Plant Molecular Biology **49:** 319–338, 2002.
Perrot-Rechenmann and Hagen (Eds.), Auxin Molecular Biology.
© 2002 *Kluwer Academic Publishers. Printed in the Netherlands.*

Evolutionary patterns in auxin action

Todd J. Cooke[1,*], DorothyBelle Poli[1], A. Ester Sztein[1] and Jerry D. Cohen[2]
[1]*Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742, USA (*author for correspondence; e-mail tc23@umail.umd.edu);* [2]*Department of Horticultural Science, University of Minnesota, Saint Paul, MN 55108, USA*

Received 17 July 2001; accepted 16 October 2001

Key words: auxin, charophytes, evolution, green algae, indole-3-acetic acid, liverworts, mosses, vascular plants

Abstract

This review represents the first effort ever to survey the entire literature on auxin (indole-3-acetic acid, IAA) action in all plants, with special emphasis on the green plant lineage, including charophytes (the green alga group closest to the land plants), bryophytes (the most basal land plants), pteridophytes (vascular non-seed plants), and seed plants. What emerges from this survey is the surprising perspective that the physiological mechanisms for regulating IAA levels and many IAA-mediated responses found in seed plants are also present in charophytes and bryophytes, at least in nascent forms. For example, the available evidence suggests that the apical regions of both charophytes and liverworts synthesize IAA via a tryptophan-independent pathway, with IAA levels being regulated via the balance between the rates of IAA biosynthesis and IAA degradation. The apical regions of all the other land plants utilize the same class of biosynthetic pathway, but they have the potential to utilize IAA conjugation and conjugate hydrolysis reactions to achieve more precise spatial and temporal control of IAA levels. The thallus tips of charophytes exhibit saturable IAA influx and efflux carriers, which are apparently not sensitive to polar IAA transport inhibitors. By contrast, two divisions of bryophyte gametophytes and moss sporophytes are reported to carry out polar IAA transport, but these groups exhibit differing sensitivities to those inhibitors. Although the IAA regulation of charophyte development has received almost no research attention, the bryophytes manifest a wide range of developmental responses, including tropisms, apical dominance, and rhizoid initiation, which are subject to IAA regulation that resembles the hormonal control over corresponding responses in seed plants. In pteridophytes, IAA regulates root initiation and vascular tissue differentiation in a manner also very similar to its effects on those processes in seed plants. Thus, it is concluded that the seed plants did not evolve *de novo* mechanisms for mediating IAA responses, but have rather modified pre-existing mechanisms already operating in the early land plants. Finally, this paper discusses the encouraging prospects for investigating the molecular evolution of auxin action.

Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; IAA, auxin (indole-3-acetic acid); NAA, naphthaleneacetic acid; NPA, *N*-(1-naphthyl)phthalamic acid; PCIB, *p*-chlorophenoxyisobutyric acid; 2,4,6-T,2,4,6-trichlorophenoxyacetic acid; TIBA, 2,3,5-triiodobenzoic acid

Introduction

It has long been appreciated that the hormone auxin (indole-3-acetic acid, IAA) is an important regulator of developmental processes in seed plants. For instance, IAA acts as the intercellular signal coupling environmental stimuli to growth responses in phototropism and gravitropism (Kaufman *et al.*, 1995; Dolan, 1998; Marchant *et al.*, 1999). For instance, the progression through different stages of angiosperm embryo development appears to require the sequential activation of two different auxin biosynthetic pathways (Michalczuk *et al.*, 1992a, b; Cooke *et al.*, 1993; Ribnicky *et al.*, 2001). A tryptophan-dependent pathway capable of high biosynthetic rates mediates

isodiametric growth of young embryos, and then polar auxin transport, in conjunction with a tryptophan-independent pathway with better homeostatic control, helps to establish polarized growth of older embryos (Schiavone and Cooke, 1987; Liu *et al.*, 1993; Steinmann *et al.*, 1999). Localized synthesis and/or polar transport establish IAA gradients that appear to be absolutely critical for positioning leaf primordia on shoot apices (Meicenheimer, 1981; Lyndon, 1998; Reinhardt *et al.*, 2000) and for initiating lateral root primordia on mature roots (Blakely *et al.*, 1988; Reed *et al.*, 1998; Casimiro *et al.*, 2001). Molecular studies of *Arabidopsis* flower development have implicated IAA-responsive genes as being crucial for the positional relationships in flowers (Sessions *et al.*, 1997; Nemhauser *et al.*, 2000). Finally, it is well documented that IAA 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 (Uggala *et al.*, 1996, 1998).

Although contemporary research on IAA action remains almost completely focused on the seed plants, there is an extensive, but scattered, older literature devoted to IAA action in other green plants. The few reviews concerning this literature are generally restricted to specific groups, such as the algae (Bradley, 1991), liverworts (Maravolo, 1980), and mosses (Christianson, 1999). By contrast, this paper takes a comparative approach in the effort to characterize IAA action in all plants, with special emphasis on the green plant lineage, including green algae, bryophytes, pteridophytes, and seed plants. It is anticipated that this approach should ultimately allow us to evaluate a number of important questions that cannot be addressed by studying a single organism or specific group. Of special interest for understanding the evolution of IAA action are the results obtained from charophycean green algae (also known as charophytes). Because these algae and land plants share many specialized characteristics, such as cell division mechanism, sperm ultrastructure, and photorespiratory enzymes, the charophytes are thought to be the green algal class most closely related to land plants (Graham, 1993; Graham *et al.*, 2000). Thus, it is assumed that extant charophytes have retained the primitive features of IAA action that were also expressed in the earliest land plants.

Given that every paper ever written about IAA action is potentially relevant to the topic of this review, we have adopted the following strategy for organizing this voluminous literature into a coherent manuscript. In the major sections, we summarize the current understanding of different processes involved in IAA action in the seed plants, and then we discuss the available literature on these processes in the algae, bryophytes, and vascular plants emphasizing the pteridophytes. Consequently, one can synthesize an appealing, albeit still incomplete, picture of the evolutionary changes in IAA metabolism and polar IAA transport, which are the principal processes responsible for regulating intracellular IAA concentration. It is also possible to draw tentative conclusions about how IAA's ability to mediate such developmental processes, such as tropisms, correlative interactions, and positional relationships, in non-seed plants evolved to regulate similar processes in seed plants. Lastly, we shall discuss the molecular events that are presumably responsible for this observed evolution of IAA-mediated development throughout the land plant lineage.

Why consider the evolutionary patterns in IAA action?

As a starting point, it is important to ask the question of why a special issue devoted to IAA molecular biology includes a paper on evolutionary patterns in IAA action. In response, we could simply cite Dobzhansky's dictum that nothing makes sense in biology except in the light of evolution, but philosophical statements do not make for compelling scientific arguments. Instead, a more appropriate justification for this paper lies in the potential of evolutionary approaches for solving certain difficult problems being faced by molecular biologists studying IAA action. For example, our current knowledge suggests that hormonal regulation in angiosperm development may frequently involve complex signal transduction networks composed of multiple, interacting, and somewhat redundant pathways (Bennett *et al.*, this issue). By contrast, it appears that the lower plants utilize simpler mechanisms of IAA regulation (Sztein *et al.*, 2000), which implies that these plants may more readily reveal how the molecular regulation of IAA action is causally related to the developmental processes responsible for plant morphology. Secondly, it is becoming increasingly obvious that due to differential rates of sequence divergence, relative sequence similarity among ho-

mologous genes is not necessarily sufficient to sort out the pattern of molecular evolution within a gene family (Eisen, 1998). Eisen (1998) has argued quite convincingly that an understanding of how different members of any gene family (e.g., the globin genes encoding oxygen-binding proteins or the homeobox genes encoding transcription factors regulating animal development) evolved over time should greatly improve the ability to make functional predictions about under-characterized members of that family. Such knowledge should also help to elucidate the roles of different homologues with overlapping functions acting in the same regulatory network. Other genomic approaches are also likely to contribute significantly to our understanding of auxin action (Theologis, this issue). Thirdly, although no molecular work has been done on IAA action in non-seed plants, there is burgeoning interest in the several model systems for these plants, including the moss *Physcomitrella patens* (Reski, 1999; Wood *et al*, 2000) and the fern *Ceratopteris richardii* (Hickok *et al.*, 1995). Thus, it is rapidly becoming feasible to apply molecular techniques for studying developmental and evolutionary problems in selected non-seed plants. *Physcomitrella* is an especially promising system due to its unique ability among all land plants studied to date to carry out homologous recombination efficient enough for gene knock-out and allele replacement studies (Schaefer and Zryd, 1997). Thus, the evolutionary perspectives contained in this review may become useful to IAA molecular biologists interested in applying the knowledge obtained from *Arabidopsis* toward the study of related problems in non-seed plants.

Because the goal of this paper is to place the knowledge available about auxin action in all plants into an evolutionary context, it is essential that the reader have an appreciation for the emerging consensus concerning the universal phylogeny of life (Baldauf *et al.*, 2000). It appears as if three lineages of photosynthetic eukaryotes were independently able to evolve multicellular growth forms: the viridiplants (green plants) being composed of the chlorophytes (green algae) and the charophytes (stoneworts and their algal relatives plus the land plants); the rhodophytes (red plants) also known as red algae; and the phaeophytes (brown plants) also known as brown algae. The rhodophytes now appear to be closely related as the sister group to the viridiplants (Baldauf *et al.*, 2000). By contrast, the lineage that gave rise to the viridiplants and rhodophytes separated as unicellular heterotrophic flagellates in deep evolutionary time from another lineage that would ultimately result in the heterokonts consisting of oomycetes and phaeophytes (see Figure 1 in Baldauf *et al.*, 2000). The rhodophytes and viridiplants appear to have acquired their chloroplasts via the primary endosymbiosis of a single cyanobacterium or a group of related cyanobacteria (Delwiche, 1999; Grzebyk *et al.*, pers. comm.). The phaeophyte lineage originated in more recent evolutionary time via secondary endosymbiosis in ancestral heterokonts in which engulfed rhodophytes were transformed into chloroplasts (Delwiche, 1999; Grzebzk *et al.*, pers. comm.). It is important in our discussion of the origins of auxin action that the reader be mindful of these contemporary perspectives on the phylogenetic relationships of multicellular photosynthetic organisms. Nevertheless, for the sake of clear communication, we shall employ the traditional organism names that were used in the primary literature on auxin action, and thus, the chlorophytes are referred to as green algae, the rhodophytes as red algae, and the phaeophytes as brown algae.

Modern phylogenetic methods have also resolved the evolutionary relationships among the simplest charophytes, with the order Charales including the genera *Chara* and *Nitella*, as being the closest living relatives to the land plants (Karol *et al.*, 2001). Comparable efforts are being made in the effort to resolve the phylogenetic relationships among the vascular land plants, including the lycophytes, horsetails, ferns, and seed plants (e.g., Pryer *et al.*, 2001). However, the evolutionary positions among the simplest land plants called bryophytes (liverworts, hornworts, mosses) remain as an unresolved and contentious issue. Certain molecular evidence is consistent with the liverworts being the first-divergent lineage (viz., the closest living relatives) of the earliest land plants (e.g., Qiu *et al.*, 1998; Qiu and Lee, 2000), while other data fit the hornworts-basal hypothesis (e.g., Nickrent *et al.*, 2000). In this paper, the sections devoted to the bryophytes emphasize the literature on liverworts and mosses for the simple reason that few published papers investigated auxin action in the hornworts.

