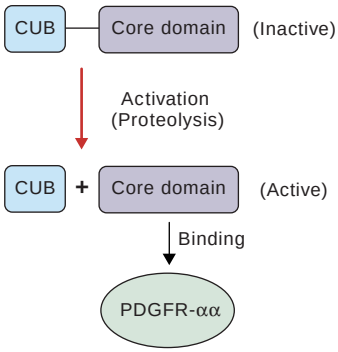

Figure 2 **PDGF-CC activation. PDGF-CC is secreted in a latent form and can be activated by proteolysis. Other PDGF isoforms (see Fig. 1) are secreted in active forms.**

bution to kidney development.

Although *PDGFR-α* is expressed throughout the kidney, the three PDGF genes, all of which encode ligands for this receptor, are expressed at different times during development and at different locations. Of the PDGF genes, *PDGF-C* is expressed first in the mesenchymal aggregates that form before the development of Henle's loop, which is where *PDGF-A* is expressed. *PDGF-B* is expressed only in the vascular endothelial cells, and is important for the recruitment of mesangial cells into the glomeruli. The important finding from the expression data is that *PDGF-C*, and not the other PDF genes, is expressed in regions adjacent to the interstitial mesenchyme of the kidney cortex, which is missing in embryos that lack *PDGFR-α*. Thus the results from expression studies and from PDGF and PDGFR gene deletions indicate that PDGF-CC may promote the migration and/or proliferation of PDGFR-α-bearing cells that populate the interstitium of the kidney. It will be interesting to see if the phenotypes of mice lacking *PDGF-C* and both *PDGF-A* and *PDGF-C* support this hypothesis.

Although further studies are needed to confirm that the *in vivo* function of PDGF-CC is distinct from those of the other PDGF isoforms, the data presented by Li *et al.* clearly show that PDGF-CC is another ligand for PDGF-α. Furthermore, PDGF-CC has the feature, unique among PDGF proteins, of being secreted in an inactive form, which consists of a CUB domain and a core PDGF domain (Fig. 2). CUB domains are present in other proteins, and have been shown to bind to proteins as well as to carbohydrates. The CUB domain may therefore function to localize and concentrate the latent PDGF-CC. The core domain specifically binds to and activates PDGFR-α, whereas the CUB domain functionally sequesters this activity. Using purified PDGF-CC and cultured cells, Li *et al.* show that PDGF-CC can be de-repressed by cleaving the CUB domain (Fig. 2). This, they argue, may also be the way in which PDGF-CC is activated *in vivo*. Transgenic mice, in which PDGF-CC is overexpressed in the heart, undergo progressive cardiac hypertrophy. This seems to be caused by excessive proliferation of cardiac fibroblasts, which express PDGFR-α. Analysis of the nature of PDGF-CC in these tissues revealed a fragment of PDGF-CC that included the core domain, but was apparently lacking the CUB domain. These observations led to the formation of a model in which PDGF-CC is proteolytically processed to generate the core domain. Together with results from tissue culture studies, which show that only the core domain is active, it seems that the cleaved form of PDGF-CC is responsible for driving the proliferation of myocardial interstitial cells. However, full-length PDGF-CC is also present in these tissues, and so the relative contribution of the core domain compared to that of full-length PDGF-CC remains to be investigated under conditions where the core domain cannot be generated from the full length protein.

As well as demonstrating the existence of PDGF-CC, and beginning to evaluate its function *in vivo*, these studies raise several interesting questions. Do the three PDGF genes have distinct functions in organs other than the kidney? Which proteases convert latent PDGF-CC to its active form, and how does the regulation of these enzymes contribute to PDGF-dependent responses? Does the CUB domain contribute to localization of the secreted PDGF-CC? Is the proteolytically liberated CUB domain biologically active, as are other proteolytically derived factors such as endostatin and angiostatin, which block angiogenesis in tumour models. Further investigation of these issues is likely to result in further surprises. □

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Morphogen diffusion: the case of the Wingless protein

Inge The and Norbert Perrimon

Understanding how morphogens promote three-dimensional patterning is an important issue in developmental biology. Recently, new insights have been gained into the mechanisms by which the *Drosophila* morphogen Wingless/Wnt is distributed in tissues.

One of the unifying goals of developmental biology is the identification of a set of evolutionarily conserved, secreted molecules that are involved in the signalling pathways that govern tissue patterning. In particular, studies of patterning of *Drosophila* and vertebrate limbs have demonstrated the critical roles of members of the Wnt, Hedgehog (Hh) and transforming growth factor-β (TGF-β) families. These molecules are often expressed in organizing centres, and can act over a long range to coordinate the patterning of an entire field of cells. Importantly, the cellular responses that these factors trigger are, in many instances, dose-dependent, which identifies them as the long-sought 'morphogens' first postulated in 1924 by Spemann and Mangold[1].

