

Evolution of growth-promoting plant hormones

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This paper is part of an ongoing series: 'The Evolution of Plant Functions'.

Abstract. The plant growth hormones auxin, gibberellins (GAs) and brassinosteroids (BRs) are major determinants of plant growth and development. Recently, key signalling components for these hormones have been identified in vascular plants and, at least for the GAs and BRs, biosynthetic pathways have been clarified. The genome sequencing of a range of species, including a few non-flowering plants, has allowed insight into the evolution of the hormone systems. It appears that the moss *Physcomitrella patens* can respond to auxin and contains key elements of the auxin signalling pathway, although there is some doubt as to whether it shows a fully developed rapid auxin response. On the other hand, *P. patens* does not show a GA response, even though it contains genes for components of GA signalling. The GA response system appears to be more advanced in the lycophyte *Selaginella moellendorffii* than in *P. patens*. Signalling systems for BRs probably arose after the evolutionary divergence of the mosses and vascular plants, although detailed information is limited. Certainly, the processes affected by the growth hormones (e.g. GAs) can differ in the different plant groups, and there is evidence that with the evolution of the angiosperms, the hormone systems have become more complex at the gene level. The intermediate nature of mosses in terms of overall hormone biology allows us to speculate about the possible relationship between the evolution of plant growth hormones and the evolution of terrestrial vascular plants in general.

Additional keywords: auxin, brassinosteroid, gibberellin, moss.

Introduction

Plant hormones play key roles in regulating many aspects of plant growth and development, including shoot elongation, plant architecture, fruit growth and seed development, all of which are crucial for food and biomass production. A key subset of the plant hormones comprises the growth-promoting compounds: auxins, gibberellins (GAs) and brassinosteroids (BRs). Through a combination of mutant analysis and molecular studies, we now possess a good understanding of the perception of each of these hormones and some of the key elements of their signal transduction pathways (Santner and Estelle 2009). We also understand GA and BR biosynthesis in several model species, such as *Arabidopsis thaliana*, tomato (*Lycopersicon esculentum* Mill.), pea (*Pisum sativum* L.) and rice (*Oryza sativa* L.) (Yokota 1997; Nomura and Bishop 2006; Yamaguchi 2008), and progress is being made in untangling auxin biosynthesis (Normanly 2010). While the presence of growth-promoting hormones across the many groups of plants has been documented extensively (e.g. GAs; MacMillan 2002), it is only in the last few years that sufficient genomes have been sequenced to allow insight into the possible evolutionary origins of the biosynthetic and signal transduction pathways.

An analysis of the evolutionary origins of plant hormone systems must determine the time and plant group in which these occurred. Inevitably, this will not be straightforward, as there are cases where these compounds exist in plants only as secondary metabolites or as pathogenic compounds. The role of a compound as a hormone probably developed by the recruitment

of existing signalling components and may have occurred independently for the same group of compounds in different phylogenetic sequences. This may be the case for the steroids in plants and animals (see the discussion of BR below). Although recent work with model species is frequently used to generalise about the roles of plant hormones, there is also a growing body of evidence that there are major differences in hormone physiology across the families represented by the different model species. This occurs both for the biosynthetic pathways involved (e.g. auxin), the specificity of the receptor systems (e.g. BRs) and the developmental processes regulated (e.g. GAs). Whether these differing characteristics have evolved independently in the separate groups or have been lost in some groups has not been explored, but certainly requires detailed examination and will be addressed later. While the evolutionary origin of these three core hormone systems has received considerable attention and is reviewed below, there are still clear gaps in our knowledge, due to the lack of sequenced genomes, which will hopefully be filled as more genomes are sequenced in the near future.

Auxin

Occurrence, biosynthesis and biological activity

A wide range of species synthesise the main naturally-occurring auxin, indole-3-acetic acid (IAA; Cooke *et al.* 2002). These species represent all the major groups of land plants, including mosses, liverworts, lycophytes, ferns, gymnosperms and angiosperms. At least some bacterial species, including

above to rapidly regulate gene transcription. Lau *et al.* (2009) could find no complete Aux-IAA or ARF genes in the genomes of several algal species, including *C. reinhardtii*, even though Palenik *et al.* (2007) previously reported the presence of two Aux-IAA genes in that species.

Turning to the mosses, Cove *et al.* (e.g. Ashton *et al.* 1988) demonstrated that the caulenmata of putative auxin-deficient *P. patens* mutants could be rescued by applied auxin. This indicates that in the moss, auxin plays a role in development and that the components of auxin signalling operate, at least to some extent. Recently, Eklund *et al.* (2010) provided further confirmation of auxin action in *P. patens* when they showed that the *A. thaliana* SHI/STY gene family (positive regulators of auxin biosynthesis genes) also regulate auxin levels, and affect growth and development in the moss. These observations are consistent with the suggestion that land plants acquired functional or potentially functional components of auxin signalling before the divergence of the bryophyte groups from the lineage leading to vascular plants, but after the algae diverged from that lineage (Lau *et al.* 2009).

