

The case for morphogens in plants

Rishikesh P. Bhalerao and Malcolm J. Bennett

Plants and animals have evolved as multicellular organisms independently of one another. This raises the intriguing question of whether plants and animals have developed similar or distinct patterning strategies to establish their body plans. Animals use concentration gradients of signals termed morphogens for tissue patterning, but whether they are also used by plants is unclear. Here we compare and contrast the plant hormone auxin with animal morphogens, and speculate as to whether plants have independently evolved similar mechanisms to regulate pattern formation.

Multicellular organisms require mechanisms to pattern their body plans. The idea that concentration gradients of signalling molecules might direct this is long established, and was originally hypothesized by mathematical modellers¹. The term ‘morphogen’ itself was coined by Turing in 1952 (ref. 1) to describe ‘form-generating substances’. In the definition that has since evolved, two criteria must be fulfilled for a signalling molecule to qualify as a morphogen². First, a morphogen must function in a concentration-dependent manner, whereby modifying its concentration gradient alters the developmental fate of target cells in its field. Second, a morphogen must directly function on target cells, rather than through a relay of intermediate signals.

Several classes of animal signalling molecules have been described as morphogens². For example, members of the transforming growth factor β (TGF β) family regulate a range of morphogenic programmes, from patterning wing disc epithelia in *Drosophila melanogaster*³ to mesoderm induction in *Xenopus laevis*⁴. The *Drosophila* TGF β homologue *Decapentaplegic*³ (*Dpp*) represents one of the best-characterized examples to date.

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Dpp is produced in a distinct group of cells in the epithelia wing disc and regulates the developmental fate of neighbouring cells through the formation of a *Dpp* concentration gradient^{3,5}. It patterns the fate of wing imaginal disc cells by activating the expression of genes such as the zinc finger transcription factors *Optomotor blind* (*Omb*) and *Spalt*^{6,7}. The developmental fate of wing disc cells differs along the *Dpp* gradient, as low *Dpp* levels activate *Omb*, whereas high concentrations activate both *Omb* and *Spalt*. Whether *Dpp* functions directly on target cells has been tested by comparing the consequences of ectopically expressing *Dpp* versus constitutively activating the *Dpp* receptor in a group of cells^{6,7}. Ectopic synthesis of *Dpp* resulted in the activation of downstream responses in source cells and surrounding cells, whereas activating the *Dpp* type-I receptor *Thickveins* (*Tkv*) in few cells confined the downstream responses to the *Tkv*-expressing cells only, thereby demonstrating that *Dpp* has a direct effect on the target cells.

Clearly, in animals the morphogen concept has been crucial in explaining the molecular basis of pattern formation in many developmental contexts, but how transferable is it to plants? Are there any signals in plants that function similarly to animal morphogens? Although several secreted peptide signals have now been described in plants^{8,9}, there is currently no evidence to suggest that they influence plant patterning through the formation of morphogen-like concentration gradients. To date, plant signalling molecules termed phytohormones represent the best potential

candidates for the plant equivalent of a morphogen¹⁰. Just as animal morphogens function in a concentration-dependent manner, phytohormones (also termed plant growth regulators or plant hormones) regulate plant cellular responses in a dose-dependent manner^{11–13}. Of these, the phytohormone auxin is the most promising plant equivalent of an animal morphogen^{14,15}. In this perspective, we examine the case for auxin functioning as a morphogen, and compare and contrast its mode of action with animal morphogens to highlight the common and distinct signalling solutions that plants have adopted to regulate pattern formation.