Evolutionary patterns in IAA metabolism

Basic characteristics in seed plants

The metabolic processes that regulate endogenous IAA levels are: biosynthesis, conjugation, and degradation (for reviews, see Normanly, 1997; Bartel, 1997;

Slovin *et al.*, 1999). The research on auxin metabolism in *Arabidopsis* is fully described in Kowalczyk *et al.* (this issue) so we shall emphasize here only those topics needed for evolutionary interpretations. IAA is synthesized via two different classes of biosynthetic pathways. One, tryptophan-mediated IAA biosynthetic pathways utilize tryptophan itself as the source of the indole ring for the IAA molecule. Earlier research had repeatedly demonstrated that excised organs, tissue sections, cultured cells, and cell-free preparations can utilize tryptophan-dependent pathways to carry out IAA biosynthesis (Wildman *et al.*, 1947; Sembdner *et al.*, 1981; Nonhebel *et al.*, 1993). This interpretation has been supported by analytical work on isolated axes of germinating bean seedlings (Bialek *et al.*, 1992; Sztein *et al.*, 2001), embryogenic cells in carrot cultures (Michalczuk *et al.*, 1992b), and excised maize coleoptiles (Koshiba *et al.*, 1995). Two tryptophan-independent pathways divert the indole ring toward IAA biosynthesis before it is catalyzed into tryptophan. Considerable evidence has been accumulating in support of the notion that these pathways are the predominant IAA biosynthetic pathways operating in intact plants. In entire *Lemna* plants (Baldi *et al.*, 1991; Rapparini *et al.*, 1999), postglobular carrot somatic embryos (Michalczuk *et al.*, 1992b), and wild-type *Arabidopsis* seedlings (Normanly *et al.*, 1993), labeled precursors common to both the tryptophan-independent and tryptophan-dependent pathways result in much greater enrichments of the IAA pool than the enrichment observed with labeled tryptophan. In addition, *Zea mays* and *Arabidopsis* mutants impaired in tryptophan synthesis exhibit much higher levels of IAA than do wild-type plants, thereby providing compelling evidence for the predominant activity of the tryptophan-independent pathway in intact plants. Finally, other experiments have led to the interpretations that the tryptophan-mediated pathway is capable of high biosynthetic rates due to the large tryptophan pool and the apparent lack of feedback inhibition, whereas the tryptophan-independent pathway serves as the low-capacity pathway subject to feedback inhibition (Ribnicky *et al.*, 1996, 2001; Sztein *et al.*, 2001). Thus, it appears that the two pathways have the potential to play distinct roles in the regulation of various developmental processes, including plant embryogenesis (Michalczuk *et al.*, 1992a, b; Ribnicky *et al.*, 2001).

IAA is usually active in developmental processes as the free molecule. Nevertheless, in the seed plants, most IAA-based metabolites accumulate as IAA-ester conjugates to inositol, co-enzyme A, sugars, polysaccharides, or glycoproteins and/or as IAA-amide conjugates to amino acids, small peptides, or proteins. IAA conjugates are generally thought to act as short-term intermediates that can be hydrolyzed to release free IAA (Cohen and Bandurski, 1982; Normanly, 1997). The competing processes of conjugate synthesis and conjugate hydrolysis are thus predicted to help further modulate the free IAA levels. Finally, IAA can be degraded via decarboxylative and oxidative pathways. In conclusion, it appears that the metabolic processes of biosynthesis, conjugation, and degradation allow plant cells to maintain precise homeostatic regulation of intracellular IAA levels (Ljung *et al.*, this issue).

Sztein *et al.* (1995, 1999, 2000) performed a comprehensive survey of IAA metabolism in the major divisions of land plants. This survey was undertaken in large part because the data available from the earlier literature were so flawed that they could not be used to devise plausible hypotheses about evolutionary patterns in IAA metabolism (for discussion, see Sztein *et al.*, 1999). Consequently, Sztein *et al.* (1995, 1999, 2000) employed similar growth conditions for all plants, excised comparable thallus or shoot tips, employed common analytical techniques, and used antibiotic treatments or axenic cultures, whenever possible. Standard isotope dilution methods were used in conjunction with GC-MS to measure the steady-state concentrations of free IAA and IAA metabolites; thus, these measurements were virtually independent of sample size, purification method, and preparative losses. Thin-layer chromatography was used to measure the rate of IAA conjugate formation from radiolabeled IAA and to tentatively identify the chemical nature of these conjugates. It is worth mentioning that the requirement for enough material for GC-MS analysis meant that the apical tips were excised from gametophytic structures in charophytes and bryophytes as opposed to from sporophytic structures in vascular plants. The significance of this limitation for evolutionary interpretations will become apparent in the later section on IAA transport. The following sections are derived in part from Sztein *et al*. (2000).

Algae

Previous work on IAA levels in a few green, red and brown algae utilized non-axenic specimens (Jacobs *et al.*, 1985; Bradley, 1991; Evans and Trewavas, 1991; Ashen *et al.*, 1999; Basu *et al.*, pers. comm.). For example, free IAA levels in thalli of the red

alga *Prionitis lanceolata* were measured at 2.5 ng per gram fresh weight (Ashen *et al.*, 1999). Similar levels were also quantified in zygotes and fruiting tips of the brown alga *Fucus distichus* (Basu *et al.*, pers. comm.). However, Evans and Trewavas (1991) cautioned that one should remain skeptical about such measurements due to microbial contamination, which is routinely present in algal cultures. No data on the concentrations of IAA conjugates and other IAA metabolites were presented in these papers.

Since charophytes are considered to represent the closest algal relatives of the land plants, it is assumed that charophytes exhibit the primitive condition for the IAA metabolism of land plants. Under steady-state conditions, the thallus tips of the charophyte *Nitella* exhibited lower absolute levels of free IAA but higher ratios of free IAA to total IAA metabolites, as compared to most land plants (Tables 1 and 2). This alga was capable of only negligible rates of IAA conjugate synthesis in the presence of radiolabeled IAA. Instead, it appeared that exogenous IAA was predominantly converted into degradation products and/or other labeled metabolites synthesized from IAA. Therefore, charophytes, at least as exemplified by *Nitella*, must primarily regulate free IAA levels via the balance between the biosynthesis of new IAA molecules and the degradation of existing molecules. The nature of the IAA biosynthetic pathway has not yet been studied in charophytes although the non-tryptophan pathway is typically observed to maintain free IAA levels similar to those measured in *Nitella* tips.

Bryophytes

What makes the bryophytes so interesting from an evolutionary perspective is that each division, namely, liverworts, hornworts, and mosses, exhibits a distinctive IAA metabolism that may underlie the profound structural differences observed among these groups.

In liverworts, GC-MS analysis showed that thallus tips produced low to intermediate levels of free IAA, as well as high ratios of free IAA to total IAA metabolites, which were similar to the levels and ratios observed in charophytes (Tables 1 and 2). Judging from the results of tryptophan-feeding experiments with *Pallavicinia* tips, IAA biosynthesis in liverworts did not involve tryptophan as its primary intermediate. Liverworts can thus be said to utilize a tryptophan-independent biosynthetic pathway. In ^{14}C-IAA labeling experiments, liverworts tended to exhibit slow, but discernable, rates of IAA conjugate formation, with most species primarily synthesizing amide conjugates. The slow conjugation rates suggest that liverworts utilize a biosynthesis/degradation strategy for regulating free IAA levels, which means that charophytes and liverworts share the putative ancestral strategy for IAA metabolic regulation.

Thallus tips of the hornwort *Phaeoceros* produced high levels of free IAA, but the ratio of free IAA to total IAA metabolites was significantly lower than those observed in charophytes and liverworts (Tables 1 and 2). ^{14}C-IAA labeling studies demonstrated that hornworts can rapidly synthesize amide conjugates so that the regulation of free IAA levels appears to involve the competing reactions of conjugate synthesis vs. conjugate hydrolysis. Hornworts exhibited IAA conjugate levels comparable to those seen in the vascular plants. However, because IAA conjugates are chemically different in these two groups, it appeared that high conjugate levels had evolved independently at least twice in the land plants. No observations on the primary IAA biosynthetic pathway have been made for hornworts.

Vegetative tips of moss gametophores maintained much lower levels of free IAA and total IAA than did hornwort tips (Tables 1 and 2). Nevertheless, the free IAA to total IAA ratios were essentially identical in these two groups. Just like the hornworts, the mosses almost exclusively accumulated amide conjugates. Lastly, given the similar rates of IAA conjugate formation, it is reasonable to conclude that mosses must also use the conjugation/hydrolysis strategy as their principal mechanism for regulating free IAA levels. Earlier work on *Funaria* chloronema cultures and cell-free homogenates demonstrated that these preparations have the potential to use several intermediates in the tryptophan-dependent pathway for inducing IAA-mediated bud formation (Lehnert and Bopp, 1983; Bhatla and Bopp, 1985; Atzorn *et al.*, 1989), and to use exogenous ^{3}H-tryptophan to synthesize IAA (Jayaswal and Johri, 1985). The principal limitation of these approaches was that they do not compare the relative contributions of the tryptophan-independent and tryptophan-dependent pathways to IAA synthesis (for further discussion, see Sztein *et al.*, 2000). By contrast, the tryptophan-feeding method permitted a direct comparison of the relative contributions of the two pathways, and it showed that mosses use the tryptophan-independent pathway, at least in gametophore tips (Sztein *et al.*, 2000).

Table 1. Major characteristics of IAA levels and biosynthetic pathways operating in the vegetative tips of green plants (Sztein *et al.* 1999, 2000). The data for the angiosperms were taken from Baldi *et al.* (1991), Wright *et al.* (1991), Michalczuk *et al.* (1992a), and Normanly *et al.* (1993).

Plants	Number of species	Free IAA level		Total IAA metabolite level ng/g FW	Number of species	Predominant IAA biosynthetic pathway
		ng/g FW	%			
Charophytes	1	11	30	38	0	unknown
Liverworts	5	10–20	20–35	35–75	1	tryptophan-independent
Mosses	4	5–10	8–12	45–80	1	tryptophan-independent
Hornworts	1	35	11	328	0	unknown
Pteridophytes	2	25–35	10–20	100–400	1	tryptophan-independent
Angiosperms	4	10–20	5–10	400–700	4	tryptophan-independent

Table 2. Major characteristics of the IAA conjugation and IAA regulation in the vegetative tips of green plants (Sztein *et al.*, 1995, 1999, 2000).

Plants	Number of species	Major conjugates	Conjugation rate	Regulatory strategy
Charophytes	1	unknown	very slow	biosynthesis/degradation
Liverworts	7	amide conjugates	slow	biosynthesis/degradation
Mosses	5	amide conjugates	intermediate to rapid	conjugation/hydrolysis
Hornworts	1	amide conjugates	intermediate to rapid	conjugation/hydrolysis
Pteridophytes	10	IAsp/Glu and/or IAgluc	rapid	conjugation/hydrolysis
Seed plants	7	IAsp/Glu and/or IAgluc	very rapid	conjugation/hydrolysis

Vascular plants

Little attention has been granted to IAA metabolism in the pteridophyte grade, which includes lycophytes, horsetails, and ferns. Shoot tips of the lycophyte *Selaginella* and the fern *Ceratopteris* exhibited high IAA metabolite levels in proportions similar to those measured in hornworts (Tables 1 and 2). Judging from tryptophan-feeding experiments, the major pathway for IAA biosynthesis in *Selaginella* tips is the tryptophan-independent pathway. Pteridophytes can rapidly synthesize IAA conjugates so that these plants appear to maintain free IAA levels by adjusting the balance between conjugation and deconjugation. The pteridophytes are distinguishable from the bryophytes with respect to the ability of the pteridophytes to synthesize several characteristic conjugates, namely, the ester conjugate IAA-glucose and the amide conjugates IAA-asparate and/or IAA-glutamate.