Pivotal for that suggestion has been the sequencing of the *P. patens* genome (Rensing *et al.* 2008). Previously, Cove *et al.* (2006) had commented that little was known about auxin-signalling pathways in moss. The sequencing indicated that auxin receptors, transporters and transcriptional regulators are encoded in the *P. patens* genome (Lau *et al.* 2008; Rensing *et al.* 2008). A total of 55 auxin-related genes were detected in *P. patens* by Rensing *et al.* (2008). Interestingly, however, there were fewer of these genes than in the flowering plants analysed: *A. thaliana* contains 174, *Populus trichocarpa* 230 and rice 175. (This represents a smaller proportion in the moss: e.g. 0.14% of the total genes compared with 0.65% in *A. thaliana*.)

There is some question, however, about whether auxin signalling components actually function in mosses to cause a 'rapid transcriptional response to auxin' (Paponov *et al.* 2009). According to Paponov *et al.* (2009), such a response, a feature of auxin signalling in angiosperms, does not occur in *P. patens*. Certainly, it appears at present that a rapid transcriptional response to auxin has not actually been demonstrated in the moss. While Paponov *et al.* (2009) acknowledge that Aux-IAAs in *P. patens* are degraded in an 'auxin-dependent manner', they suggest that this might not lead to rapid changes in gene expression. They note that Imaizumi *et al.* (2002) reported a relatively slow gene expression response to auxin in the moss; however, there appear to be no direct comparisons (in the one study) between *P. patens* and flowering plants in relation to the rate of transcriptional responses to auxin.

Paponov *et al.* (2009) suggest that a rapid transcriptional response to auxin evolved in vascular plants after their divergence from the last common ancestor shared with mosses (Fig. 2). In other words, they suggest that *P. patens* represents an intermediate stage between the green algae, which lack a functional auxin signalling pathway, and flowering plants, in which such a pathway is fully functional.

The intermediate nature of auxin signalling in *P. patens* might also be reflected in the nature and number of Aux-IAA proteins (Paponov *et al.* 2009). The moss Aux-IAAs all contain a particular motif in domain I, termed an LxLxPP motif, whereas in *A. thaliana* and other flowering plants, most Aux-

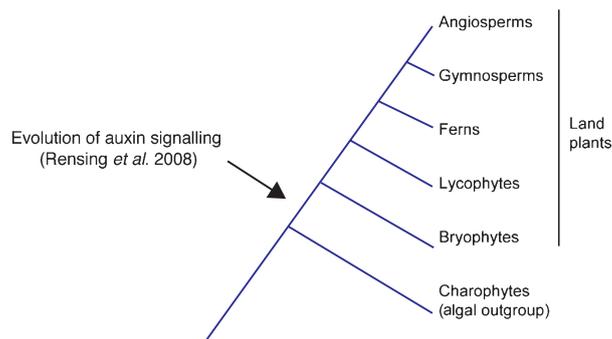


Fig. 2. Evolution of auxin signalling. Rensing *et al.* (2008) suggest that the components of auxin signalling arose before the divergence of the mosses, although Paponov *et al.* (2009) questioned whether mosses show a rapid transcriptional response to auxin (adapted from Yasumura *et al.* 2007; Rensing *et al.* 2008).

IAAs contain an LxLxL motif that appears to be essential for strong transcriptional repression (Tiwari *et al.* 2004). Paponov *et al.* (2009) suggest that in flowering plants, the LxLxPP motif has been superseded by the LxLxL domain. Furthermore, *P. patens* has only three Aux-IAA genes, many less than the flowering plant species for which the genomes have been sequenced. For example, *A. thaliana* has 29; *P. trichocarpa* 35; rice 33.

Thus the Aux-IAA family has dramatically expanded and diversified during the evolution of flowering plants from the ancestors of land plants, and this is thought to be a major reason for the great diversity of responses to the relatively simple auxin molecule. Remington *et al.* (2004) suggest that some Aux-IAA genes predate the divergence of lineages leading to *A. thaliana* and rice 136 to 168 million years ago, while other duplication events appear to have occurred more recently, e.g. after the divergence of the *Arabidopsis* and *Medicago* lineages, around 96 to 113 million years ago. It appears also that many of the more recent duplication events in *A. thaliana* have been block duplications, although Remington *et al.* (2004) note that most of the early branching points in the Aux-IAA phylogeny involved tandem duplications.

Auxin transport

In angiosperms, auxin is transported basipetally in both the shoot and root via a dedicated polar transport system (Vieten *et al.* 2007). As for other aspects of auxin biology, a crucial question is: does polar auxin transport occur in the 'lower' plants, particularly in bryophytes? Previously, Cooke *et al.* (2002) found that polar IAA transport occurs in gametophytes of the liverwort *Marchantia polymorpha* and the moss *Funaria hygrometrica*, and suggested that polar auxin transport may have helped to regulate gametophyte development in the earliest land plants. However, Fujita *et al.* (2008) could not detect polar auxin transport in gametophores of *P. patens*, although, importantly, they did find that polar auxin transport might function in sporophyte development in that species.

