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Auxin Distribution and Plant Pattern Formation: How Many Angels Can Dance on the Point of PIN?

Minireview

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The plant hormone auxin is central in patterning diverse plant tissues. The direction of auxin flow and the distribution of auxin within tissues are regulated by auxin efflux transporters that are polarly localized in cells. Feedback regulation between auxin and its transporters establishes homeostatic patterns of auxin accumulation but allows dynamic repatterning in response to developmental or environmental cues.

Introduction

The question of how many angels can dance on the point of a pin has been a touchstone in the long-running theological debate on the nature of angels. The plant hormone auxin is a less supernatural messenger, but there is an equally interesting debate about the nature of its extraordinary powers in regulating plant development. The debate about auxin also centers on PINs, in this case a family of membrane-spanning proteins that are required for auxin efflux from cells (reviewed in Paponov et al. [2005]).

PINs are central to the establishment and maintenance of the highly specific patterns of auxin distribution observed in plant tissues (reviewed in Paponov et al. [2005]). Auxins are weak acids. At the relatively low pHs of the extracellular environment (the apoplast), auxin is significantly protonated and can enter cells by diffusion (reviewed in Lomax et al. [1995]; Figure 1). Auxin uptake could be enhanced in some tissues by auxin influx carriers (Swarup et al. [2001] and references therein). Once inside the cell, the higher pH of the cytoplasm results in ionization, trapping the auxin in the cell (Figure 1). Efflux is an active, carrier-dependent process that is often dependent on PIN proteins. PINs have multiple predicted membrane-spanning domains. It is not yet clear whether they directly transport auxin or cooperate with other proteins such as a family of multidrug resistance protein homologs, which have also been implicated in auxin efflux (referenced in Paponov et al. [2005]). PIN proteins cycle between the plasma membrane and intracellular vesicular compartments (Geldner et al., 2001). On the plasma membrane, PIN proteins are frequently localized polarly to a specific cell face (Figure 1). The polar localization of the PINs correlates with the direction of auxin movement, which has long been known to be highly polar in many tissues. For example, auxin moves specifically down the stems of plants in tissues associated with the primary xylem vessels. This movement, termed the polar transport stream, correlates with the basal localization of the PIN protein in these cells (Paponov et al., 2005).

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Polar localization of auxin influx carriers is also observed in some tissues, but the efflux carrier system appears to be sufficient to generate differential auxin distribution, with the influx carriers being critical only when rapid auxin uptake is necessary to maintain gradients that would otherwise be dissipated by diffusion in the apoplast (Swarup et al. [2001], Reinhardt et al. [2003], and references therein).

Auxin Distribution and Tissue Patterning in the Root

PIN-dependent patterns of auxin distribution play multiple roles in plant growth and development. One of the best-characterized examples is the auxin maximum in the root tip, which is required for correct specification of cell fates (Figure 2; Sabatini et al., 1999). During embryogenesis, an apical-basal polarity evident in the first division of the zygote is elaborated to result in the differentiation of a root apical meristem at one end and a shoot apical meristem at the other. After seed germination, these meristems act like animal stem cells, maintaining themselves while giving rise to the entire postembryonic root and shoot systems, respectively. Establishment of the root apical meristem occurs in response to a local accumulation of auxin at the basal end of the embryo (Friml et al., 2003). The auxin maximum persists postembryonically and is required for the maintenance of the meristem (Sabatini et al., 1999). This local auxin maximum is probably interpreted by auxin-induced transcription of the *PLETHORA* (*PLT*) genes, which encode transcription factors of the AP2 class (Aida et al., 2004). Expression of *PLTs* is both necessary and sufficient for root development and maintenance and is dependent on the partially redundant transcription factors NPH4 and MP. NPH4 and MP are members of the Q-rich auxin response factor (qARF) family that activates transcription from auxin-inducible genes (reviewed in Leyser [2002]). When auxin is low, transcription from qARF-regulated genes is repressed by dimerization between the qARFs and members of the Aux/IAA family of transcriptional repressor protein. Auxin targets Aux/IAAs for degradation by the 26S proteasome, thus derepressing transcription (Leyser, 2002). Mutations and pharmacological treatments that perturb the site of auxin accumulation, auxin signaling via the Aux/IAA-qARF pathway, and *PLT* expression all perturb root meristem establishment and maintenance (Sabatini et al. [1999], Aida et al. [2004], and references therein).

