the *Drosophila* embryo reveals a new mechanism which shapes the gradient of the secreted morphogen, Wingless: Wingless protein is degraded more rapidly on one side of its source than on the other.

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Large questions often derive from simple observations. When Gonzalez, Martinez-Arias and collaborators [1] looked at the later stages of the *Drosophila* embryo and noted that antigen from the secreted protein Wingless (Wg) did not spread out evenly from the source, no-one paid much attention. And yet it was important because it raised the possibility that the spread of morphogens might, in some contexts, be allowed in one direction and blocked or impeded in another.

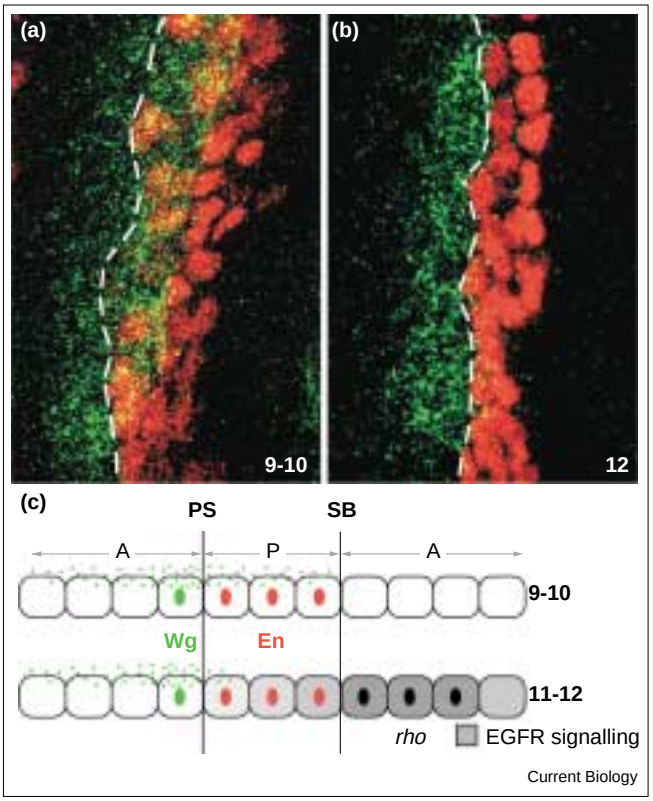
In the *Drosophila* embryo Wg is made by a line of cells at the back of the anterior (A) compartment, from there it spreads forward and patterns most of the segment, for example it specifies naked rather than denticulate cuticle in nearly all segments [2]. For this reason the view has become entrenched that Wg specifies naked cuticle as such [3] ; but I think this idea is misleading, rather I think Wg forms a concentration gradient, and it is the Wg concentration that tells the cells their location relative to the back of the A compartment. A high concentration in most segments means 'make naked cuticle', but in the first thoracic segment it means 'make denticles' [4]. In this view Wg is a morphogen that gives positional information to the A cells in front of the source, and they interpret that information according to their cell identity which is determined by selector genes such as the *bithorax* complex. But Wg also provides positional information to the posterior (P) cells behind the source, telling the P cells where they are. However in that direction it does not act with such a long reach, but appears to affect only the cells adjacent to the source [5].

So, why does Wg have a longer reach in the anterior direction than in the posterior? Jean-Paul Vincent's group have studied this matter in several papers [6–8], and now they have reported new results. Dubois *et al.* [9] have developed a method to follow the Wg protein in cells and with the electron microscope. They have fused the Wg protein to horseradish peroxidase (HRP) and transformed flies so they can express this chimaeric protein at will. The protein is functional, substituting for Wg and rescuing *wg* mutants and spreading apparently normally out from the source into nearby cells. HRP can be seen in well-fixed cells in the electron microscope and followed as a marker for Wg. However it seems that its intracellular journey lasts longer than for Wg itself as it is more resistant to degradation (compare with [10]).

