The Plant Cell, Vol. 10, 1775, November 1998, www.plantcell.org

INSIGHT

Polar Auxin Transport: New Support for an Old Model

Mark Estelle[1]

Department of Biology, Indiana University, Bloomington, Indiana 47405

In 1880, Charles Darwin noted that "some influence," later shown to be indole-3-acetic acid (IAA), moves from the tip of an oat coleoptile to the region below the tip, where it controls elongation (Darwin, 1880). Darwin's statement was probably the first description of polar auxin transport, a phenomenon that has received considerable attention in the ensuing decades for two main reasons. First, polar auxin transport is ubiquitous among higher plant species, and second, the effects of inhibiting polar auxin transport with chemical inhibitors such as naphthylphthalamic acid (NPA) are profound (Lomax et al., 1995). For example, NPA acts to disrupt tropic responses, reduce apical dominance, inhibit floral bud formation, and inhibit lateral root formation. These effects strongly suggest that auxin transport has a central role in auxin-regulated growth processes.

We now know that auxin moves basipetally at a velocity of between 5 and 20 mm per hr in the shoots and coleoptiles of a wide range of plant species (Lomax et al., 1995). We also know that acropetal transport is minimal. What is less clear is how this directionality to auxin transport is achieved.

One model, termed the chemiosmotic hypothesis, has informed our thinking on auxin transport for the past 25 years. As illustrated in Figure 1, this model, which was independently proposed by Rubery and Sheldrake (1974) and Raven (1975), posits that polar auxin transport occurs through the action of cellular auxin influx and efflux carriers located in the plasma membrane of transporting cells. To explain the strong polarity of transport, the model proposes that the efflux carrier is asymmetrically localized to the basal side of cells. In support of this aspect of the model, Jacobs and Gilbert (1983) used an immunological approach to demonstrate basal localization of a putative efflux carrier.

In 1996, Bennett et al. reported the molecular characterization of a candidate auxin influx carrier, a protein called AUX1. The *auxin resistant1* (*aux1*) mutants of Arabidopsis are resistant to IAA and have agravitropic roots, suggesting a role in some aspect of auxin physiology (Estelle, 1996). AUX1 is a presumptive membrane protein similar to amino acid permeases, suggesting that it may function to transport auxin across a membrane (Bennett et al., 1996). Because plant amino acid permeases function as proton-driven symporters, and auxin influx is also thought to occur by a proton cotransport mechanism, Bennett et al. (1996) suggested that AUX1 may be an auxin influx protein.

This model has gained support recently with the discovery that sensitivity of *aux1* seedlings to various auxins correlates with their suitability as substrates for the influx carrier (Delbarre et al., 1996; Yamamoto and Yamamoto, 1998). Thus, *aux1* seedlings are resistant to auxins that are good influx substrates, such as IAA and 2,4-D, but sensitive to NAA, a poor substrate. In fact, NAA restores gravitropism to mutant seedlings. Because NAA influx is not carrier mediated, the loss of the carrier is of no consequence (Delbarre et al., 1996).

Now, in a remarkable convergence, four groups have isolated a gene that encodes a presumptive auxin efflux carrier (Luschnig et al., 1998; Muller et al., 1998; Utsuno et al., 1998; Chen et al., 1998). The gene has several names because it was identified independently through investigations of root gravitropic mutants (*agravitropic* [*agr*] and *wavy6* [*wav6*]; Bell and Maher, 1990; Okada and Shimura, 1990), a root-specific ethylene-insensitive mutant (*ethylene insensitive root1* [*eir1*]; Roman et al., 1995), and as a member of the *PIN* family of genes (Muller et al., 1998).

Luschnig et al. (1998) first reported the cloning of the *EIR1* gene in a recent issue of *Genes and Development.* The protein has an estimated molecular weight of 69 kD and ten predicted transmembrane (TM) domains. These TM domains lie in two clusters of five each, one cluster near the N terminus of the protein and the other near the C terminus. They are separated by a region that is enriched in hydrophilic amino acids.

What is so exciting about these TM clusters? As Luschnig et al. and the other three groups show, these regions of the EIR protein exhibit sequence similarity (35% to 40% similarity in some portions) to bacterial membrane proteins that function to transport various small molecules across the plasma membrane, suggesting that EIR1 possesses a transport function. Based on their studies of the *eir1* mutant phenotype, Luschnig et al. (1998) propose that the small molecule likely to be transported by EIR1 is auxin. This is because, in addition to exhibiting ethylene resistance and agravitropic roots, *eir1* plants show reduced sensitivity to

[1]E-mail mestelle@bio.indiana.edu; fax 812-855-6705.