Auxin Distribution Is Independent of Auxin Source

The patterning of the root in response to a specific distribution of auxin has some superficial similarities to morphogen systems of animals in which a gradient of a substance, moving away from a local source, patterns morphogenesis. However, there are major differences in comparison to most morphogen systems, because patterns of auxin distribution are established actively in a PIN-dependent manner, and thus they are largely independent of both diffusion and the source of the auxin. For example, in the root tip, initially the main source of auxin is from the shoot, with auxin being transported to the root tip via both the phloem and the

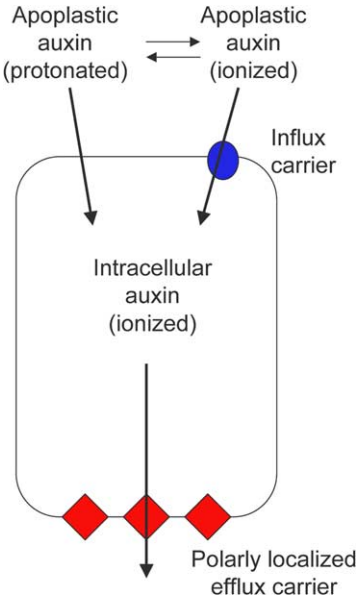

Figure 1. Auxin Movement through Plant Cells

Auxin entry into plant cells is mediated by diffusion or active uptake via an uptake carrier. Auxin export depends on an active efflux carrier.

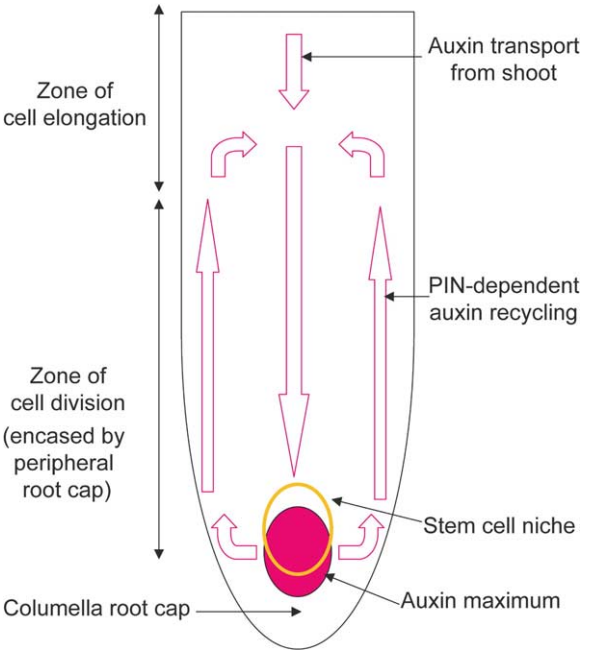

Figure 2. Auxin Accumulates in a Specific Pattern in Root Tips and Is Required for Cell Type Specification around the Stem Cell Niche

Auxin enters the auxin maximum from above and exits it through the root cap. Auxin from the root cap is channeled back up the root and can be recycled into the auxin maximum from above.