Now that morphogen molecules and their signalling pathways have been identified, the next step is to resolve the mechanisms by which they build their activity gradients. In the simplest model, these molecules diffuse into the extracellular space, and the extent of their effects correlates with a direct readout of the number of molecules that interact with membrane

receptors. Verifying this model has proved difficult, as the biochemical properties of the morphogen molecules themselves make them difficult to study (see below). Thus, because of the lack of supporting evidence, many developmental biologists have begun to question the theory that these molecules are organized into extracellular protein gradients. However, in a recently reported study, Strigini and Cohen[2] have clearly detected an extracellular gradient of Wingless protein. This observation represents direct evidence that morphogens are organized into such gradients .

Studies of the Wingless (Wg)/Wnt proteins provide a good example of the problems that have been associated with attempts to confirm that these factors are organized into extracellular gradients. Wnt proteins are closely associated with the extracellular matrix and are notoriously difficult to purify as secreted factors[3]. In addition, conventional antibody-labelling methods have detected Wg predominantly in cytoplasmic dot-like structures. Similarly, studies using electron microscopy have shown that Wg is mainly located in vesicles that are near the membrane, with very little or no protein present in the extracellular matrix[4,5]. As Wg was mainly detected in vesicle-like structures, it was previously suggested that Wg may be actively transported by transcytosis[4], a vesicle-mediated process of endocytosis and exocytosis. Interestingly, the intensity and number of Wg-containing vesicles decreases in cells further away from *wg*-expressing cells, illustrating a gradient of Wg protein throughout the tissue.

Although, before Strigini and Cohen's study, direct evidence of Wg diffusion was not forthcoming, indirect evidence indicated that Wg might fulfil the definition of a morphogen. A membrane-bound form of Wg in the *Drosophila* wing imaginal disc was shown to induce expression of several target genes as a function of its concentration[6]. This study ruled out a relay mechanism, in which Wg would induce the expression of another signalling molecule in adjacent cells that would then induce target-gene expression over a longer range. Several further findings also indicate that Wg proteins may themselves reach the extracellular space. First, one of the Wg receptors, Frizzled2 (Dfz2), has a positive effect on the long-range distribution of Wg, presumably by stabilizing extracellular Wg[7]. Second, heparan-sulphate proteoglycans, which are abundant components of the extracellular matrix, are also required for Wg signalling[8]. Despite the support provided by these observations for extracellular organization of morphogens, Strigini and Cohen's finding represents the first formal demonstration that Wg diffuses extracellularly. Using a new immunostaining method that detects extracellular proteins, they observed an extracellular gradient of Wg protein in the wing imaginal disc. This supports the idea that the Wg morphogen is organized into an extracellular protein gradient.

Strigini and Cohen then re-examined the role of dynamin, a protein involved in endocytotic and exocytotic protein transport, in Wg movement. Previous studies of the *Drosophila* dynamin homologue encoded by *shibire (shi)* showed that when Shi activity is disrupted in the embryo, Wg function is reduced, Wg accumulates in *wg*-expressing cells, and Wg-containing vesicles are not present in nearby cells[9,10]. Using the same staining protocol as for extracellular Wg in the wing imaginal disc, Strigini and Cohen were able to observe extracellular diffusion of Wg over *shi*-mutant cells, indicating that Wg protein may not require a vesicle-based mechanism to move through a field of cells (Fig. 1a). Furthermore, they found that, following removal of dynamin activity, Wg secretion is impaired and Wg protein is only present in *wg*-expressing cells, suggesting a role for *shi* in exocytosis. They then used a temperature-sensitive *shi* mutant to determine the rate of Wg movement through the wing imaginal disc. They shifted a mutant embryo to a non-permissive temperature until all of the extracellular Wg was cleared, and then incubated it at the permissive temperature. After 30–60 min of incubation, the distribution of Wg was found to resemble that of control discs, indicating that Wg travels at least 50 μm in 30 min. This rate of diffusion is consistent with that of another extracellular factor, activin (a member of the TGF-β family), in *Xenopus* embryos[11]. Together, these findings indi-