Interestingly, Mravec *et al.* (2009) show that the 'typical' PIN protein in *P. patens* localises not to the plasma membrane but to the endoplasmic reticulum (ER). PIN5 from *A. thaliana*

also localises to the ER, while other PINs from that species operate at the plasma membrane to orchestrate polar auxin transport. Mravec *et al.* (2009) suggested that an ER-based role for PIN proteins appeared very early in land plant evolution and might represent the ancestral function of these proteins. The ER-based PIN proteins are probably more concerned with intracellular than cell-to-cell transport, possibly explaining the difficulty of detecting polar auxin transport in the moss.

Gibberellins

Occurrence, biosynthesis and biological activity

GAs have been detected in a range of terrestrial vascular plants, including ferns and gymnosperms, and are also present in some fungi and bacteria (MacMillan 2002). Compared with auxin, our understanding of GA synthesis is markedly superior. We know the principal pathways involved, and most of the GA synthesis and deactivation genes have been cloned, at least in model species such as *A. thaliana*, rice and pea (Yamaguchi 2008). In angiosperms, the later stages of GA biosynthesis, and also the deactivation of bioactive GAs, are catalysed by members of the 2-oxoglutarate-dependent dioxygenase (2-ODD) group, and are known as GA 20-oxidases, GA 3-oxidases and GA 2-oxidases. Despite our extensive knowledge, however, there are very few analyses of the evolution of these genes, possibly reflecting the fact that the *P. patens* genome has not been sequenced for long. The three groups of GA-related 2-ODDs do form distinct groups in phylogenetic analyses, but it is interesting that the two synthesis groups (20-oxidases and 3-oxidases) do not cluster together in a sub-group distinct from the deactivation (2-oxidase) genes, regardless of whether the analysis is performed on genes from a large range of species (Sakakibara *et al.* 2008) or mainly on those from *A. thaliana* (Hedden and Phillips 2000). This may reflect the fact that the GA 2- and 3-oxidases oxidise neighbouring carbon atoms on the same ring of the GA molecule (the 'A ring'), even though their actions have opposite effects on the content of bioactive GA.

There appears to be little evidence from physicochemical studies that the algae produce GAs. Some fungal species, on the other hand, are copious producers of these hormones, and indeed this feature of certain pathogenic fungi led to the initial discovery of the GAs. However, consistent with the apparent gap in GA genes in algae, and the wide phylogenetic distance between fungi and land plants, it appears that the seed plants have not 'inherited' the same set of GA synthesis genes as the fungi (Bömke and Tudzynski 2009). This is because in fungi, the enzymes catalysing the later steps in GA synthesis are not 2-oxoglutarate-dependent dioxygenases but are cytochrome p450s instead (Bömke and Tudzynski 2009; Fig. 3). This is an excellent example of convergent evolution, because the phenotype in chemical terms is identical (GA₃, for example, is present in seed plants as well as fungi) but has been arrived at by different evolutionary pathways.

A further difference concerns the biosynthesis of the early GA precursor *ent*-kaurene. In fungi (Hedden *et al.* 2002) and the moss *P. patens* (Hayashi *et al.* 2006), a single bifunctional enzyme catalyses the two steps involved in converting geranylgeranyl diphosphate to *ent*-kaurene (Davidson *et al.* 2006). In contrast, in the angiosperms, there is a separate monofunctional enzyme

for each step (Davidson *et al.* 2006). Recently, it was shown that the gymnosperm white spruce (*Picea glauca*) employs two enzymes (Keeling *et al.* 2010), like angiosperms, and it appears that the two-enzyme system in plants evolved after the divergence of the mosses but before the divergence of the angiosperms from gymnosperms. Keeling *et al.* (2010) suggest that duplication and subfunctionalisation of the ancestral gene have resulted in the monofunctional enzymes found in seed plants.

The observation that in a phylogram of GA 2-ODD genes (Sakakibara *et al.* 2008), there are clustered representatives from *P. patens* in the 2-oxidase and 20-oxidase groups, suggests that these two groups originated and began to differentiate from each other before the divergence of the moss from the vascular lineage. In other words, the 2-ODD GA genes are very ancient. It should be noted, however, that there is no published evidence as yet that the *P. patens* 'GA' genes actually encode functional proteins. Two of these genes have been tested in this respect, with no functional activity detected (Hirano *et al.* 2007). Consistent with that, GAs appear not to have been detected in mosses, even though state-of-the-art physicochemical techniques have been used for *P. patens* gametophytes (Hirano *et al.* 2007). It has been suggested, however, that it is the spores or sporophyte – not the gametophyte – that might contain GAs in the moss (Anterola *et al.* 2009). A bioactive GA (GA₄) has been detected in the lycophyte *S. moellendorffii* (Hirano *et al.* 2007) and several GAs have been found in ferns (e.g. *Cibotium glaucum*; MacMillan 2002).

It is interesting to review information on the functions of GAs and GA-related compounds in the various plant groups. Anterola (2008) develops the theme that as plants evolved, there were changes in the roles played by GAs, from promoters of spore germination in mosses, to antheridiogens in ferns and to growth promoters in angiosperms. Possibly, this should be amended to include GA-related compounds and GA precursors, since GAs themselves have not yet been identified in *P. patens*. The evidence for the involvement of GA-related compounds in the moss comes from the effects of AMO1618, which inhibits the formation of the early GA precursor *ent*-kaurene. AMO1618 inhibits spore germination in *P. patens* (Anterola *et al.* 2009). *Ent*-kaurene was able to substantially, but not completely, reverse this effect, while GA₃ did not reverse the inhibition at all.

