Morphogens: how big is the big picture?

focus on
development

Peter A. Lawrence

Morphogens are in the front line just now. Here I trace how the concept of a morphogen has evolved over the past 100 years and step a little beyond what we already know.

There has been much recent discussion about morphogens[1–8]. A true morphogen is a molecule that spreads out from a localized source to form a concentration landscape, typically a monotonic gradient. The heart of the concept is that the concentration landscape determines, point by point, the responses of all cells in the field. These responses will include the activation of particular genes which then determine the pattern of cell differentiation. Thus the concentration landscape prefigures the pattern formed. If the landscape is changed, for example by experiment, the pattern changes correspondingly. There has been a great deal of debate about the minimal definition of a morphogen, but I think most would agree with this one.

Earlier views of morphogens were a bit different; a morphogen gradient was seen as fixing only a single organizing centre. For example during regeneration of a hydroid, the gradient might position a new head, and this head would then organize the rest of the body plan. This older idea of a morphogen began with Morgan[9] and Boveri[10]. Morgan was studying regeneration in worms after decapitation. He found that, although all parts of the body could regenerate the head, each part did so at a different rate — the nearer to the head, the faster regeneration occurred. Morgan thought that this graded response might stem from the organization of the body itself — maybe there was a gradient of some organizing substance, high at the front and low at the back.

The hypothesis that a morphogen could spread by diffusion probably began with a discussion by Dalcq and Pasteels[11] of results obtained by Yamada[12] on the vertebrate mesoderm. They noted that placing "high" (somatic) and "low" (splanchnic) types of mesoderm together resulted in the induction of an "intermediate"(kidney) mesoderm in between. They proposed that a morphogen could diffuse from where its concentration was normally high into tissue where it was low making a different kind of mesoderm in the middle of the sandwich where an intermediate concentration of morphogen existed; kidney would be formed by these mesodermal cells.

Some of the best and most analytic experiments were done on insects by Sander[13] in the 1950s. At that time, insects were believed to be members of a large group of animals having "mosaic development". However, in his experiments on the insect embryo, Sander built an incontrovertible case that the body pattern is not programmed point-by-point in the unfertilized egg. Instead, the body plan is progressively elaborated as a result of interactions between a few localized determinants. The first step is the establishment of one or more anteroposterior gradients of pervasive morphogens that are then interpreted by individual cells in the axis (reviewed in ref. 13). Interpretation would involve turning on specific genes at particular concentrations, a key concept originally proposed by von Ubisch[14] (for a discussion of this history, see ref. 15).

Sander's hypotheses gained concrete support when a gradient of bicoid protein was found to determine the body plan of the *Drosophila* embryo[16,17]. There was evidence that the concentration at each point in the embryo was somehow interpreted to specify anatomy — and this indeed meant turning on different sets of genes at each level in the anteroposterior axis. The *bicoid* gradient is only the first of many steps that deploy those genes[18], and later steps involve

