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# Did auxin play a crucial role in the evolution of novel body plans during the Late Silurian–Early Devonian radiation of land plants?

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## Introduction

The overall objective of this chapter is to identify plausible developmental mechanisms that might have contributed to the rapid diversification of early land plant lineages during the Late Silurian to Early Devonian Periods (Kenrick and Crane, 1997). An even more rapid diversification of bilateral animal lineages seems to have occurred during the well-known

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Cambrian radiation (Gould, 1989; Raff, 1996; Conway Morris, 2000). Given that the lineages that ultimately gave rise to animals and to plants are most likely to have diverged as ancient lineages of unicellular flagellates (Baldauf *et al.*, 2000), the origins of early land plants occurred via different macroevolutionary processes than those operating in ancient animals (Meyerowitz, 2002). Nonetheless, as an archetypal example of organismal radiation, our current understanding of the Cambrian radiation can be used as a conceptual framework for considering the analogous radiation of early land plants.

## Brief overview of Cambrian radiation of bilateral animals

### Rapid diversification of animal phyla

Although well-preserved fossils of the soft-bodied Ediacaran fauna are widely distributed in Late Precambrian rocks, it has been very problematical to trace the evolution of the simple features of Ediacaran fossils into the more complex body plans of Cambrian metazoa (Knoll and Carroll, 1999; Conway Morris, 2000). Therefore, palaeontologists have focused on the Cambrian radiation that resulted in the rapid (or 'explosive' with respect to geological time) appearance of the crown phyla of bilateral animals, including molluscs, arthropods, echinoderms and chordates, around 550–530 million years ago (Mya) (Raff, 1996; Erwin *et al.*, 1997; Valentine *et al.*, 1999; Conway Morris, 2000). In marked contrast to the fossil record, molecular clock estimates predict that initial divergences of these lineages occurred much earlier in the Precambrian era (Bronham *et al.*, 1998; Heckman *et al.*, 2001). Even if a long Precambrian fuse was needed to ignite the Cambrian explosion (Fortey, 2001), it is still undeniable that earliest bilateral animals diversified into the crown metazoan lineages during a rather narrow slice of deep geological time, with the result that all extant animal phyla appeared before the end of the Cambrian. Some authors attribute the Cambrian radiation to the occurrence of large-scale environmental perturbations resulting in new adaptive landscapes at the Precambrian-Cambrian boundary (Knoll and Carroll, 1999), while others emphasize the possible role of new ecological selection pressures associated with the rise of filter feeding and macroscopic predation (Conway Morris, 2000). Of particular relevance to this chapter are the putative developmental mechanisms discussed below that may be responsible for generating new phylum-specific body plans in the presence of those selective pressures.

### Characteristic body plan of each phylum

Traditionally, animal biologists have recognized different phyla on the basis of their fundamental *Baupläne* (or body plans in English), which encapsulate the nature and organization of tissues and organs within the animal body. For example, the arthropods comprise a diverse assemblage of bilateral organisms, including horseshoe crabs, spiders, millipedes, crustaceans and insects. Yet the arthropod phylum as a whole exhibits a common body plan with such unifying features as a segmented exoskeleton, jointed legs, ventral nerve cord and dorsal heart with an open circulatory system. Interestingly, the characteristic body plans of all extant animal phyla were already expressed in their marine ancestors by the end of the Cambrian radiation (Raff, 1996). While certain phyla underwent major structural innovations accompanying their post-Cambrian invasion of the land environment, these terrestrial adaptations occurred within the conserved patterns of their pre-existing body plans.

## Early establishment of body plan

For two related reasons, until the recent innovations in molecular analyses, phylogenetic schemes for animal phyla were typically based on the comparative morphology of embryos and larvae. One, the basic body plan of each phylum is first expressed at these early stages of animal development (Gilbert, 2000). Ever since von Baer (1828), it has been recognized that the general features of each phylum appear in embryonic and/or early postembryonic development as opposed to the more specialized features of individual classes that develop at later stages. Indeed, it is the common embryonic or larval features that disclose the close evolutionary relationships within those phyla like molluscs or arthropods with divergent adult forms. Furthermore, the common body plan of vertebrate embryos makes them also appear remarkably similar even though the specialized features of the adults are distinctly different. Two, the structures developing in the early, so-called phylotypic stage are more evolutionarily conserved than those formed at later stages (Raff, 1996). For example, in insects, the germ band or early larval stages have a segmented worm-like appearance that resembles the body plans of other taxa, such as the onychophorans, related to the arthropods (Ballard *et al.*, 1992). As another intriguing example, living echinoderms develop free-swimming bilateral larvae that undergo a complicated metamorphosis to become adults exhibiting 5-parted radial symmetry. Peterson and Davidson (2000) have hypothesized that ancestral stem-groups of bilateral animals developed simple larva similar to those characteristic of extant echinoderms. These larvae are then envisioned to have served as the structural platform for the activity of 'set-aside cells' required to generate the complex body plans of different crown bilateral animals.

## Altered expression of embryonic genes resulting in new body plans

The basic approach in evolutionary developmental biology ('evo-devo') is to study the genetic regulation of embryonic and larval development of extant organisms belonging to various lineages in order to elucidate the developmental mechanisms responsible for the origin and/or diversification of those lineages (Raff, 1996; Gilbert, 2000; Arthur, 2002). This research is based on the reasonable, albeit untestable, assumption that genetic regulation of embryonic development is extraordinarily stable over vast geological time. The justification for this assumption is that any disruption in embryonic regulation should result in an avalanche of disruptive, and inevitably fatal, consequences for postembryonic development (Raff, 1996). Similar arguments have been also put forth to explain the failure of any new metazoan body plans to evolve following the Cambrian radiation (Gilbert, 2000).

The paradigmatic case of genetic regulation of metazoan body plan involves a conserved group of homeobox (*Hox*) genes (Erwin *et al.*, 1997; Gellon and McGinnis, 1998: Valentine *et al.*, 1999; Carroll *et al.*, 2001). *Hox* genes play crucial regulatory roles in various aspects of metazoan axis specification, such as external segment identity in arthropods and internal body segmentation in vertebrates. Molecular analyses of *Hox* gene diversity have shown that these genes were gradually duplicated during the ancient evolution of non-bilateral animals, with the result that the stem group of bilateral animals appear to have evolved a fully-fledged cluster consisting of a minimum of seven *Hox* genes (de Rosa *et al.*, 1999; Peterson and Davidson, 2000). Some workers have proposed that the *Hox* genes were responsible for encoding the original anterior-posterior axis in the common ancestor of all animals (e.g. Slack *et al.*, 1993), while others argue that the *Hox* cluster was recruited for pattern formation of the pre-existing anterior-posterior axis during the evolution of the stem group of bilateral animals (e.g. Peterson and Davidson, 2000). In either

event, it appears that the evolution of different body plans in the lineages of bilateral animals did not depend on the evolution of new *Hox* clusters or other developmental master genes. Instead, the diversification of bilateral animals required various innovations in regulatory networks controlling the expression of these genes (Gellon and McGinnis, 1998; Knoll and Carroll, 1999; Carroll *et al.*, 2001). The molecular palette of developmental genes was already present in primordial bilateral animals before these genes were rewired to sculpt novel bilateral body plans.

