and functional organization throughout the central nervous system.

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Evolution and Development: PINpointing the Origins of Auxin Transport Mechanisms

Specialists and non-specialists alike know that auxin regulates plant development, but the role of auxin transport mechanisms in the context of land plant evolution has been controversial. Two recent studies resolve the controversy by demonstrating that PIN-mediated auxin transport regulates morphogenesis in a moss.

Jane A. Langdale

Auxin (derived from the Greek word ‘auxein’ to grow) was the first plant growth regulator to be discovered. In now classic experiments, Fritz Went placed agar blocks under oat stems and then after a period of time transferred the blocks to the top of newly decapitated stems [1]. The decapitated stems resumed growth because auxin had moved downwards from the first stem into the agar and had then moved from the agar into the second stem. It was nearly 50 years before an active mechanism of polar auxin transport was first suggested [2] and another 24 years before the molecular basis of the transport process was revealed [3]. Key to the polar transport mechanism is the asymmetric location of PIN-FORMED (PIN) efflux carriers on the plasma membrane. PIN transmembrane proteins are mostly located on the basal side of cells and thus contribute to the general trend of moving auxin from the shoot down to the root. Phylogenetic analyses revealed the presence of a family of PIN genes in the flowering plant *Arabidopsis* and showed that representatives of the family can be found in all available land plant genomes [4,5]. Despite this observation, the origin of auxin transport mechanisms and the contribution of those mechanisms to the evolution of land plant form remained obscure, not least because of reports based on pharmacological studies which suggested that polar auxin transport does not occur in the leafy shoots of mosses [6]. Two papers in this issue of *Current Biology* resolve any uncertainty about the origins of PIN function by showing perturbed shoot development in *pin* mutants of the moss *Physcomitrella patens*. Polar auxin transport therefore regulates shoot development in one of the earliest divergent land plant lineages.

Land plants evolved from aquatic green algae ~470 million years ago, with phylogenetic analyses positioning charophytes as the land plant sister group [7]. Charophyte algae exhibit a range of vegetative body plans, ranging from single cells to highly branched multicellular structures, but these are all found in the haploid (gametophyte) generation of the lifecycle [8]. The diploid (sporophyte) generation of the lifecycle is invariant and unicellular. The alternation of haploid gametophyte and diploid sporophyte generations, both of which are multicellular, is a shared feature of all land plant lifecycles. However, the relative dominance of each generation changed as land plants evolved (Figure 1). In the earliest divergent bryophyte grade (liverworts, mosses and hornworts) the dominant generation is the gametophyte. In mosses, leafy shoots are characteristic of the gametophyte generation whereas the sporophyte develops just a single unbranched axis that subtends the spore-containing

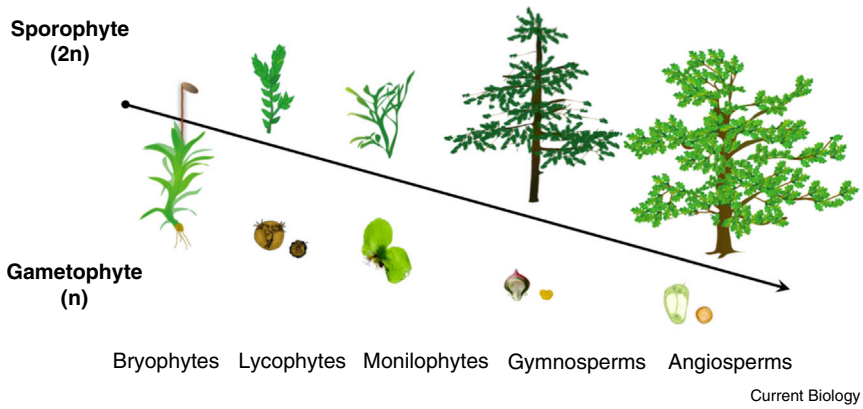

Figure 1. Alternation of generations in land plants.
Leafy shoots develop in the gametophyte generation of mosses but in the sporophyte generation of more recently diverged vascular plant lineages.

sporangium. By contrast, in vascular plants (lycophytes, monilophytes, gymnosperms and angiosperms) the gametophytes comprise just a few cells whereas the sporophyte develops complex shoots. Since the divergence of mosses and vascular plants over 450 million years ago, developmental mechanisms that elaborate shoot architecture have therefore evolved independently in the gametophyte of mosses and the sporophyte of vascular plants.

In angiosperms (flowering plants), polar auxin transport is a major player in the regulation of many developmental processes, including the elaboration of shoot architecture. For example, auxin is produced in developing leaves and PIN-mediated polar transport leads to the dynamic accumulation of auxin maxima in the shoot apex, the positions of which determine the placement of subsequent leaf primordia [9]. As such, polar auxin transport regulates the characteristic phyllotactic patterns that are observed in which leaves are arranged on the shoot in spiral, opposite or paired configurations [10]. In mosses, polar auxin transport has been reported to function in the sporophyte and in the filamentous stage of the gametophyte generation. However, using pharmacological inhibitors and auxin reporter constructs, no evidence for polar auxin transport could be found in the leafy shoots of the gametophyte [6], despite a known role for auxin in leafy shoot development [11]. On the basis of these observations, the assumption was made that the polar auxin transport pathway and its role in shoot patterning arose *de novo* to regulate shoot development in the sporophyte generation of the land plant lifecycle, and that independent mechanisms evolved to pattern the leafy shoots of moss gametophytes [6].