The IAA metabolism of gymnosperms and angiosperms is thoroughly described in several recent reviews (Normanly, 1997; Bartel, 1997; Slovin *et al.*, 1999; Kowalczyk *et al.*, this issue). Pteridophytes and seed plants manifest only quantitative differences in their IAA metabolism (Tables 1 and 2). In general, seed plants produced the highest levels of total IAA metabolites, with exceptional levels of IAA-glucose, IAA-aspartate, and/or IAA-glutamate. Seed plants must also use the equilibrium between conjugation and hydrolysis for controlling free IAA levels.

Summary

The major features of auxin metabolism in green plants can be organized into an evolutionary framework by placing the features listed in Tables 1 and 2 on a simplified land plant cladogram (Figure 1), which represents a common, but not universally accepted land plant phylogeny deduced from recent molecular and morphological work (Kendrick and Crane, 1997; Qiu *et al.*, 1998; Duff and Nickrent, 1999; Nickrent *et al.*, 2000). It appears plausible that the tryptophan-independent pathway is the predominant class of IAA biosynthetic pathway in growing shoot tips in the

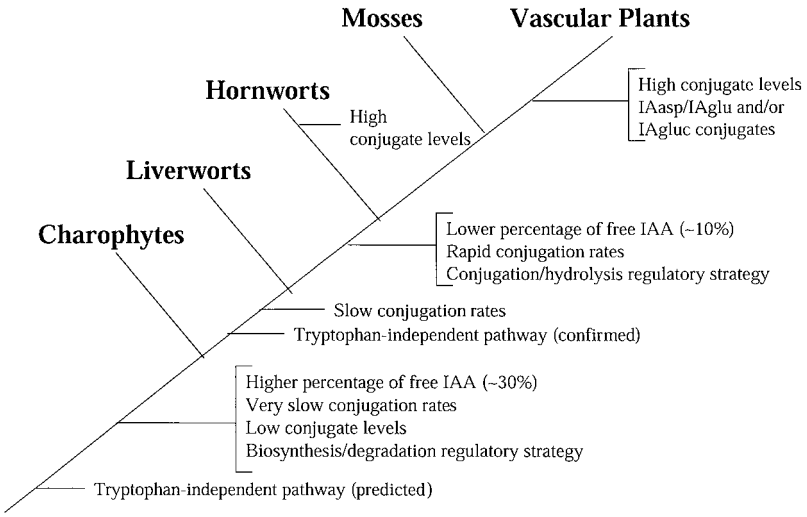

Figure 1. A character map of the major evolutionary events in IAA metabolism of green plants. The characters derived from Tables 1 and 2 are placed on a simplified version of a land plant cladogram in order to illustrate the likely positions of the major evolutionary events in IAA metabolism. Modified from Sztein *et al.* (2000) with permission.

charophytes and land plants. However, a major innovation in the IAA metabolic regulation occurred within the bryophyte grade. Liverworts have retained the putative ancestral strategy for regulating free IAA levels that depends on the biosynthesis of new IAA molecules and the degradation of existing molecules. The nested suite of allied characteristics seen in charophytes and liverworts included a higher percentage of free IAA, slow conjugation rates, and low levels of total IAA metabolites. The other bryophytes and vascular plants evolved the potential to regulate free IAA levels by adjusting the equilibrium between conjugate synthesis and conjugate hydrolysis. It is presumed that this one-step strategy permitted the more precise spatial and temporal regulation of free IAA levels.

Evolutionary patterns in IAA transport

Basic characteristics

IAA transport is characterized by its polarity, direction, distance, and transporting cells (Goldsmith, 1977; Lomax *et al.*, 1995). Most research attention has been devoted to polar IAA transport, which is defined as IAA movement in a specific, often basipetal direction. Polar IAA transport has been extensively studied in seed plants for several reasons: (1) it appears to be causally involved in the polarized growth observed in many plant structures, (2) it can easily be measured in plant explants with a simple apparatus, and (3) it is sensitive to several inhibitors that act on the IAA-efflux-carrier complex, such as *N*-(1-naphthyl)phthalamic acid (NPA) and 2,3,5-triiodobenzoic acid (TIBA). In angiosperms, NPA acts as a strong phytotropin in that it reduces the lateral IAA transport associated with tropic responses in addition to its antagonism of polar transport, while the inhibitory effects of TIBA are restricted to polar transport (Lomax *et al.*, 1995). Since NPA acts as competitive inhibitor of the binding of certain flavonoids such as quercitin, which are the presumptive *in vivo* inhibitors of polar IAA transport, it is generally thought that NPA inhibition of any process is a diagnostic indicator of the involvement of polar IAA transport (Jacobs and Rubery, 1988; Brown *et al.*, 2001). Although a thorough discussion of the mode of action of these inhibitors would exceed the scope of this review, recent work (Steinmann *et al.*, 1999) can be interpreted to mean that NPA may function as a general inhibitor of secretory processes as opposed to being a specific inhibitor of auxin transport per se. This review will follow the convention of using these inhibitors as diagnostic indicators of IAA transport, because it is premature to assess the validity of that new perspective.

According to the chemiosmotic model for polar IAA transport, electrochemical H^+ gradients across plasma membranes are the ultimate driving force for polar transport (Rubery and Sheldrake, 1974; Raven, 1975; Goldsmith, 1977). Apoplastic IAA in the cell

wall (pH 5) is envisaged to cross the plasma membrane either through passive diffusion of protonated IAA (pK_a 4.7) or via IAA influx carriers acting as proton symporters. Unprotonated IAA in the cytosol (pH 7) is transported back into the cell wall via IAA efflux carriers. The pronounced polarity of IAA transport is proposed to result from the asymmetric localization of IAA carriers at different ends of transporting cells. Recent molecular evidence has lent considerable support to this model (Estelle, 1998; Palme and Galweiler, 1999; Swarup *et al.*, 2000). Bennett *et al.* (1996) isolated a putative IAA influx carrier known as AUX1, which shares considerable homology with amino acid permeases. Immunolocalization studies suggest that influx carrier proteins are sometimes positioned at the apical ends of transporting cells (Swarup *et al.*, in press). Moreover, the IAA efflux carriers called PIN proteins, which exhibit significant sequence similarity to bacterial proteins responsible for transporting small molecules, are preferentially localized at the basal ends of transporting cells (Muller *et al.*, 1998; Steinmann *et al.*, 1999). Current knowledge of the molecular regulation of polar IAA transport in *Arabidopsis* is reviewed elsewhere (Galweiler and Palme, this issue).

A few reports on IAA transport can be found in the literature on lower plants. This research utilized conventional agar-block techniques to characterize IAA transport in multicellular structures. The polarity of IAA transport was routinely determined by switching the positions of the donor and receiver blocks and comparing the ratio of basipetal (apex to base) vs. acropetal (base to apex) transport at a selected time. In addition, flux experiments were conducted to quantify the rates of IAA transport across the plasma membranes in vesicle fractions, single cells, and small tissue pieces. In flux experiments where polar IAA transport inhibitors mediated an increase in net IAA accumulation, this result is interpreted to mean that the IAA efflux carriers were active in those systems. Although these experiments disclose the biochemical potential of a multicellular structure to transport IAA in a polar manner, the question of whether the intact structure carries out polar transport depends on whether the transport components are actually localized at specific intracellular sites.

Frankly, the entire literature on lower plants is unfortunately plagued by methodological problems and idiosyncratic units that prevent rigorous quantitative comparisons of IAA transport rates among different structures in different plants. However flawed the data on transport rates are, polarity ratios calculated from those data are, in general, independent of the methodologies employed. These ratios when considered with inhibitor studies allow us to discern qualitative differences in the abilities of various lower plant structures to carry out IAA transport (Tables 3 and 4).

Algae

In the brown alga *Fucus*, the addition of NPA to the medium mediated a 1.6-fold increase in accumulated IAA in the rhizoids, which can be taken to indicate that the IAA efflux carrier is present in brown algal rhizoids (Basu *et al.*, pers. comm.) (Table 3). Interestingly, NPA caused those rhizoids to undergo precocious branching, which differed from the multiple rhizoids observed in IAA treatments. These results suggested that NPA has developmental effects on *Fucus* rhizoids that cannot be attributed to the direct inhibition of IAA efflux alone.

Insofar as the charophytes are assumed to have retained the basal (or pleisiomorphic) state of the IAA transport system in the land plant lineage, it was hoped that the existing literature would have presented a definitive picture of IAA transport in the charophytes. Dibb-Fuller and Morris (1992) compared IAA transport capabilities of unicellular green alga *Chlorella* vs. thallus tips of the multicellular charophyte *Chara* (Table 3). It was concluded that IAA accumulation in *Chlorella* cells did not depend on specific IAA carriers, but rather appeared to involve the pH-sensitive diffusion of IAA across the plasma membrane. The apical portions of *Chara* thalli did exhibit saturable, i.e., carrier-dependent, IAA fluxes in both directions that were competitively inhibited by unlabeled IAA. IAA efflux was almost totally unaffected by NPA and TIBA, with the exception that the highest TIBA concentration caused a slight inhibition that was attributed to secondary pH effects. Surprisingly, other workers reported that decapitated *Chara* explants with growing rhizoids showed substantial net IAA accumulation in the presence of NPA (Klambt *et al.*, 1992). The most straightforward way to reconcile the results of these two papers is to assign the NPA effect to the possibility that an active NPA-sensitive IAA efflux carrier is localized in the rhizoids that are attached to the thallus explants used in Klambt *et al.* (1992). However, these authors observed that another NPA effect, namely the inhibition of rhizoid growth, was abolished by the coincidental application of IAA. Because this result suggested that NPA had additional effects unrelated

Table 3. A summary of flux experiments used to characterize transmembrane IAA fluxes and the potential for polar IAA transport in green plants. IAA accumulation increase was calculated as the ratio of the difference in net IAA accumulated in inhibitor vs. control experiments over the net IAA accumulated in control experiments times 100, as reported in the cited references.

Group	Species	Generation	Structure	Inhibitor	IAA accumulation increase (%)	References
Algae	*Fucus distichus*	sporophyte	zygotes	5×10^{-5} M NPA	58	Basu *et al.* (pers. comm.)
	Chorella vulgaris	gametophyte	unicells	3×10^{-6} M NPA	0	Dibb-Fuller and Morris (1992)
Charophytes	*Chara globularis*	gametophyte	thallus segments with rhizoids	1×10^{-4} M NPA	67	Klambt *et al.* (1992)
	Chara vulgaris	gametophyte	thallus	1×10^{-5} M NPA	0	Dibb-Fuller and Morris (1992)
				1×10^{-5} M TIBA	0	Dibb-Fuller and Morris (1992)
Mosses	*Funaria hygrometrica*	gametophyte	protonemal protoplasts	1×10^{-5} M NPA	32	Geier *et al.* (1990)
				1×10^{-5} M TIBA	29	Geier *et al.* (1990)
			protonemata	1×10^{-5} M TIBA	27	Rose *et al.* (1983)
			rhizoids	1×10^{-5} M TIBA	96	Rose and Bopp (1983)
Angiosperms	*Zea mays*	sporophyte	coleoptile	1×10^{-5} M NPA	140	Sussman and Goldsmith (1981)
			segments	1×10^{-5} M TIBA	64	Sussman and Goldsmith (1981)
	Cucurbita pepo	sporophyte	hypocotyl	1×10^{-5} M NPA	80	Hertel *et al.* (1983)
			vesicles	1×10^{-5} M TIBA	84	Hertel *et al.* (1983)

to its ability to increase intracellular IAA levels, further research is certainly needed to characterize IAA transport in the charophytes.