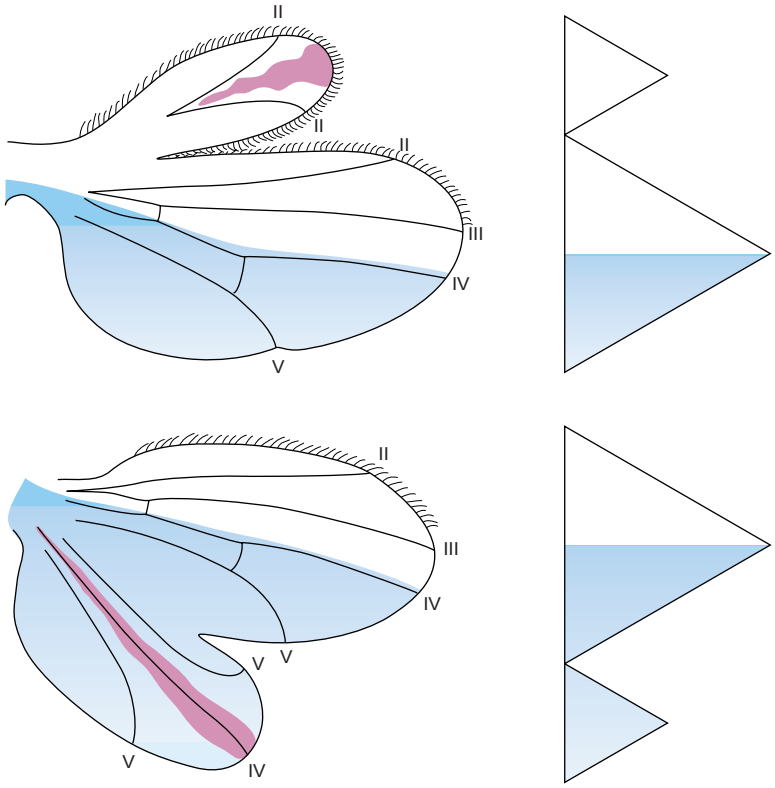

Figure 1**Patterning and morphogen expression.** Clones expressing the Decapentaplegic morphogen (pink) repattern the surrounding wing cells and create new well-proportioned winglets with veins (numbered). Note also the gradient interpretation in which the scale represents the concentration of morphogen. All posterior compartment territory (*engrailed* gene is on) is shown in blue; the *engrailed* gene is off in the anterior territory. The figure was adapted from ref.19.

more morphogens such as hunchback. Hunchback is locally activated above a certain concentration of bicoid, and it also diffuses, setting up a more local gradient than bicoid and leading to finer patterning. This understanding of hunchback leads to ideas of cascading gradients: a primary one with a long range, turning on subsidiary ones that would either divide up the same field into smaller domains or extend beyond the initial reach of the primary gradient[19].

So, morphogens had been identified in a syncytial egg in which proteins could diffuse from nucleus to nucleus in a common cytoplasm and affect different nuclei. What about multicellular systems? There was evidence that the *Drosophila* wing disc depends on the *decapentaplegic* (*dpp*) gene, and it was tested whether Dpp might be a morphogen that operated in the epithelium[20] — an experiment that was conceptually simple but technically difficult. Why not make a small group of marked cells secrete excess morphogen *in vivo*? If the morphogen really does pattern the wing, such a localized source of morphogen would have predictable effects, with localized reiterations of pattern radiating outwards beyond the source itself. The results were spectacular, and indicated that Dpp is the morphogen of a theoretician's dream (Fig. 1), and not only in insects: conceptually comparable experiments in the brain of chick embryo gave similarly eloquent results[21,22].

But how does the morphogen work? Does it spread throughout the tissue, acting directly on cells far away from the source or does it deploy a 'bucket brigade' of intermediate messengers that would relay the signal further and further away? A series of experiments in *Drosophila*, in which receptors were manipulated in the receiving cells, showed that Dpp does act directly at a distance[23,24]. The next question is how does it spread *in vivo*? Is it extracellularly, intracellularly, by simple diffusion, or are specific carriers involved, or could it be transported by long thin cell extensions (cytonemes)[25]? The answers are still not clear, but there are indications that the spread of Dpp somehow depends on the endocytic cycle; however it is not known whether the morphogen itself must cycle in and out of cells to spread from cell to cell. And there is no reason why other morphogens, such as Wingless (Wg), should move in the same way as Dpp[6,26]. More questions include: how exactly is the concentration read, how many levels can be distinguished by an individual cell, and how are thresholds of response determined?

In the old days, we used to imagine that the range of a morphogen would be primarily determined by its chemical nature, but this now seems to be too simplistic. There are other ways of varying range. To take just one clear example, the secreted protein Hedgehog (Hh) normally has a range in the wing of a few cells (and this distance is so short that other factors may be operating — a protein would be expected to diffuse at about 100 μm per minute, that is, 5–10-cell diameters). If the receptor is removed from those cells, Hh passes easily through them, with very little attenuation[27]: in other words, the range of this morphogen ligand *in vivo* depends, in part, on the amount and distribution of its receptor. This has turned out to be just the beginning of the story; the effective range of a morphogen could be varied by several factors, including inhibitors, competitors and its interaction with the extracellular matrix (reviewed in ref. 6).

Morphogens and affinity

Gradients do not just affect pattern, they also determine cell adhesiveness or 'affinity'. If groups of cells are transplanted up and down the proximal–distal axis of the wing, then the further they are transplanted, the more they tend to sort out from their neighbours[28]. The molecular basis of this kind of affinity is little understood, but cadherins are chief among the usual suspects[29].

We have looked at a similar gradient of affinity in the *Drosophila* abdomen. By manipulating the Hh pathway in a clone of cells, it is possible to change the type of cell produced. Each of these clones is tantamount to transplantation, like moving a patch of cells up or down the anteroposterior axis. These clones mix well with their neighbours if they resemble them; however, when the clones are made of cells that normally would belong elsewhere, they sort themselves so that the clones become displaced towards their original 'home'. The bigger the discrepancy between their position and their home, the greater the sorting, indicating that cell affinity is normally graded in the anteroposterior axis. Thus the experiments[30] show that cells sort out autonomously, only according to their identity and, as this depends on the Hh pathway, it follows that their affinity is determined by this pathway itself.

