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A short history of auxin-binding proteins

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Introduction

Plant hormone receptors have proved to be elusive research targets. The successes of describing receptors from animals and bacteria have not yet been matched for plants. Nevertheless, where candidate receptors have been identified, they have been subjected to detailed examination. One such is the protein known as ABP1, an auxin-binding protein first described from maize (*Zea mais* L.).

The first detection of ABP1 was as an auxin-binding activity in crude membrane preparations of etiolated coleoptiles (Hertel *et al.*, 1972). Over the next decade this binding activity was characterized in detail for ligand specificity (Ray *et al.*, 1977), affinity (Ray, 1977a; Batt *et al.*, 1976) and cellular compartmentalization (Ray *et al.*, 1977). In 1985, the binding protein was purified for the first time (Löbler and Klämbt, 1985) leading on to functional studies of ABP1, examination of its cell biology and its structure. This review summarizes the advances made in each of these areas.

Photolabelled proteins

It would be incorrect to suggest that all auxin binding in plants could be ascribed to ABP1. There have been numerous reports of other binding sites for indole-3-acetic acid. Most have been discovered through the use of tritiated azido IAA, a photoactive auxin analogue (Melhado *et al.*, 1982). A number of labelled proteins from a range of plants have been traced and sequenced, almost all turning out to be enzymes (Venis and Napier, 1995).

Maize ABP1 is on the list of photolabelled proteins and we will return to the activities of this below. For the remainder, the interaction with auxin at physiologically relevant concentrations has failed to alter the protein's activity. As such, the binding fails to satisfy one of the key criteria of receptors, namely that ligand binding initiates a biologically relevant response. Although these proteins are not likely to be receptors, this certainly does not mean that they are not relevant to auxin biochemistry or physiology. Indeed, photolabelling has identified conjugate hydrolases, glutathione *S*-transferases and, possibly, components of the auxin transport machinery (Venis and Napier, 1995).

Auxin affinity chromatography

Affinity purification has added a number of new auxin-binding proteins to the list recently. A phenylacetic acid column is often used to purify maize ABP1, but it has also been shown to select for a 44 kDa protein from pea (Reinard *et al.*, 1998). Further characterisation revealed that this protein is an isovaleryl-CoA dehydrogenase, an enzyme necessary for leucine catabolism in mammals and targeted to mitochondria (Reinard *et al.*, 2000).

A number of soluble auxin-binding proteins have been reported by Sakai's group (Sugaya and Sakai, 1996). Their 2,4-D matrix has been shown to purify a glutathione-dependent formaldehyde dehydrogenase. A 2,4-D matrix was also used to purify peach auxin-binding proteins (Ohmiya *et al.*, 1993), later shown to be members of the protein superfamily known as the germins (Dunwell *et al.*, 2000). It is interesting to note that ABP1 is also a member of this superfamily,

although outside the germin motif boxes (see below) there is very low homology (less than 5%) between the proteins from peach and ABP1. The same group has identified another 2,4-D binding protein (labelled Pp60) as protein disulphide isomerase (PDI) (Sugaya, *et al.*, 2000). The affinity of the site was K_D 2,4-D = 3.5×10^{-5} M (similar to that for the other peach proteins, 4×10^{-5} M) which is not very high. Competition with other auxins showed that IAA was a poor ligand. It seems unlikely that protein disulfide isomerase, an ubiquitous endoplasmic reticulum (ER) folding enzyme, is also an auxin receptor. Nevertheless, it clearly does have a binding capacity for auxin, as it does for other hormones, including 3,3[1],5-triiodothyronine in animal ER (Primm and Gilbert, 2001). It is interesting to note that PDI, ABP1, the peach germins and the IAA-amino acid hydrolases (Davies *et al.*, 1999) co-localize in the ER, although the functional significance of this observation remains unclear.