To meet the first criteria of a morphogen², it must be clear that auxin functions in a concentration gradient to direct a developmental gradient. One of the best-characterized experimental systems in which auxin has been suggested to function as a morphogen is the developing xylem in the wood-forming tissues of trees. Here, the cambial meristem gives rise to xylem mother cells, which undergo cell division, cell expansion and secondary wall formation in a sequential manner to generate a developmental gradient in the secondary xylem of trees (Fig. 1a). The concentration of indole-3-acetic acid (IAA) — the major form of auxin found in higher plants — has been measured in single cells from the secondary xylem using mass spectrometry¹³. This study revealed the presence of an IAA concentration gradient that peaked in the meristem where cells are dividing, and declined towards the edges of the developing xylem and near the phloem (Fig. 1a). Along this IAA concentration gradient, there were

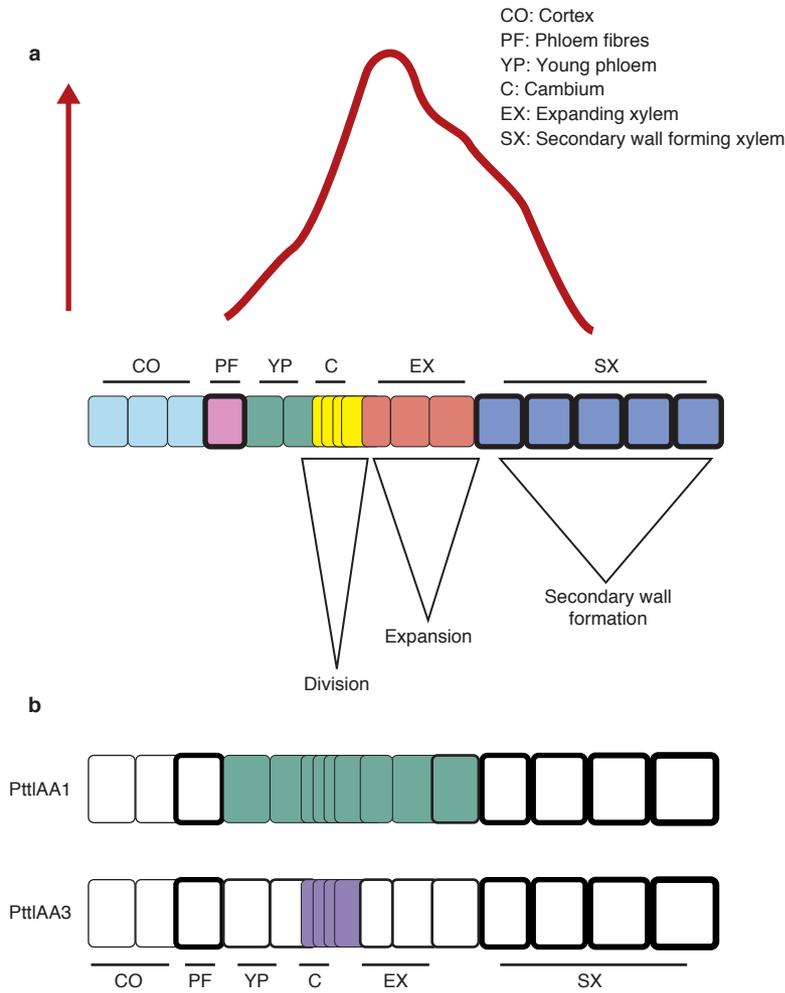


Figure 1 Auxin concentration gradient and *Aux/IAA* gene expression in wood-forming tissues. **(a)** Cell layers and the auxin gradient in wood-forming tissue. The distinct zones of cell division, expansion and secondary wall formation comprising the developing xylem are shown. The concentration gradient of the auxin IAA (shown in red at the top of the figure) overlaps with the developmental gradient of secondary xylem. Thick black boxes indicate secondary cell walls. **(b)** The expression domains of two auxin genes, *PttIAA1* and *PttIAA3*, in the cells of developing xylem and phloem fibre cells are shown. *PttIAA3* expression (purple) coincides with the highest level of auxin, whereas *PttIAA1* (green) appears to be induced at a lower auxin concentration threshold.

distinct zones of developing xylem cells, each at a specific stage of maturation. These observations therefore suggest that auxin may function as a morphogen that organizes the developing xylem cells into distinct zones of division, expansion and secondary wall formation. Moreover, direct IAA measurements¹⁶ and use of auxin-responsive reporter genes¹⁴ have identified a differential auxin distribution with an concentration maximum centred on columella initial (stem) cells in the *Arabidopsis thaliana* primary root apex (Fig. 2b), inferring that an auxin maximum may function as an organizer for cell patterning in plant tissues.