In summary, it seems reasonable to conclude from this brief description that the Cambrian radiation of bilateral animals can be viewed as having arisen from the rapid emergence of new expression patterns of pre-existing developmental regulatory genes. These altered expression patterns resulted in a wide range of new body plans for bilateral animals. These new animals were presumably subjected in turn to ecological selection pressures that favoured those animals with body plans best adapted for surviving in Cambrian oceans.

## Silurian–Devonian radiation of land plants

Using the conceptual framework derived from our consideration of the Cambrian radiation of bilateral animals, we intend here to discuss plausible developmental mechanisms underlying the evolution of novel plant body plans and hence the macroevolution of different plant lineages in the Late Silurian to Middle Devonian periods. The hormone auxin appears to regulate the organizational features comprising body plans of contemporary plants; therefore, particular attention is granted to the hypothesis that evolutionary changes in auxin action were causally involved in the generation of different body plans during the radiation of early vascular plants.

### Did early land plants diverge in a rapid evolutionary radiation?

Recent work has provided cogent molecular, biochemical and cellular evidence that ancient charophycean green algae (also called charophytes), whose living descendents include the orders Zygnematales, Coleochaetales and Charales, represent the primordial group giving rise to land plants (Graham, 1993; Graham *et al.*, 2000; Karol *et al.*, 2001). Living charophytes, sometimes derided as 'pond scum', have haplobiontic life cycles, with a dominant haploid gametophyte and a diploid phase solely consisting of the zygote that undergoes meiosis to produce four haploid cells. These algae exhibit a diverse range of growth forms, including unicells, tip-growing filaments, margin-expanding discs and complex shoot-like axes consisting of alternating large multinucleate internodal cells and multicellular nodes bearing lateral branches. This diversity of growth forms in the charophytes is thought to reflect the more permissive nature of aquatic habitats (Niklas, 2000).

According to molecular clock estimates, ancient charophycean green algae are predicted to have invaded the land during the Precambrian around 600 Mya (Heckman *et al.*, 2001). Dating from the Middle Ordovician around 470 Mya, the oldest microfossils, which are considered to have originated from genuine land plants, are obligate spore tetrads with sporopollenin-impregnated walls, imperforate cuticles and narrow tubes (Gray, 1985; Edwards and Wellman, 2001; Graham and Gray, 2001). Indeed, the ability of these first land plants successfully to colonize terrestrial environments is often attributed to the desiccation-resistant coverings observed on these microfossils. This microfossil evidence suggests that the earliest land plants are likely to have exhibited a bryophyte-grade of structural organization,

at least with respect to spore morphology (Gray, 1985; Edwards and Wellman, 2001; Graham and Gray, 2001). Available molecular sequence information is also consistent with the perspective that the three extant bryophyte lineages diverged earlier than the monophyletic lineage giving rise to the vascular plants (Qiu *et al*., 1998; Nickrent *et al*., 2000; Karol *et al*., 2001).

It is sometimes postulated that the first land plants had quickly evolved the embryo, via the intercalation of mitotic divisions of the zygote prior to the occurrence of sporic meiosis (Graham and Wilcox, 2000). However, the lack of any confirming fossil evidence makes it conceivable that land plants did not develop embryos until long after the successful colonization of terrestrial habitats (Niklas, 1997). The earliest mesofossils with possible bryophyte affinities have been identified as miniature branching axes in Lower Devonian rocks (Edwards *et al*., 1995; Edwards, 2000; Edwards and Axe, 2000). However, very few macrofossils, including *Sporogonites, Tortilicaulis* and *Pallaviciniities* from Devonian strata appear to exhibit the characteristics of dorsiventral thalli and/or monosporangiate sporophytes that disclose their likely affinities to extant bryophytes (Taylor and Taylor, 1993). Thoughtful discussions about the inadequacy of the fossil record for elucidating the major events in bryophyte evolution can be found in many sources (e.g. Kenrick and Crane, 1997; Niklas, 1997; Edwards, 2000; Kenrick, 2000).

It is irrefutable that a considerable time interval exists between the first putative land plant spores in mid-Ordovician strata and the first protracheophyte *Cooksonia* in Upper Silurian rocks. In marked contrast to the bryophyte enigma, the fossil record for vascular plants provides compelling evidence that the appearance of *Cooksonia* was followed by the rapid radiation of numerous vascular plant lineages including those lineages that evolved into extant lycophytes, ferns and horsetails (Taylor and Taylor, 1993; Kenrick and Crane, 1997; Niklas, 1997). This radiation of vascular plants is dated to have occurred in the Late Silurian (424–409 Mya) according to cladistic analysis (Kenrick and Crane, 1997: Figure 7.15) or in the Early to Middle Devonian (409–381 Mya) according to macrofossil stratigraphy (Taylor and Taylor, 1993; Kenrick and Crane, 1997). The rapid diversification of early vascular plant lineages culminated with the origin of the progymnosperms (i.e. the progenitor lineage for seed plants) in the Middle Devonian, as indicated by the fossil *Svalbardia* (Taylor and Taylor, 1993; Berry and Fairon-Demaret, 2001).

The fossil record indicates that the Late Silurian to Middle Devonian was indeed the most innovative interval in the morphological diversification of land plants. During this interval, land plants appear to have evolved many features often assumed to serve as crucial terrestrial adaptations, including multicellular primary and secondary meristems, vascular tissues, vegetative organs (roots, stems, and leaves), and sporangium-bearing organs (sporophylls and sporangiophores) (Graham, 1993; Taylor and Taylor, 1993; Kenrick and Crane, 1997). Of particular interest to this chapter, it is often presumed that the first sporophyte consisted of a spherical embryo that was directly modified to form a simple sporangium embedded in the archegonium (Niklas, 1977; Graham and Wilcox, 2000). Its evolutionary elaboration into the complex vascular plant sporophyte with elevated sporangium-bearing axes must have been a critical adaptation for flourishing in terrestrial environments, because this plant body is well-constructed to produce numerous meiospores for effective aerial dispersal.

In parallel to the Cambrian radiation of bilateral animals, the rapid diversification of vascular plants starting in the Late Silurian hints at the possibility that the ancestral stem group for all vascular plants had just previously experienced a limited number of genetic innovations, which permitted and/or facilitated the rapid origin of new body plans. Contrary

to the selection pressures operating on the first bilateral animals in Cambrian oceans, it is reasonable to propose that during the Silurian–Devonian radiation of land plants, natural selection acted to favour those body plans that enabled these plants to survive in terrestrial environments. The following sections will address the developmental mechanisms responsible for generating the body plans of land plants.

