Action of plant hormones

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With the evolution of specific perception mechanisms, molecules such as auxins, cytokinins, gibberellins, abscisic acid and ethylene, acquired a hormonal role during the evolution of land plants. These hormones induce cell division, cell elongation and cell differentiation by regulating the expression of transcription factors and cell type specific genes. A limited number of modules and signalling pathways have been utilized during hormonal signal transduction. The specificity arises at the level of receptors, transcription factors, post-translational modifications of signalling agents and crosstalk between different hormones. The signalling pathways are turning out to be highly conserved in plants and animals.

THE phytohormones regulate and integrate the overall growth, development and reproduction in plants. This involves the regulation at the level of size of individual parts, integration of their function and ultimately the generation of the final form. The morphogenesis devolves basically around the processes of cell division, cell elongation and cell differentiation and hormones are involved in regulating all of them. Auxin was among the first hormone to be discovered and subsequently gibberellins, cytokinins, abscisic acid and ethylene were identified. These five hormones, often referred to as the classical five, represent five distinct groups. Additional ones such as jasmonates, brassinosteroids and systemin have been added to the list and new ones continue to be discovered. Although several phytohormones were first discovered in fungi, they are now known to occur more or less ubiquitously in most of the plants, including the cryptograms. Each phytohormone evokes several responses so that, finally, a cell must be integrating the output signals of a variety of intracellular and extracellular signals in responding and acquiring the final fate.

Auxins are typically associated with cell elongation, while auxin and cytokinin act synergistically to regulate the process of cell division. Depending on the ratio of auxin and cytokinin, the organogenesis of roots and shoots is specified and the best evidence for these effects derives from the analysis of *Arabidopsis* mutants, either with altered levels of hormones or altered signalling[1]. The gibberellins (GA) regulate the mobilization of soluble sugars from the starch in the cereal grains and transform genetic dwarfs of corn, pea and rice into phenotypically tall plants. Abscisic acid (ABA) is involved in the regulation of stomatal closure, adaptation to various stresses, induction of dormant structures, e.g. winter buds in deciduous trees and seeds in several species of higher plants. Seed maturation and synthesis of storage proteins is also regulated by ABA. In several responses GA and ABA act antagonistically and their relative amounts have been found to specify either seed maturation or seed germination[2]. Ethylene is involved in inhibitory responses, evokes the classical triple response in the young seedlings and is associated with ripening of fruits.

The topics on hormonal action discussed in this review are selective, as we wish to bring into focus the themes of current research emphasis. Signalling pathways involving calcium, phospholipids, phosphatases and G-proteins are discussed in another article in this issue by Clark *et al.*[3] The action of a hormone involves its perception, initiation of a specific response – which tends to be independent of transcriptional or translational controls – and finally the sustained response under the new steady-state growth conditions. In many cases the early responses evoked within a few minutes are at the level of ion fluxes and generation of a second messenger such as calcium ions, protons, IP3, DAG or cyclic ADP ribose, etc. Such messengers, besides mediating the response, also amplify the signal to sustain the response. The transcriptional as well as translational regulations seem to occur after a lag of a few minutes. In many cases more than one messenger or signalling cascade seem to be utilized and the signalling flux though a particular cascade seems to depend on the physiological age or the competence of the responding tissue and crosstalk between different hormones. There is now evidence that both antagonistic as well as stimulatory steps are involved during hormonal action and the balance of these determines the final effect of even a single hormone.

Auxin-binding proteins and their role

The elongation of coleoptile or hypocotyl sections has been extensively used to understand the auxin effects at

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the level of wall loosening and cell elongation. The excised coleoptile sections undergo auxin-induced cell elongation after 10 to 25 minutes. It has been known for over fifty years that acidic buffers (or pH) can substitute auxins for elongation; auxin evokes the secretion of protons by activating a proton ATPase[4,5]. Following the demonstration of specific auxin-binding sites in the plasma membranes of maize coleoptiles, the auxin-binding protein – ABP1 was identified. Whether this protein is a true auxin receptor is still debated, but it functions as a putative receptor in ABP1-mediated membrane-associated ion fluxes and cell enlargement. Exogenously added purified corn ABP1 confers the competence in tobacco protoplasts to respond to auxin, while the antibodies against ABP1 block the auxin-induced hyperpolarization[6]. The antibodies directed against the putative auxin-binding site of ABP1 mimic the auxin effect and induce the membrane hyperpolarization[7]. These observations provide strong functional evidence that auxin is perceived at the outer face of the plasma membrane (PM) and ABP1 is involved in mediating the rapid responses at the level of ion fluxes[8].