Bryophytes

The charophytes are capable of producing large haploid thalli, but zygotes represent the only diploid cells in their life cycles. One major innovation that occurred during bryophyte evolution is the origin of the embryo with its subsequent elaboration into the macroscopic sporophyte body. The bryophyte life cycle is thus said to consist of alternating haploid gametophytic and diploid sporophytic generations. Indeed, the embryo is frequently cited as the quintessential morphological adaptation of land plants, in large part because a multicellular diploid generation is much better suited to produce abundant meiospores for aerial dispersal in terrestrial environments (Graham, 1993; Taylor and Taylor, 1993; Kendrick and Crane, 1997; Niklas, 1997). Given this evolutionary history, it is important in this review to distinguish between the nature of IAA transport in gametophytes vs. sporophytes of different bryophyte groups.

Almost all the published work on IAA transport in bryophytes has focused on the gametophyte generation (Tables 3 and 4). In thallus midribs of the liverwort *Marchantia*, the initial front of labeled IAA moved at similar rates in the basipetal and acropetal directions (Maravolo, 1976; Gaal *et al*., 1982). However, the polarity ratio of total transported IAA (i.e., basipetal transport over acropetal transport) exceeded 5.0. Basipetal transport was significantly inhibited by a median ring of TIBA-containing lanolin ring around the thallus explant (Maravolo, 1976), which provided further evidence that liverwort gametophytes can carry out typical polar IAA transport. This interpretation is consistent with other observations that anaerobic conditions and metabolic inhibitors could suppress basipetal transport in liverwort thalli (Gaal *et al.*, 1982). No information concerning IAA transport in liverwort rhizoids has been published in the literature.

In protonemata of the moss *Funaria* grown under low light, high levels of applied IAA appeared to saturate IAA uptake, and TIBA mediated a 27% increase in net IAA accumulation (Rose *et al.*, 1983). These observations demonstrated that this stage of moss gametophyte development produces both influx and efflux carriers associated with polar IAA transport. Identical conclusions were drawn from protoplasts isolated from *Funaria* protonemata grown under similar light conditions (Geier *et al.*, 1990). By contrast, both characteristics of saturable influx and inhibitor sensitivity were not observed in high-light-grown protonemata, which was attributed to the possibility that such pro-

Table 4. A summary of agar-block experiments used to characterize polar IAA transport in green plants. Polarity (B/A) ratio was calculated as the ratio of the basipetal transport over the acropetal transport rates reported in the cited reference. Transport inhibition was calculated as the ratio of the difference in basipetal transport rates in control vs. inhibited structures over the basipetal rate in control structures times 100.

Group	Species	Generation	Structure	Polarity (B/A) ratio	Inhibitor	Transport inhibition (%)	References
Liverworts	*Marchantia polymorpha*	gametophyte	thallus	5.3	10^{-3} M TIBA	62	Maravolo (1976); Gaal *et al.*, 1982
	Pellia epiphylla	sporophyte	seta	0.9–1.1	10^{-5} M NPA	0	Thomas (1980); Poli *et al.* (unpub. obs.)
Hornworts	*Phaeoceros pearsoni*	sporophyte	immature sporangium	0.9	10^{-5} M NPA	0	Poli *et al.* (unpub. obs.)
Mosses	*Funaria hygrometrica*	gametophyte	rhizoid	11.4	10^{-4} M TIBA	54	Rose and Bopp (1983)
	Polytrichum ohioense	sporophyte	seta	9.1	10^{-5} M NPA	11	Poli *et al.* (unpub. obs.)
Pteridophytes	*Selaginella willenovi*	sporophyte	stem	2.1	None applied	–	Wochok and Sussex (1973)
	Osmunda cinnamomea	sporophyte	rachis	190.0	None applied	–	Steeves and Briggs (1960)
	Regnellidium diphyllum	sporophyte	rachis	52.0	None applied	–	Walters and Osborne (1979)
Angiosperms	*Zea mays*	sporophyte	coleoptile	609.3	10^{-5} M NPA	99	Poli *et al.* (unpub. obs.)
	Cucurbita pepo	sporophyte	hypocotyl	20.0	10^{-5} M TIBA	68	Jacobs and Hertel (1978)

tonemata might have higher endogenous IAA levels. In *Funaria* rhizoids, TIBA mediated a pronounced 96% increase in net IAA accumulation, which established that the inhibitor-sensitive IAA efflux carrier is also present in moss rhizoids (Rose and Bopp, 1983). Due to the multicellular nature of moss rhizoids, these authors were able to confirm that moss rhizoids carry out polar IAA transport with a polarity ratio of 11.4.

Because polar IAA transport is an important mechanism for regulating developmental events in the sporophytes of seed plants (for references, see Introduction), our lab decided to examine IAA transport in bryophyte sporophytes in the attempt to gain some insights into the evolutionary origins of IAA transport in land plant sporophytes (Poli *et al.*, unpublished observations). Bryophyte sporophytes are especially intriguing because they undergo polarized growth to form linear tripartite axes (Bold *et al.*, 1987; Crum, 2001). Both liverwort and moss sporophytes develop an apical capsule, a basal foot, and an intermediate seta, although developmental processes responsible for generating these sporophytes are strikingly different in the two groups. The hornwort sporophyte consists of an elongated apical sporangium and a basal foot with an intervening intercalary meristem that divides to generate new sporangial cells throughout sporophytic growth.

In liverworts, elongation rates of IAA-treated setae were more than twice the rates observed in control setae (Schnepf *et al.*, 1979; Thomas, 1980). In addition, the anti-auxin PCIB caused marked reductions in elongation rates of *Pellia* setae, which suggests very strongly that seta elongation is principally regulated by endogenous IAA under normal conditions. Nevertheless, agar-block studies involving long-term equilibration (Thomas, 1980) and repeated sampling (Poli *et al.*, unpublished observations) demonstrated that IAA must diffuse through developing setae, because these workers found no evidence for either transport polarity or NPA sensitivity (Table 4). In the hornwort *Phaeoceros*, short segments of immature sporangia that were cut from just above their intercalary meristems maintained a slight, non-significant, polarity ratio favoring acropetal transport that was completely resistant to NPA (Table 4). The only structures from bryophyte sporophytes capable of measurable polar IAA transport were young setae of the moss *Polytrichum*, which manifested a polarity ratio favoring basipetal transport of 9.1 (Table 4). Surprisingly, polar IAA transport in this moss was only slightly inhibited by NPA, in marked contrast to its strong inhibition of polar IAA transport in seed plants (Lomax *et al.*, 1995).

Vascular plants

Although there are numerous reports of polar IAA transport in coleoptiles, stems, roots, and other organs

of seed plants (for reviews, see Goldsmith, 1977; Lomax *et al.*, 1995), we were able to locate just four reports on IAA transport in pteridophytes (Table 4). Stem segments from the lycophyte *Selaginella* carried out both basipetal and acropetal transport, with a polarity ratio approaching 2.1 (Wochok and Sussex, 1973). Albaum (1938) demonstrated that IAA could move from the apex toward the base in the gametophytes of the fern *Pteris*, but he did not measure the potential for acropetal transport so that it is unknown if IAA transport in this gametophyte was preferentially basipetal. IAA transport was strongly polar in the elongating rachis of the leaves from the ferns *Osmunda* (Steeves and Briggs, 1960) and *Regnellidium* (Walters and Osborne, 1979).

Summary

The known features of polar IAA transport in green plants can be organized into an evolutionary framework by placing the features listed in Tables 3 and 4 on a simplified land plant cladogram (Figure 2), as was done for IAA metabolism in Figure 1. However, for several reasons, Figure 2 should be considered as being provisional. Only a few species represent each lineage in Figure 2; in particular, it includes the observations from two liverworts, one hornwort, and two mosses for the entire bryophyte grade. Furthermore, the limited work available on the Charales means that the origins of transport characteristics must be assigned rather uncertain positions at the base of this cladogram. Keeping these provisions in mind, one can still speculate about broad evolutionary patterns in polar IAA transport, which can, in turn, serve as testable hypotheses for future research. The molecular potential for IAA transport, i.e., IAA influx and efflux carriers, was apparently already present in the charophyte lineage before the divergence of the Charales. The capacity for polar auxin transport in the Charales, at least as judged by the sensitivity to efflux-carrier inhibitors, is apparently restricted to the rhizoids. Thus, there is no present evidence to support the notion that polar IAA transport might be involved in the generation of the branched *Chara* thallus. By contrast, polar IAA transport occurs in all structures of land plant gametophytes tested to date so that it becomes plausible to assert that polar transport may have helped to regulate the development of the gametophytes of the earliest land plants. By contrast, judging from the extant bryophyte lineages, the primitive bryophytes evolved various developmental mechanisms for producing axial sporophytes. Only mosses can apparently exploit polar IAA transport as the mechanism for regulating the axial growth of their sporophytes. Although it is appealing to hypothesize that this developmental mechanism evolved in the common ancestor of the moss and vascular plant lineages, the alternative explanation of independent origins in both lineages can not be eliminated by the limited observations available in the literature.

Evolutionary patterns in IAA regulation of plant development

The lower-plant literature contains numerous papers on the developmental effects of IAA; synthetic auxin analogues such as 2,4-dichlorophenoxyacetic acid (2,4-D) and naphthaleneacetic acid (NAA), and so-called anti-auxins such as *p*-chlorophenoxyisobutyric acid (PCIB) and 2,4,6-trichlorophenoxyacetic acid (2,4,6-T) dating back to the 1930s. This literature is typically surveyed in occasional reviews devoted to specific groups, as noted above. In addition, these reviews tend to focus on the hormonal regulation of developmental processes that are unique to that specific group such as the chloronema-to-caulonema transition and subsequent bud formation in mosses (Bopp, 1980; Cove and Ashton, 1984; Bhatla *et al.*, 1996; Christianson, 1999). The present review will instead describe the roles that IAA plays in several developmental processes that occur throughout the land plant lineage. These widespread IAA responses are somewhat arbitrarily separated into IAA responses involving tropisms, correlative interactions, and positional relationships. The following text is thus not intended as an exhaustive review of all IAA responses in non-seed plants, but instead cites selected papers in order to provide a general overview of certain IAA responses.

IAA responses involving tropisms

In the early land plants, novel physiological processes are thought to have evolved as specific adaptations to the localized distributions of essential resources and to the non-buoyant atmosphere found in the terrestrial environment. One topic of considerable interest to this paper is the evolution of tropistic growth responses that can serve to orient multicellular plant structures relative to various environmental stimuli such as light and gravity. Multicellular algae tend not to exhibit

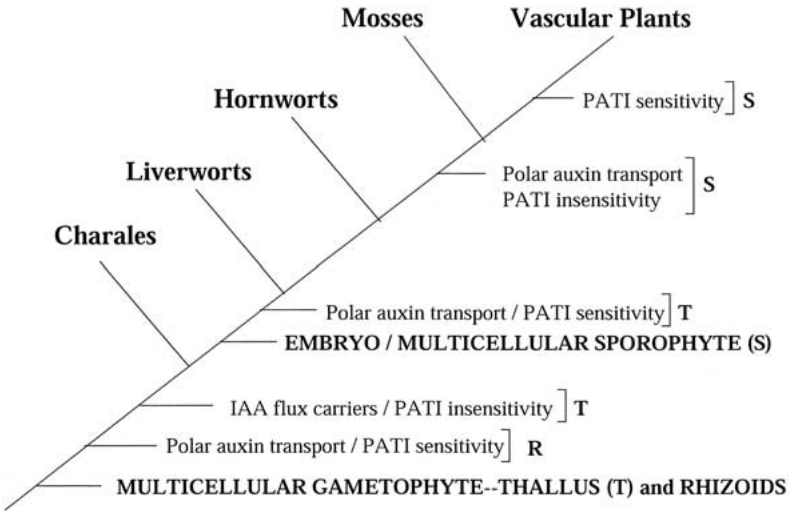

Figure 2. A provisional character map of the major evolutionary events in IAA transport in green plants. The characters derived from Tables 3 and 4 are placed on a simplified version of a land plant cladogram in order to illustrate the provisional positions of the major evolutionary events in IAA transport. The character of polar IAA transport is recorded for those plants that exhibit polar transport in agar-block experiments and/or significant sensitivity to polar transport inhibitors (PATI) in flux experiments. The data on PATI sensitivity is based on the responses to at least one inhibitor. Not enough lower plant structures have been exposed to PATIs to discriminate between NPA and TIBA sensitivities at this time. The letters R, T, and S refer to the first appearance of the bracketed traits in the gametophytic rhizoids, gametophytic thallus, and sporophyte, respectively. For further discussion of this figure, see its description in the text.

tropistic responses but rather they employ buoyancy mechanisms involving air bladders that cause the algae to assume upright orientations in the water column toward the light (Bold and Wynne, 1985). In filamentous charophycean algae and in land plant protonemata, the entire tropic response, including environmental perception, signal transduction, and directional growth, occurs within individual cells (Wada and Kadota, 1989; Sievers *et al.*, 1996; Braun, 1997; Staves, 1997). The evidence reported in those papers indicates that these plants do not use IAA as the intracellular signal for either phototropism or gravitropism.