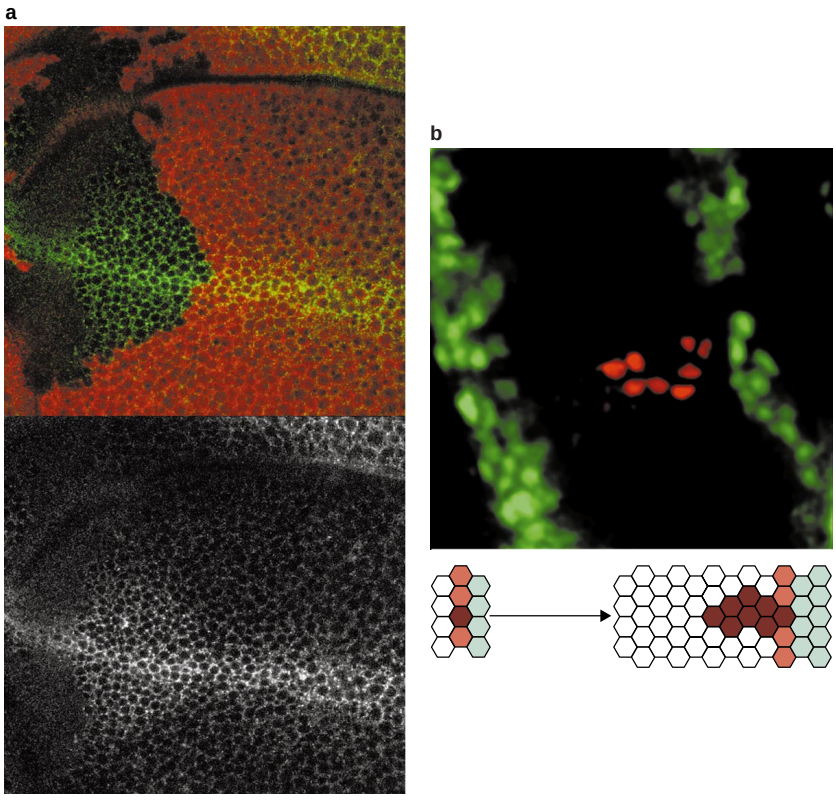
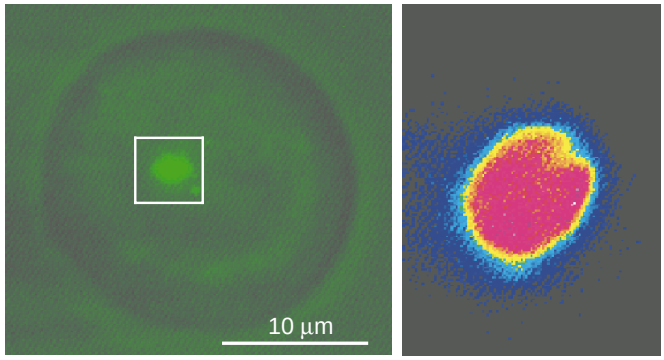


Figure 1 a, **Wg does not require vesicle-mediated transport to diffuse across a field of cells. Extracellular Wg protein (top panel, green) is spread over a large clone of *shi*-mutant cells, as identified by the absence of β-galactosidase (top panel, β-galactosidase shown in red). Bottom panel, Wg expression shown separately in greyscale. Reproduced from Strigini and Cohen[2].** b, **Wg protein can be distributed by anterior migration. Top panel, stage-11 embryo carrying a photoactivatable lineage tracer, after earlier activation of caged rhodamine. Progeny of a marked single cell are shown in red; nuclear GFP expressed under the control of *engrailed-Gal4* in cells posterior to wg-expressing cells is shown in green. A cell originating from the wg-expression domain gives rise to eight daughters that apparently spread anteriorly. Bottom panel, diagram of the proposed cell migration. Reproduced from Pfeiffer *et al.*[12]. Green, *en*-expressing cells; red, *wg*-expressing cells; brown, anteriorly migrating progeny of *wg*-expressing cells.**

It's good to talk: cell–cell communication by gap junctions



FROM BUKAUSKAS *ET AL. PROC. NATL. ACAD. SCI. USA*

Gap junctions facilitate the exchange of solutes, metabolic precursors and electrical currents between neighbouring cells. They appear as clusters (or plaques) of tightly packed particles, in which each particle is a single channel. Although it was known that the appearance of such plaques is associated with the electrical coupling of two cells, previous methods measured only the average conductance over an entire cell population and not the properties of a single junction.