Several other auxin-binding proteins such as 23 kDa auxin efflux carrier protein, 40 and 42 kDa proteins from uptake carrier, 60 kDa β-glucosidase and 31 kDa endo-1,3-β-glucanase have been characterized from plants. However, ABP1 from the site I binding sites in corn microsomes is the best-characterized and as already mentioned, it plays a role in the initiation of hyperpolarization response[9]. Other proteins are involved in the transport and control of auxin levels. The mature ABP1 is a glycoprotein, exhibits a glycosylation site, exists in several isoforms and is a dimer with each subunit being 22 kDa. About 90–95% of ABP1 is localized in the lumen of endoplasmic reticulum (ER) in the Golgi stacks and shows the KDEL ER-retention signal at the C-terminal end. Low levels of ABP1 have been reported to be associated with the plasma membrane and cell wall. The 22 kDa corn ABP1 proteins are encoded by a small gene family[10]. The recent experiments on transgenic tobacco expressing the *Arabidopsis* ABP1 under an inducible promoter and on maize cell lines exhibiting constitutive expression of ABP1, provide strong evidence for its putative receptor function[11]. On inducing the expression of ABP1, the tobacco leaves exhibited the typical auxin response – the epinastic curvature due to a localized increase in cell expansion. Moreover, in the transgenic tobacco plants, the entire leaf acquires the capacity to respond to auxin as opposed to the developmentally acquired graded response in the wild type leaf. In young leaves from the wild type plant, the tip is the most responsive region to auxin-induced cell expansion, while the basal lamina does not respond at all. These results indicate that ABP1 mediates auxin-dependent cell expansion in the intact plant. In a maize endosperm cell line lacking a detectable level of ABP1, constitutive expression of ABP1 led to an auxin-dependent increase in cell size. The formation of large-sized cells is not an artifact as the control line also shows this feature at a low frequency. These results do demonstrate the receptor role of ABP1 in cell expansion. Despite these compelling arguments, the overall picture of auxin perception and action is complex and seems to involve more than one mechanism. The precise role of ABP1 in ER and its mode of interaction with H^+-ATPase are unknown as yet. The auxin-induced increase in plasma membrane H^+-ATPase activity followed by hyperpolarization and the inhibition of auxin response by specific antibodies indicate the presence of ABP1 on the outer face of the plasma membrane. The ER and the PM pools are not regulated, but ABP1 seems to be secreted from the ER at a very low rate to the PM[12–14]. It is not known if the ABP1 is internalized following vesicle formation and is degraded. In case the ABP1 is also internalized similar to some of the receptors known in animals and as the endomembrane system turns over, it is not too difficult to visualize that the ABP1 pools in the PM and ER basically represent a dynamic situation.

A possible relationship has been suggested between the auxin-induced proton secretion resulting in the acidification of the cell wall and subsequent increase in extensibility and growth; however, the overall mechanism is still unresolved[15–19]. Most interestingly, McQueen-Mason *et al.*[20] isolated from the actively growing regions of cucumber hypocotyl, two proteins named as expansins of about 29 kDa, that seem to play a role in the acid-induced expansion of cell walls. The expansin genes have also been isolated from a large number of plants and are now known to constitute a large superfamily of two subgroups, the α- and β-expansins. The molecular basis of wall loosening by expansins is not yet understood[21].

The auxin induces initially a slight depolarization believed to be due to the movement of chloride ions followed by hyperpolarization, changes in membrane potential, decrease in cytosolic pH and an increase in free calcium levels. In fact, IAA treatment of maize coleoptiles has been found to evoke in-phase oscillations of these responses with a period of 20 to 30 minutes[22–24]. A kinetic analysis of these responses and auxin uptake mechanisms seems to provide interesting leads to understand the signal transduction process. The auxin uptake is strongly dependent on the proton motive force and additional hyperpolarization further stimulates auxin accumulation[25]. The studies on the uptake of auxin by *Cucurbita* vesicles show the involvement of three components in auxin transport: (i) a low level membrane permeability for the undissociated IAA molecules through hydrophobic, lipophilic interactions, (ii) cotransport of $IAA^-/2H^+$ mediated by a symport influx carrier, and (iii) an IAA anion efflux carrier. The

transport of IAA anions and protons will thus result in the accumulation of net positive charges, a slight depolarization and the lowering of the cytosolic pH. This has important consequences and will result in an elevation of intracellular calcium level. The stimulation of a proton pump will lead to hyperpolarization and extracellular acidification. The most important feature of this line of reasoning is that the cytosolic pH and free calcium levels are related[26]. It is noteworthy that both cytosolic pH and calcium ions have been proposed to be the messengers in early auxin action; the transport of calcium across intracellular membranes with protons as driver ions is responsible for the observed phenomenon and probably serves both in calcium regulation and signal transduction[23].