In the wing disc, cell affinity also depends on the Hh pathway, but it has not yet been determined whether there are any gradients of affinity, and whether these might depend on the three main wing morphogens so far discovered, Hh, Dpp and Wg. However, results already indicate that the Dpp morphogen gradient does have part of its readout as cell affinity. One example: clones that cannot respond to Dpp (because they have no receptor) activate downstream genes as if they are located far from the source of Dpp (where the ambient concentration of Dpp should be minimal). Clones of this genotype are more likely to sort themselves away from their surroundings if they are made near the source of Dpp, whereas clones far away grow and survive[31]. One explanation is that Hh might act in the wing through Dpp to change affinity in a graded manner, rather as it does more directly in the abdomen[1]. If gradients of affinities do exist in the wing, then current interpretations of the behaviour of clones in the wing[32] may have to be modified.

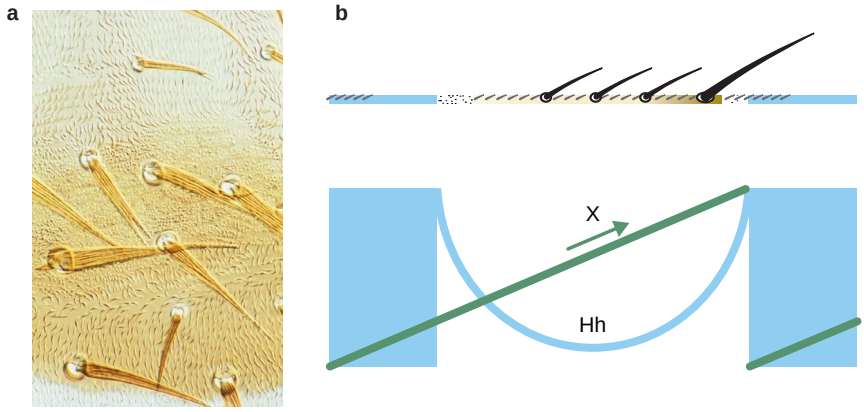

Figure 2 **Different morphogens may determine pattern and polarity in the segment. a**, Portion of a clone of cells (PKA) with activated response pathway to Hedgehog. The clone of cells is marked so that each cell gives numerous scrappy hairs. In front of the clone, the hairs are normally oriented, but behind the clone two or three rows of hairs point anteriorwards (towards the top of the picture). For further information see ref. 42. **b**, The *Drosophila* abdomen. One model representing the relationship between a morphogen (Hedgehog), the gradient of which patterns cell identity, and a different morphogen (X), the vector of which might orient polarity. X would emanate from the cells located at the back of the anterior compartment (white) that received most Hh. X would have a much longer reach than Hh and would spread into the anteriorward posterior compartment to polarize cells there. The arrangement and orientation of hairs is indicated above (see ref. 43 for further details).

Figure 3 **Genes determine shape and proportion with precision.** In black and white, Joy Liebert (born 1914; photographed in 1934) and in colour her grand-daughter Bea Chater (born 1981; photographed in 2001).

Morphogens and polarity

But morphogens might do more than just turn on genes in stripes and make affinity gradients. Planar polarity[33] is a feature of many cells, perhaps of all cells in epithelia[34]. I suspect that cells always know their orientation in the organ, even if they do not always show it to us by the orientation of beating cilia, by the arrangement of chromosomes[35] or by the secretion of anisotropic structures. In the late 1950s, it was shown[36] that the segment of a hemipteran insect was patterned by a 'gradient' that affected cell behaviour, and later it was argued[37,38] that this gradient was actually a diffusing morphogen. Checking and confirming this model, Stumpf did an eloquent experiment that illustrated how one level in a concentration gradient could specify a particular ridge in the cuticle of a moth[18,39].

But these experiments were mainly about cell polarity. Insects are excellent material because the cuticle is frequently decorated with hairs and bristles that amount to little arrows, telling the observer the polarity of each cell (for review, see ref. 40). It was suggested that the vector of a morphogen gradient might inform each cell of the anteroposterior axis, rather like a magnetic field orients iron filings[37,38]: thus, the local slopes in the landscape of concentration of a morphogen would prefigure the pattern of pointing hairs and bristles. We have now revisited this old problem with new methods, looking at the abdomen of *Drosophila*[30,41–43]. Hh, the morphogen that is graded in the anterior compartment of the segment, is read directly by the cells and directs the type of cuticle they secrete. However, it seems that Hh cannot be the morphogen that determines polarity: if the activation of the Hh-signalling pathway is manipulated intracellularly and within a group of cells, the type of cuticle made by those cells alone is changed, as expected, but some influence spreads outside the group to change the polarity of cells nearby (Fig. 2a).