Despite the fact that the above studies demonstrate the existence of a concentration

gradient of auxin, is this important for its influence on cell fate? Several lines of evidence suggest this is the case. First, during vascular tissue differentiation, localized application of auxin can elicit distinct developmental responses at specific distances from the point of application^{11,12}. Second, in the *Arabidopsis* root apex, altering the position of the auxin concentration gradient results in a corresponding shift in distal element positioning¹⁴. Third, mutations in the presumptive *Arabidopsis* auxin transporter PIN4 result in a wider auxin maximum at the root apex, causing nearby cells to acquire new developmental characteristics¹⁵.

Although a gradient of a signalling molecule can often be measured in a biological system

that can be correlated with a specific cell fate pattern, this does not mean that it can be classified as *bona fide* morphogen, as a signalling molecule may function through a relay of intermediate signals within the target cells². An additional criterion central to proving whether a signalling molecule is a morphogen is the demonstration that it functions directly on the target cell, thereby implying that each cell in the field of the morphogen gradient can directly respond to a certain morphogen threshold concentration. As discussed above, the animal morphogen Dpp meets this criterion^{6,7}. Until recently, however, conducting an equivalent experiment for auxin was impossible, as we lacked key knowledge about the factors that synthesize, and function downstream of, this phytohormone. Therefore, there is little evidence to date that would unequivocally dismiss a relay mechanism and conclusively show that auxin functions directly on target cells. However, there are hints that this may be the case: isolated plant cells (termed protoplasts) conditionally expressing the putative auxin receptor ABP1 can be induced to elongate in response to auxin in an ABP1-dependent manner¹⁷, and auxin can induce protoplast cell division¹⁸. As protoplasts are individual cells, one can probably exclude the role of cell-to-cell interaction or signal relay mechanisms.

How do individual plant cells interpret a concentration gradient of auxin to elicit specific developmental responses? One intriguing possibility is that individual plant cells might monitor the slope of an auxin gradient, rather than a finite concentration. A precedent for this comes from motile unicellular eukaryotic cells that can detect chemo-attractant concentration differences as low as 2% between their front and back ends¹⁹. Similarly, *Dictyostelium discoideum* cells can gauge their position in a gradient of the chemo-attractant cAMP by comparing receptor occupancy on different parts of their membrane²⁰. For a spatial sensing mechanism to work, cells must be large enough and have a mechanism to compare receptor occupancy at both ends²¹. This mechanism would therefore, in our view, be a particularly suitable way for immobile plant cells to measure auxin gradients, although this has not yet been shown definitively. Although plant cells such as root trichoblasts, which exhibit auxin-dependent changes in cell polarity, certainly fulfill the former criteria (elongating to ~160 μm before morphological signs of polar root hair initiation²²), studies to monitor auxin receptor occupancy are currently limited by our knowledge about early steps in the auxin transduction pathway.

A central element of classical morphogen theory is that cells exposed to particular

concentration thresholds of a morphogen adopt distinct developmental fates². Although evidence exists that plant cell fate is influenced by auxin gradients^{11–15}, what has been lacking until now is how a concentration threshold of auxin is interpreted by individual plant cells to elicit specific developmental responses. Animal morphogens such as Dpp have been demonstrated to activate downstream genes such as *Omb* and *Spalt* in a concentration-dependent manner^{6,7}. So can auxin control gene expression in a similar fashion? In our view, recent studies of auxin autoregulation on the *Aux/IAA* gene have shown that it can. Significantly, *Aux/IAA* protein abundance is also receptive to changes in cellular auxin concentrations^{23,24}. The addition of auxin negatively regulates the level of *Aux/IAA* proteins and, equally importantly, the degree to which gene expression is repressed by *Aux/IAA* proteins in transient protoplast transfection assays depends on the auxin concentration²⁴.