INSIGHT

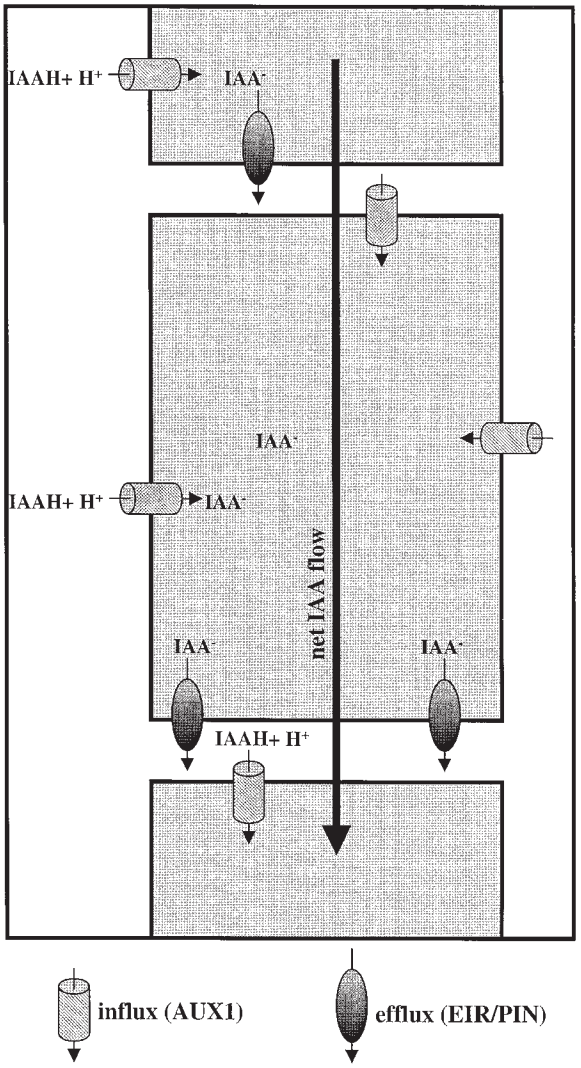

Figure 1. Model for Polar Auxin Transport Through a File of Transporting Cells.

Movement of IAA into cells is mediated by AUX1 and related proteins via a symport mechanism whereby protonated IAA is cotransported with a single proton ($IAAH + H^+$). This activity is dependent upon a proton gradient across the plasma membrane, which is maintained by a proton pumping ATPase (not shown). IAA efflux is mediated by members of the EIR/PIN family. Net directional movement of IAA is due to localization of EIR/PIN on the basal plasma membrane.

2,3,5-triiodobenzoic acid (TIBA), another inhibitor of auxin transport.

Further evidence for a role of EIR1 in auxin transport comes from Luschnig et al.'s experiments with the *altered lateral root1* (*alf1*) mutant, in which elevated endogenous auxin levels lead to decreased root elongation and increased lateral root formation (Celenza et al., 1995). The double mutant *eir1 alf1* does not display the root-elongation defect, suggesting that *eir1* acts to suppress the effects of high endogenous auxin levels conferred by *alf1*. Importantly, the *eir1* mutation does not confer resistance to auxin that is simply added to the medium, a characteristic that experiments with the *aux1* mutant suggest is more likely to be associated with defects in an auxin influx carrier.

Perhaps the most compelling evidence offered by Luschnig et al. (1998) that the *eir1* mutation causes defects in an auxin efflux carrier comes from their experiments with *EIR1*-expressing yeast cells, which are resistant to toxic fluoroindoles. Although other explanations are possible, resistance to these compounds is probably due to their EIR1-mediated transport out of the yeast cells. Chen et al. (1998) report similar results using their *AGR1* clone. They also perform two additional experiments that support the efflux model. First they show that root tips of *agr1* seedlings preloaded with radiolabeled IAA retain the label longer than do wild-type root tips. Second, *AGR1*-expressing yeast cells release preloaded IAA more rapidly than do control cells, suggesting that AGR1 transports IAA out of the yeast cell.