In a recent study, Bukauskas *et al.* (*Proc. Natl Acad. Sci. USA* **97,** 2556–2561; 2000) used GFP-tagged connexin 43 (Cx43–EGFP) and dual whole-cell patch clamps to investigate the relationship between clusters and junctional conductance (g_j) in cell–cell pairs. When this construct was transfected into cells that were defective in communication, Cx43–EGFP fluorescence was observed throughout, except at areas of cell-cell contact, where punctate staining was present at the cell membrane. Most pairs of cells with large plaques (>0.2 µm in diameter) showed electrical coupling, with g_j values ranging from 11–60 nS, whereas those joined by smaller plaques were frequently uncoupled.

As the intensity of fluorescence in a large plaque is essentially constant (see picture), Bukauskas *et al.* were able to correlate the activity measured across a pair of cells with the activity of a single channel. The fluorescence per unit area within the plaque, together with previous calculations of channel density in plaques, was used to estimate the fluorescence intensity of a single channel. Next, pairs of cells with a single gap junction were identified, and the total fluorescence of each plaque was determined. Using their estimate of single-channel fluorescence intensity, the authors could then estimate the number of channels within the plaque. They also measured the conductance between the pairs of cells. Armed with this information, they calculated the g_j of a single channel within a plaque, and identified three categories. Small plaques (90–330 channels) showed no electrical coupling and had no active channels. Slightly larger plaques (200–400 channels) exhibited weak conductance (g_j values of 0.05–0.7 nS) and contained only 1–2 active channels. Large plaques (≥500 channels), however, possessed 35 or more active channels and had g_j values of ≥4 nS.

These findings were unexpected in several ways. First, it seems that a minimum cluster size, in terms of the number of channels, is required to open a gap junction. Second, only a fraction of channels within a gap junction are active at any given time. Third, in gap junctions above a certain critical size, the proportion of channels that are active does not seem to increase significantly with an increasing number of channels. Thus, gating by gap junctions seems to be an all-or-nothing phenomenon that occurs only when a certain channel concentration is attained, but in which an overlying regulatory step limits the number of channels that are active. These results offer new insights and also raise several questions — how is clustering initiated? What senses when the threshold number of channels has been reached? And how is channel activity regulated so that only a certain proportion of the channels within a junction is active at any one time?

ANGELA EGGLESTON

cate that, in the wing imaginal disc, transcytosis may not be involved in Wg diffusion, and that Shi activity is critical for secretion of Wg. It is worth noting, however, that because the level of expression of Wg-targeted genes was not examined in *shi*-mutant cells, it is possible that Shi also has a role in receiving cells for implementation of the Wg signal.

In another recent study of Wg distribution, Pfeiffer *et al.*[12] have proposed another mechanism underlying the movement of Wg. In the embryo, Wg is responsible for determining the fate of naked regions of cuticle in the epidermis, which incorporates three to four rows of cells anterior to *wg*-expressing cells, and one row posterior to them. Consistent with its function, Wg can be detected in three to four rows of cells anterior to *wg*-expressing cells, and in one to two rows posterior to them. Pfeiffer *et al.* observed that membrane-bound Wg protein could rescue *wg*-null mutants when expression was limited to *wg*-expressing cells. As the membrane-bound Wg protein is not believed to be secreted and the *wg*–Gal4 promoter used in this experiment is not expressed in an area wider than a single row of cells, they investigated whether *wg*-expressing cells moved anteriorly in an epidermal segment. First, they marked a single cell anterior to the parasegment boundary after photoactivation of caged rhodamine (Fig. 1b), and observed a cluster of rhodamine-marked cells anterior to the *wg*-expressing cell. Second, they marked *wg*-expressing cells with either a nuclear or a cytoplasmic β-galactosidase, and found that these non-secreted markers could spread anteriorly. Together, these results indicate that cells originating from the *wg*-expressing domain may be displaced anteriorly, while still retaining the ability to secrete Wg. The anterior movement of originally *wg*-expressing cells may therefore contribute to the patterning of the embryonic cuticle by Wg. However, it is important to note that the inheritance model proposed by Pfeiffer *et al.* is based on Wg-overexpression studies, and it remains to be established whether the amount of endogenous Wg inherited is sufficient for signalling activity.

Since the morphogen-gradient hypothesis was first formulated, positive demonstration that these factors actually diffuse into the extracellular space has been difficult. In the case of Wg, diffusion in the extracellular matrix seems to be the primary mechanism in *Drosophila* imaginal discs. Although extracellular diffusion may be the principal mechanism in cases where Wg acts at a distance of several cells, further mechanisms, such as cell movement, may also function in short-range signalling by Wg in tissues. During development, it is possible that a combination of several mechanisms is used, depending on the cell

type and the range of action of the extracellular signals involved. □

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PLC fills a GAP in G-protein-coupled signalling

Craig Montell

An emerging theme in G-protein-coupled signalling is that effectors for G proteins, such as phospholipase C-β, also function as GTPase-activating proteins. Such dual roles may contribute to high temporal resolution in G-protein-coupled signalling cascades.