Early auxin-regulated genes, cell division and cell elongation

The second messengers such as calcium ions, either themselves and or along with calcium binding proteins, e.g. calmodulin, activate the protein kinase cascade which in turn activates other proteins, including the transcription factors. These factors presumably interact with the auxin-response elements and regulate the expression of auxin-inducible or auxin-responsive genes. Auxin-induced elongation in the coleoptile sections, detectable 10–25 minutes after treatment requires continuous synthesis of RNA and proteins to sustain the elongation growth. The analysis of the early auxin-inducible genes has been particularly fruitful and had led to finding several new features in common with some of the stress-induced proteins. The members of Aux/IAA class were the first ones to be identified and at present, several families of early auxin-regulated genes are known (reviewed by Abel and Theologis[27]). The Aux/IAA gene, SAUR gene, and ACS gene (ACC synthase) families share similar promoters consisting of two auxin-response domains. The two other, GH3 and GST-like, gene families have different auxin-response elements. In most of the cases, the expression of auxin-responsive genes is due to transcriptional activation, and the steady state transcript level increases within 5–60 minutes.

The expression of SAUR (small auxin up-regulated RNA) genes is very rapid and the mRNAs are detectable within 2–5 minutes in the auxin-responsive tissues. In the cell elongation zone of soybean hypocotyls, SAURs are present uniformly in the epidermis and cortical cells of the unstimulated hypocotyl[28,29]. Following gravistimulation, SAURs seem to turnover more rapidly and disappear from the upper region followed by an accumulation in the lower portion of the hypocotyl where the epidermal and cortical cells would undergo a more rapid elongation growth. The asymmetric distribution of SAURs occurs before the actual onset of the elongation growth thus providing strong evidence for their involvement in the auxin response. SAUR genes are now known to occur in several other plants also.

Several auxin-resistant mutants (*axr1, tir1, axr4* and *sar1*) in *Arabidopsis* have been isolated. The *axr1* plants show pleiotropic defects such as reduced apical dominance, shorter stature, reduced or even inhibited expression of several early auxin-inducible genes and defects in protein degradation (*axr1, tir1*)[27,30–32]. The expression of *TIR1* and *AXR1* genes is highest in the actively dividing and elongating cells[32,33]. The *AXR1* gene encodes a protein, which is related to the N-terminal half of the ubiquitin-activating enzyme E1 of the targeted-protein degradation pathway and is visualized to determine the stability of an early acting signalling component[34]. The E1 protein can form *in vitro* heterodimers with the ECR1 (E1 C-terminus-related 1) protein and the complex in turn can activate the proteins related to ubiquitin[35]. Ubiquitin-mediated degradation pathways are involved in regulating the cell division cycle, apoptosis, immune response and metabolism. It is possible that the AXR1 protein stimulates cell division by degrading a cell division kinase (CDK) inhibitor[36,37]. The *AXR1* gene may thus not be involved directly in the signalling pathway, but may function in regulating the levels of a rapidly turning-over negative regulator.

Auxin activation of MAP kinase cascade

Among the early responses, an activation of the MAP kinase cascade by 2,4-D-treated tobacco BY-2 cells has been found. A 46 kDa MBP-kinase activity is transiently activated in cells treated with 2,4-D for 5 minutes[38]. This transient activation has been reported to be due to the cytosolic acidification rather than due to the auxin[39]. Since these reports, the NPK1 (*Nicotiana* protein kinase1) has been identified to be a MAPKKK[40]. The *NPK1* gene is actively transcribed in the meristematic cells undergoing rapid cell divisions suggesting that its expression is cell cycle-dependent[41]. Preliminary studies indicate that it is localized in the central region of the mitotic spindle and the phragmoplast[42]. The activation of the MAPK cascade by NPK1 in the maize protoplasts assay system using the early auxin inducible promoter GH3 from soybean and *GFP* as reporter gene, abolishes the auxin activation of the GH3 promoter[40]. The expression of auxin-insensitive promoters was, however, not affected. The transgenic tobacco plants overexpressing the NPK1 kinase domain showed defects in embryo and endosperm development and thus the expression of NPK1 protein is tightly regulated. These results bring out a new feature and show that the competence or the sensitivity to respond to auxin depends on the antagonistic and stimulatory signalling steps.