One other affinity matrix has been used to purify a putative auxin receptor, tryptophan linked to sepharose through the primary amine group (Kim *et al.*, 1998). The reported affinity of a protein purified from rice was high, K_D IAA $= 1.9 \times 10^{-8}$ M, with four auxin sites. Evidence for receptor activity was presented, although the half-saturating concentration for IAA in the assay was in the micromolar range and no activity was detected below 10^{-7} M IAA. Therefore, the apparent affinity and biological activity data are at variance. In the presence of the purified protein (named ABP_{57}) and IAA, proton translocation across plasma membrane was promoted. Tobacco plasma membrane H^+-ATPase activities have been linked to auxin in similar assays before (Laporte and Rossignol, 1997), but in this case an increase in proton translocation was seen only when both purified protein and auxin were present together. The protein can also be purified with antibodies specific for bovine serum albumin (Kim *et al.*, 2001). The only auxin to have activity in the assay was IAA and data for tryptophan were not presented, raising questions on the importance of the protein in auxin perception. The protein has yet to be cloned or fully sequenced.

Mutant screens for receptors

Affinity labelling and affinity purification have yielded a long list of auxin-binding proteins, although few remain candidate receptors. The same is true for molecular genetic approaches to receptor isolation. Many screens of mutant populations have been carried out and many auxin-insensitive plants identified. Once again, these huge programmes have been reviewed widely and readers are referred to Hobbie and Estelle (1994), Leyser (1997) and Luschnig and Fink (1999). Again, there are new mutants and cloning reports, but as these do not have any details of auxin binding they will not be covered here. Indeed, it is pertinent to point out that of the many genes cloned through these programmes, all important for auxin action (Dharmasiri and Estelle, Liscum and Reed, Delong *et al.*, and Friml and Palme in this issue), none has been shown to have a protein product which binds auxin. It is clear that plant hormone receptors generally (with the exceptions of ethylene, cytokinins, and perhaps, brassinosteroids) have proved recalcitrant to molecular genetics so far. Screening activation-tagged lines might change this, but so far no auxin receptors have been derived from direct mutant screens. Forward screens have also failed to pull out ABP1 mutants. However, an ABP1 knock-out mutant has been isolated recently by reverse genetics (Chen *et al.*, 2001). The homozygote is lethal due to defects during early embryogenesis and the implications of this knock-out are discussed below.

ABP1

APB1 is ubiquitous in the vascular plants, including the pteridophytes and bryophytes. A pile-up of full-length translated cDNA sequences is shown in Figure 1, and this includes the sequence of ABP1 from the moss *Ceratodon purpureus.* ABP1 ESTs from a fern and other monocots are also available but are not included here. Very recently, ESTs have become available from *Chlamydomonas*, a lower plant of the green algae. The occurrence of ABP1 homologues in the green algae predates the evolution of both auxin carriers (Dibb-Fuller and Morris, 1992) and IAA catabolic pathways (Sztein *et al.*, 1995). Auxin binding in this homologue has not yet been demonstrated. There are no close homologues to ABP1 outside the plant kingdom, nor in the blue-green alga *Synechocystus*, fungi or yeast. One sequence motif, the germin motif, is partially conserved and this could place ABP1 in a protein superfamily common to all kingdoms (Dunwell *et al.*, 2000) but, apart from this, ABP1 is special to green plants.

The full sequence of ABP1 itself has revealed little about the protein. Sequences from a range of plants (Figure 1) show a high level of residue conservation throughout the mature protein. However, the signal peptide, cleaved co-translationally as the polypeptide enters the ER, is highly variant. Three domains of 13 to 20 amino acids, labelled boxes A (or D16), B and C, are highly conserved amongst all higher plant ABP1s. All ABP1s also contain an ER lumen retention motif (with the possible exception of the *Ceratodon* sequence, discussed below), three cysteines and one conserved N-glycosylation site. Throughout the dicots there is a second conserved N-glycosylation site close to the N-terminus (Massotte *et al.*, 1995). Strawberry ABP1, for example, has an additional third site (Lazarus and Macdonald, 1996).

Secondary structure algorithms suggest the polypeptide folds into β sheets and β turns with only a small stretch of α helix at the C-terminus. The C-terminus carries the KDEL-ER retention motif, the consequences of which are discussed below.