Another GA synthesis inhibitor, paclobutrazol, inhibited growth of the leafy *P. patens* gametophyte, an effect that again was not reversed by GA₃. A third GA synthesis inhibitor, uniconazole, inhibited elongation in *S. moellendorffii* sporophytes and, yet again, a bioactive GA, GA₄ could not reverse that inhibition.

One explanation for these observations is that a GA-like compound or compounds, derived from *ent*-kaurene, exhibits bioactivity in these species. This compound(s) is not *ent*-kaurene itself, because paclobutrazol inhibits growth but not *ent*-kaurene production. Neither does it appear to be a 'normal' bioactive GA, because GA₃ could not restore growth or spore germination, and such GAs have not been identified in the moss. Possibly, the capacity of *ent*-kaurene to stimulate the germination of *P. patens* spores is attributable to a conversion by those spores of *ent*-kaurene to the bioactive compound. Further discussion on *ent*-kaurene-derived bioactive compounds was recently provided by Hayashi *et al.* (2010).

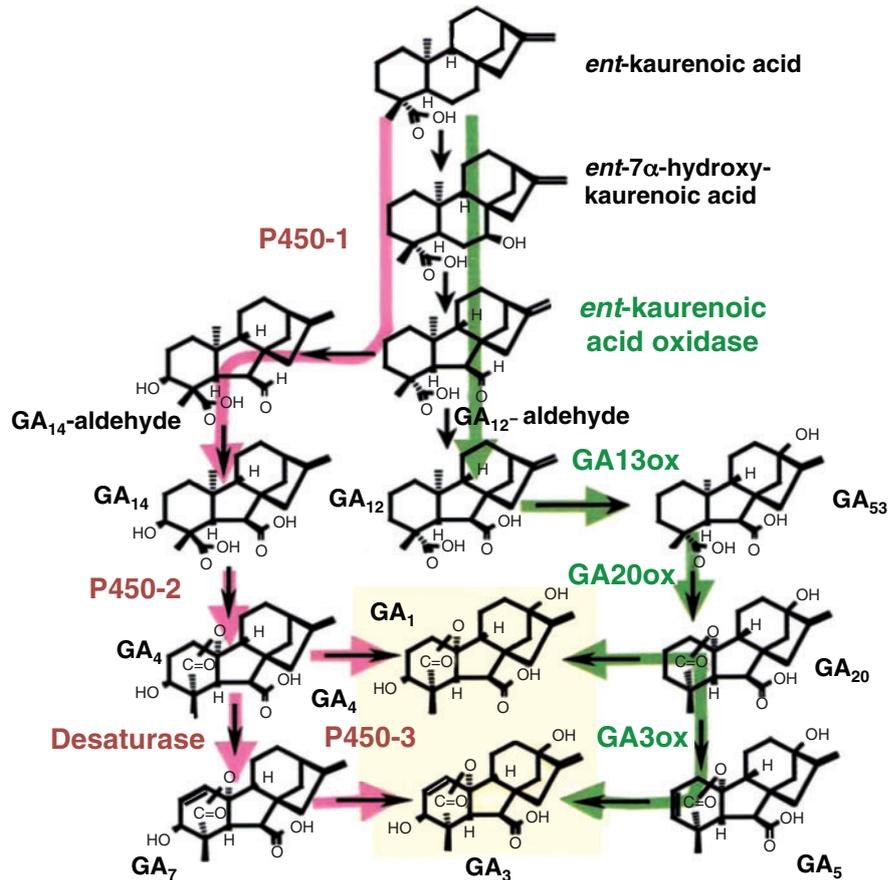


Fig. 3. Convergent evolution in GA biosynthesis pathways. In both fungi (pink) and angiosperms (green), the same early precursors (e.g. *ent*-kaurenoic acid) are converted to the same bioactive GAs (e.g. GA₁ and GA₃, shown in the primrose box), but different enzymes catalyse the steps involved (adapted from Hedden *et al.* 2002).

It is also relevant that the bifunctional *ent*-kaurene-producing enzyme in *P. patens*, when expressed in *Escherichia coli* (Anterola *et al.* 2009) or in a different insect cell-based system (Hayashi *et al.* 2006), produced *ent*-kaurene and *ent*-16 α hydroxykaurene (called 16 α hydroxykaurane in Anterola *et al.* 2009). It appears that *P. patens* produces large amounts of this latter compound, which has been implicated in plant defence (von Schwanzenberg *et al.* 2004).

In the ferns, there are also responses to GAs that are normally considered inactive in flowering plants. For these plants, unlike for mosses, there is no doubt that the GAs themselves are detectable (MacMillan 2002). In the ferns, the main role of the GA-related compounds is to promote the formation of antheridia at the expense of archegonia; the name given to such compounds is 'antheridiogen'. In the ferns *Lygodium circinnatum* and *Lygodium flexuosm*, the main antheridiogen is GA₇₃ methyl ester (Yamauchi *et al.* 1996). Other antheridiogens are GA₉ methyl ester and 3-*epi*-GA₆₃ (Yamauchi *et al.* 1995).