polar transport stream (Figure 2; Bhalerao et al., 2002; Swarup et al., 2001). However, once the seedling is established, the root tip itself can synthesize auxin (Bhalerao et al., 2002). The pattern of auxin distribution in the root tip is maintained regardless of the source of auxin. Adding to this, auxin can leave the auxin maximum via the root cap from where it is transported by PINs back up the root away from the tip in the epidermal tissues (Friml et al. [2002a], Ottenschläger et al. [2003], and references therein). This redistributed auxin is required to regulate elongation of cells as they exit the meristem and enter the elongation zone. Recent results suggest that auxin can be recycled from the elongation zone, rejoining the polar transport stream down the center of the root and reentering the site of auxin accumulation at the tip (Figure 2; Blilou et al., 2005). Thus, rather than a defined source and a defined sink with auxin distributed between them, patterns of auxin accumulation are actively maintained by the PIN protein network, regardless of the origin of the auxin. Consistent with this, auxin added exogenously can be rapidly redistributed to maintain the pattern of auxin accumulation (Friml et al., 2002b). Such treatments affect growth but have relatively limited effects on patterning. Treatments that inhibit auxin efflux, or treatments with auxin analogs that are not exported efficiently, result in much more severe effects on patterning (Sabatini et al., 1999; Friml et al., 2002b).

Auxin Patterns Can Be Reconfigured

The active nature of PIN-mediated auxin distribution has the added effect of allowing major reconfiguration of auxin distribution patterns in response to developmental or environmental cues. For example, as described above, during embryogenesis, a basal accumulation of auxin is required to establish the root meristem. However, this is not the first pattern of auxin distribution observed during embryogenesis. In *Arabidopsis*, following division of the zygote into a small apical cell and a larger basal cell, auxin accumulates in the apical cell. This correlates with the localization of PIN7 to the apical membrane of the basal cell (Friml et al., 2003). The apical pattern of PIN7 localization and auxin distribution persists until the 32 cell stage and is required to specify shoot meristem fates. At the 32 cell stage, PIN7 polarity flips completely to contribute to the establishment of the basal site of auxin accumulation that specifies root fate (Friml et al., 2003).

It is likely that PIN-mediated alterations in auxin distribution can also be triggered by the environment, with changes in both light intensity and the gravity vector correlating with changes in PIN localization (Friml et al., 2002a; Ottenschläger et al., 2003). It is predicted that consequent changes in auxin distribution in elongating tissues mediate the differential cell elongation required for tropic growth.

The impressive dynamism in auxin distribution patterns coupled with the diversity in tissue responses to them argues against auxin possessing another key feature of a morphogen, namely a tight causal link between different concentrations of the morphogen and the adoption of specific cell fates. The ability of auxin to specify apical fates and then, immediately afterward, to specify basal fates does not support a simple concentration-based auxin read out. Instead, it suggests a model in which auxin accumulation at a specific site triggers activation of a developmental pathway pos-

sible in those cells because of additional fate-specification factors. In the context of the root meristem, these additional factors are likely to include the SCR and SHR transcription factors that show a specific, auxin-independent pattern of expression in the root tip that is required for root meristem maintenance (Sabatini et al. [1999], Aida et al. [2004], and references therein).

Another example illustrating these points is found at the shoot apical meristem (SAM). The SAM is a dome of cells, which maintains itself while producing cells that give rise to the growing shoot beneath it. Leaves are initiated in an ordered pattern on the flanks of the SAM, in many species in a spiral with successive leaves initiating on average 137° apart. This pattern is generated by successive, PIN-dependent, local sites of auxin accumulation that trigger leaf differentiation (Reinhardt et al. [2003] and references therein; Benkova et al. [2003]). In *Arabidopsis*, polar localization of PIN1 in the SAM epidermis directs auxin toward a point on the flank of the dome, concentrating it there while depleting it from surrounding tissues. Leaf differentiation coincides with establishment of polarly localized PIN1, positioned in a way predicted to pump auxin from the center of the epidermal auxin maximum into the interior of the SAM. This new transport conduit probably starts the process of patterning of leaf vascularization. Meanwhile, the next leaf can initiate at a site sufficiently far from the previously initiated leaves that auxin can begin to accumulate there.

The patterning of leaf formation at the SAM again illustrates the powerful effects of dynamic PIN-dependent auxin gradients on plant morphogenesis. The evidence that auxin selects the site for leaf differentiation is compelling. However, here again, the responding tissues are prepatterned to the extent that the ability to make a leaf is restricted to a ring of cells on the flanks of the SAM, with cells above and below being unable to response to auxin in this way (references in Reinhardt et al. [2003]). In this case, it is likely that this prepattern is at least partly established by a homeodomain transcription factor, WUSCHEL (WUS), the expression of which is restricted to the meristem core (reviewed in Carles and Fletcher [2003]). Patterning of WUS is auxin independent.