The work of Utsuno et al. (1998) also includes physiological studies that suggest that *AGR1* functions in auxin efflux. These authors observed that when *agr1* mutants are grown on vertically oriented agar medium containing either IAA or NAA, the roots grow into the medium, whereas wild-type seedlings grow along the surface. Both IAA and NAA are good substrates for the efflux carrier (Delbarre et al., 1996). In contrast, 2,4-D, which is not effectively transported by the efflux carrier, does not elicit this response in either mutant or wild-type plants. One explanation for these results is that because *agr1* seedlings are deficient in an efflux carrier, cells adjacent to the medium accumulate IAA and 2,4-D and consequently do not elongate as much as cells on the other side of the root. In a manner similar to *aux1* and NAA, loss of the efflux

carrier has little effect on 2,4-D accumulation.

That EIR1 is an efflux carrier is strongly supported by a paper that will appear shortly in *EMBO Journal.* Here, Muller et al. describe the cloning of a gene called *PIN2*, so named because the encoded protein is 64% identical to PIN1. The *pin1* mutant is characterized by inflorescences that terminate in pin-like structures with little or no initiation of floral buds (Okada et al., 1991). This defect is associated with reduced polar auxin transport in the inflorescence, suggesting that the PIN1 protein is required for polar auxin transport (Okada et al., 1991).

To study the function of *PIN2*, Muller et al. (1998) isolated a *pin2* insertion mutant by reverse genetics using lines that had been mutagenized with the maize transposon *En-1.* The mutant has defects in root gravitropism similar to those exhibited by the *eir1* mutant. Sure enough, a complementation test between *pin2* and *wav6-52* (an allele of *eir1* and *agr1*) showed that the two mutants are allelic, and sequence comparisons confirm that *PIN2* is the same gene as *EIR1.*

Physiological studies indicate that there are actually two polar auxin transport streams in the root—auxin moves in a polar fashion from the shoot down to the root tip through cells in, or adjacent to, the stele (Lomax et al., 1995). However, auxin is also transported away from the root tip toward the top of the root through epidermal and cortical cell files. It is this second transport stream that is thought to be crucial for mediating gravitropic responses (Evans, 1991).

Is the cellular location of PIN2 consistent with participation in either of these auxin transport streams? Yes, it is. When Muller et al. (1998) performed whole-mount immunolocalization studies, they found that the EIR1/PIN2–specific signal was predominantly associated with the basal end (i.e., distal to the root tip) of epidermal and cortical cells in the elongation zone of the root. This striking result is precisely what the chemiosmotic hypothesis would predict. Moreover, this location for EIR1/PIN2 is also consistent with the gravitropic defects observed in the *eir1* and *pin2* mutants.

Now that good candidates for auxin influx and efflux carriers have been identified, the way is clear for detailed genetic and biochemical investigations of polar auxin transport. Three issues stand out. First, it is incumbent upon these workers to directly establish the biochemical function of these proteins using robust assays for IAA transport. The available evidence strongly suggests roles in auxin influx and efflux but more direct evidence is still required. Second, how is auxin transport regulated? Physiological studies indicate that spatial and temporal regulation of auxin transport is a key aspect of many growth processes (see, e.g., Kaufman et al., 1995).

The third issue relates to the specific functions of the more than ten different members of the *EIR*/*PIN* gene family that have been identified to date in Arabidopsis (K. Palme, personal communication). The genetic studies reported in the papers discussed in this article have already shown that *EIR1*/*PIN2* and *PIN1* have distinct functions in the root and shoot, respectively. Thus, it seems likely that some or all of the other members of the *EIR*/*PIN* family will also be shown to have unique functions. I expect that the continued genetic, molecular, and biochemical studies of these genes, which is now in progress in a number of laboratories around the world, will provide rich new insights into the role of auxin in plant growth and development.

REFERENCES

Bell, C.J., and Maher, P.E. (1990). Mutants of *Arabidopsis thaliana* with abnormal gravitropic responses. Mol. Gen. Genet. **220,** 289–293.

Bennett, M.J., Marchant, A., Green, H.G., May, S.T., Ward, S.P., Millner, P.A., Walker, A.R., Schulz, B., and Feldmann, K.A. (1996). *Arabidopsis AUX1* gene: A permease-like regulator of root gravitropism. Science **273,** 948–950.

