PERSPECTIVES: BOTANY

Auxin Transport: Down and Out and Up Again

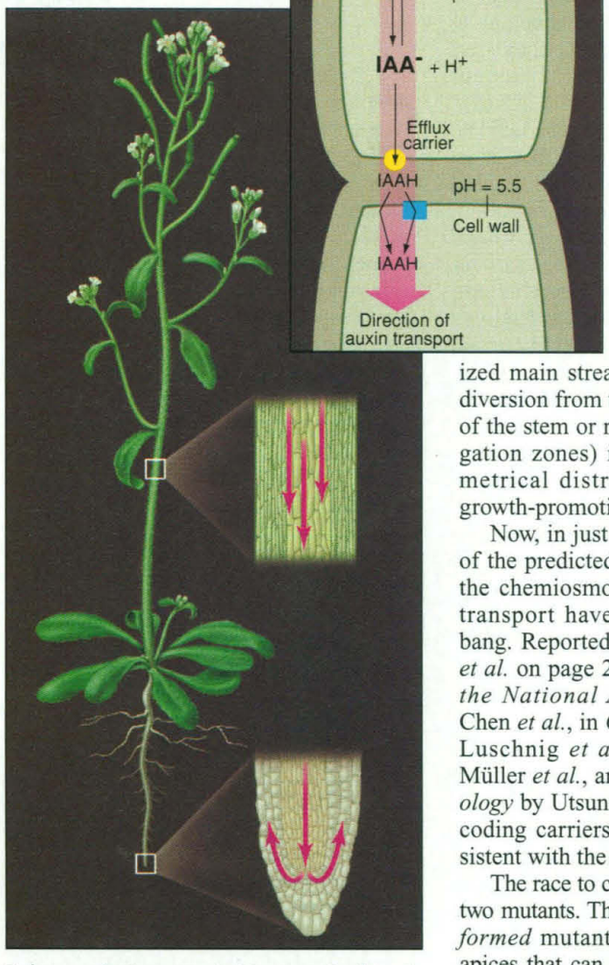
Alan M. Jones

Almost with clairvoyance, Charles and Francis Darwin in *Power of Movement in Plants* described in the last century a growth-promoting "influence" which, by its asymmetric distribution in plant organs, causes asymmetrical growth (tropism) after stimulation by gravity or light (*1*). Fifty years later, this "influence" was identified as indole-acetic acid (IAA), an endogenous member of the class of growth hormones called auxins (*2*). Auxins are actually morphogens, not hormones, because they are distributed in spatial gradients within plant organs. This gradient conveys to the developing cells their location within the morphogenic field—and their fate. The different concentrations of auxin along this gradient, in combination with other signals, trigger different responses—elongation at one position and differentiation at another, for example (*3*).

Morphogen gradients in other systems are established by diffusion, but in plants spatial gradients of auxin in tissues are actively maintained by a polar auxin transport system operating in cells. The molecular means by which cells establish and maintain this gradient has captured the attention of many plant physiologists since the Darwins. Thus, by the mid-1970s a coherent model of polar auxin transport emerged, and it has stood the test of time with only slight revision (*4*). Now a flurry of reports in this issue and elsewhere have put a molecular face on the components predicted in the model (*5–9*)

In the model (*10*), known as the chemiosmotic model of polar auxin transport, auxins in their acid (protonated) forms enter cells electrogenically through a carrier. Once in the cell, where the pH is higher than the extracellular space, the anionic form is favored and accumulates to about a 20-fold greater concentration than extracellular auxin. Auxin exits the cell at its basal membrane through an anion transporter, moving down its chemical concentration gradient. This process of uptake and basal efflux iterated along the flanks of cells in roots and shoots is the basis of the polar movement of auxin (see the figure). Energy is expended only to maintain the chemical (ΔpH) and electrogenic ($\Delta\Psi$) gradients of the plasma membrane.

Diagnostic tools can distinguish the two carriers to some extent. First, auxin transport inhibitors block auxin transport at the efflux carrier. Second, the uptake and efflux carriers have different auxin transport profiles. The synthetic auxin 2,4-dichlorophenoxy-acetic acid (2,4-D) is transported by the uptake carrier but not the efflux carrier, whereas the opposite is true for the auxin naphthalene-1-acetic acid (1-NAA). Thus, mutants in the uptake and efflux carrier should be rescued with 1-NAA and 2,4-D, respectively.



Polar auxin transport. Movement of auxin basipetally (from the apex toward the base) through the center of shoots and roots occurs by an iterated movement through cells—inward at the apical membrane and outward at the basal membrane. After auxin reaches the root tip, it moves acropetally (from the base toward the apex) in the cortical tissues.

The pathways of auxin transport through tissues are complex and poorly understood, but it is clear that there is a major stream of auxin in the central vascular tissues running from the shoot apex to its base where it joins the root at the root-shoot junction (*11*). At this junction, the auxin pathway becomes uncertain, but below the root-shoot junction, into the root itself, auxin travels predominantly down through the central stele, then upon reaching the root tip is distributed back upward along the root in the epidermis and subtending cortical cells, tracing an "inverted umbrella" pathway (see the figure). There are plenty of places along the way where auxin can be diverted from this polarized main stream and, in fact, the lateral diversion from the central stele to one side of the stem or root (especially in the elongation zones) is the basis for the asymmetrical distribution of the Darwins' growth-promoting influence.

Now, in just a few weeks, the identities of the predicted molecular components of the chemiosmotic model for polar auxin transport have been announced with a bang. Reported in this issue by Gälweiler *et al.* on page 2226, in the *Proceedings of the National Academy of Sciences* by Chen *et al.*, in *Genes and Development* by Luschnig *et al.*, in *EMBO Journal* by Müller *et al.*, and in *Plant and Cell Physiology* by Utsuno *et al.* (*5–9*) are genes encoding carriers that have properties consistent with the chemiosmotic model (*10*).