In this issue, Bennett *et al.* [12] and Viaene *et al.* [13] demonstrate that PIN-mediated auxin transport is essential for the development of filaments, leafy shoots and sporophytes of the moss *P. patens*. There are four *PIN* genes in *P. patens*, two of the three canonical 'long' PINs (*PpPINA-C*) are shown to be plasma membrane targeted (*PpPINA* and *PpPINB*) and the fourth (*PpPIND*) is a short version that is probably targeted to the endoplasmic reticulum [12,13]. The efflux function of the canonical *Pp*PINA and *Pp*PINB proteins was demonstrated using an *in vivo* assay that measures the amount of radiolabelled auxin secreted from filaments into the growth media (intriguingly up to 90% of auxin is secreted from wild-type moss filaments into the immediate environment) [14]. In *P. patens* lines overexpressing long *Pp*PIN proteins, an increased amount of auxin was exported into the media as compared to wild-type, whereas reduced amounts were exported from filaments of loss of function mutants. The export function was associated with polar localization of PINA and PINB proteins to the tips of growing filaments, suggesting a central role for polar auxin transport in tip growth [13]. This observation is somewhat surprising given that auxin plays no role in tip growth of root hairs or pollen tubes in flowering plants.

Loss of function double *pinApinB* mutants of *P. patens* exhibit perturbed development of both the filamentous and leafy shoot stages of gametophyte development. Leafy shoot formation occurs prematurely in the absence of PIN function, suggesting that polar auxin transport may establish a gradient of auxin along the growing filament [13]. In this scenario, perturbation of the gradient would lead to altered levels of auxin in the tip cell and would trigger leafy shoot formation in the cells below it. The effects of altered auxin levels on leafy shoot development can also be seen following the application of exogenous auxin to growing shoots. The range of phenotypic perturbations (from the production of more leaves than normal to the termination of shoot growth) indicates the significance of distinct threshold levels of auxin in different developmental contexts [12] — a phenomenon also seen with auxin responses in flowering plants [15]. Importantly, the range of phenotypic defects identified after auxin application can be replicated by inhibition of polar auxin transport (either though application of chemical inhibitors or in *pinApinB* double mutants). As such, polar auxin transport plays a crucial role in patterning leafy shoots of the *P. patens* gametophyte. This discovery suggests either that the PIN-mediated polar auxin transport pathway operating in the moss gametophyte was co-opted into the sporophyte generation after the divergence of mosses from other land plants, or that the pathway was recruited on at least two independent occasions — once in the gametophyte of mosses and once in the sporophyte of vascular plants.

The exact role of polar auxin transport in *P. patens* can be inferred from *pin* mutant phenotypes. In this regard, two features are noteworthy. The first is the discovery that as in flowering plants [16–18], both gravitropic and phototropic responses are disrupted in leafy shoots of *P. patens pinApinB* mutants [12]. Therefore, the physiological role of polar auxin transport is conserved in gametophyte and sporophyte shoots despite very different morphological contexts. The second is that *P. patens pinB* mutant sporophytes occasionally bifurcate to form branched structures [12]. Assuming that the unbranched sporophyte axes of extant bryophytes

are representative of the earliest land plants, the transition from an unbranched to a branched form was one of the most significant steps in land plant evolution, paving the way for indeterminate shoot growth in the sporophyte. The *P. patens pinB* mutant phenotype suggests that this transition may have been facilitated by altering polar auxin transport processes in the sporophyte generation. An alternative view, based on the discovery of branched fossils that predate vascular plants, suggests that the earliest land plants were branched and that extant bryophytes lost branching function [19]. Either way, the modification of PIN-mediated polar auxin transport can now be proposed as a major driver of morphological novelty during land plant evolution.

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Animal Evolution: Stiff or Squishy Notochord Origins?

The notochord is considered an evolutionary novelty and one of the defining characters of chordates. A new study of an annelid challenges this view and proposes an earlier evolutionary origin in the most recent common ancestor of chordates and annelids.

Andreas Hejnol[1,2] and Christopher J. Lowe[1,2]

Chordates (sea squirts, lancelets and vertebrates) are one of the animal groups with the most complex body plans. One of the defining characters of this group is a rod-like elastic structure on the dorsal side of their body that is commonly known as the 'notochord' or *chorda dorsalis* [1]. This structure stiffens the body and functions also as an attachment site for lateral muscle packages, called 'somites', which helps with undulating swimming movements. The notochord develops in the embryo from a dorsal mesodermal population of cells that form a rod. In some lineages (ascidian larvae, hagfish, coelacanth), these cells become vacuolarized, while in others (*Branchiostoma*) they become muscular. The notochord has also an important developmental signaling function, for instance as a source of BMP antagonists during the formation of the overlying neural plate and as the initial source of the signaling molecule Sonic Hedgehog (Shh) to ventralize the forming nerve cord [2]. In most craniates (bony fish, birds, mammals), however, the notochord is a transient structure that disappears after it has accomplished its signaling function and is replaced by the backbone composed of vertebrae made out of cartilage or bones.

What is the evolutionary origin of this defining chordate character? Are there any comparable structures in more closely related deuterostome lineages that might hint to its origin or can it be that it is an evolutionary novelty (Figure 1)? The closest group to chordates are the Ambulacraria, comprising hemichordates (acorn worms) and echinoderms (sea urchins, sea stars and sea cucumbers) and perhaps *Xenoturbella* [3]). As there are no strong contenders for notochord-like structures in these animals, the notochord is generally considered an evolutionary innovation of chordates [4,5]. However, a recent paper by Lauri and coauthors [6] challenges this widely held view.

The authors [6] searched for cells that resemble the notochord in the polychaete worm *Platynereis dumerilii*, a member of the distantly related