Moyle *et al.*²⁵ have recently demonstrated that, in the developing xylem of poplar trees, individual members of the *Aux/IAA* family have both overlapping and non-overlapping patterns of expression. Fig. 1b illustrates the expression of two such poplar *Aux/IAA* genes, whose expression correlates with an auxin concentration threshold. On the basis of the above results it is now possible to see how the auxin concentration gradient could pattern the developing xylem. The auxin concentration gradient could set up a corresponding gradient of *Aux/IAA* protein and thereby affect the expression of downstream genes. Alternatively, the expression of different *Aux/IAA* genes could itself be concentration-threshold-dependent, with the expression of *PttIAA1* requiring low auxin concentration but *PttIAA3* requiring a high auxin concentration threshold. In turn, these *Aux/IAA* genes could regulate gene expression to set up auxin concentration-dependent domains of gene expression patterns that, in turn, regulate xylem development.

Although evidence exists in both plants and animals that cell fate is influenced by gradients of auxin and morphogens, respectively, are these gradients formed by similar mechanisms? Classic models presume that morphogens simply diffuse extracellularly within their field of target cells^{2,3}. However, recent evidence suggests that, in animals, target cells may have a much more active role in setting up morphogen gradients²⁶. Wingless (Wg), for example, binds tightly to membrane-associated proteins²⁸ and appears to be transported in specialized membrane vesicles, termed argosomes²⁹, that move between source and target cells in the *Drosophila* wing