Auxin Regulates Its Own Distribution

These data combine to suggest a model in which dynamic patterns of auxin distribution trigger the activation of incipient developmental pathways at specific locations in the plant. PINs are therefore the conductors of an assembled orchestra of developmental events. When the auxin baton points your way, it's your turn to play whatever musical instrument you happen to be holding. A pressing question, then, is what controls the polarization of PINs and hence the direction of auxin flow? Here is where the debate becomes dangerously similar to the one about the nature of angels and their ability to be in multiple places at once, because one of the main contenders for regulating auxin flow is auxin itself.

The idea of positive feedback in establishing conduits for auxin flow during, for example, vascular differentiation has been around for quite some time. However, more recent data have provided a long list of far more complex sets of interactions between auxin and its movement. PIN transcription, accumulation, and subcellular localization all seem to be regulated by auxin. This can have either a stabilizing effect on patterns of auxin distribution or a destabilizing effect. In the root tip, for example, there is a strong stabilizing effect. The transcription patterns of the PINs that are required to maintain the auxin distribution pattern are themselves auxin regulated (Blilou et al., 2005). As a result of this, null mutations in single PIN family members have little or no phenotypic effect because the changes in auxin distribution that result from PIN loss appear to change the transcription of other PINs to compensate (Blilou et al., 2005).

The likely existence of auxin-regulated destabilizing effects on auxin distribution comes from several lines of evidence. First, there is evidence that auxin can trigger PIN protein degradation (Sieberer et al., 2000). Even more dramatically, analysis of the PINOID (PID) gene of *Arabidopsis*, which encodes a serine/threonine protein kinase, suggests that auxin can trigger changes in the direction of its flow (Friml et al., 2004). Mutations in PID result in a phenotype similar to that of *pin1* mutants, including similar defects in organ formation at the primary shoot apex (Benjamins et al. [2001] and references therein). However, while the *pin1* phenotype results from lack of efflux at the shoot apex, the *pid* phenotype correlates with mislocalization of PIN1. Furthermore, overexpression of *PID* can flip the polarity of PIN localization in various tissues (Friml et al., 2004). Since *PID* transcription is upregulated by auxin, this suggests a mechanism whereby a local accumulation of auxin could reach a threshold that activates PID transcription, changes PIN polarity, and dissipates the auxin maximum (Benjamins et al., 2001). This mechanism could operate to pattern successive leaf positions at the SAM flanks.

In both these cases, auxin regulates the amount and direction of its efflux from cells by regulating the transcription of genes involved in auxin efflux. The known transcriptional responses to auxin are mediated by Aux/IAA degradation, as mentioned above. The receptors for this pathway are intracellular, providing further feedback, since efflux of auxin from a cell will reduce auxin-regulated transcriptional effects on PINs and PID. In addition, there is good evidence for at least one extracellular site for auxin perception. This may involve the auxin binding protein ABP1 and is likely to regulate membrane responses to auxin such as proton pumping (Bauly et al. [2000] and references therein). The effect of this would be to acidify the apoplast, leading the increased protonation of apoplastic auxin and increased auxin influx. These multiple points of interaction between auxin and the machinery that distributes it allow for robustly maintained but rapidly adjustable patterns of auxin accumulation.

Conclusions

The mechanism of auxin patterning seems in tune with the continuous and environmentally responsive nature of plant development. It also presents a major challenge for plant developmental biologists. Qualitative models derived from feedback systems such as these can easily account for all kinds of patterns of auxin accumulation. However, formalization of the models and quantification of their parameters are necessary to de-

termine if such models can actually deliver the outcomes observed. This will take some time to achieve, especially since mechanistic details of central processes, such as that of the polar localization of the auxin efflux complex, are lacking and many key parameters are not currently measurable. Perhaps the most pressing need is for improved, sensitive, direct methods for measurement for auxin levels, both inside and outside cells.

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