## Are the characteristic body plans of land plants established during embryonic development?

Although not many plant biologists make explicit reference to the concept of body plan, its occasional appearance in the botanical literature has led to some confusion because it has been applied to different levels of plant organization. For instance, those plant biologists focusing on algal life forms have referred to different types of cellular organization, such as unicellular, siphonous, colonial and multicellular, as representing the basic body plans of photosynthetic eukaryotes (e.g. Niklas, 2000). The present chapter adopts an alternative perspective from the discipline of plant morphology, where the body plan concept, when used, is generally restricted to vascular plant sporophytes (e.g. Troll, 1943; Groff and Kaplan, 1988). The multicellular sporophyte of each land plant division can be said to exhibit a characteristic body plan that is based on such features as meristem organization, vascular tissue arrangement, positional relationships among vegetative organs and positional relationships of reproductive structures on vegetative organs (Bold *et al.*, 1987; Gifford and Foster, 1989). In this section, the sporophytes from representative divisions of extant land plants are illustrated in order to evaluate whether or not the embryo or the young postembryonic plant expresses the fundamental body plan of each division.

As an example of the bryophyte grade of plant organization, the mature sporophyte of liverwort *Marchantia polymorpha* L. consists of three parts: absorptive foot, elongated seta and spore-producing capsule (Figure 5.1A–D) (Smith, 1995; Bold *et al.*, 1987; Crum, 2001). This sporophyte is initiated as a zygote that develops into a small spherical embryo inside the archegonium (Figure 5.1B). Then the spherical embryo differentiates into three regions in which different cell shapes reveal the ultimate fate of each region (Figure 5.1C). The small basal cells destined to form the foot are generally oriented perpendicular to the future growth axis. The presumptive seta is composed of enlarged isodiametrical cells, whereas the immature cells of the capsule are greatly elongated and oriented parallel to the future axis. Although each region is capable of a limited number of additional cell divisions, the embryo does not develop any localized region of cell division recognizable as a genuine meristem. Instead, the embryo expands and differentiates into the mature sporophyte illustrated in Figure 5.1D, which displays the typical foot and capsule found in almost all but a few semi-aquatic liverworts. The *Marchantia* seta is similar to other liverwort setae in that it elongates via diffuse growth over its entire length, but it is considerably shorter. This reduced length is attributed to *Marchantia* sporophyte developing suspended from an elevated archegoniophore, which does not arise on most other liverwort thalli. Because liverwort sporophytes never develop an apical meristem capable of producing additional organs, they do not exhibit the modular organization of reiterated units that is characteristic of vascular plant sporophytes.

In essence, the liverwort sporophyte can thus be said to exhibit a tripartite body plan that is first expressed in the young embryo. Hornworts and mosses, the other two bryophyte divisions, also exhibit tripartite body plans; however, the cellular activities associated with axial growth are distinctly different among bryophyte divisions (Smith, 1955; Wardlaw, 1955;

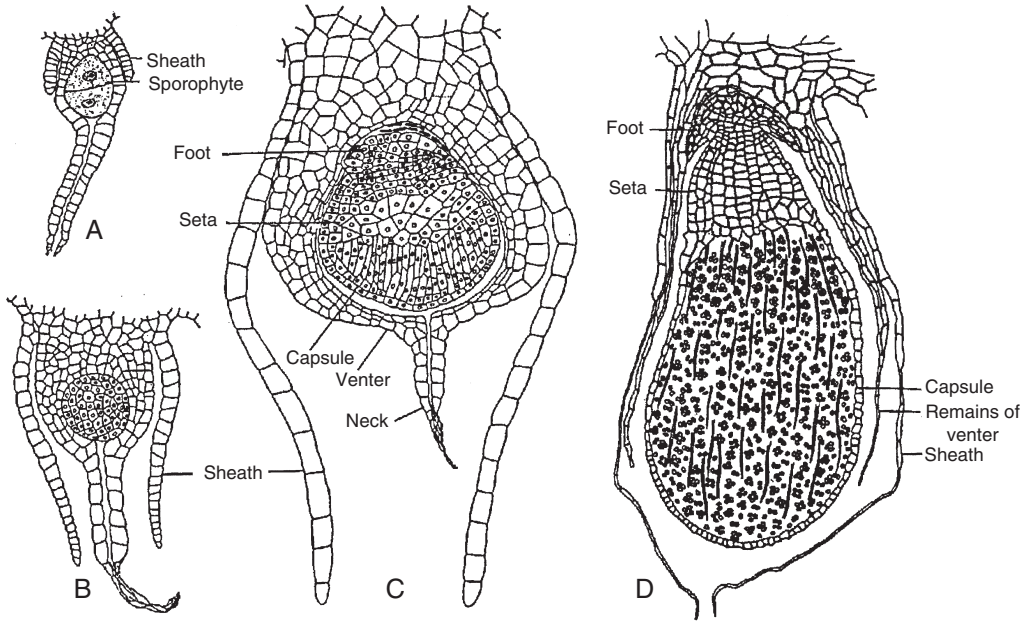


**Figure 5.1** The development of the embryo and sporophyte of the liverwort *Marchantia polymorpha*. (A) Two-celled embryo inside the archegonium. (B) Young spherical embryo lacking obvious cellular differentiation. (C) Expanded spherical embryo exhibiting tripartite organization. (D) Nearly mature sporophyte with well-defined foot, seta and capsule. Redrawn from Smith (1955) with permission from McGraw-Hill Companies.

Bold *et al.*, 1987; Crum, 2001). The embryo of the hornworts is also composed of a basal tier destined to become the foot and an apical tier representing the future apicalmost cells of the capsule (Campbell, 1918; Smith, 1955; Wardlaw, 1955; Crum, 2001). The intermediate tier develops into a narrow band of dividing cells called an intercalary meristem that remains positioned just above the foot. The intercalary meristem undergoes unifacial divisions on its capsule side, with the result that these new cells compose almost the entire linear capsule that rises above the gametophyte. Thus, the three-tiered hornwort embryo is directly enlarged into a tripartite body plan. In most mosses, it is difficult to recognize three cellular tiers in the first stages of embryonic development (Smith, 1955; Wardlaw, 1955; Lal and Bhandari, 1968; Bold *et al.*, 1987; Crum, 2001). A transient apical cell differentiates into the upper hemisphere and then this apical cell and its derivatives act to generate a spindle-shaped embryo. This embryo exhibits three well-defined regions that are destined to develop into the foot, seta and capsule of the mature moss sporophyte. In marked contrast to the diffuse growth of the liverwort seta, a unifacial intercalary meristem arises in the moss seta just beneath the immature capsule and its activity generates most of the cells composing the elongating seta.