Thus, throughout evolutionary history, there may have been considerable change with regard to the roles played by GAs and related compounds, as well as in the actual compounds that activate the responses.

Regulation of GA levels by auxin and by GA signalling

In seed plants, it is well known that GA levels can be tightly regulated (Yamaguchi 2008), and we can speculate about the antiquity of the regulatory mechanisms involved. Two such regulatory factors are auxin and the 'DELLA' proteins, both of which upregulate GA synthesis and downregulate GA deactivation by controlling gene transcription. The effect of auxin is one of the clearer examples of plant hormone interactions. It appears to be ancient within the angiosperms, occurring in both monocots (Wolbang *et al.* 2004) and eudicots (Ross *et al.* 2000). One possible scenario is that the capacity of auxin to upregulate the GA genes arose only once, before the divergence of the three ODD groups; for the synthesis genes, this effect has persisted throughout subsequent evolution. After the divergence of the 2-oxidases, modification occurred such that at least some members of this group are now downregulated, not upregulated, by the level of auxin normally found in the plant. Interestingly, the putative ancestral condition (upregulation by normal auxin levels) can still be observed for some 2-oxidase genes in some circumstances (O'Neill and Ross 2002). A similar scenario might also apply in the case of the DELLA proteins, which mediate the capacity of bioactive GA to downregulate its synthesis and to upregulate its deactivation. The ancient nature of

this phenomenon is indicated by evidence that it occurs in *S. moellendorffii* (Hirano *et al.* 2007).

GA signalling

Our understanding of GA signalling, as for GA synthesis, is well advanced, again enabling an examination of evolutionary trends. GAs operate by first interacting with a GA receptor, termed GID1 in rice, to form a complex that destabilises the growth-inhibitory DELLA proteins. This degradation occurs after the DELLAs are targeted to the 26S-proteasome (Ueguchi-Tanaka *et al.* 2007). Clearly, there are interesting parallels between GA and auxin signalling: GID1 is analogous to TIR1 and the DELLAs to Aux-IAA proteins, and protein destabilisation is a major feature of both systems (Santner *et al.* 2009). It has been claimed, somewhat controversially, that DELLA stability is affected also by hormones other than GA. Auxin is included in that category, but the evidence comes from a single report (Fu and Harberd 2003) that has yet to be confirmed. More recently, it has been suggested that usually, hormones other than GAs affect DELLA stability indirectly, by first affecting GA levels (Achar and Genschik 2009).

It appears that the lycophyte *S. moellendorffii* has a GID1-DELLA GA response system resembling that of seed plants (Hirano *et al.* 2007; Vandenbussche *et al.* 2007), whereas in the moss *P. patens*, such a system is not functional, even though genes for GID1-like proteins and for DELLAs are present. Yasumura *et al.* (2007) also concluded that there were significant developments in GA signalling between the divergences of the bryophytes and the lycophytes (Fig. 4). They found that the moss receptor and moss DELLA proteins did not interact with each other in a yeast two-hybrid assay, even when bioactive GA (GA_3) was present. However, the corresponding proteins from the lycophyte *Selaginella kraussiana* did interact with each other, and this interaction

was enhanced by GA_3 . Next, Yasumura *et al.* (2007) showed that the moss receptor interacted with the *S. kraussiana* DELLA (although this was not GA_3 -dependent), but the reciprocal interaction between lycophyte receptor and moss DELLA did not occur. They interpreted this observation as showing that the ancestral form of receptor, as present in the moss, was capable of interacting with DELLAs and that this capacity has subsequently persisted. The DELLAs, on the other hand, had to acquire the capacity to interact, and this occurred between the bryophyte and lycophyte divergences (Yasumura *et al.* 2007). Consistent with that, Hirano *et al.* (2007) also found that moss DELLAs did not interact with any receptors in the yeast two-hybrid assay.

However, Hirano *et al.* (2007) found that the same *P. patens* receptor as used by Yasumura *et al.* (2007) did not interact with any DELLA that they tested, including one from *S. moellendorffii*, although they did not test the *S. kraussiana* DELLA. Therefore, the interesting assertion by Yasumura *et al.* (2007) that the ancestral receptor possessed the capacity to interact with DELLAs does rest on only one interaction (with a DELLA from *S. kraussiana*). Furthermore, Hirano *et al.* (2007) reported that the rice DELLA protein SLR1 can interact with the lycophyte receptor but not with the moss receptor, suggesting that the moss receptor might not, after all, possess a 'pre-existing' capacity to interact with DELLAs.