However, in multicellular plant structures, IAA was apparently recruited early in land plant evolution to serve as the intercellular signal connecting apical cells best positioned for environmental perception to subapical cells most capable of undergoing rapid directional growth. For instance, several workers have investigated the phototropic responses of *Pellia* setae (for review, see Thomas, 1980). Ellis and Thomas (1985) demonstrated that shaded sides of these setae became more acidic prior to the onset of phototropic curvature. This acid efflux and the subsequent curvature were inhibited by both neutral buffers and IAA antagonists. These observations are consistent with the interpretation that seta phototropism is mediated by lateral IAA transport resulting in enhanced proton efflux and wall loosening on the shaded side. This mechanism appears identical to the mechanism proposed to operate in seed plants (Kaufman *et al.*, 1995), with the intriguing exception that the polar transport inhibitor TIBA blocks seta phototropism in *Pellia* (Ellis and Thomas, 1985) but has no apparent effect on higher plant phototropism (Lomax *et al.*, 1995). In the stems of the lycophyte *Selaginella* (Bilderback, 1984), directional light induced asymmetric IAA accumulation resulting in marked phototropic curvatures. Moreover, the localized application of IAA on either side of *Selaginella* stems mediated tropic curvature independent of the light conditions. TIBA could also block the phototropic responses of these stems. Thus, it can reasonably be concluded that lateral IAA gradients act as the common intercellular signaling mechanism for phototropic responses throughout the land plant lineage; however, the differential sensitivity to transport inhibitors implies that the regulation of these gradients may differ in non-seed vs. seed plants.

IAA responses involving correlative interactions

Among the charophytes, the Charales develop the most complex bodies, which are composed of a shoot-like main axis consisting of alternating single-celled internodes and multicellular nodes bearing lateral secondary branches in a regular pattern. In many species,

these secondary branches resemble miniaturized reiterations of the main axis. Thus, given regular morphological patterns of charophyte thalli, it is not unexpected that these algae do not tend to express correlative interactions where one plant part affects the growth of another part.

If one defines apical dominance as the ability of IAA from a growing region to maintain the quiescence of preexisting meristematic regions and/or to inhibit the formation of new meristematic regions, then it follows that this IAA response must also have arisen early in the evolution of land plants. For example, considerable evidence suggests that IAA regulates apical dominance and/or equivalent phenomena in liverworts. Exogenous IAA was observed to suppress the growth of isolated vegetative propagules known as gemmae in several species of liverworts, which led to the hypothesis that IAA transported from the parent thallus blocks the germination of gemmae still attached to that thallus (LaRue and Narayanaswami, 1957; Maravolo and Voth, 1966; Stange, 1971). Davidonis and Munroe (1972) presented surgical evidence that IAA transported from the larger branch of *Marchantia* thalli inhibited the growth of adjacent smaller branches. Several instances of genuine apical dominance were reported for the leafy gametophores of mosses. For example, in *Splachnum*, decapitating gametophore apices resulted in vigorous growth of reactivated lateral buds (MacQuarrie and von Maltzahn, 1959). The application of IAA to the cut apex suppressed bud activation, whereas kinetin overcame the IAA inhibition of bud activation (von Maltzahn, 1959), in a manner very similar to the hormonal regulation of apical dominance observed in seed plants (Tamas, 1995). When lanolin rings containing the polar transport inhibitor TIBA were placed around intact *Plagiomnium* gametophores, bud activation occurred below the rings, as might be expected from reduced IAA transport to those buds (Nyman and Cutter, 1981). However, the simultaneous application of IAA and a cytokinin was required to suppress all microscopic indications of bud activation in this moss. Applied IAA was also seen to block the development of quiescent lateral buds below decapitated shoots in several ferns (Wardlaw, 1946), but its effects were less pronounced in another fern, *Davallia* (Croxdale, 1976). Using ELISA techniques, Pilate *et al.* (1989) attempted to quantify the levels of IAA and several cytokinins in growing, quiescent, and activated buds of the fern *Marsilea*. In general, growing buds exhibited much higher hormone levels than quiescent buds. Interestingly, the relative ability of lateral buds to become activated upon apical bud removal was directly correlated with the measured levels of inactive cytokinin precursors in those buds. It was hypothesized that these precursors are metabolized into active molecules as an initial step in bud activation (Pilate *et al.*, 1989).

In conclusion, because IAA and cytokinins act as the primary regulators of apical dominance in seed plants (Tames, 1995), the evidence from bryophytes and pteridophytes suggests that this hormonal regulation of apical dominance is widespread in the land plant lineage.

IAA responses involving positional relationships

Each division of land plants can be said to exhibit a characteristic body plan based on such organizational features as axial polarity, embryo structure, meristematic activity, vascular tissue organization, positional relationships among vegetative organs, and positional relationships of reproductive organs on vegetative organs (Bold *et al.*, 1987; Gifford and Foster, 1989). The evidence cited in the Introduction demonstrates that IAA acts as an important regulator of the body plans of seed plants. The limited research available on non-seed plants hints that IAA may also help to regulate the body plans of these plants, at least with respect to the positions of absorptive structures and the differentiation of vascular tissue.

Insofar as IAA mediates increased rhizoid formation on cut sections of *Chara* thalli (Sievers and Schröter, 1971), it appears that this particular IAA response evolved prior to the evolution of land plants. It should therefore be expected that IAA was frequently observed to control the amount and sites of rhizoid initiation in the gametophytes of liverworts (e.g., Kaul *et al.*, 1962; Maravolo and Voth, 1966; Stange, 1977; Kumra and Chopra, 1987), mosses (e.g., Nyman and Cutter, 1981; Chopra and Vashistha, 1990), and pteridophytes (e.g., Haupt, 1957; Kato, 1957; Hickok and Kiriluk, 1984).

The more interesting question is whether or not the IAA response system responsible for rhizoid induction in land plant gametophytes was co-opted in early vascular plant evolution to regulate root initiation. The evidence available from extant pteridophytes is consistent with the hypothesis that endogenous IAA exercises primary control over lateral root initiation in these plants, much like its effects in seed plants. In ferns, IAA mediated the formation of both lateral roots on excised *Pteridium* roots (Partanen and Partanen,

1963) and adventitious roots on *Matteuccia* rhizomes (Ma and Steeves, 1992). In *Selaginella*, leafless cylindrical axes called rhizophores emerge at the sites of shoot branching. Although a discussion of the morphological nature of the rhizophore extends far beyond the scope of this review (see Lu and Jernstedt, 1996), the formation of *Selaginella* roots via either direct rhizophore transformation or endogenous root initiation was greatly enhanced by the application of IAA and the IAA analogue indole-3-butyric acid (Williams, 1937; Webster, 1969; Wochok and Sussex, 1975). Furthermore, TIBA induced all lateral meristems to develop as shoots (Wochok and Sussex, 1975). It is expected that recent advances in the molecular regulation of lateral root formation in *Arabidopsis* (e.g., Casimiro *et al.*, 2001) may soon provide the opportunity to address the issue of whether the IAA response genes for root formation were co-opted from the preexisting system for regulating rhizoid formation or from another IAA response system.

Given that IAA is the primary hormone for regulating vascular tissue development in seed plants (for references, see Introduction), it is surprising that this topic has rarely been investigated in non-seed vascular plants, namely the pteridophytes. For instance, Steeves and Briggs (1960) did observe that IAA, when applied in place of excised pinnae, was sufficient to mediate the final stage of xylem maturation in *Osmunda* leaves. Moreover, the limited research available suggests that IAA may also help to regulate the patterning of vascular bundles in pteridophytes, with some intriguing features that require further investigation. Ma and Steeves (1992) studied IAA effects on the primary vascular tissues in *Matteuccia* stems, which are typically arranged as a dictyostele composed of isolated vascular bundles. Suppressing leaf primordia on these apices resulted in the formation of additional vascular tissue so that the vascular tissue was organized as a complete ring or siphonostele in the underlying stem (Ma and Steeves, 1992). The application of IAA-soaked beads to suppressed apices caused the stele to exhibit parenchymatous leaf gaps similar to those formed in the dictyosteles of untreated plants. Thus, while IAA acted to reduce the total amount of vascular differentiation in the suppressed apices of this fern, these experiments could also be interpreted to suggest that IAA was involved in the positioning of the remaining vascular bundles, in a manner reminiscent of its ability to position primary vascular tissues in seed plants (Sachs, 1991; Berleth *et al.*, 2000). Nothing is known about the hormonal regulation of the simple vascular tissues observed in certain bryophytes (Hébant, 1977; Ligrone *et al.*, 2000).

Discussion

IAA is an ancient signaling molecule

The presence of IAA responses in both the charophyte *Chara* and the brown alga *Fucus* suggests that IAA is an ancient signaling molecule in photosynthetic aquatic organisms. One can imagine that a prototypical IAA effect might be similar to the group effect of *Fucus* rhizoids where IAA appears to act as the pheromone that coordinates the mutual attraction of the rhizoids emerging from adjacent zygotes (Jaffe, 1968). IAA does indeed exhibit several features that could conceivably make it predisposed to serve as a pheromone for communicating among small multicellular aquatic organisms. IAA is a small organic molecule with a pK_a of 4.7, which means that it has the potential for rapid diffusion in aquatic environments and across cell membranes. Its deprotonated form is soluble at low concentrations in aquatic environments with typical pH values. It becomes protonated in acidic cell walls and, thus, it can readily diffuse across adjacent cell membranes. Due to its structural similarity to tryptophan, IAA has some inherent affinity for pre-existing amino acid transporters. It seems quite reasonable to hypothesize that natural selection might have therefore favored the evolution of generalized amino-acid transporters into more specific IAA transporters. Indeed, the IAA influx carrier AUX1 in *Arabidopsis* has apparently evolved from an amino acid permease (Bennett *et al.*, 1996). Finally, IAA can easily be synthesized as a product of amino acid degradation. (Clearly, once IAA was adopted as a signaling molecule, it became advantageous to control its biosynthesis in a more regulated manner, which might have provided the selection pressure favoring the evolution of the tryptophan-independent pathway for synthesizing IAA.)