Maize ABP1 expressed in the baculovirus system (Macdonald *et al.*, 1994) has now been crystallised and preliminary X-ray diffraction data collected (Woo *et al.*, 2000). Three crystal types were grown, each showing the basic unit of structure was a homodimer, in agreement with early gel filtration data (Venis, 1977). The resolution of a high-definition protein structure should follow shortly. The protein used for crystallisation has been shown to be indistinguishable from ABP1 purified from maize seedlings, except at the C-terminus where the ER targeting sequence has been modified to KEQL to promote secretion and facilitate purification. Post-translational modifications and binding kinetics of the mutated version remained unchanged from wild type ABP1 and so the crystal structure should be illuminating.

Mass spectrometry analysis of ABP1 purified from maize coleoptiles has recently been reported (Feckler *et al.*, 2001). In the experimental conditions used by the authors to purify the protein and perform mass spectrometry analysis, a disulfide bridge was found between C_2 and C_{61} (in box A, Figure 1) and no intermolecular disulfide bridge involving the remaining C_{155} (in the C-terminal domain) has been revealed. This is at variance with crystallographic data which show that a single disulfide links C_2 with C_{155}, stabilizing the protein both in the presence and absence of bound auxin (Napier and Pickersgill, unpublished). It was recently shown that mutation C158 of tobacco ABP1 (equivalent to C155 in maize) into serine alters the folding of the protein, its capacity to interact with auxin and its activity at the plasma membrane (David *et al.*, 2001). These results demonstrated the importance of this in ABP1 action, but a discrepancy remains at the structural level between experimental results and this needs to be resolved.

An attractive model is that intramolecular disulfide bridge shuffling occurs *in vivo* to control ABP1 folding, targeting and signalling, but it is necessary to test this hypothesis.

Auxin-binding site

The key to the activity of an auxin receptor is its auxin-binding site. Long before ABP1 was purified, models to predict the relationship between the molecular structure of auxins and hormone activity were developed (Thimann, 1963; Farrimond *et al.*, 1978; reviewed by Napier, 2001). These were complemented by mechanistic models for the recognition site (Kaethner, 1977; Katekar, 1979) which became more sophisticated with increasing computational capacity and knowledge (Tomic *et al.*, 1998). In parallel with models based on auxin structures, biochemistry identified amino acids likely to be involved in auxin binding (Venis, 1977; Navé and Benveniste, 1984). Determination of the sequence of ABP1 indicated that the biochemist's data matched a string of residues in the linear peptide. This peptide became known as the D16 box (Venis *et al.*, 1992) or Box A (Brown and Jones, 1994) (Figure 1).

Antibodies raised to the Box A sequence were shown to have auxin-like activity (Venis *et al.*, 1992; Walther *et al.*, 1997). With the antibody able to substitute for auxin in physiological assays, it seemed likely that this part of ABP1 was a major contributor to the auxin site. As new sequences from diverse plants became available, it was also found that this region of ABP1 is fully conserved over 15 residues.

Two other parts of ABP1 are also highly conserved, labelled Box B and C, respectively (Figure 1). The latter overlaps the site at which a photolabelled IAA was found to bind, labelled peptide 11 (Brown and Jones, 1994), this too might be involved in the binding site, but the role of this part of the protein has yet to be fully discovered. A monoclonal antibody named mAb12 has been raised to tobacco ABP1 and found to recognize a discontinuous epitope embracing residues in both boxes A and C (Leblanc *et al.*, 1999a). Crystallography illustrates that β sheets from

Figure 1. Pile-up of ABP1 sequences. ABP1s are listed against their genus names. Conserved residues are shown in bold type. A consensus sequence is shown at the foot and lower case letters represent residues not conserved. Two boxes of completely conserved residues are outlined, Boxes A and B, and a third box with high identity is labelled Box C. Several sequence motifs are listed; glc represents N-glycosylation sites, KDEL the ER retention motif, the residues listed in the shaded boxes represent the core cupin motif and the peptide reported to carry photo-activated IAA (Brown and Jones, 1994) is labelled peptide 11.

each conserved box fold around the ligand-binding site (Napier and Pickersgill, unpublished), both sets of results being consistent with the photolabelling experiments.