So why is Wg antigen not seen to spread backwards after a certain time (see Figure 1a,b)? Maybe there is a block to spread of the Wg protein? Not apparently so, because the HRP can be seen in P cells in which the Wg antigen is no longer detected, suggesting that Wg spreads backwards but is promptly degraded [9]. It had been shown long ago that Wg antigen is found in large cellular inclusions which in the electron microscope appear to be multivesicular bodies [11]. Now Dubois *et al.* [9] have evidence that these are probably in the degradative pathway as they also see HRP in lysosomes. Furthermore they find that, anterior to the Wg source, there are only a few vesicles containing only HRP without attendant Wg, whereas posteriorly, there are many such vesicles [9]. They conclude that Wg is sent for degradation much more readily behind, while it persists for longer in front. They also show that reduction in the activity of two genes needed for endocytosis and generation of lysosomes, *clathrin* and *deep orange*, increases the activity of Wg, presumably because degradation is compromised [9]. These studies argue that degradation could be an important means of regulating the action of a morphogen.

Some years ago two papers [2,12] showed that activation of the epidermal growth factor (EGF) pathway antagonises the effects of Wg, and pointed out that the EGF receptor, EGFR, is activated behind the source of Wg but not in front. Everyone seems to agree that Spitz, a ligand for EGFR, is produced by the most anterior A cells, spreading forwards and backwards. They also seem to agree that just anterior to the source of Spitz — the most posterior P cell — Spitz effectively blocks the effects of Wg, but further anterior — the most anterior P cell — it is subjugated by the higher concentration of Wg present (Figure 1c). Dubois *et al.* [9] have now looked to see if the EGF pathway might be responsible for driving degradation of Wg and indeed find that, if this pathway is blocked, degradation is reduced. Taking this thesis at its simplest, one might expect that Wg should not be degraded in the most anterior P cells but should be degraded in the posterior P cells, giving a step in the concentration of Wg-containing vesicles within the P compartment. However, this may be hard to see because there are always more, perhaps secretory, vesicles in the actual domain of expression of the gene,

Figure 1



Wingless persists anterior, but not posterior, to its source. (a,b) Wingless (Wg, green) is made at the back of the anterior (A) compartment, just anterior to the posterior (P) compartment cells. The P cells express and contain Engrailed protein (En, red). At earlier embryonic stages (9-10) Wg is evenly distributed on both sides of its source. Later (Stage 11) Wg is found mostly anterior to the source and little protein persists behind it. (c) A summary of the situation showing secreted Wg protein (green dots) and the cell that secretes it (green nucleus); En protein is shown in red. The level of EGF signalling is shown by shading. Cells expressing the *rhomboid* (*rho*) gene have black nuclei; these cells are thought to secrete the EGF-like ligand Spitz. PS, parasegment boundary; SB, segment boundary. Adapted from [9].

and the sharpest step that is visible is therefore at the back of the expression domain, at the A/P border. Perhaps the outcome depends also on where and on which processes the Wg and EGF pathways compete. The earlier papers emphasised competition at the level of transcriptional control, but Dubois *et al.* [9] now argue that, in addition, the competition may affect the sorting of vesicles.

Like all good observations those of Dubois *et al.* [9] raise more questions than they answer: for example is the Wg receptor, Frizzled, internalised with its ligand? If so, does the HRP becomes dissociated from the Wg antigen at the same time and place that the receptor separates from the ligand? Where is the Wg antigen when it is outside the cell? When does the Wg ligand set in train the downstream events, is it when it binds to Frizzled, or when it is internalised? Is endocytosis part of the process whereby Wg spreads from cell to cell, as has been suggested for another important morphogen in *Drosophila*, Decapentaplegic? [3,13]

As is commonplace in the fly field nowadays more is known about the genes involved and the hierarchy of genes required for a process than what the process really is, or what the genes actually do. How does the EGF pathway regulate degradation? Is it by accelerating the transfer of vesicles to lysosomes as Dubois *et al.* [9] suggest, and, if so, what is involved. These are problems in Cell Biology, and while the genetic approach *in vivo* is good at posing such questions, it is not so good at answering them. Clearly one way forward is for fly people to go down the corridor and use the electron microscope [14], just as Dubois *et al.* [9] have done.

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