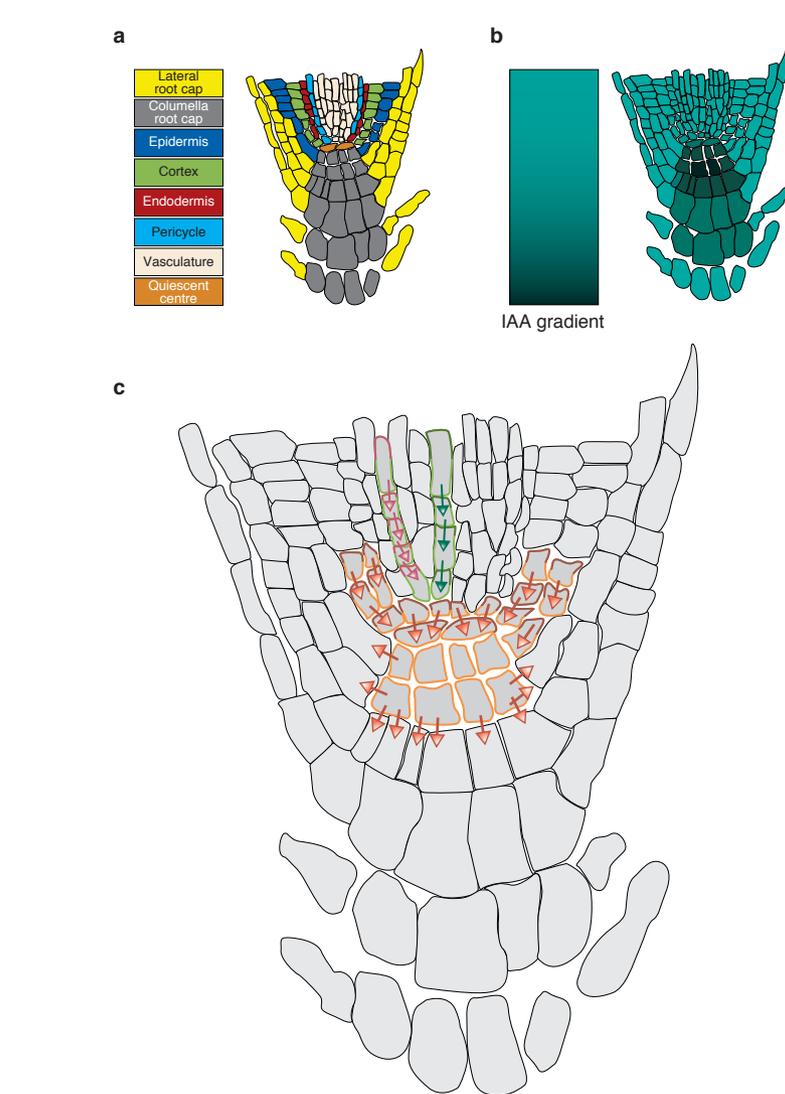


Figure 2 Tissue patterning, auxin gradients and polar auxin transport in the *Arabidopsis* root apex. (a) Organization of *Arabidopsis* root apical tissues. (b) Auxin distribution in root apex, as visualized by the auxin-responsive reporter DR5-GUS¹¹, revealing an auxin maximum centred on columella initial cells. (c) Polar and non-polar localization of the presumptive auxin influx carrier AUX1 (purple bars; ref. 22) and the putative auxin efflux carriers PIN1 (green bars; refs 12, 22) and PIN4 (orange bars; ref. 12). Coloured arrows illustrate the polar movement of auxin through the protophloem (purple arrows), other stele tissues (green arrows) and root cells close to the quiescent centre (orange arrows).

through a repeated sequence of exocytic and endocytic events. Another morphogen Hedgehog is frequently lipid modified²⁷, although whether this is important for setting up its concentration gradient is unclear.

In plants, IAA transport similarly relies on the cellular endocytic machinery. However, auxin (a small molecule) movement in and out of cells also requires specialized carriers³⁰. Several *Arabidopsis* genes encoding presumptive auxin carriers have been identified during the past decade. The amino acid permease-like gene *AUX1* encodes a presumptive auxin

influx carrier³¹, whereas a family of bacterial transporter-like *PIN* genes encodes presumptive auxin efflux carriers^{15,32–37}. The *GNOM* gene encodes an ARF-GEF that transports membrane proteins such as PIN1 between plant endosomal compartments and the plasma membrane³⁸. It is currently unclear whether *GNOM*-dependent trafficking of PIN1 is required solely to target the presumptive auxin efflux carrier to the plasma membrane or whether it could facilitate the vesicular transport of auxin in a manner akin to morphogen secretion^{38,39}.

What determines the concentration of a morphogen gradient? The range and slope of a gradient are determined by the rate of morphogen synthesis in source cells, its rate of transport and its rate of degradation within target cells. Traditionally, morphogen gradients in animal tissues were considered to be source driven². However, recent evidence suggests that gradients of animal morphogens such as Wg form because a proportion of the protein is degraded through the endocytic pathway as it is transported between target cells within its field⁴⁰. Similarly, auxin gradients in plant tissues also seem to be sink-driven¹⁵, but here gradient formation seems to be regulated by components of their transport (rather than degradation) machinery. For example, the auxin maximum in the *Arabidopsis* root apex depends on the transport activity of the presumptive auxin efflux carrier protein PIN4 (ref. 15).