Binding site models

Models of the auxin receptor site have taken a number of forms since that of Kaethner (1977). It should be remembered that all but one of these are based on biological activity measurements and do not necessarily reflect the binding site of ABP1. The exception is the study of Edgerton *et al.* (1994) who used auxin binding data from maize microsomal membranes (Ray,

1977b), later shown to represent the activity of ABP1. The characteristics of this model binding site are very similar to those models based solely on biological data, although specific predictions differ. Essentials include a carboxylic acid binding group, a hydrophobic transitional region and a hydrophobic platform capable of accommodating the π electrons of the aromatic ring system. Details vary, particularly about whether the auxin side chain, the carboxylate group, needs to adopt different confirmations when bound and unbound, unnecessary for the ABP1 model (Edgerton *et al.*, 1994), incorporated in that of (Kaethner, 1977).

By their nature, these binding site models describe the space, hydrophobicity and charge expected around a bound auxin molecule. The first attempt to construct a molecular model of ABP1 itself has just been published (Warwicker, 2001). Crystallographic coordinates of two germins were used as structural framework into which the most homologous stretch of ABP1 sequence was mounted. The model predicts some features with good probability, such as a coordination site for a metal ion within a β barrel structure. Both these predictions have been confirmed by crystallography (Napier and Pickersgill, unpublished). The protein structure of one other germin, oxalate oxidase, has also been published recently and this was shown to have a similar metal site containing manganese. The manganese is likely to contribute to the oxidase and superoxide dismutase activities of oxalate oxidase. A metal ion in ABP1 would form an ideal carboxylic acid coordination group for the binding pocket. However, the metal ion in ABP1 is likely to be Zn^{2+}, not Mn^{2+}, and ABP1 has no oxalate, or IAA oxidase activity (Napier and Marshall, unpublished).

The metal ion in the model is complexed by a cluster of three histidine residues and a glutamic acid. All these are residues of the germin motif, part of which is found in Box A HRHSCEE (Figure 1). The third histidine is contributed by the second part of the germin motif, H106. Further, a tryptophan (W44), ten residues upstream of Box A, was suggested to face into the binding pocket and contribute the π electron acceptor platform for the indole-aromatic ring of auxin. This is a different tryptophan than the one hypothesized to be the hydrophobic platform by Brown and Jones (1994b) (W_{136} in peptide 11, Figure 1), but it is likely that both hypothesis will be corrected once ABP1 crystal data have been analysed (Woo *et al.*, 2000). It is worth noting that Warwicker's tryptophan platform is not conserved in the *Arabidopsis* sequence.

Warwicker was not able to model either the N- or C-termini of ABP1 due to low sequence homology. However, he did note two tryptophan-aspartic and glutamic acid tripeptides, DDW136 in Box C and WDE153 in the C-terminal domain, and hypothesized that either one of these could occupy the auxin-binding site in the absence of ligand. Displacement of this tripeptide by free auxin would induce a conformation change to initiate signalling. One of these tripeptides, DDW, is adjacent to the residue considered photolabelled by azido-IAA (Brown and Jones, 1994b). This is an attractive model but, again, one that needs to be tested. The acidic residues of the other tripeptide, WDE, were shown by site directed mutagenesis to play a critical role in protein folding and functional activity of ABP1 at the plasma membrane (David *et al.*, 2001).

In addition to the homology with germins, Box A has been shown to share features with some peroxidases (Savitsky *et al.*, 1999). Peroxidases, particularly some plant peroxidase isozymes, share the HxH sequence, for example. In total, five fragments of ABP1 sequence were shown to have some homology, although three of these are overlapping homologies to the same Box A of ABP1 using different fragments of the peroxidases. The protein structure of the peroxidase family has been solved and shown to be principally α-helix different from the β-barrel structure of ABP1α The important observation from the paper is not that ABP1 will have peroxidase activity (it has no haem group) but that plant peroxidases (and not other peroxidases which do not share the homologies) might have a site for auxin binding and oxidation. There is a conserved tryptophan residue in plant peroxidases, its orientation and distance from the HxH motif will be interesting to compare with the coordinates of analogous residues around the auxin-binding site of ABP1. It should be noted, however, that no peroxidase has been found labelled or purified by auxin (see above) and so the affinity and specificity of such a site are likely to be low.