The fern *Pityrogramma triangularis* (Kaulf.) Maxon serves here as a representative example of the pteridophyte-grade of plant organization (Figure 5.2). The fern zygote within the archegonium undergoes a series of segmentation divisions resulting in the formation of a globular-shaped embryo (Campbell, 1918; Smith, 1955; Wardlaw, 1955; Gifford and Foster, 1989). This embryo is often said to be composed of four quadrants representing the shoot apex, first leaf, first root and foot (Figure 5.2A–D). The shoot apex is solely responsible

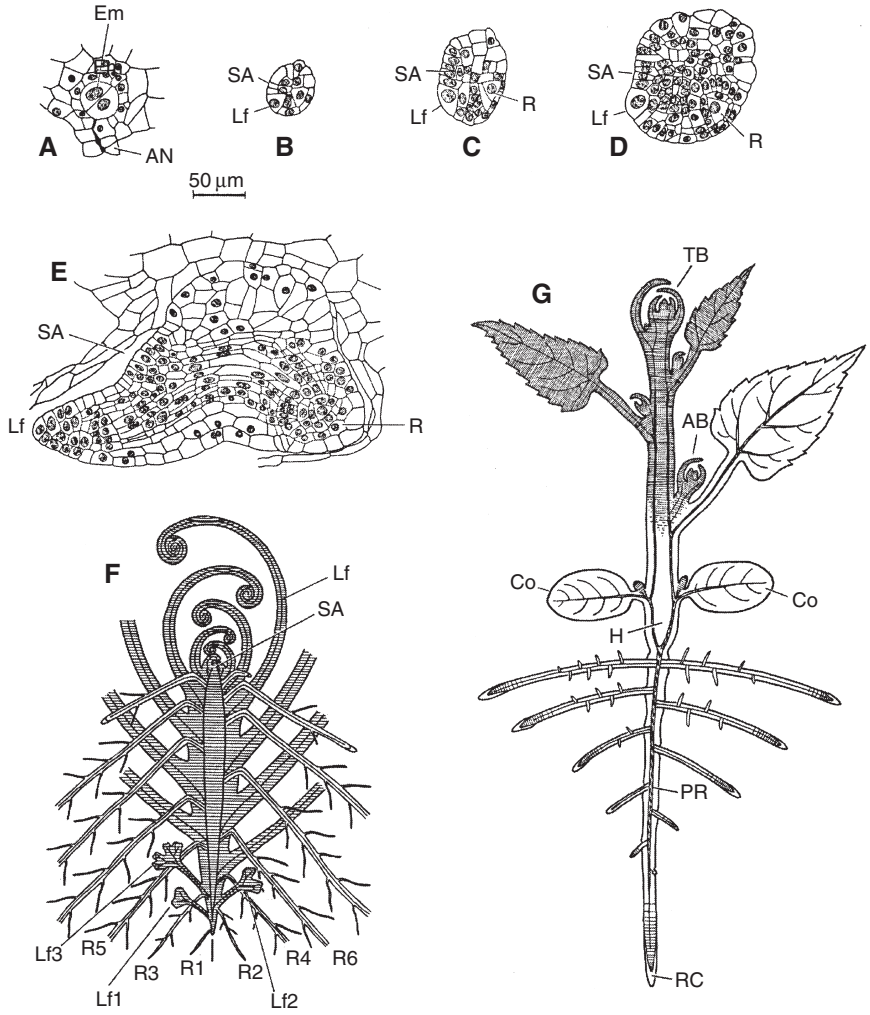


**Figure 5.2** Embryonic development of the fern *Pityrogramma triangularis* (A–E) and the body plans of the ferns (F) and the dicots (G). A. Two-celled embryo inside the archegonium. (B) Young spherical embryo exhibiting characteristic apical cells for shoot apex and first leaf in apical hemisphere. (C–E) Successive stages of the four-quadrant embryo composed of shoot apex, first leaf, first root and foot (unlabelled). The first root arises in a lateral position near the base of the first root. Panels A–E were redrawn with permission from unpublished work of W. Hagemann. (F) Diagrammatic illustration of typical fern body plan, which shows that the positional relationships first expressed in the unipolar embryo are reiterated in postembryonic development. (G) Diagrammatic illustration of the model dicot body plan, which shows that the positional relationships first expressed in the bipolar embryo are reiterated in postembryonic development. This situation does not hold true for all dicots. Panels (F) and (G) were redrawn from Troll (1959) with permission of Georg Thieme Verlag. Abbreviations: Em, embryo; AN, archegonial neck; SA, shoot apex; Lf, leaf; R, root; TB, terminal bud; AB, axillary bud; Co, cotyledon; PR, primary root; RC, root cap.

for generating the growth axis of the postembryonic plant; hence, the fern embryo is referred to as being unipolar (Groff and Kaplan, 1988). The first root arises near the base of first leaf (Figure 5.2E). All postembryonic roots are also observed to originate near leaf bases and thus, fern roots are consistently lateral with respect to the longitudinal axis of

the growing plant (Figure 5.2F), which has been termed the homorhizic condition (Troll, 1943; Groff and Kaplan, 1988). The horsetails (*Equistem*), which are thought to comprise a monophyletic group with the ferns (Pryer *et al*., 2001), exhibit similar organographic arrangements, with the first root subtending the first leaf and all postembryonic roots arising at the nodes (Gifford and Foster, 1989).

The relative positions of embryonic organs illustrated in Figure 5.2 foreshadow the postembryonic body plan in almost all ferns (Eames, 1936; Smith, 1955; Bierhorst, 1971; Gifford and Foster, 1989). In even those ferns exhibiting unusual habits, the body plan is irrevocably fixed by its embryonic organization. In marked contrast to the subterranean root systems of dicot trees, the tree ferns from such genera as *Dicksonia* and *Cyanthea* develop buttress-like coverings of interwoven roots that arise at the bases of lower leaves and grow down the outside of the trunk and into the ground (Smith, 1955; Gifford and Foster, 1989). Additional roots continue to form at the bases of higher tree fern leaves, even though these roots are destined to remain short and never penetrate the soil. Mature plants of the aquatic floating fern *Salvinia* do not develop roots and this rootless feature can be traced back to its embryo, where the non-growing sector subjacent to the first leaf is often interpreted as a vestigial first root (Eames, 1936; Bierhorst, 1971). Even the problematic whisk ferns (Psilotales), which are apparently closely related to eusporangiate ferns according to molecular analyses (Pryer *et al*., 2001), exhibit an embryonic organization that correlates with its mature morphology, at least with respect to the absence of any roots. In *Tmesipteris tannensis* Bernh. and *Psilotum nudum* (L.) Pal. Beauv., the embryos display a two-parted organization consisting of a distal shoot apex and a proximal foot with no evidence at all for leaf or root quadrants (Holloway, 1921, 1939; Bierhorst, 1971). The embryonic shoot apex develops into a branched plagiotropic rhizome lacking roots, which may be an adaptive response to the epiphytic habit frequently adopted by whisk ferns. (Eventually, the rhizome produces aerial axes bearing reduced dorsiventral structures called enations which are actually homologous to genuine leaves (for resolution of this controversial issue, see Kaplan, 2001).) A rare exception to the general rule about the embryonic encapsulation of fern body plan is seen in certain *Ophioglossum* species where roots develop subterminal buds capable of growing into new shoots (Peterson, 1970); nevertheless, the roots of the daughter shoots also emerge at the base of their fronds, thereby replicating the positional relationships of the original shoot.