The responses of animals to external stimuli, such as light and odours, are initiated with great speed through the engagement of G-protein-coupled signalling cascades. Once these responses are activated, they must be terminated quickly to enable a fast recovery from the initial stimulus and to ensure that the responses to subsequent stimuli are reproducible and achieved with high temporal resolution. While the biochemical steps leading to the rapid activation of such cascades are relatively well established, the molecular events that facilitate rapid termination are poorly understood.

Drosophila phototransduction has served as an excellent model for dissecting the mechanisms underlying rapid G-protein-coupled signalling, partly as a result of the wealth of mutants affecting visual transduction[1], and partly because this response utilizes one of the fastest known G-protein-mediated cascades. Cation influx is activated within 20 ms of exposure to light, and termination of the response occurs in less than 100 ms. It was shown many years ago that the effector for the heterotrimeric G protein, G_q, is phospholipase C (PLC), which is encoded by the *norpA* locus[2]. However, in this issue of *Nature Cell Biology*, Cook *et al.*[3] report that PLC also functions in photoreceptor cells as a GTPase-activating protein (GAP) for G_q. This not only represents the first *in vivo* evidence that PLC functions as a GAP, but also offers a solution to the vexing problem concerning the identity of the mechanism by which responses are quickly terminated upon cessation of external stimuli.

Phototransduction, in both vertebrates and invertebrates, is initiated by the light activation of a seven-pass transmembrane receptor, rhodopsin, and its subsequent association with a heterotrimeric G protein, consisting of a Gα subunit bound to GDP and a Gβγ dimer[1,4]. This interaction of the G protein with light-activated rhodopsin catalyses the release of GDP. GTP then binds to Gα, which decreases the affinity between the Gα and Gβγ subunits, and allows these dissociated subunits to interact with their effector molecules. In *Drosophila* phototransduction, the G protein is referred to as G_q and its effector is PLC, whereas the mammalian G protein is called Gt (or transducin), and couples to cGMP-phosphodiesterase (PDE). In each case, inactivation of the Gα subunits requires hydrolysis of GTP, which facilitates reassociation of the Gα and Gβγ subunits, thereby preventing further activation of their effector molecules.

Termination of the light response seems to involve several steps. However, the inactivation of G protein as a result of GTP hydrolysis seems to be the rate-limiting event[5]. A conundrum is that the known rates for termination of the response *in vivo* are typically faster, and in the case of phototransduction, several orders of magnitude faster, than the rate of GTP hydrolysis measured *in vitro*[4]. It was therefore suggested that the intrinsic GTPase activity of the Gα subunit may be stimulated by a GAP.

A major breakthrough in resolving the difference in the rates of GTP hydrolysis *in vitro* and *in vivo* was the finding that mammalian PLC accelerates GTP hydrolysis *in vitro* by a factor of about 2000 (ref. 6). This development has important ramifications, as the $G\alpha_q$ subunit can, in the presence of PLC, hydrolyse the bound GTP with a half-time of 25–75 ms, a time frame that is consistent with physiological measurements of *Drosophila* phototransduction. These *in vitro* data provide a potential mechanism whereby responses to agonists that utilize G-protein-coupled cascades and PLC are rapidly terminated. However, there is not yet any *in vivo* evidence to support a dual role for PLC as an effector and as a GAP.

To determine whether PLC functions as a GAP in *Drosophila* phototransduction, Cook *et al.* took advantage of mutant *norpA* alleles that express reduced concentrations of PLC[3]. In addition to causing a reduction in the amplitude of the light response, these mutations cause a decrease in PLC concentrations that result in slow response termination following cessation of the light stimulus. However, the molecular mechanism underlying this latter defect was unknown. Cook *et al.* have addressed this issue in a series of experiments that began by showing that the level of GTPase activity specifically due to the $G\alpha_q$ subunit, is directly proportional to PLC concentration. This is the first evidence to indicate that PLC, which is the direct effector of the $G\alpha_q$ subunit, may also function as a GAP *in vivo*.

The finding that the protein encoded by *norpA* is both a GAP and an effector molecule raises the question of the functional significance of this combination of roles in one protein. The two activities are not coupled in order to facilitate signal amplification, the phenomenon whereby the