Cell biology

ABP1 has been detected in many tissues of the plant. To those catalogued before in maize (reviewed by Jones, 1994; Napier and Venis, 1995) can be added spores of fern, wheat shoots and roots, barley pre-anthesis flower spikes and developing caryopses, maize tassels and immature embryos, tomato seed,

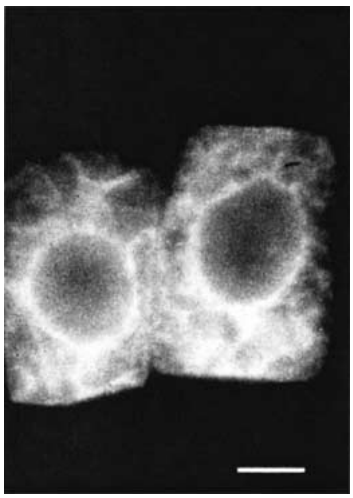

Figure 2. Immunofluorescence image of the distribution of ABP1 in a maize root cell (Henderson *et al.*, 1997).

ovary and flower buds and *Arabidopsis* rosette leaves all according to the available EST databases. Indeed, where ABP1 has been looked for it has been found, suggesting expression throughout the plant even if its expression is weak. The recent ABP1 knockout data (Chen *et al.*, 2001) also suggests a critical role in early embryo development. In maize coleoptiles, the protein is long-lived and expression levels are low, but largely unregulated (Oliver *et al.*, 1995).

All higher-plant ABP1s contain both a signal peptide and an ER retention motif and immunofluorescence, immunogold and biochemical tests have confirmed that most ABP1 is accurately retained (Figure 2). It is unclear whether or not ER retention is a feature of the moss sequence, no characteristic retention motif is present, but there is an abundance of acidic residues downstream of the conserved C-terminal cysteine (not shown, terminated early in Figure 1), one feature common in luminal ER proteins. The N-terminus also has features consistent with being a signal peptide and so it remains probable that *Ceratodon* ABP1 enters the ER and is retained there. The possibility that it is secreted cannot be ruled out, however. The data for the fern *Ceratopteris* (not shown) is incomplete, being derived from an expressed sequence tag clone and neither N- or C-termini are represented. Such ER targeting has added a layer of complexity to the cell biology of ABP1 in that most of the physiological data demonstrating activity as a receptor also place the site of action ABP1 on the plasma membrane.

Despite the ER retention motif, data suggest that ABP1 does pass along the constitutive secretion pathway to the plasma membrane and cell surface (Jones and Herman, 1993; Diekmann *et al.*, 1995; Henderson *et al.*, 1997). The bulk of the protein remains in the ER and this is consistent with all the biochemical data showing characteristic ER-type glycosylation (Henderson *et al.*, 1997) and localization (Ray, 1977b; Tian *et al.*, 1995; Henderson *et al.*, 1997). That detected at the cell surface is only a small fraction of the total (Bauly *et al.*, 2000; Henderson *et al.*, 1997). Yet, a number of different approaches have suggested that ABP1, and peptides based on the C-terminus of ABP1, added to intact cells without auxin complement reconstitute auxin-like physiological responses (Barbier-Brygoo *et al.*, 1991; Thiel *et al.*, 1993; Gehring *et al.*, 1998; Leblanc *et al.*, 1999b). These experiments add to other earlier work demonstrating that maize ABP1 was able to potentiate the auxin responsiveness of tobacco protoplasts when added in the medium at low concentrations, whereas polyclonal antibodies to the maize protein were shown to impair the same auxin-dependent response (Barbier-Brygoo *et al.*, 1989; Rück *et al.*, 1993). More recently, the use of a large panel of 9 distinct monoclonal antibodies were used on the functional tobacco protoplast assay to confirm that ABP1, and not a related protein, is involved in early auxin responses at the plasma membrane (Leblanc *et al.*, 1999a). In addition, polyclonal antibodies to ABP1 have been used to show that the auxin signal for protoplast swelling is perceived by extracellular ABP1 (Steffens *et al.*, 2001).