The other major pteridophyte group, the lycophytes, also manifests unipolar embryonic organization, which is largely reproduced in the body plans of adult plants (Groff and Kaplan, 1988; Gifford and Foster, 1989). However, postembryonic roots in the lycophytes do not exhibit the same positional relationships as the ephemeral first root. For example, the embryos of different *Selaginella* species exhibit wide variation in the position of the first root relative to other embryonic organs, which are not reflected in the organographic arrangements of their postembryonic plant bodies (Bower, 1935; Gifford and Foster, 1989). Subsequent roots in many *Selaginella* species originate from leafless axes called rhizophores that form at stem bifurcations. The distal ends of these rhizophores bear typical roots with root caps. Some workers suggest that rhizophores are considered as true roots (e.g. Gifford and Foster, 1989), while others view them as unique root-bearing structures (e.g. Lu and Jernstedt, 1996). A similar uncoupling of embryonic root position and postembryonic organization is observed in other lycophytes. In most *Lycopodium* species, postembryonic roots originate close to the shoot tip and traverse down the cortex before emerging into the soil (Gifford and Foster, 1989). The postembryonic roots of *Isoetes* and its extinct relatives are borne on different specialized structures called rhizomorphs of uncertain homology

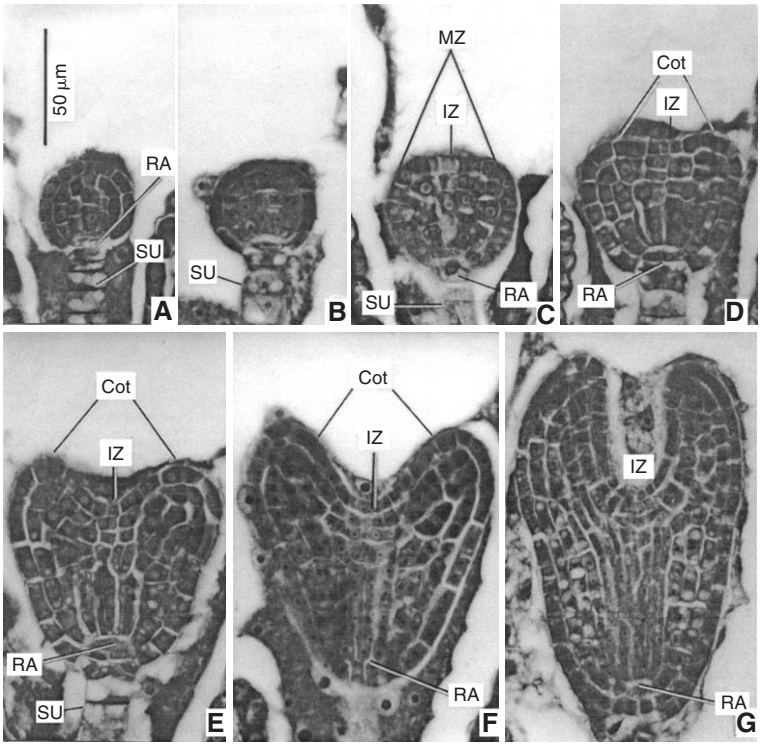


**Figure 5.3** Embryonic development of the dicot *Capsella bursa-pastoris*. (A) and (B) Young embryos exhibiting well-defined suspensor and embryo proper. The incipient root apical meristem can be recognized by the periclinal cell divisions delimiting the future root cap. (C) Mid-globular embryo displaying incipient shoot apical meristem with a lighter staining central (or initial) zone and a darker staining lateral (or morphogenetic) zone. (D–F) Early to late heart embryos showing cotyledon emergence from the lateral zones. (G) Early torpedo stage. Reprinted from Kaplan and Cooke (1997) with permission from American Society of Plant Biologists. Abbreviations: RA, root apex; Su, suspensor; MZ, morphogenetic zone; IZ, initial zone; Cot, cotyledon.

(Paolillo, 1963, 1982). In conclusion, it appears that the fundamental pteridophyte body plan is fully manifested in either developing embryos (ferns and horsetails) or young post-embryonic plants (lycophytes) and then it is reiterated throughout the life of the plant.

Characteristically, seed plant embryos exhibit bipolar (or allorhizic) organization with the embryonic shoot and root apices arising at opposite poles (Troll, 1943; Groff and Kaplan, 1988). In many, but not all, embryos, these incipient meristems act to perpetuate the bipolar organization, with the result that the primary plant body exhibits a central axis with opposite shoot and root systems, as is diagrammatically illustrated in Figure 5.2G. For example, in the dicot *Capsella bursapastoris* (L.) Medic. (Figure 5.3), the incipient root and shoot apical meristems arise during the globular stage of embryonic development (Kaplan and Cooke, 1997). These meristems can first be recognized by the cellular activities that accompany the formation of their distal or lateral structures. In particular, the origin of the root apical meristem is disclosed by periclinal divisions that delimit the distal root cap from the more proximal root body (Figure 5.3A–D). This first root will then generate the root system of the mature plant. The embryonic shoot apical meristem is revealed by the presence of cytohistological zonation at the shoot pole of the late globular embryo (Figure 5.3C–G).

The dark-staining lateral regions of this incipient meristem are clearly distinguishable from the light-staining central region. The lateral regions give rise to two cotyledons, which are homologous to the first leaf of pteridophyte embryos. The central region is destined to become the epicotylar shoot apical meristem that is ultimately responsible for generating the entire shoot system. Thus, the bipolar organization of the *Capsella* embryo establishes the positional relationships that are expressed throughout postembryonic development.

Although Figure 5.2G was proposed as the model for the bipolar condition in dicots (Troll, 1943), it is clear that the body plans of individual seed plants may represent either direct reiterations or modified arrangements of the original embryonic organization. The dicots in particular exhibit the greatest variation in the relationship between embryonic organization and mature body plan (Groff and Kaplan, 1988). Although many dicots maintain the bipolar embryonic organization throughout postembryonic development, the embryonic organization of numerous other species is subsequently modified by: (1) the shoots bearing lateral roots (e.g. many vines like *Vitis vinifera* L. and *Hedera helix* L.); (2) the roots bearing new shoots (e.g. saprophytic plants like *Monotropa uniflora* L.); or (3) both shoot-borne roots and root-borne shoots (e.g. many perennial herbs and certain trees) (Groff and Kaplan, 1988). It can be argued that root-borne shoots are simply mimicking the original bipolar axis, with the new shoot apex at one pole and the bud-producing root at the other. However, the origin of lateral roots from the shoot does not reflect the embryonic organization but rather represents a postembryonic modification of the bipolar body plan.