The influence is probably another morphogen that is produced when cells receive Hh. Maybe this morphogen has a longer range than Hh, forming a gradient across the whole segment (Fig. 2b). The nature of this morphogen and whether it exists at all outside our fevered imaginations is still unclear. But if you allow the model, it means that we already have two overlapping systems of morphogens in the anterior compartment of the segment — with the matter of the posterior compartments, and how they are patterned and polarized, still unknown. Perhaps the patterning of the posterior compartments depends on yet another morphogen that is graded there, just like Dpp in the *Drosophila* wing. If so, the next question would be whether this morphogen both patterns and polarizes the posterior compartment, or could there be another morphogen for polarization?

The nature and the behaviour of the polarizing morphogen in the anterior compartment are still unknown. The involvement of the Wnt receptors (the *frizzled* genes) in planar polarity[40] indicates that the polarizing morphogen might be a Wnt.

Morphogens and growth

The determination of shape and size is one of the biggest unsolved problems in developmental biology[1]. Our own experience tells us, time and again, that shape and proportion are precisely controlled by genes — look at people who are genetically related and wonder at the precision with which the human face is sculpted (Fig. 3).

Early regeneration studies of a cockroach limb segment by Bohn[44] revealed that when pieces of the segment were removed and the distal piece grafted back onto the proximal stump, intercalary regeneration occurred to restore the lost piece. He described an illuminating experiment where he confronted distal and proximal parts of one segment to make a compound segment that was already too long, but intercalation occurred even so, giving a reversed polarity to the intervening piece (and again illustrating the link between pattern and polarity). He concluded that intercalation must depend on local interaction between confronting tissues — the further apart they originated, the more intercalation occurred[44]. These experiments begin to answer the question of how cells normally know when to stop growing, and how they know when the organ has reached the right size.

There are several good examples to show that growing organs do not count up the number of cells, but respond to dimension itself[1]. So how is information of dimension conveyed to each cell? Bohn's experiments indicate the involvement of a gradient. I have pointed out that if the boundaries of morphogen gradients are fixed, the steepness of the intervening territory is a reflection of the length of the axis, so that each cell could retrieve some estimate of that length by measuring the steepness of the gradient across its own boundaries[18]. This is not so far-fetched as it might seem, for it is now known that a cell can compare receptor occupancy on different parts of the membrane, and use this to determine its polarity[45,46]. If the reader will grant me this speculation, then in the *Drosophila* abdomen, which morphogen is the one used to provide a measure of dimension? I guess (as the question cannot yet be answered) that it is more likely to be one of the gradients downstream of Hh, such as the morphogen responsible for polarity. Why? Because evidence from studies of growth in the wing of *Drosophila* indicates that the control of growth may occur within compartmental units[47], and therefore, one would expect measurement of dimension in the abdomen to depend on a single monotonic gradient (as we imagine it determines polarity), and not on a U-shaped gradient such as Hh. Note also that local removal of receptors to Hh makes the cells blind to Hh, yet they are polarized and grow normally, again pointing to the

involvement of another morphogen.

Part of the process of growth control is based on competition. In a population of cells that make an organ, the choice of which cells will divide and give surviving progeny is not random. Indeed, there is some comparison between the cells in the growing compartment; the relatively stronger are chosen to divide further and these kill the relatively weaker ones[48]. It is not clear how this poorly understood but crucially important process can be linked to control of size, but it has to be. □

Peter A. Lawrence is at the MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, UK.
e-mail: pal@mrc-lmb.cam.ac.uk