There is increasing evidence that other hormones such as GA, ethylene and ABA also activate transiently a MAP kinase cascade. The ABA response is turning out to be complex and both Ras (a small molecular weight G-protein) and type 2C phosphatases have been implicated in the MAP kinase activation[43].

Role of Myb-related transcriptional factors in the action of GA and ABA

The induction of α-amylase by GA in the aleurone layers of cereals has been extensively investigated. In oat aleurone protoplasts, GA-attached to Sepharose beads induces the synthesis of α-amylase and thus the perception of GA occurs at the level of external face of the plasmalemma[44,45]. There are several reports indicating the possible involvement of heterotrimeric G proteins, cyclic GMP and inositol 1,4,5-triphosphate in the transduction of the GA response in the aleurone cells (reviewed in Jones *et al.*[46]). The analysis of specific mRNAs shows that whereas GA regulates the synthesis of both high- and low-pI α-amylases at the transcriptional level, ABA completely inhibits the mRNA accumulation[47]. Three highly conserved *cis*-acting elements are present in the α-amylase genes from cereals – C/TCTTTTC/T – the pyrimidine box and TAACAAA and TATCCAC boxes[45,48]. The promoter analysis of several GA-induced genes indicates that all three elements are required for the optimal GA response and models have been proposed to explain the possible role of these boxes in the transcriptional initiation[49]. The similarity of the second element sequence with the promoters in the high- and low-pI amylases with the c-Myb consensus binding sequence led to the suggestion that these motifs could interact with Myb-like transcription fractors[45]. This reasoning has ultimately led to the demonstration that GA up-regulates the expression of a transcription factor, the GAmyb, prior to the GA-induced α-amylase gene expression. The expression of GAmyb is independent of fresh protein synthesis and a GA-regulated post-translational activation of pre-existing protein/s seems to be involved. The recombinant GAmyb specifically binds *in vitro* to the TAACAA box and in the transient expression assays, GAmyb activates the transcriptional expression of high-pI α-amylase promoter fused to a reporter gene in the absence of exogenous GA[45]. Thus the activation of GAmyb is an early step in the expression of GA-induced α-amylases in barley aleurone.

MYB proteins are now known to occur in fungi and photoautrophic plants and are widely distributed in plants and have also been implicated in the ABA response[50–52]. MYB protein can interact with other transcription factors also and a complex interaction has been found in corn with the regulatory gene C1 protein and the ABA-regulated viviparous gene[53], *VP1*. The *C1* gene codes a myb-related protein whose expression is regulated by ABA and the VP1 protein[54]. The *c1* gene regulates the expression of anthocyanin genes in the grains, while the *vp1* mutants show sensitivity to ABA, a viviparous development of embryos and lack of anthocyanin biosynthesis[55]. The *VP1* gene encodes a protein of 691 amino acids whose N-terminal region functions as a transcription activation domain. In maize protoplasts, an overexpression of VP1 in the presence of ABA has been shown to activate the expression of a C1 promoter-driven reporter gene[53]. In *Arabidopsis thaliana* also the protein encoded by the *Atmyb2* gene shows considerable homology to the C1 myb-related protein and is up regulated by ABA, dehydration and salt stress[52].

The Myb is a helix–turn–helix motif type of DNA binding protein, which contains typically three repeats (R1, R2 and R3) in animals. In higher plants most of the Myb proteins reported so far typically contain the R2 and R3 repeats and the GAmyb (now designated as HvMYBGA), is also of the R2R3 type[50]. A recent study across the different plant groups suggests that the R1R2R3-type of MYB sequences are present in liverwort and mosses, ferns and higher plants[51]. The *A. thaliana* gene *AtMYB3R-1* also contains three repeats and it has been suggested that the R2R3-MYB sequences could have been derived from the more ubiquitous R1R2R3-type[51].

