Evolution of growth-promoting plant hormones

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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 \textit{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, \textit{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 \textit{Selaginella moellendorffii} than in \textit{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 \textit{Arabidopsis thaliana}, tomato (\textit{Lycopersicon esculentum} Mill.), pea (\textit{Pisum sativum} L.) and rice (\textit{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...
pathogens, also produce IAA, and there are also reports that certain fungi can synthesise this compound (Gay et al. 1994; Spilvallo et al. 2009). However, evidence for IAA in microalgae, including Chlamydomonas spp., is not convincing at present (Dutcher et al. 1992). Consistent with that, Rensing et al. (2008) reported that genes thought to be related to auxin homeostasis were not found in the genomes of the aquatic unicellular green algae Ostreococcus tauri, Ostreococcus lucimarinus and Chlamydomonas reinhardtii. However, IAA has been identified in multicellular algae, including Fucus distichus and Ectocarpus siliculosus (Basu et al. 2002; Le Bail et al. 2010), and importantly, Nitella spp. (Sztein et al. 2000). Nitella is a member of the Charophyta, the algal group that is thought to have included the ancestors of land plants (Judd et al. 2002). It is possible that unicellular algae ancestral to all multicellular plants may have once had the capacity to synthesise auxin but that capacity has since arisen independently in at least two different multicellular algal groups (and hence in land plants).

Lau et al. (2009) reviewed the evidence that auxin can have physiological effects on algal growth, as well as evidence to the contrary, and concluded by doubting the functional relevance of auxin in algae, suggesting instead that IAA may simply be a by-product of tryptophan. However, a recent paper on the brown alga E. siliculosus suggests a role for IAA in development, although the response pathway appears to be different to that in vascular plants (Le Bail et al. 2010).

The actual presence or absence of IAA itself is not difficult to establish, since the compound is amenable to physicochemical analysis. However, our understanding of auxin synthesis pathways is still far from satisfactory. For angiosperms, five pathways have been proposed: one independent of and four dependent on the amino acid tryptophan (Fig. 1; Woodward and Bartel 2005; Sugawara et al. 2009). The main difficulty is determining which pathway or pathways predominate in a given species or plant part, or in a given environment. Another problem is that each pathway remains incompletely characterised at the biochemical and molecular levels.

In view of these gaps in our knowledge, it is difficult to discern evolutionary trends in auxin synthesis pathways. However, recent evidence indicates that two tryptophan-dependent pathways that operate in bacteria may also play significant roles in plants. One of these involves indole-3-pyruvic acid as the first product of tryptophan (Koga et al. 1991; Stepanova et al. 2008; Tao et al. 2008), and the other, indole-3-acetamide (Pollmann et al. 2009; Mano et al. 2010). Genes encoding enzymes for converting indole-3-acetamide to IAA in plants are phylogenetically related to those from bacteria (albeit distantly), indicating their ancient origin (Mano et al. 2010).

A third tryptophan-dependent pathway, via tryptamine, was brought to researchers’ attention in 2001 by the discovery of the YUC genes, which are thought to encode the enzyme for converting tryptamine to the next product in the pathway, N-hydroxytryptamine (Zhao et al. 2001). The YUC genes appear to be widespread in plants, including moss (Zhao 2010), although they appear not to have been identified in bacteria. Mutations in or overexpression of YUC genes result in auxin-related phenotypes, but there is some doubt about N-hydroxytryptamine as an intermediate (Quittenden et al. 2009) and about whether tryptamine is the in vivo substrate for the YUC proteins (Zhao 2010).

A fourth pathway, with indole-3-acetaldoxime as the first product of tryptophan, appears to be restricted to the Brassicaceae (Quittenden et al. 2009; Sugawara et al. 2009) and may therefore be of relatively recent origin. Indole-3-acetaldoxime is also a precursor of the defence compounds, indole glucosinolates (Grubb and Abel 2006).

Recently, Eklund et al. (2010) suggested that the tryptamine and indole-3-pyruvic acid pathways might operate in Physcomitrella patens, based on the presence of genes usually associated with these pathways (YUCCA genes for the tryptamine pathway and TAA1-like genes for the indole-3-pyruvic acid pathway).

**Auxin signalling**

Much of the recent literature on evolutionary aspects of auxin concerns the development of auxin signalling and, to a lesser extent, auxin transport, rather than auxin synthesis. This might, to some extent, reflect our better understanding of these aspects.

Genome sequencing in a range of species has permitted searches to be made for the auxin perception and signal transduction genes originally identified in angiosperms. Key proteins from those plants are the TIR1-AFB family, which function in auxin reception, and the Aux-IAA proteins, which, when auxin levels are low, interact with proteins known as auxin response factors (ARFs) to prevent these ARFs from regulating genes required for auxin responses (Santner et al. 2009). When auxin levels are high, the auxin–TIR1 complex destabilises Aux-IAA proteins, and the resulting low level of Aux-IAAAs cannot prevent ARFs from regulating the auxin response genes.

The central issue is whether these auxin signalling components are also present in plants such as algae and mosses, and, if so, whether they interact in the way described...
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 caulenomata 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 Imazum 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-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 diphasphate 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 phylogeny 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).
It is also relevant that the bifunctional ent-kaurene-producing enzyme in \textit{P. patens}, when expressed in \textit{Escherichia coli} (Anterola et al. 2009) or in a different insect cell-based system (Hayashi et al. 2006), produced ent-kaurene and ent-16\(\alpha\) hydroxykaurene (called 16\(\alpha\)hydroxykaurane in Anterola et al. 2009). It appears that \textit{P. patens} produces large amounts of this latter compound, which has been implicated in plant defence (von Schwartzenberg 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 \textit{Lygodium circcinatum} and \textit{Lygodium flexuosum}, the main antheridiogen is GA\(_7\) methyl ester (Yamauchi et al. 1996). Other antheridiogens are GA\(_8\) methyl ester and 3-epi-GA\(_{63}\) (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.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig3.png}
\caption{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. GA\(_1\) and GA\(_3\), shown in the primrose box), but different enzymes catalyse the steps involved (adapted from Hedden et al. 2002).}
\end{figure}

\textbf{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 DELLAAs 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 (Santer 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 (Achard 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 DELLAAs 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 (GA3) was present. However, the corresponding proteins from the lycophyte *Selaginella kraussiana* did interact with each other, and this interaction was enhanced by GA3. Next, Yasumura et al. (2007) showed that the moss receptor interacted with the *S. kraussiana* DELLA (although this was not GA3-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 DELLAAs and that this capacity has subsequently persisted. The DELLAAs, 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 DELLAAs 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 DELLAAs 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 DELLAAs.

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 DELLAAs, 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 DELLAAs evolved after the capacity to interact with the GA receptor. They contend that as far as the evolution of the DELLA growth-inhibiting
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 GA17 and GA8, 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 3β position. GA4 (with a hydroxyl group at the 3β 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 and Bishop 2006; Bajguz and Tretyn 2003). BRs have been identified from many angiosperm and gymnosperm families and also from a pteridophyte (Equisetum avense), a bryophyte (Marchantia polymorpha) and a chlorophyte (Hydrodictyous 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 steroid hormones and the removal of cholesterol in mammals, showing strong evolutionary conservation in pathways that metabolise potentially dangerous steroids (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 5α-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 (Fujita 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, bri1 (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 (Tori 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). Santer 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 perception 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, BRI 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. 5α 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 response 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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References


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