All together, the data have shown that ABP1 is active in auxin responsiveness at the surface of the plasma membrane despite carrying a targeting motif (Figure 3). Other ER-directed proteins are also being shown to escape and have biological activities outside this compartment (Xiao *et al.*, 1999; Okazaki *et al.*, 2000); As such, ABP1 is not unusual in being both targeted to the ER and found to have activities elsewhere. The KDEL motif has been shown to be involved in the stability of ABP1 but not to be important for auxin binding and interaction with the plasma membrane (David *et al.*, 2001). Up to now, no experimental data have indicated a functional role of ABP1 inside of the ER, apart from supplying the cell surface with ABP1 (Figure 3). The possible influence of auxin on the targeting of ABP1 at the cell surface has been investigated but no significant change in ABP1 exportation has been observed consecutively to auxin treatment (Henderson *et al.*, 1997).

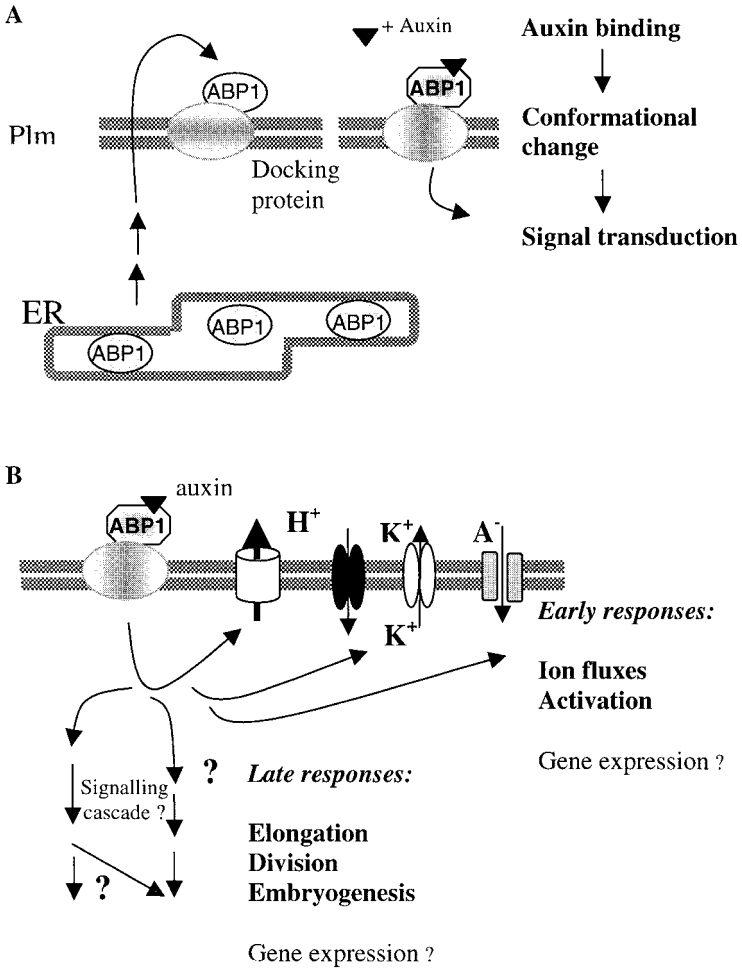

Figure 3. A. ABP1 is located both in the ER and at the outer face of the plasma membrane. Auxin binding induces a conformational change of ABP1 modifying the interaction with the plasma membrane (Plm) resulting in the activation of the signalling cascade. B. Experimental evidences support that the fraction located at the plasma membrane is involved in the control of early auxin electrophysiological responses. Correlation between such early responses and late cellular or developmental responses is not established yet. ABP1 seems to be implicated directly or indirectly in both cell elongation and cell division. No evidence has been provided so far that ABP1 mediates gene expression of early or late auxin-responsive genes.