Ethylene receptor and signal transduction

As stated earlier, ethylene evokes the triple response in the *Arabidopsis* seedlings grown in dark. These are characterized by an exaggerated curvature of the apical hook, a radial swelling of the hypocotyl and an inhibition of hypocotyl and root elongation. The classical genetic screens have been particularly fruitful to isolate the mutants with altered response to ethylene and have led to the elucidation of ethylene perception and signalling pathway. Two classes of mutants have been identified: those, which are insensitive to ethylene (*ein* or *etr1*), and those, which show the triple response constitutively, even in the absence of ethylene. The second class included mutants that are either overproduces of ethylene and could be rescued by the inhibitors of ethylene biosynthesis or those that had lesions in the perception mechanism (constitutive triple response *ctr1*) and were not rescued by inhibitors. The analysis of genes in these mutants has been particularly instructive.

Ethylene receptors have been found to be homologous to the histidine kinases of the two-component signalling systems found in some of the prokaryotic and eukaryotic species and function in osmoregulation, light and oxygen sensing and cyclic AMP synthesis[56]. This

signalling system consists of two components, the sensor and the response regulator. The sensor consists of a signal-input domain and a catalytic histidine kinase transmitter domain. Activation of the sensor by a stimulus results in the autophosphorylation of a conserved histidine in the transmitter domain and subsequent phosphotransfer to a conserved aspartate residue in the receiver domain of the second component, the response regulator. The different receptor genes characterized from *A. thaliana* consists of two sub-families, the ETR1, ERS1 group and the ETR2, ERS2, EIN4 group[57]. The functional receptor seems to be a homodimer. The ethylene-binding domain consists of an N-terminal hydrophobic region containing the three transmembrane sub-domains. This is followed by the GAF domain of yet-unidentified function, a histidine kinase domain and a C-terminal receiver domain[56]. The ETR1, ETR2 and EIN4 are the hybrid kinases and have fused sensor and response regulators in the same polypeptide. The ERS1 and ERS2 lack the C-terminal response regulator and are the simple type of ethylene related histidine kinases. The response regulators can also exist as separate polypeptide and their homologues have been found in *Arabidopsis*[58] (ARR). The expression pattern of ethylene receptors is stage- and tissue-specific in tomato, suggesting that the hormonal response can also be regulated at the level of the receptor expression[59].

The transgenic yeast cells expressing the N-terminal hydrophobic regions of ETR1 and ERS1 bind ethylene, suggesting that both of them can function as receptors[60,61]. The experiments with yeast cells expressing ETR1 also show that copper is associated with ethylene binding[62]. Downstream of ETR1, a negative regulator of ethylene response, the *CTR1* gene has been identified. This has similarities to the mammalian Raf kinase which is a MAPKKK[63]. This kinase presumably phosphorylates a MAPKK, which in turn phosphorylates a MAP kinase. The MAP kinase cascade ultimately modulates the activity of transcription factors. The CTR1 kinase negatively modulates the ethylene pathway because all the *ctr1* alleles are recessive and result in loss or reduction of function. It is believed that in the absence of ethylene, CTR1 is activated and acts as a negative regulator to represses the downstream events, while in the presence of ethylene, the activation of ethylene receptor prevents the activation of CTR1, whereby the downstream factors are turned on[61,64].

Two-component regulators, cytokinin and regulation of cell division

As already stated the cytokinins act synergistically with auxins in inducing cell division in plants. The characterization of genes in the cytokinin-independent (CKI) mutants has been particularly fruitful and has ultimately led to the identification of the putative cytokinin receptor and the role of cytokinin in the induction of cyclin D3. The CKI1 mutant calli of *A. thaliana* proliferate rapidly, turn green and produce roots in the cytokinin-free medium[65]. The *CKI1* gene has been found to be homologous to the histidine kinase and receiver domains of the prokaryotic two-component regulators[65]. The predicted amino acid sequence indicates that the *CKI1* gene codes for a 125 kDa protein which at the N-terminal region has two membrane-spanning sequences of hydrophobic amino acids, followed by the histidine kinase domain and at the C-terminal end the receiver domain. Based on the bacterial model systems, the region of polypeptide between the two hydrophobic domains is predicted to be extracytoplasmic and involved in sensing the cytokinin[66]. Its binding with a cytokinin is yet to be demonstrated, but the structural features of the CKI1 polypeptide suggest that it might be a cytokinin receptor.

In *Arabidopsis*, two genes *IBC6* and *IBC7* are induced by cytokinin within minutes of treatment[67]. Their DNA sequence has been found to be homologous to the prokaryotic two-component response regulators. These are believed to mediate the cytokinin signalling and may be involved in regulating the expression of cyclins. Besides cytokinin, the expression of *IBC6* and *IBC7* genes is also induced by cycloheximide, suggesting that these are regulated by a rapidly turning-over protein, which either represses transcription or degrades the mRNA[67]. Similar features had earlier been reported for the auxin primary-response genes such as SAUR and it has been suggested that *IBC6* may also be a cytokinin primary-response gene.