Yasumura *et al.* (2007) suggest that the capacity of GA to stimulate the receptor–DELLA interaction also originated between the bryophyte and lycophyte divergences. Interestingly, the moss DELLAs, when transformed into certain *A. thaliana* genotypes, were able to inhibit growth but they did not do so in their native species; at least, not in the gametophyte. On the basis of this observation, Yasumura *et al.* concluded that the inhibitory function of the DELLAs evolved after the capacity to interact with the GA receptor. They contend that as far as the evolution of the DELLA growth-inhibiting

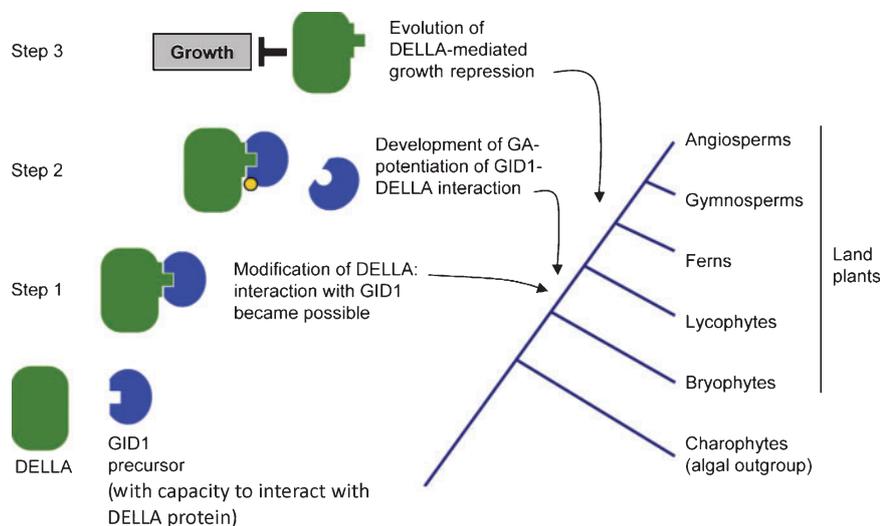


Fig. 4. Evolution of GA signalling. According to the model shown (adapted from Yasumura *et al.* 2007), early DELLA proteins lacked the capacity to interact with receptor (GID1) proteins. This capacity arose after the divergence of the bryophytes. Next came the capacity of GA (yellow dot) to promote the DELLA–GID1 interaction, followed by changes in the system that respond to DELLAs, resulting in the ability of these proteins to inhibit growth. However, Hirano *et al.* (2007) questioned whether the early GID1 protein possessed the capacity to interact with DELLAs, and noted that since the divergence of mosses, DELLAs themselves have undergone changes that enhance their growth inhibitory property.

capacity is concerned, changes in the responding system (involving the transcription of DELLA-regulated genes) have been more important than changes in the DELLA proteins themselves. Nevertheless, the DELLA proteins may also have undergone changes to enhance their growth-inhibiting capacity, as lycophyte DELLAs, but not those of moss, inhibited growth when overexpressed in rice (Hirano *et al.* 2007).

Interestingly, several GAs that are classified as inactive in flowering plants were highly effective at stimulating the receptor–DELLA interaction in *S. moellendorffii* (Hirano *et al.* 2007). Chief amongst these were GA₃₇ and GA₉, which were much more effective than GA₁ or GA₃, especially in the case of one of the two lycophyte receptors (SmGID1b). GA₁ and GA₃ meet the structural requirements for activity in flowering plants, in part because they have a hydroxyl group at the 3 β position. GA₄ (with a hydroxyl group at the 3 β position but not at the 13 position) was more effective than GA₁, in the *S. moellendorffii* system, leading to the suggestion that SmGID1b can discriminate between GAs based on the presence or absence of hydroxylation at the 13 position (Hirano *et al.* 2008). In contrast, however, in *S. kraussiana*, GA₁, GA₃ and GA₄ were equally effective at stimulating a receptor–DELLA interaction (Yasumura *et al.* 2007).

Shimada *et al.* (2008), reporting on the crystal structure of GID1, noted that in the course of evolution from the more ancestral SmGID1-type receptors, replacement of certain key amino acid residues refined the receptor to give high affinity and specificity to bioactive seed plant GAs such as GA₄ and GA₁. The GID1-like receptors from the lycophytes and mosses appear to have evolved from proteins known as hormone-sensitive lipases, an ancient group present in both plants and animals. These enzymes have, during the course of evolution, lost their catalytic activity and instead have evolved a pocket in which the GA molecule fits. An amino acid ‘lid’, which holds the GA in place, has also developed, at least in angiosperms (Shimada *et al.* 2008).

Brassinosteroids

Occurrence, biosynthesis and biological activity

BRs have been identified from ~60 plant species (Bajguz and Tretyn 2003). While the majority of the recent work has been performed in the model species *A. thaliana*, tomato, rice and pea, many of the early identifications were from cultured cells of *Catharanthus roseus* or tissues with high concentrations of the compounds (e.g. pollen) (Yokota 1997). Over 65 BRs have now been identified but elegant work using mutants late in the biosynthetic pathway suggests that only two C28 BRs are active, brassinolide and its precursor, castasterone (Nomura *et al.* 2005; Nomura and Bishop 2006). BRs have been identified from many angiosperm and gymnosperm families and also from a pteridophyte (*Equisetum avense*), a bryophyte (*Marchantia polymorpha*) and a chlorophyte (*Hydrodictyon reticulatum*) (Kim *et al.* 2002; Bajguz and Tretyn 2003). However, P450 genes with reasonable homologies to those involved with BR biosynthesis in angiosperms have not been found in *S. moellendorffii* or *P. patens* even though BRs were present (T. Yokota, pers. comm.).