It is thus plausible to speculate that IAA had originally evolved in photosynthetic aquatic organisms to serve as a pheromone for regulating the growth of nearby members of the same species. What is unexpected, or perhaps even shocking, is that the rhizoids of *Chara* and *Fucus* exhibit similar IAA regulation and inhibitor sensitivities. Such similarity can be attributed to either a common origin or convergent evolution. In the case of a common origin, it must be appreciated that the viridiplantae and heterokont lineages,

which ultimately gave rise to charophytes and brown algae, respectively, separated as unicellular flagellates in deep evolutionary time (Baldauf *et al.*, 2000), as described in the Introduction. It seems unlikely that the molecular architecture for an IAA response system for rhizoid regulation would have been functioning in ancestral unicellular flagellates prior to the evolutionary divergence of the viridiplantae and heterokont lineages over a billion years ago. Given the closer phylogenetic relationship between the viridiplants and the red algae (Baldauf *et al.*, 2000), another remote possibility is that the IAA response system was transferred from the red algal lineage into the heterokont lineage during the secondary endosymbiosis that created the brown algal lineage (Delwiche, 1999; Grzebyk *et al.*, pers. comm.). In the case of convergent evolution, it is possible to construct a plausible argument that the chemical properties of IAA mentioned above favored its independent co-optation as a signaling molecule in different lineages. Molecular characterization of the IAA response systems in the brown, red (if present), and green algae will be necessary to distinguish among the alternative explanations of common origin via direct transmission, common origin via secondary endosymbiosis, and convergent evolution.

IAA as an ancient regulator of green plant development

It must first be acknowledged that we have a rather sketchy picture of IAA regulation of developmental processes in several basal groups in the land plant lineage. In particular, the only developmental research published on the charophytes studied the ability of IAA to promote rhizoid initiation in *Chara*; moreover, no research has been done on IAA regulation of hornwort development. Nevertheless, what emerge from the present survey are several surprising perspectives about the evolutionary patterns in auxin action in green plants. One, the entire land plant lineage (from charophytes to angiosperms) exhibits the same fundamental metabolic and transport mechanisms for regulating intracellular IAA levels, with the only significant differences being the increasing regulatory sophistication in IAA metabolism (e.g., conjugation reactions) and IAA transport (e.g., inhibitor sensitivity) observed in certain bryophytes and all vascular plants. Two, several IAA responses, such as tropisms and apical dominance, are seen to play critical roles in the development of bryophytes, pteridophytes, and seed plants. The evidence gathered from the bryophytes suggests that their tropistic responses involve an asymmetric IAA distribution across the stimulated organs and that their apical dominance results from IAA secretion from the apical meristem. Thus, it appears that both these responses depend on the same regulatory mechanisms in all land plants. Moreover, IAA appears to mediate root initiation and vascular tissue differentiation in the pteridophytes via regulatory mechanisms that are reminiscent of those operating in the seed plants. In summary, it is quite plausible that the seed plants did not evolve *de novo* mechanisms for mediating IAA responses, but have rather modified pre-existing mechanisms already present in the early land plants.

Future prospects for studying the molecular evolution of IAA action

But an important question remains: how does one reconcile the fundamental commonality of IAA action observed throughout the charophyte and land plant lineage with the increasing morphological complexity expressed in this lineage? The tentative answer to that question comes from the new field of evolutionary developmental genetics (Raff, 1996). Its insights into how genetic changes in developmental mechanisms seem to underlie the evolution of animal body plans are truly amazing, at least to most plant biologists gazing in awe over the kingdom boundary.

In essence, the primary working hypothesis in evolutionary developmental genetics is that certain genes responsible for regulating developmental processes in simple organisms experienced repeated duplication and altered transcriptional regulation, with the result that these genes were able to specify more complex body plans. Of course, the paradigmatic example for the genetic regulation of animal body plans involves a conserved group of homeobox (*Hox*) genes known as the *Hox* clusters that encode transcription factors involved in diverse developmental processes (Erwin *et al.*, 1997; Valentine *et al.*, 1999; Knoll and Carroll, 1999; Peterson and Davidson, 2000). It is proposed that a single 'primordial' *Hox* gene present in ancient sponges underwent a series of duplication events that resulted in two *Hox* clusters arising in the basal bilateral animal group in the early Cambrian period. During the Cambrian radiation, rapid diversification and altered regulation of these genes is thought to have greatly contributed to the evolution of the different body plans of bilateral animals.

In plants, initial work on the molecular evolution of developmental control genes is beginning to yield similar interpretations. For instance, the diversity of the MADS-box gene family encoding another group of eukaryotic transcription factors appears to correlate with morphological complexity during plant evolution (Theissen *et al.*, 2000). Two MADS-box genes have been isolated from the moss *Physcomitrella* (Krogan and Ashton, 2000) in contrast to 15 genes from the fern *Ceratopteris* and to even larger numbers from several angiosperm species (Theissen *et al.*, 2000) . The ability of *Physcomitrella* to perform homologous recombination should allow these investigators to determine the precise roles of the MADS-box genes in the mosses (Theissen *et al.*, 2001). Of particular importance to angiosperm development are the MADS-box gene subfamilies that act as homeotic selector genes for controlling floral organ identity. On the other hand, although certain other MADS-box genes are seen to regulate various developmental processes in angiosperms, it is not clear at present whether they are involved in the fundamental organization of the angiosperm body plan. Finally, the correlation between gene family size and morphological complexity is also manifested by the actin gene family that encodes one of the major cytoskeletal proteins in plants (Bhattacharya *et al.*, 2000). These authors do, however, note that the actin gene family may be more useful for investigating the mechanisms of gene duplication in green plants as opposed to for elucidating the relationship between gene duplication and morphological complexity.

Aside from the research on the MADS-box genes acting in floral development, current work on the evolutionary significance of plant MADS-box genes is largely focused on the effort to assign developmental functions to these putative regulatory genes. Plant biologists interested in the molecular evolution of IAA action face the opposite problem: we know that IAA responses are critical to the overall organization of the body plans of different land plants, but we must now start to characterize the gene families responsible for regulating those responses throughout the plant lineage. Given the increasing complexity observed in the gene families described above, it seems quite conceivable that gene families involved in IAA action may also be composed of one or a few genes in charophytes, with a progressive increase in the number of homologous genes from the early-divergent bryophytes to the late-divergent angiosperms. Just to cite one of many possible examples of IAA regulatory genes, the *Arabidopsis* genome contains 16 sequences that share considerable similarity to the AtPIN1 gene encoding the auxin efflux carrier. Although some of these sequences are certainly pseudogenes, a substantial number must be expressed as functional genes because they exhibit mutant phenotypes (Palme and Galweiler, 1999; Galweiler and Palme, this issue). Since there is no evidence to suggest that the charophytes use auxin gradients to establish their body plans, one might expect that the auxin efflux carrier reported to function in *Chara* thalli (Dibb-Fuller and Morris, 1992) is encoded only by a single PIN homologue. It follows from this prediction that the number of PIN gene family members should correlate with increasing IAA transport capability and morphological complexity observed in land plant lineages from charophytes to angiosperms. Similar arguments can be constructed for most other gene families involved in IAA regulation. As a final consideration, we anticipate that the characterization of the molecular evolution of IAA action will also provide considerable insight into the rapid diversification of early land plants during the late Silurian through middle Devonian periods, as is discussed elsewhere (Cooke *et al.*, 2001).

References

Albaum, H.G. 1938. Inhibition due to growth hormones in fern prothallia and sporophytes. Amer. J. Bot. 25: 124–133.

Aloni, R. 1995. The induction of vascular tissues by auxin and cytokinin. In: P.J. Davies (Ed.) Plant Hormones: Physiology, Biochemistry, and Molecular Biology, 2nd ed., Kluwer Academic Publishers, Dordrecht, Netherlands, pp. 531–546.

Ashen, J.B., Cohen, J.D. and Goff, L.J. 1999. GC-SIM-MS detection and quantification of free indole-3-acetic acid in bacterial galls on the marine red alga *Prionitis lanceolata* Harvey. J. Phycol. 35: 493–500.

Atzorn, R., Bopp, M. and Merdes, U. 1989. The physiological role of indole acetic acid in the moss *Funaria hygrometrica* Hedw. II. Mutants of *Funaria hygrometrica* which exhibit enhanced catabolism of indole-3-acetic acid. J. Plant Physiol. 135: 526–530.

Baldauf, S. L., Roger, A. J., Wenk-Siefert, I. and Doolittle, W.F. 2000. A kingdom-level phylogeny of eukaryotes based on combined protein data. Science 290: 972–977.

Baldi, B.G., Maher, B.R., Slovin, J.P. and Cohen, J.D. 1991. Stable isotope labeling, *in vivo*, of D- and L- tryptophan pools in *Lemna gibba* and the low incorporation of label into indole-3-acetic acid. Plant Physiol. 95: 1203–1208.

Bartel, B. 1997. Auxin biosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. 48: 51–66.

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.

Berleth, T., Mattsson, J., and Hardtke, C.S. 2000. Vascular continuity and auxin signals. Trends Plant Sci. 5: 387–393.

Bhatla, S.C. and Bopp, M. 1985. The hormonal regulation of protonemal development in mosses. III. Auxin-resistant mutants of the moss *Funaria hygrometrica* Hedw. J. Plant Physiol. 120: 233–243.

Bhatla, S.C., Kapoor, S., and Khurana, J.P. 1996. Involvement of calcium in auxin-induced cell differentiation in the protonema of the wild strain and auxin mutants of the moss *Funaria hygrometrica*. J. Plant Physiol. 147: 547–552.

Bhattacharya, D., Aubry, J., Twait, E.C. and Jurk, S. 2000. Actin gene duplication and the evolution of morphological complexity in land plants. J. Phycol. 36: 813–820.

Bialek, K., Michalczuk, L. and Cohen, J.D. 1992. Auxin biosynthesis during seed germination in *Phaseolus vulgaris*. Plant Physiol. 100: 509–517.

Bilderback, D.E. 1984. Phototropism of *Selaginella:* the role of the small dorsal leaves and auxin. Am. J. Bot. 71: 1330–1337.

Blakely, L.M., Blakely, R.M., Colowit, P.M. and Elliott, D.S. 1988. Experimental studies on lateral root formation in radish seedling roots. II. Analysis of dose-response to endogenous auxin. Plant Physiol. 87: 414–419.

Bold, H.C. and Wynne, M. J.1985. Introduction to the Algae. Prentice-Hall, Englewood Cliffs, NJ.

Bold, H.C., Alexopoulos, C.I. and Delevoryas, T. 1987. Morphology of Plants and Fungi, 5th ed. Harper & Row, New York.

Bopp, M. 1980. The hormonal regulation of morphogenesis in mosses. In: F. Skoog (Ed.) Plant Growth Substances 1979, Springer-Verlag, Berlin, pp. 351– 361.

Bradley, P.M. 1991. Plant hormones do have a role in controlling growth and development in algae. J. Phycol. 27: 317–321.

Braun, M. 1997. Gravitropism in tip-growing cells. Planta 203: S11–S19.

Brown, D.E., Rashotte, A.M., Murphy, A.S., Normanly, J., Tague, B.W., Peer, W.A., Taiz, L. and G.K. Muday. 2001. Flavonoids act as negative regulators of auxin transport *in vivo* in *Arabidopsis*. Plant Physiol. 126:524– 535.

Casimiro, I., Marchant, A., Bhalerao, R.P., Beeckman, T., Dhooge, S., Swarup, R., Graham, N., Inzé, D., Sandberg, G., Casero, P.J. and Bennett, M. 2001. Auxin transport promotes *Arabidopsis* lateral root initiation. Plant Cell 13: 843–852.

Chopra, R.N. and Vashistha, B.D. 1990. The effect of auxins and antiauxins on shoot-bud induction and morphology in the moss, *Bryum atrovirens* Will. ex Brid. Aust. J. Bot. 38: 177–184.