1. Day, S. J. & Lawrence, P. A. *Development* **127,** 2977–2987 (2000).
2. Lawrence, P. A. & Struhl, G. *Cell* **85,** 951–961 (1996).
3. McDowell, N. & Gurdon, J. B. *Semin. Cell Dev. Biol.* **10,** 311–317 (1999).
4. Podos, S. D. & Ferguson, E. L. *Trends Genet.* **15,** 396–402 (1999).
5. Strigini, M. & Cohen, S. M. *Semin. Cell Dev. Biol.* **10,** 335–344 (1999).
6. Teleman, A. A., Strigini, M. & Cohen, S. M. *Cell* **105,** 559–562 (2001).
7. Tickle, C. *Semin. Cell Dev. Biol.* **10,** 345–351 (1999).
8. Vervoort, M. *Bioessays* **22,** 460–468 (2000).
9. Morgan, T. H. *Wilhelm Roux's Archive Dev. Biol.* **5,** 582 (1897).
10. Boveri, T. *Verh. Phys-med. Ges.Würzburg* **34,** 145–175 (1901).
11. Dalcq, A. & Pasteels, J. *Bull. Acad. Roy. Méd.Belg.* **6,** 261–308 (1938).
12. Yamada, T. *Okajimas Folia Anat. Jap.* **19,** 131–197 (1940).
13. Sander, K. *Ciba Foundation Symposium* **29,** 241–263 (1975).
14. von Ubisch, L. *Entwicklungsprobleme* (Gustav Fischer, Jena, 1953).
15. Sander, K. *Landmarks in Developmental Biology* 1883–1924 (Springer, Berlin, 1997).
16. Driever, W. & Nusslein-Volhard, C. *Cell* **54,** 95–104 (1988).
17. Driever, W. & Nüsslein-Volhard, C. *Cell* **54,** 83–94 (1988).
18. Lawrence, P. A. *The Making of a Fly: The Genetics of Animal Design* (Blackwell Scientific Publications, Oxford, 1992).
19. Struhl, G., Johnston, P. & Lawrence, P. A. *Cell* **69,** 237–249 (1992).
20. Zecca, M., Basler, K. & Struhl, G. *Development* **121,** 2265–2278 (1995).
21. Crossley, P. H., Martinez, S. & Martin, G. R. *Nature* **380,** 66–68 (1996).
22. Dupe V. and Lumsden A. *Development* (in press).
23. Lecuit, T. *et al. Nature* **381,** 387–393 (1996).
24. Nellen, D., Burke, R., Basler, K. & Struhl, G. *Cell* **85,** 357–368 (1996).
25. Ramirez-Weber, F. A. & Kornberg, T. B. *Cell* **97,** 599–607 (1999).
26. Strigini, M. & Cohen, S. M. *Curr. Biol.* **10,** 293–300 (2000).
27. Chen, Y. & Struhl, G. *Cell* **87,** 553–563 (1996).
28. Nardi, J. B. & Kafatos, F. C. J. *Embryol. Exp. Morphol.* **36,** 469–487 (1976).
29. Nose, A., Nagafuchi, A. & Takeichi, M. *Cell* **54,** 993–1001 (1988).
30. Lawrence, P. A., Casal, J. & Struhl, G. *Development*, **126,** 2441–2449 (1999).
31. Burke, R. & Basler, K. *Development* **122,** 2261–2269 (1996).
32. Dahmann, C. & Basler, K. *Trends Genet.* **15,** 320–326 (1999).
33. Nübler-Jung, K. *Wilhelm Roux's Archive Dev. Biol.* **183,** 17–40 (1977).
34. Drubin, D. G. *Cell Polarity* (Oxford University Press, Oxford, 2000).
35. Hochstrasser, M., Mathog, D., Gruenbaum, Y., Saumweber, H. & Sedat, J. W. *J. Cell Biol.* **102,** 112–123 (1986).
36. Locke, M. *J. Exp. Zool.* **36,** 459–477 (1959).
37. Stumpf, H. F. *Nature* **212,** 430–431 (1966).
38. Lawrence, P. A. J. Exp. Biol. **44,** 607–620 (1966).
39. Stumpf, H. F. *J. Exp. Biol.* **49,** 49–60 (1968).
40. Shulman, J. M., Perrimon, N. & Axelrod, J. D. *Trends Genetics* **14,** 452–458 (1998).
41. Lawrence, P. A., Casal, J. & Struhl, G. *Development*, **126,** 2431–2439 (1999).
42. Struhl, G., Barbash, D. A. & Lawrence, P. A. *Development* **124,** 2155–2165 (1997).
43. Struhl, G., Barbash, D. A. & Lawrence, P. A. *Development* **124,** 2143–2154 (1997).
44. Bohn, H. J. *Embryol. Exp. Morph.* **32,** 81–98 (1974).
45. Parent, C. A. & Devreotes, P. N. *Science* **284,** 765–770 (1999).
46. Zigmund, S. H. *J. Cell Biol.* **88,** 644–647 (1981).
47. Simpson, P. *Dev. Biol.* **69,** 182–193 (1979).
48. Simpson, P. & Morata, G. *Dev. Biol.* **85,** 299–308 (1981).

ACKNOWLEDGEMENTS
I thank Klaus Sander for years of education.