The asymmetric distribution of both animal morphogens and auxin as they move away from their source is important for generating a polarized signalling response. For example, asymmetric Wg distribution confers polarity during later stages of wing epidermal development⁴¹: it is synthesized in a one-cell-wide ectodermal stripe in each wing segment and is asymmetrically distributed across five cells anteriorly, but only one cell to the posterior side. This asymmetric distribution seems to be caused by an enhanced rate of lysosomal degradation in posterior (versus anterior) cells⁴⁰. Auxin also exhibits asymmetric tissue distribution, which is important for its action (Figs 1, 2). However, auxin gradients depend on the asymmetric distribution of auxin carriers (rather than degradation enzymes). Differential intracellular targeting of the auxin efflux carrier PIN4 in selected root apical cells (Fig. 2c) results in the formation of an auxin maximum in these tissues (Fig. 2b)¹⁵. Maximal auxin abundance is visualized (using a reporter gene) in root columella cells that exhibit non-polar PIN4 localization, whereas cells in other tissues on the flanks of the auxin gradient target PIN4 to the plasma membrane domain(s) facing the columella cells (Fig. 2c). Localization of AUX1 and PIN1 proteins to the upper and lower plasma membranes of root protophloem cells⁴², respectively, illustrates that plants can facilitate the vectorial movement of auxin by asymmetrically targeting auxin influx and/or efflux carrier proteins to opposite ends of cells (Fig. 2c). Indeed, the apico-basal patterning defect exhibited by the *gnom* embryo has been attributed to impaired targeting of auxin efflux carrier PIN1 to the basal plasma membrane of developing embryo cells, thereby disrupting the movement of auxin⁴³.

In summary, plant and animals seem to have evolved different mechanisms to pattern tissues through the asymmetric distribution of auxin and morphogens, respectively. These distinct solutions probably reflect, at least in part, the nature of the signals themselves. Irrespective of these differences, it is clear that both kingdoms have evolved complex mechanisms to regulate the graded distribution of these signalling molecules, raising the intriguing possibility that plants and animals may have independently developed comparable mechanisms to pattern their tissues as they evolved into multicellular organisms.

From the evidence to date, auxin seems to have all the necessary attributes that are associated with morphogens. These include: concentration gradients of auxin that seem to regulate pattern formation; the ability of auxin to function directly on single cells; and activation of gene expression by auxin in a dose-dependent manner. But does this mean that auxin can now be classified as a morphogen? The answer to this question depends on the stringency applied to the definition of a morphogen. If the definition of a morphogen is a substance whose gradient determines pattern formation, then auxin could be considered a morphogen. However, when a more stringent condition is applied, namely that of direct auxin action on responding cells, the evidence is currently weak. One major practical problem in proving that auxin is a morphogen is mainly caused by the lack of a single experimental system in which all the criteria required to classify auxin as a morphogen can be met. Although concentration gradients of auxin can be demonstrated in one system, its ability to induce gene expression and function directly on cells has been proven in another. To make a firm case for auxin as a morphogen, one would need to demonstrate all these properties in a single experimental system and specific developmental context. This aim has been aided by the recent identification of several components of the auxin signalling⁴⁴ and biosynthetic⁴⁵ machinery, which will now make it possible for researchers to test whether auxin functions directly on plant cells, as has been described for Dpp and other animal morphogens^{6,7}.

Current evidence suggests that auxin represents the closest equivalent to animal morphogens in higher plants. But what evidence is there for other morphogen-like signals in plants? It is hard to imagine that such signals do not exist in plants, but could we be barking up the wrong tree by rigidly superimposing the signalling concepts established for morphogen gradients in animals? Indeed, plants are likely to have developed distinct patterning solutions

as they independently evolved to become multi-cellular organisms. For example, fossil records suggest that the early land plants evolved a central conducting tissue (termed the stele) that performed support and transport functions, surrounded by a ring of photosynthetic cells just beneath the surface⁴⁶. Therefore, these early land plants must have developed radial patterning mechanisms to organize their green tissues around the central stele. Regulatory proteins such as Shortroot (Shr) represent likely candidates that evolved to radially pattern plant tissues. Although *SHR* is transcribed in *Arabidopsis* root stele tissues, the SHR protein moves into the adjacent cell layer where it is required for formation and specification of endodermal tissue identity⁴⁷. SHR therefore patterns the radial organization of root (and shoot) ground tissues in a binary fashion, rather than requiring the formation of a gradient. This example highlights the pitfalls of importing concepts from the animal field and illustrates that plants represent far more than just green animals when it comes to developing novel signalling solutions to regulate pattern formation. □

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