Christianson, M.L. 1999. Control of morphogenesis in bryophytes. In: A.J. Shaw and B. Goffinet (Eds.) Bryophyte Biology, Cambridge University Press, Cambridge, UK, pp. 199–224.

Cohen, J.D. and Bandurski, R.S. 1982. Chemistry and physiology of the bound auxins. Annu. Rev. Plant Physiol. 33: 403–430.

Cooke, T.J., Racusen, R.H. and Cohen, J.D. 1993. The role of auxin in plant embryogenesis. Plant Cell 5: 1494–1495.

Cooke, T.J., Poli, DB., Sztein, A.E. and Cohen, J.D. 2001. Evolutionary trends in auxin regulation and their potential role in the rapid diversification of land plants. Evolution of Plant Physiology Symposium Abstracts, Linnean Society, London, p. 12.

Cove, D.J. and Ashton, N.W. 1984. Hormonal regulation of gametophytic development in bryophytes. In: A.F. Dryer and J. F. Duckett (Eds.) The Experimental Biology of Bryophytes, Academic Press, London, pp. 177–201.

Croxdale, J. 1976. Hormones and apical dominance in the fern *Davallia*. J. Exp. Bot. 27: 801–815.

Crum, H. 2001. Stuctural Diversity of Bryophytes. University of Michigan Herbarium, Ann Arbor, MI.

Davidonis, G.H. and Munroe, M.H. 1972. Apical dominance in *Marchantia*: correlative inhibition of neighbor lobe growth. Bot. Gaz. 133: 177–184.

Delwiche, C.F. 1999. Tracing the thread of plastid diversity through the tapestry of life. Am. Nat. 154: S164–S177.

Dibb-Fuller, J.E. and Morris, D.A. 1992. Studies on the evolution of auxin carriers and phytotropin receptors: transmembrane auxin transport in unicellular and multicellular Chlorophyta. Planta 186: 219–226.

Dolan, L. 1998. Pointing roots in the right direction: the role of auxin transport in response to gravity. Genes Dev. 12: 2091–2095.

Duff, R.J. and Nickrent, D.L. 1999. Phylogenetic relationships of land plants using mitochondrial small-subunit rDNA sequences. Am. J. Bot. 86: 372– 386.

Eisen, J.A. 1998. Phylogenomics: improving functional predictions for uncharacterized genes by evolutionary analysis. Genomic Res. 8: 163–167.

Ellis, J.G. and Thomas, R.J. 1985. Phototropism of *Pellia*: evidence for mediation by auxin stimulated acid efflux. J. Plant Physiol. 121: 259– 264.

Erwin, D., Valentine, J. and Jablonski, D.. 1997. The origin of animal body plans. Am. Sci. 85: 126–137.

Estelle, M. 1998. Polar auxin transport: new support for an old model. Plant Cell 10: 1775–1778.

Evans, LV. and Trewavas, A.J. 1991. Is algal development controlled by plant growth substances? J. Phycol. 27: 322–326.

Gaal, D.J., Dufresne, S.J. and Maravolo, N.C. 1982. Transport of ^{14}C-indoleacetic acid in the hepatic *Marchantia polymorpha*. Bryologist 85: 410–418.

Geier, U., Werner, O. and Bopp, M. 1990. Indole-3-acetic acid uptake in isolated protoplasts of the moss *Funaria hygrometrica*. Physiol. Plant. 80: 584–592.

Gifford, E.M. and Foster, A.S. 1989. Morphology and Evolution of Vascular Plants, 3rd ed. Freeman, New York.

Goldsmith, M.H.M. 1977. The polar transport of auxin. Annu. Rev. Plant Physiol. 28: 439–478.

Graham, L.E. 1993. Origin of Land Plants. Wiley, New York.

Graham, L.E., Cook, M.E. and Busse, J.S. 2000. The origin of plants: body plan changes contributing to a major evolutionary radiation. Proc. Natl. Acad. Sci. USA 97: 4535–4540.

Haupt, W. 1957. Die Induktion der Polarität bei der Spore von *Equisetum*. Planta 49: 61–90.

Hébant, C. 1977. The Conducting Tissues of Bryophytes. J. Cramer, Vaduz.

Hertel, R., Lomax, T.L., and Briggs, W.R. 1983. Auxin transport in membrane vesicles from *Cucurbita pepo* L. Planta 157: 193–201.

Hickok, L.G. and Kiriluk, R.M. 1984. Effects of auxins on gametophyte development and sexual differentiation in the fern *Ceratopteris thalictroides* (L.) Brongn. Bot. Gaz. 145: 37–42.

Hickok, L.G., Warne, T.R., and Fribourg, R.S. 1995. The biology of the fern *Ceratopteris* and its uses as a model system. Int. J. Plant Sci. 156: 332–345.

Jacobs, M. and Hertel, R. 1978. Auxin binding to subcellular fractions from *Cucurbita* hypocotyls: *in vitro* evidence for an auxin transport carrier. Planta 142: 1–10.

Jacobs, M. and Rubery P.H. 1988. Naturally occurring auxin transport regulators. Science 241: 346–349.

Jacobs, W.P., Falkenstein, K. and Hamilton, R.H. 1985. Nature and amount of auxin in algae. IAA from extracts of *Caulerpa paspaloides* (Siphonales). Plant Physiol. 78: 844–848.

Jaffe, L.F. 1968. Localization in the developing *Fucus* egg and the general role of localizing currents. Adv. Morphogen. 7: 295–328.

Jayaswal, R.K. and Johri, M.M. 1985. Occurrence and biosynthesis of auxin in protonema of the moss *Funaria hygrometrica*. Phytochemistry 24: 1211– 1214.

Karol, K.G., McCourt, R.M., Cimino, M.T., and Delwiche, C.F. 2001. The closest living relatives to the land plants. Science 294: 2351–2353.

Kato, Y. 1957. The effects of colchicine and auxin on rhizoid formation of *Dryopteris erythrosora*. Bot. Mag. 70: 258–263.

Kaufman, P.B., Wu, L., Brock, T.G. and Kim, D. 1995. Hormones and their orientation of growth. In: P.J. Davies (Ed.) Plant Hormones: Physiology, Biochemistry and Molecular Biology, 2nd ed., Kluwer Academic Publishers, Dordrecht, Netherlands, pp. 547–571.

Kaul, K.N., Mitra, G.C. and Tripathi, B.K. 1962. Responses of *Marchantia* in aseptic culture to well-known auxins and antiauxins. Ann. Bot. 26: 447–467.

Kenrick, P. and Crane, P.R. 1997. The Origin and Early Diversification of Land Plants: A Cladistic Study. Smithsonian Institute Press, Washington, D.C.

Klambt, D., Knauth, B. and Dittmann, I. 1992. Auxin dependent growth of rhizoids of *Chara globularis*. Physiol. Plant. 85: 537–540.

Knoll, A.H. and Carroll, S.B. 1999. Early animal evolution: emerging views from comparative biology and geology. Science 284: 2129–2137.

Koshiba, T., Kamiya, Y. and Iino, M. 1995. Biosynthesis of indole-3-acetic acid from l-tryptophan in coleoptile tips of maize (*Zea mays*). Plant Cell Physiol. 36: 1503–1510.

Krogan, N.T. and Ashton, N.W. 2000. Ancestry of plant MADS-box genes revealed by bryophyte (*Physcomitrella patens*) homologues. New Phytol. 147: 505–517.

Kumra, S. and Chopra, R.N. 1987. Callus initiation, its growth and differentiation in the liverwort *Asterella wallichiana* (Lehm. et Lindenb.) Groelle. I. Effect of auxins and cytokinins. J. Hattori Bot. Lab. 63: 237–245.

LaRue, C.D. and Narayanswami, S. 1957. Auxin inhibition in the liverwort *Lunularia*. New Phytol. 56: 61–70.

Lehnert, B. and Bopp, M. 1983. The hormonal regulation of protonema development in mosses. I. Auxin-cytokinin interaction. Z. Pflanzenphysiol. 110: 379–391.

Ligrone, R., Duckett, J.G. and Renzaglia, K.S. 2000. Conducting tissues and phyletic relationships of bryophytes. Phil. Trans. R. Soc. Lond. Ser. B 355: 795–813.

Liu, C.-M., Xu, Z.-H. and Chua, N.-H. 1993. Auxin polar transport is essential for the establishment of bilateral symmetry during early plant embryogenesis. Plant Cell 5: 621–630.

Lomax, T.L., Muday, G.K. and Rubery, P.H. 1995. Auxin transport. In: P. J. Davies (Ed.) Plant Hormones: Physiology, Biochemistry, and Molecular Biology, 2nd ed., Kluwer Academic Publishers, Dordrecht, Netherlands, pp. 509–530.

Lu, P. and Jernstedt, J.A. 1996. Rhizophore and rood development in *Selaginella martensii*: meristem transitions and identity. Int. J. Plant Sci. 157: 180–194.

Lyndon, R.F. 1998. The Shoot Apical Meristem: Its Growth and Development. Cambridge University Press, Cambridge, UK.

Ma, Y. and Steeves, T.A. 1992. Auxin effects on vascular differentiation in Ostrich fern. Ann. Bot. 70: 277–282.

MacQuarrie, G. and von Maltzahn, K. 1959. Correlations affecting regeneration and reactivation in *Splachnum ampullaceum* (L.) Hedw. Can. J. Bot. 37: 121–134.

Maravolo, N.C. 1976. Polarity and localization of auxin movement in the hepatic, *Marchantia polymorpha*. Am. J. Bot. 63: 529–531.

Maravolo, N.C. 1980. Control of development in hepatics. Bull. Torrey Bot. Club 107: 308–324.

Maravolo, N.C. and Voth, P.D. 1966. Morphogenic effects of three growth substances on *Marchantia* gemmalings. Bot. Gaz. 127: 79–86.

Marchant, A., Kargul, J., May, S.T., Muller, P., Delbarre, A., Perrot-Rechenmann, C. and Bennett, M. J. 1999. AUX1 regulates root gravitropism in *Arabidopsis* by facilitating auxin uptake within root apical tissues. EMBO J. 18: 2066–2073.

Meicenheimer, R.D. 1981. Changes in *Epilobium* phyllotaxy induced by N-1-naphthylphthalmic acid and α-4-chlorophenoxyisobutric acid. Am. J. Bot. 68: 1139–1154.

Michalczuk, L., Cooke, T.J. and Cohen, J.D. 1992a. Auxin levels at different stages of carrot somatic embryogenesis. Phytochemistry 31: 1097– 1103.

Michalczuk, L., Ribnicky, D.M., Cooke, T.J. and Cohen, J.D. 1992b. Regulation of indole-3-acetic acid biosynthetic pathways in carrot cell cultures. Plant Physiol. 100: 1346–1353.

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. 17: 6903– 6911.

Nemhauser, J.L., Feldman, L.J. and Zambryski, P.C. 2000. Auxin and ETTIN in *Arabidopsi*s gynoecium morphogenesis. Development 127: 3877–3888.

Nickrent, D.L., Parkinson, C.L., Palmer, J.D. and Dugg, R.J. 2000. Multigene phylogeny of land plants with special reference to bryophytes and the earliest land plants. Mol. Biol. Evol. 17: 1885–1895.

Niklas, K.J. 1997. The Evolutionary Biology of Plants. University of Chicago Press, Chicago.

Nonhebel, H.M., Cooney, T.P. and Simpson, R. 1993. The route, control and compartmentation of auxin synthesis. Aust. J. Plant Physiol. 20: 527–539.

Normanly, J. 1997. Auxin metabolism. Physiol. Plant. 100: 431–442.