The bipolar organization of gymnosperm embryos is characteristically maintained during postembryonic growth, with very few reported examples of root-borne shoots or vice versa (Groff and Kaplan, 1988). By contrast, resolving the nature of positional relationships in monocot embryos represents an extraordinary challenge that can only cursorily be addressed here. The traditional perspective is that the single cotyledon occupies the terminal position in the developing monocot embryo (Souèges, 1931; Gifford and Foster, 1989), which suggests that the shoot apex should be viewed as a lateral structure. However, the weight of morphological evidence argues that the monocot shoot apex does indeed arise in the terminal position (Haccius, 1952, 1960; Swamy and Laksmanan, 1962), but it is subsequently displaced into what appears to be a lateral position by the pronounced growth of its overarching cotyledon (for further discussion, see Gifford and Foster, 1989). As an additional complication, the orientation of the first root relative to the central axis shows considerable variability in monocot embryos. The first root of many monocots is positioned at the opposite embryonic pole, thereby displaying the allorhizic organization of the typical bipolar embryo (Troll, 1943). However, the first roots of certain monocots such as *Zea mays* L. (Randolph, 1936) arise at the opposite pole but much later in embryo development, which resembles the timing of the first root initiation in unipolar fern embryos, while the first roots of still other monocots such as *Aponogeton madagascariensis* Mirbel (Yamashita, 1976) are reported to originate as genuine lateral structures. Nevertheless, the embryonic first root of almost all monocots is short lived or else poorly developed so that their postembryonic root system is almost entirely derived from shoot-borne roots. Therefore, although the monocot embryo is generally interpreted to have bipolar organization, the postembryonic monocot body plan is regarded as being secondarily homorhizic, due to the origin of subsequent roots as shoot-borne organs (Troll, 1943).

This section has established the following generalizations: embryonic pattern is amplified to form the postembryonic body in bryophytes, or it is reiterated to generate the postembryonic body in vascular plants. The most noteworthy exception to these generalizations

is that in some vascular plants, the positional relationships of the all roots except the embryonic root are established in the young postembryonic body.

## What developmental mechanisms act to generate plant body plans?

It must be appreciated that the botanical research on this question is far less advanced than comparable research in animal development. However, the initial evidence reviewed below suggests that the hormone auxin (indole-3-acetic acid) acts as a critical developmental mechanism for generating the body plans of land plants.

As discussed in a previous section, the current theory for explaining the developmental mechanisms underlying the Cambrian radiation is that the genes responsible for regulating embryonic development in basal animals experienced repeated duplication and altered transcriptional regulation, with the result that these genes were able to specify more complex body plans. A similar working hypothesis is now being adopted by the emerging disciple of plant evolutionary developmental biology (for an excellent overview, see Cronk, 2001). For instance, plant homeobox genes can be classified into at least three subfamilies: *KNOTTED1*-like (*KNOX*) genes affecting meristematic cell fates; homeodomain-leucine zipper (*HD-ZIP*) genes regulating later developmental and physiological processes; and *GLABROUS2*-like genes specifying epidermal cell fates (Chan *et al*., 1998; Williams, 1998; Bharathan *et al*., 1999). The archetypal example of the first subfamily is the so-called *SHOOTMERISTEMLESS* gene (Barton and Poethig, 1993) that is expressed in the central region of the globular embryo of *Arabidopsis thaliana* (L.) Heynh. (Long *et al*., 1996). The identical stage in the related *Capsella* embryo is illustrated in Figure 5.3C. Although the name of this gene implies that it is essential for the origin of the shoot apical meristem, an alternative interpretation is that it maintains the proliferative activity of the embryonic central region in order to generate the epicotylar shoot apical meristem (Kaplan and Cooke, 1997). The recent report of two *KNOX*-like genes in the moss *Physcomitrella patens* (Hedw.) B. S. G. indicates that this subfamily started diverging early in the evolution of land plants (Champagne and Ashton, 2001). The ability of *Physcomitrella* to perform homologous recombination should allow these investigators to determine the precise roles of homeobox genes in mosses (Theissen *et al*., 2001). However, research to date has not revealed any direct relationship between evolutionary changes in homeobox genes and new body plans in plants. Since the primeval protist lineages evolving into animals and plants had probably diverged as single-celled eukaryotes, it should come as no surprise that plants might depend on different genetic mechanisms to organize their body plans (Meyerowitz, 2002).

On the other hand, it is quite intriguing that the molecular diversity of the MADS-box family of transcription factors correlates with the morphological complexity of land plants (Theissen *et al*., 2000; Vergara-Silva *et al*., 2000; Cronk, 2001). Seven MADS-box genes have been isolated from the moss *Physcomitrella patens* (Krogan and Ashton, 2000; Henschel *et al*., 2002) in contrast to 15 genes from the fern *Ceratopteris richardii* Brogn. and to even larger numbers from several angiosperm species. Of particular importance to angiosperm reproductive development are the MADS-box genes that act as homeotic selector genes for controlling floral organ identity. Indeed, the ABC model provides molecular validation for the classical morphological concept of serial homology, i.e. all lateral dorsiventral structures from cotyledons to carpels are leaf homologues (Goethe, 1790), because the triple mutant in all three functions exhibits foliage leaf-like structures in place of floral organs (Coen and Meyerowitz, 1991). The molecular evolution literature expresses considerable optimism that the MADS-box gene diversity underlies the evolution of plant body plans

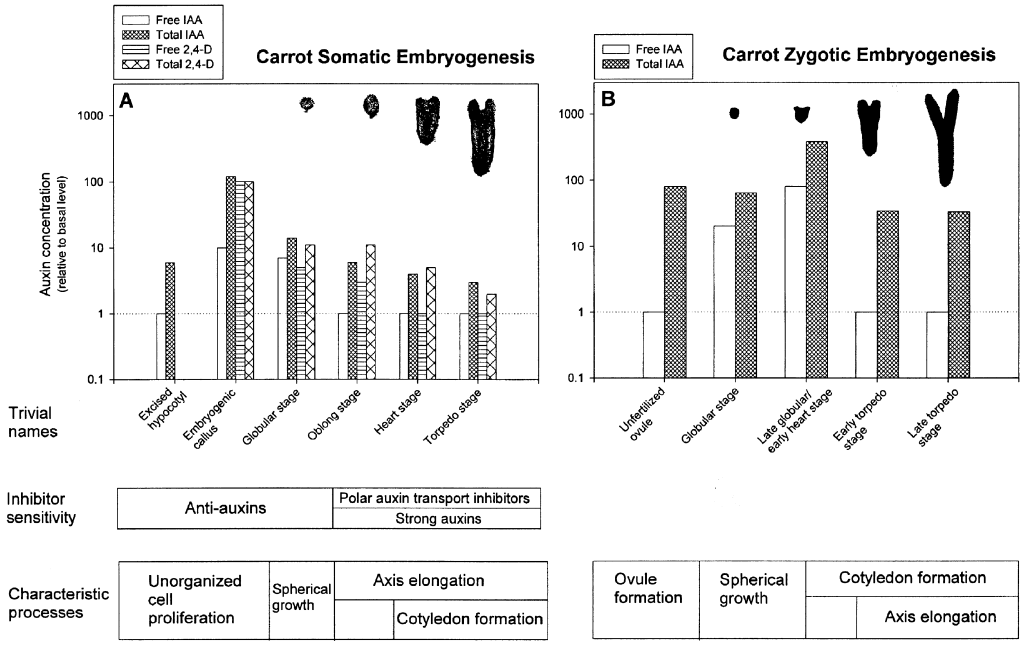


**Figure 5.4** Auxin regulation of the somatic (A) and zygotic (B) embryogenesis of the carrot *Daucus carota* (for details, see Schiavone and Cooke, 1987; Michalczuk *et al.*, 1992a; Ribnicky *et al.*, 2001). Trivial names refer to the shapes of each embryonic stage; characteristic processes refer to the morphogenetic processes occurring at each stage; and inhibitor sensitivity refers to the auxin agonists or antagonists active at each stage. For the purpose of normalizing the data for free auxin concentrations between embryo types, all data are expressed relative to basal levels, which are 7 ng IAA/g FW and 14 ng 2,4-D/g FW in oblong to torpedo stages in somatic embryos and 26 ng IAA/g FW in unfertilized ovules and torpedo stages in zygotic embryos. Redrawn from Ljung *et al.* (2002) with permission from Kluwer Academic Publishers. Abbreviations: IAA, indole-3-acetic acid = endogenous auxin; 2,4-D,2,4-dichlorophenoxyacetic acid = synthetic auxin.