Celenza, J.L., Grisafi, P.L., and Fink, G.R. (1995). A pathway for lateral root formation in *Arabidopsis thaliana.* Genes Dev. **9,** 2131–2142.

Chen, R., Hilson, P., Sedbrook, J., Rosen, E., Caspar, T., and Masson, P.H. (1998). The *Arabidopsis thaliana AGRAVITROPIC1* gene encodes a component of the polar-auxin-transport efflux carrier. Proc. Natl. Acad. Sci. USA, in press.

Darwin, C. (1880) The Power of Movement in Plants (assisted by F. Darwin). (London: John Murray).

Delbarre, A., Muller, P., Imhoff, V., and Guern, J. (1996). Comparison of mechanisms controlling uptake and accumulation of 2,4-dichlorophenoxyacetic acid, naphthalene-1-acetic acid, and indole-3-acetic acid in suspension-cultured tobacco cells. Planta **198**, 532–541.

Estelle, M. (1996). Plant tropisms: The ins and outs of auxin. Curr. Biol. **6,** 1589–1591.

Evans, M.L. (1991). Gravitropism: Interaction of sensitivity, modulation and effector redistribution. Plant Physiol. **95,** 1–5.

Jacobs, M., and Gilbert, S.F. (1983). Basal localization of a presumptive auxin transport carrier in pea stem cells. Science **220,** 1297–1300.

Kaufman, P.B., Wu, L.-L., Brock, T.G., and Kim, D. (1995). Hormones and the orientation of growth. In Plant Hormones: Physiology, Biochemistry, and Molecular Biology. P.J. Davies, ed (Dordrecht, The Netherlands: Kluwer Academic Publishers), pp. 547–571.

Luschnig, C., Gaxiola, R., Grisafi, P., and Fink, G.R. (1998). EIR1, a root-specific protein involved in auxin transport, is required for gravitropism in *Arabidopsis thaliana.* Genes Dev. **12,** 2175–2187.

Lomax, T.L., Muday, G.K., and Rubery, P.H. (1995). Auxin transport. In Plant Hormones: Physiology, Biochemistry, and Molecular Biology. P.J. Davies, ed (Dordrecht, The Netherlands: Kluwer Academic Publishers), pp. 509–530.

INSIGHT

Muller, A., Guan, C., Galweiler, L., Tanzler, P., Huijser, P., Marchant, A., Parry, G., Bennett, M., Wisman, E., and Palme, K. (1998). *AtPIN2* defines a locus of *Arabidopsis* for root gravitropism control. EMBO J., in press.

Okada, K., and Shimura, Y. (1990). Reversible root tip rotation in *Arabidopsis* seedlings induced by obstacle-touching stimulus. Science **250,** 274–276.

Okada, K., Ueda, J., Komaki, M.K., Bell, C.J., and Shimura, Y. (1991). Requirement of the auxin polar transport system in early stages of *Arabidopsis* floral bud formation. Plant Cell **3,** 677–684.

Roman, G., Lubarsky, G.B., Kieber, J.J., Rotheneberg, M., and Ecker J.R. (1995). Genetic analysis of ethylene signal transduction in *Arabidopsis thaliana*: Five novel mutant loci integrated into a stress-response pathway. Genetics **139,** 1393–1409.

Raven, J.A. (1975). Transport of indole-3-acetic acid in plant cells in relation to pH and electrical potential gradients, and its significance for polar IAA transport. New Phytol. **74,** 163–172.

Rubery, P.H., and Sheldrake, A.R. (1974). Carrier-mediated auxin transport. Planta **188,** 101–121.

Utsuno, K., Shikanai, T., Yamda, Y., and Hashimoto, T. (1998). *AGR*, an *Agravitropic* locus of *Arabidopsis thaliana*, encodes a novel membrane protein family member. Plant Cell Physiol. **39,** in press.

Yamamoto, M. and Yamamoto, K.T. (1998). Differential effects of 1-naphthaleneacetic acid, indole-3-acetic acid and 2,4-dichlorophenoxyacetic acid on the gravitropic response of roots in an auxin-resistant mutant of Arabidopsis, *aux1*. Plant Cell Physiol. **39,** 660–664.

Editor's note: This is the first in a new series of peer-reviewed front-section articles that has been designed to offer our readers insight into findings from any area of the biological sciences that are likely to have a particularly pronounced impact on our understanding of plant growth and development.