The structure of BRs is derived from the 5-cholestone skeleton and BRs have strong similarities to the steroid hormones of

animals. They consist of four rings and a variable-length side chain with a wide range of hydroxylation states (Yokota 1997; Bajguz and Tretyn 2003). The biosynthetic pathways for sterols from the cycloartenol precursor appear to be conserved across all land plants (Morikawa *et al.* 2009). The steps leading to the BRs have also been clarified recently with the predominant C28 pathway leading from 24-methylenecholesterol to castasterone, and then to brassinolide in certain species and tissues (Fujita *et al.* 2006; Nomura and Bishop 2006; Jager *et al.* 2007). While a complex matrix of steps is possible, recent work has suggested a likely dominant pathway. This has been possible, as the genes involved with this pathway have been identified in several model angiosperms, with cytochrome P450 monooxygenases being responsible for the changing hydroxylation patterns. Indeed, C26 hydroxylation, one of the key deactivation processes for the BRs, may also result in the deactivation of insect steroidal hormones and the removal of cholesterol in mammals, showing strong evolutionary conservation in pathways that metabolise potentially dangerous sterols (Meaney 2005).

The isolation and characterisation of dwarf BR mutants was fundamental to the acceptance of BRs as growth-promoting hormones during the 1990s. Indeed, it was the complementation of the *det2* mutant in *A. thaliana* by an animal 5 α -reductase gene that really confirmed the hormonal status of BRs in plants (Li *et al.* 1997). Clear evidence was provided by the dark green dwarf phenotype of BR-deficient mutants in several angiosperm species (e.g. *A. thaliana*, pea, rice and tomato; Li *et al.* 1996; Bishop 2003; Nomura *et al.* 2004). However, while many processes have been linked with BR function (e.g. cell elongation, cell division, reproductive and vascular development, stress responses, senescence and etiolation), relatively few of these have been examined in sufficient depth to show a clear endogenous physiological role for BRs. This requires evidence that changes in endogenous BR levels, caused by environmental factors or autonomously during development, regulate the process in both a positive and negative fashion. Such evidence is only present for a few developmental processes including stem elongation (e.g. Nomura *et al.* 1997), xylem differentiation (Yamamoto *et al.* 2001) and fruit development (Symons *et al.* 2006), and then only in a select number of angiosperm species. Such physiological evidence is lacking from other plant groups.

In fact, information on BRs in plants other than the angiosperms is too patchy to indicate whether the biosynthetic pathways are identical to those in angiosperms but does suggest a similar range of compounds are present, especially in the gymnosperms (Fujioka 1999). In most cases, one or both of the known active molecules, castasterone or brassinolide, is (are) present. This at least provides the potential for BRs to be acting as a hormone in these systems, although they could simply be present as secondary metabolites. However, at present, we do not possess BR mutants in plant groups other than the angiosperms. There do not appear to be reports of BRs in fungi or bacteria, or in plant pathogens in general, which contrasts to what is known for auxin and the GAs.

BR signalling

The BR receptor was identified at a fairly early stage from *A. thaliana* via the isolation of the BR-insensitive mutant, *brl1* (Clouse *et al.* 1996; Li and Chory 1997). Similar mutants have

subsequently been identified in other species including pea (Nomura *et al.* 2003) and rice (Yamamuro *et al.* 2000; Bai *et al.* 2007), providing confirmation of a similar mode of perception in monocots and eudicots. Substantial progress on identifying elements of the transduction pathway has also been made using mutant analysis and molecular studies in *A. thaliana*, and it has emerged that the transduction pathway directly influences the expression of response genes (Vert and Chory 2006; Li and Jin 2007). The BRI1 receptor is a leucine-rich repeat receptor-like kinase that is located at the cell membrane, with the receptor domain on the external side of the membrane and with an intracellular kinase domain that is activated by BR binding. This domain is capable of transferring the external perception of the BR signal to the intracellular transduction pathway, which consists of several genetically-defined components, including a soluble glycogen synthase kinase 3-like kinase (BIN2), a phosphatase (BSU1) and two transcription factors (BZR1 and BZR2) (Bai *et al.* 2007; Li and Jin 2007). Bai *et al.* (2007) demonstrate that this transduction pathway from the cell surface to the regulation of transcription is also conserved between monocots and eudicots, although there has been some duplication of components in the different lineages. The BR receptor system is related to the transmembrane receptor kinase signal transduction pathways in animals (Torii 2004). There are similar receptor systems in bacteria and components may well have been recruited from elements of such systems. The BRI1 receptor in tomatoes also binds the proteinaceous hormone systemin, as well as BRs, but does not appear to be essential for systemin-induced responses (Scheer and Ryan 2002; Holton *et al.* 2008). Santner and Estelle (2009) conclude that signalling systems for BRs probably did not evolve until after the evolutionary split of mosses and vascular plants. Consistent with this view, Sasaki *et al.* (2007) did not show any relationship of plant receptor-like kinase genes in *M. polymorpha* to BR receptors. While several *M. polymorpha* gene sequences contained domains related to those in the BRI1 receptor, they did not contain the key extracellular domain involved with BR binding (S. E. Davidson, unpubl. data).