in general (for enthusiastic advocacy, see Vergara-Silva *et al.*, 2000). We wish to point out to the contrary that no compelling evidence yet exists to support the notion that MADS-box genes play even minor roles in establishing the fundamental tripartite, unipolar or bipolar organization of any division of land plants, including the angiosperms.

In our opinion, auxin is causally involved in the establishment of plant body plans, at least in the seed plants (Figure 5.4). The progression through both somatic and zygotic embryogenesis of the carrot *Daucus carota* L. appears to require the sequential activation of two different auxin biosynthetic pathways (Michalczuk *et al.*, 1992a,b; Ribnicky *et al.*, 1996, 2002). During the initial stages of embryo growth, a tryptophan-dependent pathway for auxin biosynthesis produces high levels of free (i.e. active) auxin, which are apparently critical for mediating the rapid cell divisions needed to generate the globular embryo. Then the embryo switches to a tryptophan-independent pathway for auxin biosynthesis that appears capable of exercising greater homeostatic control over the free auxin levels. The action of this pathway results in much lower free auxin levels that may be a necessary precondition for establishing auxin gradients regulating the polarized growth of older embryos. The results from inhibitor experiments are entirely consistent with these concepts: both synthetic auxins and polar auxin transport inhibitors are able to block or alter the polarized growth, but not the initial isodiametric expansion, of all angiosperm embryos examined

(Schiavone and Cooke, 1987; Liu *et al.*, 1993; Fischer *et al.*, 1997; Hadfi *et al.*, 1998). It remains critical to characterize auxin levels and biosynthetic pathways in other embryos than those of carrots. Auxin levels are reported to increase between the initial embryonic and subsequent postembryonic development in the wheat (*Triticum aestivum* L.) caryopsis (Fischer-Iglesias *et al.*, 2001). It is clear that these results are not directly comparable to those obtained from carrot embryo work because wheat embryos undergo prolonged postembryonic growth while still retained inside the caryopsis.

Molecular research is beginning to reveal the molecular basis for auxin action in *Arabidopsis* embryos (Souter and Lindsey, 2000; Hamann, 2001). For instance, a homozygous null mutation in the putative auxin receptor gene (*ABP1*) results in embryo development being blocked at the early globular stage (Chen *et al.*, 2001). Of even greater importance are the observations on *gnom* mutant embryos, which become enlarged spherical structures unable to initiate a polarized growth axis. The molecular basis for the mutant phenotype is that the embryos fail to localize the auxin efflux carrier PIN1 in the proper position for carrying out polar auxin transport (Steinmann *et al.*, 1999).

Moreover, auxin acts as a critical regulator of postembryonic body plan of seed plants. In particular, localized synthesis and/or polarized transport are thought to establish auxin gradients that appear absolutely critical for the positioning of new leaf primordia on the shoot apex (Meicenheimer, 1981; Reinhardt *et al.*, 2000) and of new lateral root primordia along developing roots (Reed *et al.*, 1998; Casimiro *et al.*, 2001). Finally, it is well documented that auxin exercises predominant control over many aspects of vascular tissue development, including the induction of primary vascular tissues (Roberts *et al.*, 1988; Aloni, 1995); the positioning of primary vascular bundles (Sachs, 1991; Berleth *et al.*, 2000); and the activity of vascular cambia (Uggla *et al.*, 1996, 1998). All this evidence taken together demonstrates that auxin acts as a very important regulator of the body plans of seed plants.

Much less research has been devoted to the auxin regulation of developmental processes in bryophytes and pteridophytes (for review, see Cooke *et al.*, 2002). In fact, to the best of our knowledge, no published work has directly studied auxin action in body plan organization in these plants. Nonetheless, three arguments can be advanced in support of the prediction that auxin must also help to regulate the body plans of non-seed plants. One, it seems quite plausible that the seed plants would not have evolved *de novo* development mechanisms for generating body plans but rather would have modified pre-existing mechanisms already operating in the common ancestor of non-seed and seed plants. Two, we have noted elsewhere that the nature of metabolic regulation of auxin levels appears to have evolved in concert with the increasing morphological complexity in the land plant lineage (Sztein *et al.*, 2000). Three, certain auxin-mediated processes, including tropisms, apical dominance and axis elongation, are widespread among all land plants, including bryophytes (Cooke *et al.*, 2002). Among the positional relationships subject to auxin regulation in non-seed plants are: rhizoid initiation in bryophyte gametophytes (Kaul *et al.*, 1962; Kumra and Chopra, 1987; Nyman and Cutter, 1981: Chopra and Vashistha, 1990), root initiation in pteridophyte sporophytes (Wardlaw, 1957; Partanen and Partanen, 1963; Wochok and Sussex, 1975) and vascular differentiation in fern sporophytes (Ma and Steeves, 1992). It is also worthwhile here to mention the provocative modelling work of Stein (1993) who attempted to relate predicted patterns of auxin concentration in the shoot apex to the observed arrangements of primary vascular tissues in a wide range of fossil plants. Although this model is based on several assumptions about auxin biosynthesis, movement and accumulation that have never been evaluated with real meristems, it does result in a close correspondence between predicted hormone distributions and underlying stelar patterns for many

fossil plants, which suggests that altered patterns of auxin action may have been involved in the diversification of stelar patterns throughout the land plant lineage.

## Did major changes in auxin regulation occur prior to the Silurian–Devonian radiation?

The approach adopted in evolutionary development biology to address such questions involves: (1) the use of the fossil record and/or molecular phylogenies to identify those groups that diverged from the stem group prior to the radiation; and (2) the characterization of the developmental mechanisms in extant organisms from those early diverging lineages (Raff, 1996; Arthur, 2002). The ultimate goal is to predict those developmental mechanisms operating in the stem group that may have contributed to body plan diversification during the radiation. The critical assumption discussed earlier is that the developmental mechanisms for generating embryonic body plans are assumed to remain extraordinarily stable over geological time. This assumption has been validated in non-bilateral animals by the direct relationship between their homeobox gene diversity and their phylogenetic relationships (Peterson and Davidson, 2000), but we have no evidence to evaluate its validity for plants.