Based on the analysis of DNA sequence of expressed sequence tags (EST) in *A. thaliana*, a G-protein coupled receptor (GCR1) has also been isolated, which shows sequence similarity to the seven transmembrane (7TM) receptors[68]. The *Arabidopsis* plants expressing the antisense GCR1 gene with 35S promoter show reduced sensitivity to cytokinins, but a normal response to other plant hormones. These results have been implied to mean that *GCR1* has a functional role in cytokinin signal transduction pathway[68]. Considering the homology with the 7TM receptors known in other organisms, it is conceivable that *GCR1* is also a candidate receptor for cytokinin, but more information is needed, especially about its effect on the expression of cytokinin-regulated genes, e.g. cyclins and cytokinin primary-response genes.

The activity of cyclins and cyclin-dependent kinases (CDKs) has been found to regulate the passage of cells through G1/S and G2/M transitions or checkpoints during a cell cycle. The level of D-type cyclins (CycD) increases several-fold during the G1/S phase transition as the quiescent cells enter the proliferative phase[69]. In *Arabidopsis* cell suspension cultures as well as the pro-

liferating cells of plants, the cyclin CycD3 has been shown to be up-regulated at the transcriptional level by the cytokinins[70]. The *Arabidopsis* mutants with a high level of endogenous cytokinin showed two- to three-fold higher steady state level of CycD3 mRNA compared to the wild type. Likewise, the transgenic plants constitutively expressing the CycD3 upon culture formed green calli and continued to maintain cell divisions on the cytokinin-free medium. These results provide unequivocal evidence for modulation of CycD3 levels by cytokinin.

Other hormones have also been reported to modulate the expression of cell cycle components. The role of auxin in possible regulation of cell cycle has already been mentioned. Gibberellins have been found to induce the differential expression of a CDK and cyclins in rice[71]. The expression of a cyclin-dependent protein kinase inhibitor (ICK1) which can interact *in vitro* with Cdc2a and CycD3 has been reported to be induced by ABA[72]. Thus, control of cell cycle is turning out to be an important target of control by plant hormones.

It is possible that cytokinins may also play a role in the G2/M transition also[73]. The Cdc2-like kinase, a 34 kDa protein was identified from the tobacco parenchymatous cells. These cells remain arrested in the G2 phase unless a cytokinin is provided. 1-NAA and 6-BAP induce more than 40-fold increase in the amount of the Cdc-2 protein. The induced kinase protein is inactive in the auxin-containing medium and cytokinin was found to stimulate the dephosphorylation leading to an activation of the kinase activity. During cell cycle, the dephosphorylation is catalysed by the Cdc25 phosphatase and thus cytokinins could also be involved in regulating the G2/M transition by regulating a dephosphorylation step[73,74].

Possible organization of signalling components and pathways into modules

Several signalling components seem to be organized as multifunctional complexes or functional modules. MAP kinase signal transduction cascade (MAPKKK, MAPKK, MAPK) is often described as MAP kinase module in a functional sense. Its interaction with other proteins of the signalling pathway is thought to be brought about by scaffold proteins that serve as interaction platforms. COP9 (constitutive photomorphogenesis in *Arabidopsis*) protein is organized into regulatory complexes known as Signalosome and such complexes have been reported in cauliflower, yeast (*S. pombe*), *Drosophila* and mammals[75]. These are believed to be involved in the control of cell cycle and regulation of MAP kinase signalling. It is conceivable that the other components for signalling (e.g. G-protein coupled receptors and IP3 cycle components, Ca/CAM and PKs or other calcium sensing proteins and CDPKs) could also be organized in functional modules. Such an organization can circumvent the problem of delays due to the slow diffusion of the signalling components.

Concluding thoughts

The analysis of DNA sequences and new information of extinct plants suggests that the present group of plants had a monophyletic origin and as the ancestral form migrated from an aquatic to terrestrial habitat, the recruitment of secondary metabolites as hormones probably arose as a biological necessity to integrate the overall development during the diversification of land plants[76]. The role of hormones in vascular plants probably arose from pre-existing elements of primary metabolism in charophycean algae and bryophytes[76,77]. The existing information on the occurrence and function of hormones in bryophytes is in accordance with this proposal[78] and a further study of such plants could even be more incisive and telling about the origin of hormonal responses.

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