Normanly, J., Cohen, J.D. and Fink, G.R. 1993. *Arabidopsis thaliana* auxotrophs reveal a tryptophan-independent biosynthetic pathway for indole-3-acetic acid. Proc. Natl. Acad. Sci. USA 90: 10355–10359.

Nyman, L.P. and Cutter, E.G. 1981. Auxin-cytokinin interaction in the inhibition, release, and morphology of gametophore buds of *Plagiomnium cupidatum* from apical dominance. Can. J. Bot. 59: 750–760.

Palme, K. and Galweiler, L. 1999. PIN-pointing the molecular basis of auxin transport. Curr. Opin. Plant Biol. 2: 375–381.

Partanen, J.N. and Partanen, C.R. 1963. Observations on the culture of roots of the Bracken fern. Can. J. Bot. 41: 1657–1661.

Peterson, K.J. and Davidson, E.H. 2000. Regulatory evolution and the origin of the bilaterians. Proc. Natl. Acad. Sci. USA 97: 4430–4433.

Pilate, G., Sossountzov, L. and Miginiac, E. 1989. Hormone levels and apical dominance in the aquatic fern *Marsilea drummondii* A. Br. Plant Physiol. 90: 907–912.

Pryer, K.M., Schneider, H., Smith, A.R., Cranfill, R., Wolf, P.G., Hunt, J.S. and Sipes, S.D. 2001. Horsetails and ferns are a monoplyletic group and the closest living relatives to seed plants. Nature 409: 618–622.

Qiu, Y.-L., Cho, Y., Cox, J.C. and Palmer, J.D. 1998. The gain of three mitochondrial introns identifies liverworts as the earliest land plants. Nature 394: 671–674.

Qiu, Y.-L. and Lee, J. 2000. Transition to a land flora: a molecular perspective. J. Phycol. 36: 799–802.

Raff, R.A. 1996. The Shape of Life: Genes, Development, and the Evolution of Animal Form. University of Chicago Press, Chicago.

Rapparini, F., Cohen, J.D. and Slovin, J.P. 1999. Indole-3-acetic acid biosynthesis in *Lemna gibba* studied using stable isotope labeled anthranilate and tryptophan. Plant Growth Regul. 27: 139–144.

Raven, J.A. 1974. Transport of indoleacetic acid in plant cells in relation to pH and electrical potential gradients, and its significance for polar IAA transport. New Phytol. 74: 163–172.

Reed, R.C., Brady, S.R. and Muday, G.K. 1998. Inhibition of auxin movement from the shoot into the root inhibits lateral root development in *Arabidopsis*. Plant Physiol. 118: 1369–1378.

Reinhardt, D., Mandel, T and Kuhlemeier, C. 2000. Auxin regulates the initiation and radial position of plant later al organs. Plant Cell 12: 507– 518.

Reski, R. 1999. Molecular genetics of *Physcomitrella*. Planta 208: 301–309.

Ribnicky, D.M., Ilic, N., Cohen, J.D., and Cooke, T.J. 1996. The effects of exogenous auxins on endogenous indole-3-acetic acid metabolism. The implications for carrot somatic embryogenesis. Plant Physiol. 112: 549–558.

Ribnicky, D.M., Cohen, J.D., Hu, W.-S. and T.J. Cooke. 2001. An auxin surge following fertilization in carrots: a general mechanism for regulating plant totipotency. *Planta*, in press.

Roberts, L.W., Gahan, P.B. and Aloni, R.1988. Vascular Differentiation and Plant Growth Regulators. Springer-Verlag, Berlin.

Rose, S. and Bopp, M. 1983. Uptake and polar transport of indoleacetic acid in moss rhizoids. Physiol. Plant. 58: 57–61.

Rose, S., Rubery, P.H. and Bopp, M. 1983. The mechanism of auxin uptake and accumulation in moss protonemata. Physiol. Plant. 58: 52–56.

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

Sachs, T. 1991. Cell polarity and tissue patterning in plants. Development (Suppl.) 1: 83–93.

Schaefer, D.G. and Zryd, J.P. 1997. Efficient gene targeting in the moss *Physcomitrella patens*. Plant J. 11: 1195–1206.

Schiavone, F.M. and Cooke, T.J. 1986. Unusual patterns of somatic embryogenesis in the domesticated carrot: developmental effects of exogenous auxins and auxin transport inhibitors. Cell. Diff. 21: 53–62.

Schnepf, E., Herth, W. and Morre D.J. 1979. Elongation growth of setae of *Pellia* (Bryophyta): effects of auxin and inhibitors. Z. Pflanzenphysiol. 94: 211–217.

Sembdner, G., Gross, D., Liebisch, W. and Schneider, G. 1981. Biosynthesis and metabolism of plant hormones. In. J. MacMillan (Ed.) Hormonal Regulation of Plant Development. I. Molecular Aspects of Plant Hormones. Encyclopedia of Plant Physiology, Vol. 9, Springer-Verlag, Berlin, pp. 281–444.

Sessions, A., Nemhauser, J.L., McColl, A., Roe, J.L., Feldmann, K.A. and Zambryski, P.C. 1997. ETTIN patterns the *Arabidopsis* floral meristem and reproductive organs. Development 124: 4481–4491.

Sievers, A. and Schröter, K. 1971. Versuch einer Kausalanalyse der geotropischen Reaktionskette im *Chara*-Rhizoid. Planta 96: 339–353.

Sievers, A., Buchen, B. and Hodick, D. 1996. Gravity sensing in tip-growing cells. Trends Plant Sci. 1: 273–279.

Slovin, J.P., Bandurski, R.S. and Cohen, J.D. 1999. Auxin. In: P.J.J. Hooykaas, M.A. Hall and K.R. Libbenga (Eds.) Biochemistry and Molecular Biology of Plant Hormones, Elsevier Science, Amsterdam, pp. 115–140.

Stange, L. 1971. Effects of morphactins and of auxin on the formation of meristematic centres in *Riella helicophylla*. Ind. J. Plant Physiol. 14: 44–54.

Stange, L. 1977. Meristem differentiation in *Riella helicophylla* (Bory et Mont.) Mont. under the influence of auxin and antiauxin. Planta 135: 289–295.

Staves, M.P. 1997. Cytoplasmic streaming and gravity sensing in *Chara* internodal cells. Planta 203: S79–S84.

Steeves, T.A. and Briggs, W.R. 1960. Morphogenetic studies on *Osmunda cinnamomea* L. The auxin relationships of expanding fronds. J. Exp. Bot. 11: 45–67.

Steinmann, T., Geldner, N., Grebe, M., Mangold, S., Jackson, C. L., Paris, S., Galweiler, L., Palme, K. and Jurgens, G. 1999. Coordinated polar localization of auxin efflux carrier PIN1 by GNOM ARF GEF. Science 286: 316– 318.

Sussmann, M.R. and Goldsmith, M.H.M. 1981. The action of specific inhibitors of auxin transport on uptake of auxin and binding of N-1-naphthylphthalamic acid to a membrane site in maize coleoptiles. Planta 152: 13–18.

Swarup, R., Marchant, A. and Bennett, M.J. 2000. Auxin transport: providing a sense of direction during plant development. Biochem. Soc. Trans. 28: 481– 485.

Swarup, R., Friml, J., Marchant, A., Ljung, K., Sandberg, G., Palme, K. and Bennett, M. Localisation of the auxin permease AUX1 in the *Arabidopsis* root apex reveals two novel functionally distinct hormone transport pathways. Genes Dev., in press.

Sztein, A.E., Cohen, J.D., Slovin, J.P. and Cooke, T.J. 1995. Auxin metabolism in representative land plants. Am. J. Bot. 82: 1514–1521.

Sztein, A.E., Cohen, J.D., de la Fuente, I.G. and Cooke, T.J. 1999 Auxin metabolism in mosses and liverworts. Am. J. Bot. 86: 1544–1555.

Sztein, A.E., Cohen, J.D. and Cooke, T.J. 2000. Evolutionary patterns in the auxin metabolism of green plants. Int. J. Plant Sci. 161: 849–859.

Sztein, A. E., Cohen,J.D. and T. J. Cooke. 2001. Indole-3-acetic acid biosynthesis in isolated axes from germinating been seeds: the effect of wounding on biosynthetic pathway. Plant Growth Regul., in press.

Tamas, I.A. 1995. Apical dominance. In: P. J. Davies (Ed.) Plant Hormones: Physiology, Biochemistry, and Molecular Biology, 2nd ed. Kluwer Academic Publishers, Dordrecht, Netherlands, pp. 572-597.

Taylor, T.N. and Taylor, E.L. 1993. The Biology and Evolution of Fossil Plants. Prentice Hall, Englewood Cliffs, NJ.

Theissen, G., Becker, A., Di Rosa, A., Kanno, A., Kim, J.T., Munster, T., Winter, K.-U. and Saedler, H. 2000. A short history of MADS-box genes in plants. Plant Mol. Biol. 42: 115–149.

Theissen, G., Munster, T and Henschel, K. 2001. Why don't mosses flower? New Phytol. 150: 1–5.

Thomas, R.J. 1980. Cell elongation in hepatics: the seta system. Bull. Torrey Bot. Club 107: 339–345.

Uggla, C., Mellerowicz, E.J. and Sundberg, B. 1998. Indole-3-acetic acid controls cambial growth in Scots pine by positional signaling. Plant Physiol. 117: 113–121.

Uggla, C., Moritz, T., Sandberg, G. and Sundberg, B. 1996. Auxin as a positional signal in pattern formation in plants. Proc. Natl. Acad. Sci USA 93: 9282–9286.

Valentine, J.W., Jablonski, D. and Erwin, D.H. 1999. Fossils, molecules and embryos: new perspectives on the Cambrian explosion. Development 126: 851– 859.

von Maltzahn, K.E. 1959. Interaction between kinetin and indoleacetic acid in the control of bud reactivation in *Splachnum ampullaceum* (L.) Hedw. Nature 183: 60–61.

Wada, M. and Kadota, A. 1989. Photomorphogenesis of lower green plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 40: 169–191.

Walters, J. and Observe, D.J. 1979. Ethylene and auxin-induced cell growth in relation to auxin transport and metabolism and ethylene production in the semi-aquatic plant, *Regnellidium diphyllum*. Planta 146: 309–317.

Wardlaw, C.W. 1946. Experimental and analytical studies of pteridophytes. VIII. Further observations on bud development in *Matteucia struthiopteris*, *Onoclea sensibilis*, and species of *Dryopteris*. Ann. Bot. 10: 117–132.

Webster, T.R. 1969. An investigation of angle-meristem development in excised stem segments of *Selaginella martensii*. Can. J. Bot. 47: 717– 722.

Wildman, S.G., Ferri, M.G. and Bonner, J. 1947. The enzymatic conversion of tryptophan to auxin by spinach leaves. Arch. Biochem. 13: 131–144.

Williams, S. 1937. Correlation phenomena and hormones in *Selaginella*. Nature 139: 966.

Wochok, Z.S. and Sussex, I.M. 1973. Morphogenesis in *Selaginella*: auxin transport in the stem. Plant Physiol. 51: 646–650.

Wochok, Z.S. and Sussex, I.M. 1975. Morphogenesis in *Selaginella*. III. Meristem determination and cell differentiation. Dev. Biol. 47: 376–383.

Wood, A.J., Oliver, M.J. and Cove, D.J. 2000. New frontiers in bryology and lichenology: bryophytes as model systems. Bryologist 103: 128–133.

Wright, A.D., Sampson, M.B., Neuffer, M.G., Michalczuk, L., Slovin, J.P. and Cohen, J.D. 1991. Indole-3-acetic acid biosynthesis in the mutant maize *orange pericarp*, a tryptophan auxotroph. Science 254: 998–1000.