A comparable approach toward the Silurian–Devonian radiation of vascular plants would involve an investigation of the genes responsible for regulating auxin action in the three extant bryophyte lineages, which diverged from the vascular plant stem group prior to this radiation, according to morphological and molecular phylogenies (Kenrick and Crane, 1997; Qiu *et al*., 1998; Nickrent *et al*., 2000; Renzaglia *et al*., 2000; Karol *et al*., 2001). It must be acknowledged that this effort is hampered by several limitations. For instance, it is probably premature to attempt a comparative study of auxin regulatory genes in land plants because few non-seed plants, with the notable exception of *Physcomitrella patens*, are readily amenable to molecular manipulation. Moreover, the phylogenetic relationships among the bryophyte lineages remain unresolved, although the research cited above does frequently, but not always, place the mosses as the sister group to the vascular plants. Lastly, since macrofossils exhibiting the morphological characteristics of modern bryophytes (e.g. monosporangiate sporophytes and heteromorphic gametophyte-dominant generations) appear relatively late in the fossil record, one must always keep in mind the possibility that one or more modern bryophyte lineages may be derived from isomorphic, polysporangiate ancestors (Kenrick and Crane, 1997).

What can be accomplished here is that we can discuss the distribution of auxin regulatory processes in charophytes and bryophytes (Table 5.1) in order to speculate about developmental mechanisms that may have been operating in the stem group before the diversification of vascular plants in the Late Silurian–Early Devonian radiation. The tryptophan-independent pathway has definitively been identified as the predominant auxin biosynthetic pathway in the liverwort *Marchantia polymorpha* L., the moss *Polytrichum ohioense* Ren. & Card., and several vascular plants, which indicates that the capacity for tightly regulating auxin biosynthesis may be ubiquitous in the land plant lineage (Sztein *et al*., 2000). Because the charophyte *Nitella* spp. maintains auxin levels that are comparable with those measured in bryophytes, it is likely that the tryptophan-independent pathway is also operative in this group. Insofar as charophytes and liverworts carry out metabolic interconversions between active free and inactive conjugated forms of auxin at slow rates, it appears that the free auxin levels are maintained in these groups via the balance between biosynthetic and degradative reactions. However, hornworts and mosses share the ability for rapid auxin conjugation with vascular plants, which provides these lineages the potential

**Table 5.1** Phylogenetic distribution of critical processes involved in auxin action in green plants. The occurrence of polar auxin transport (PAT) was assessed by the direct measurement of PAT in agar-block experiments and/or by the sensitivity of auxin efflux to PAT inhibitors

| Process | Charophytes | Liverworts | Hornworts | Mosses | Vascular plants |
|---|---|---|---|---|---|
| Tryptophan-independent pathway | Predicted | Yes | n.d. | Yes | Yes |
| Auxin conjugation rates | Very slow | Slow | Intermediate to rapid | Intermediate to rapid | Rapid to very rapid |
| Predicted mechanism for regulating auxin levels | Biosynthesis/ degradation | Biosynthesis/ degradation | Conjugation/ hydrolysis | Conjugation/ hydrolysis | Conjugation/ hydrolysis |
| PAT in gametophyte rhizoids | Yes? | n.d. | n.d. | Yes | n.d. |
| PAT in gametophyte thalli | No | Yes | n.d. | Yes | n.d. |
| PAT in young sporophytes | n.s. | No | No | Yes | Yes |

n.d., no data; n.s., non-existent structure. For references, see text.

for even more precise regulation of auxin levels (Sztein *et al*., 1995, 1999, 2000). One significant difference is that almost all vascular plants produce two specific conjugates, IAA-aspartate (or -glutamate) and IAA-1-*O*-glucose, that are not accumulated in bryophytes.

Membrane proteins capable of mediating the transmembrane auxin transport also evolved before the origin of the land plants, as evidenced by their activity in the thalli of the charophyte *Chara vulgaris* L. (Dibb-Fuller and Morris, 1992). However, there is contradictory evidence about whether charophytes carry out the more sophisticated process of intercellular auxin transport, as characterized by basipetal polarity and inhibitor sensitivity. The standard inhibitors of polar auxin transport do not affect auxin efflux from intact thalli (Dibb-Fuller and Morris, 1992), but these inhibitors are reported to have significant effects on decapitated thalli with growing rhizoids (Klambt *et al*., 1992). Intercellular auxin transport with strong basipetal polarity and inhibitor sensitivity has been measured in liverwort thalli (Maravolo, 1976; Gaal *et al*., 1982); moss protonemata (Rose *et al*., 1983; Geier *et al*., 1990), and moss rhizoids (Rose and Bopp, 1983). The sporophytes of hornworts and liverworts do not exhibit polar auxin transport (Thomas, 1980; DB. Poli, unpublished observations). By contrast, young setae of the moss *Polytrichum* maintain significant fluxes of basipetal polar transport that are even higher than those measured in corn coleoptiles (DB. Poli, unpublished observations). If mosses are the actual sister group of the vascular plants, then this observation suggests that their common ancestor evolved the auxin-dependent mechanism for generating polarized axes that is still being utilized in the sporophytes of extant members of both groups. Finally, bryophytes exhibit many of the auxin-mediated responses, such as apical dominance, phototropism and axis elongation also found in vascular plants (Cooke *et al*., 2002). In summary, the range of auxin-regulated processes reported in extant bryophytes lends some credibility to the hypothesis that auxin was intimately involved in establishing the body plans of ancestral Silurian plants prior to the diversification of vascular plant lineages.

## Conclusions

Using the conceptual framework derived form the study of the Cambrian radiation of bilateral animals, this chapter attempted to address four questions concerning the evolution of the early land plants. One, the evidence assembled indicates that the early land plants did undergo a rapid evolutionary radiation during the Late Silurian to Early Devonian periods. Two, the characteristic body plans of different divisions of extant land plants are established during embryonic and early postembryonic development, which means that the regulatory mechanisms operating in embryonic development are also critical for generating these body plans. Three, the research evidence surveyed in this chapter establishes that the hormone auxin serves as a primary mechanism for regulating embryo and postembryonic development, at least in vascular plants. Four, judging from our current knowledge of auxin action in early-divergent bryophyte lineages, it appears that major changes in auxin action occurred in the earliest land plants prior to the Late Silurian. Therefore, the evidence available to date lends support to the appealing perspective that genetic changes in auxin action in early land plants were instrumental in the subsequent diversification of body plans in vascular plants. It follows that increased knowledge of the auxin regulation of embryonic mechanisms in extant plants should help to elucidate the developmental events that generated novel body plans during the Silurian–Devonian radiation of new vascular plant lineages.

Our enthusiasm for these interpretations is dampened somewhat by the realization that due to the limited number of relevant papers, we are skating over the conceptual equivalent of thin ice. A rigorous evaluation of the question posed in the title requires that much greater research effort be devoted toward the characterization of: (1) the phylogenetic relationships among the lineages of both extant bryophytes and putative bryophyte fossils; (2) the genetic regulation of auxin action in embryo development; and (3) the developmental mechanisms operating in charophytes, bryophytes and pteridophytes. Regardless of the ultimate answer, that question will be worth considering if it inspires further research on those three subjects.

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