The race to clone these genes began with two mutants. The first, the *Arabidopsis pin-formed* mutant (*pin1-1*), has pin-shaped apices that can be phenocopied with auxin transport inhibitors. *pin1* mutants have greatly reduced auxin transport in the inflorescence shoot. The second, *agravitropic* (*agr1*, allelic to *wav6-52* and *eir1*), lacks the root gravitropism response (*12*).

PIN1 was cloned by transposon tagging and found to encode a membrane

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protein similar to that of a number of bacterial membrane transporters (*5*). The PIN1 predicted structure is a hydrophilic central domain flanked by five to six predicted membrane-spanning domains (*5*). Consistent with the model, PIN1 is most abundant on the basal membrane of cells in the central vascular region of the shoot. Although direct biochemical evidence that PIN1 transports auxins outwardly is still lacking, the indirect evidence presented is compelling.

From the complexity of the auxin pathway, multiple auxin efflux carriers are to be expected that are specific to tissues, individual cells, and even plasma membrane locations. On the basis of the large number of similar *PIN* genes already in the sequence database, these expectations will certainly be met soon, and the new results point to the first realization. *AGR1* (*6*)—now independently cloned by four labs—can be added to the list of putative auxin efflux carriers, and the evidence indicates that its function is specific to roots. *AGR1,* also called *EIR1* (*7*) and *PIN2* (*8*), encodes a membrane protein that is 64% identical to PIN1, but unlike PIN1, it is found on the apical, rather than basal, membrane of cells. But this distribution actually fits the model: "Out" in cortical root cells is "up" because in this part of the root, the auxin pathway is moving upward toward the shoot (see the figure). Further support for AGR1's identity as an efflux carrier is that yeast cells expressing AGR1 transport out auxin (*6*) and the toxin 5-fluoro-indole (*6*, *7*) more rapidly than do control yeast cells. In addition, *agr1* roots retain preloaded auxin longer than wild-type roots (*6*), and Utsuno *et al.* (*9*) report that *agr1* roots are sensitive to 2,4-D but not IAA or NAA (a characteristic of the efflux carrier). Müller *et al.* (*8*), however, did not observe convincing differences in the auxin sensitivity profile among these inhibitors, so all the expectations for an efflux carrier are not yet unequivocally met.

The work by these labs marks a breakthrough in the field and has transformed the Darwins' recalcitrant topic of gravitropisms and phototropisms to one where answers to the next set of questions are within reach: In which cell type is each carrier located, and how do they operate to establish the longitudinal and radial auxin gradients? Which are the lateral carriers predicted to set up asymmetrical auxin gradients, and how do extracellular signals act upon them? What are the endogenous regulation mechanisms? New mutant genes, soon to be cloned (*13*), will certainly shed light on these questions with gravity.

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PERSPECTIVES: PROTEIN STRUCTURE

Pumping Iron Through Cell Membranes

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Gated protein channels allow ions and small molecules to flow across a cell membrane in a regulated way. Despite the importance of these proteins, the three-dimensional structure at atomic resolution has been determined for only a few gated channel proteins, such as channel-forming bacterial protein toxins and a partial bacterial potassium channel. On page 2215 of this issue, Ferguson *et al.* describe the complete structure of a gated channel from the outer membrane of *Escherichia coli* (*1*).

The protein studied by Ferguson *et al.*, designated FhuA, has a number of extraordinary properties. It serves as a transporter for the uptake of an iron complex (ferrichrome) that belongs to a class of microbial compounds (siderophores) that solubilize Fe^{3+}, a structurally related antibiotic (albomycin), a protein toxin (colicin M), and a peptide toxin (microcin 25), and it functions as a receptor for a number of viruses (phages T1, T5, ϕ80, and UC-1) that multiply in *E. coli* (see the figure). Moreover, the activity of FhuA is dependent on energy input that is provided by the proton motive force of the cytoplasmic membrane because there is no energy source in the outer membrane.

Energy is transmitted from the cytoplasmic membrane to the outer membrane by a complex that consists of the three proteins TonB, ExbB, and ExbD, of which TonB and ExbD are located in the periplasm between the cytoplasmic membrane and the outer membrane with their NH_2-termini inserted in the cytoplasmic membrane (see the figure) (*2–4*). It has been genetically and biochemically shown that TonB interacts with FhuA, suggesting that TonB assumes an energized conformation in response to the proton motive force and allosterically opens the FhuA channel. Energy coupling is required for all FhuA ligands except phage T5. Only T5 infects TonB mutants, binds to isolated FhuA, releases its DNA, and opens a channel in FhuA (*5*) similar in size to a FhuA deletion mutant in which residues 322 to 355 were genetically excised (*6*).

The crystal structure consists of 22 antiparallel transmembrane β strands extending from residues 161 to 723 that form a β barrel. The β-barrel strands are interconnected by large loops at the cell surface and small turns in the periplasm. Such a β-barrel structure is also the principal arrangement of the outer membrane porins. However, in contrast to the porins, no loop folds inside the barrel to restrict the permeability of the channel. Rather, the FhuA barrel is entirely closed by the NH_2-terminal cork domain that enters the β barrel from the periplasmic side. More than half of the molecule is located above the bilayer membrane. It contains a cavity that is open to the external medium and separated from a periplasmic cavity by a thin layer of amino acid residues. It is tempting to propose that the channel is opened in this region by a conformational transition that is caused by the interaction of FhuA with TonB in the energized conformation. The region of FhuA (TonB box, residues 7 to 11) that according to a genetic suppression analysis in-

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