The system of perception and signal transduction for plant steroid hormones is often contrasted with that of animal systems, where an intracellular receptor–steroid complex is formed and directly regulates gene expression in the nucleus (Revelli *et al.* 1998). However, while some recent reports have also suggested that steroid reception may occur at the cell membrane in certain animal systems (Thomas *et al.* 2007), preliminary searches of animal genome databases do not reveal *BRI1*-like sequences. Further, receptor sequences similar to those in animals have not been found in plants (Clouse 2002). These observations imply that although similar compounds (steroids) are used by both plants and animals to regulate development, signalling systems for the compounds may have originated independently in the two life forms. This is possible, as it is hypothesised that recruitment of a control mechanism can occur backwards from the functional gene level.

Another difference between the plant and animal steroid receptors is that the main BR receptor gene in plants, *BRI1*, is expressed across all tissue types (Nomura *et al.* 2003), whereas in animals, the expression of the steroid receptors is tightly regulated in distinct tissue types (Williams 1997). However,

BRI1 homologues in plants have gained functional specificity in the control of some developmental processes such as vascular differentiation (Caño-Delgado *et al.* 2004; Kim and Wang 2010). There is clearly an interesting area of research needed to clarify and confirm that the steroid hormone systems in plants and animals have evolved independently, even though some of the genes involved in BR biosynthesis in plants are similar to those in human systems (e.g. 5 α reductase; Li *et al.* 1997).

There appears to be little interaction between bioactive GA and BR levels, in contrast to the effect of IAA on bioactive GA content (Jager *et al.* 2005). However, there have been numerous reports of interactions between IAA and BRs. Certainly, the expression of some genes is regulated by both hormones, although the number affected in this way is a small subset of the genes affected by the individual hormones (Goda *et al.* 2004). There is also evidence that bioactive BRs influence auxin transport (Li *et al.* 2005; Symons *et al.* 2008). A recent paper suggests that a member of the BR response pathway, BIN2 kinase, inactivates ARF2, leading to increased expression of auxin-induced genes (Vert *et al.* 2008). However, the purported synergistic enhancement of auxin-induced elongation by BR (Vert *et al.* 2008) is only weakly supported by the growth response data, raising questions about the overall conclusions of the study. Whatever the auxin–BR interactions, if any, prove to be, it is too early to speculate on their evolutionary origin until a clear understanding of the interactions is achieved in model angiosperms.

Conclusions and future directions

Our understanding of plant hormone evolution has improved rapidly over the last decade due to the identification of many of the hormone receptors and elements of the response pathways, and the availability of genome sequences for several model species. As next generation sequencing tools become widespread, we will see a plethora of genome sequences from all plant groups over the next few years that, when combined with detailed molecular data on the synthesis and response genes from angiosperm models, will allow a precise understanding of the evolution of all the plant hormone systems. It will be interesting to see how these systems have evolved to suit the different adaptive traits of the various life cycles that occur across the plant groups. Furthermore, it will be important to determine whether there are major differences between the gametophyte and sporophyte generations.

One intriguing question is whether the development of hormone systems was driven by adaptation to the terrestrial environment, was an essential precursor to terrestrial colonisation or was driven by the development of advanced multicellular growth. The recent identification of auxin and its proposed actions in a brown algal model, *Ectocarpus siliculosus* (Le Bail *et al.* 2010), may lend support to the latter hypothesis. However, this raises the question of how the systems were recruited in different phylogenetic groups (e.g. auxin in brown and green algae, and steroids in plants and animals). Once sufficient genomes are available, answers to these questions will be forthcoming. However, the lack of certain hormone-related genes in unicellular plants has already been suggested in some cases (e.g. for auxin synthesis genes; Rensing *et al.* 2008),

probably arguing strongly for independent evolution of the systems in different multicellular groups.

What does appear to be clear is that many of the plant hormone systems have been exploited by phylogenetically distinct pathogens (e.g. auxin by *Agrobacterium* and certain insects, and GAs by *Gibberella fujikuroi*). While co-evolution of hosts and pathogens has been examined in detail for many processes, especially those related to resistance mechanisms, some hormone groups (e.g. BRs) still require examination. Given the central role that the plant growth hormones play in plant development, they are an ideal system for pathogen manipulation, since a plant is unlikely to be able to easily evolve resistance to major disruptions in these hormone systems. Furthermore, pathogens need to produce only small amounts of the hormone in question in order to dramatically affect resource allocation or structural development.

A further productive line of research will be to confirm the impression, drawn from the limited number of genomes sequenced outside the angiosperms, that, at least for auxin and the GAs, there has been considerable expansion of the number of genes involved with these hormone systems. Not only do the processes influenced by these groups of hormones appear to change, but the system has become genetically more complex and, possibly, the responses more rapid. Either the developmental processes themselves have been recruited and adapted to new roles, with the original hormone response system remaining in place, or the hormone response has been recruited by pre-existing developmental processes previously not involved with hormones. Future research should resolve this question.

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