The possibility that ABP1 has a role as chaperone when in the ER has recently been evoked (Chen *et al.*, 2001). Expression data showed that one other ER chaperone, BiP, was up-regulated in transcription by applied stresses, conditions likely to require more chaperone activity (Oliver *et al.*, 1995). ABP1 was not; indeed, heat stress down-regulated translation. However, chaperone-like interactive with specific partners cannot be ruled out. Polypeptides have been co-precipitated with ABP1, but these remain unidentified. Identification of ABP1 interacting components, at both the plasma membrane and in the ER, is one of the challenges of the years to come, but a challenge that will need to be met before we can fully understand ABP1 behaviour.

As for most phytohormones, the site of auxin perception has long been contentious (Vesper and Kuss, 1990; Claussen *et al.*, 1996). The role of ABP1 in the perception of auxin at the plasma membrane is no longer controversial. The existence of an intracellular auxin receptor remains an open question, such internal receptor could act either in concert or independently to ABP1 in response to changes in cellular auxin content. None can exclude the possibility that auxin carriers (Friml and Palme, this issue), which control auxin fluxes through plant cells and tissues, could also act as auxin receptors. Among the large

number of auxin binding proteins identified in the plast 20 years, none is presently considered as a possible intracellular auxin receptor.

ABP1 and downstream auxin responses

The involvement of ABP1 in early auxin responses at the plasma membrane has already been mentioned above. These early responses consist of modifications of ion fluxes across the plasma membrane, reflecting activation or deactivation of ion channels or transporters in response to auxin (Figure 3). The electrical responses are monitored by electrophysiological measurements which reflect either the overall magnitude of ion gradients (e.g. hyperpolarization) or, more specifically, the flux of a given ion (e.g. H^+, K^+ or anions). Auxin has been shown to act via ABP1 on the activation of the proton pump ATPase (Rück *et al.*, 1993), potassium channels (Thiel *et al.*, 1993) and voltage-dependent anion channels (Zimmermann *et al.*, 1994; Barbier-Brygoo *et al.*, 1996). An enhanced auxin sensitivity of the inward and outward rectifying K^+ channels was reported in guard cells of tobacco plants overexpressing different forms of ABP1 (Bauly *et al.*, 2000). Mesophyll protoplasts isolated from transgenic plants over-expressing the maize ABP1 were also shown to exhibit a shift of the hyperpolarization response to the lower auxin concentrations (H. Barbier-Brygoo and K. Palme, personal communication). These responses correspond to initial steps of a signalling pathway, but their correlation with other molecular or cellular auxin responses is not established yet.

Another early auxin cellular response, namely protoplast swelling, was shown to be mediated by extracellular ABP1, reinforcing the idea that the functional role of the protein in auxin responses involves the plasma membrane located protein (Steffens *et al.*, 2001). Protoplast swelling is a rapid and specific cellular response to auxin and shares common features with auxin-mediated growth at the organ level (Steffens and Lüthen, 2000). The study of transgenic tobacco plants over-expressing *Arabidopsis* ABP1 under the control of an inducible promoter has provided other experimental evidence supporting ABP1-mediated, auxin-dependent cell expansion (Jones *et al.*, 1998). This has been achieved by measuring the degree of curvature of leaf strips, or the size of mesophyll protoplasts, in samples treated or not with the inducer and auxin. No phenotype was observed at the whole-plant level when grown in the presence of the inducer (Jones *et al.*, 1998).

Constitutive expression of antisense ABP1 in BY2 cells was shown to reduce cell growth (Chen *et al.*, 2001). The reduction was interpreted as evidence for the role of ABP1 in both auxin-induced cell expansion and, directly or indirectly, cell proliferation. However, further experiments will be necessary to elucidate distinct roles for ABP1 in the control of cell expansion versus cell division. It is not clear whether more severe growth alteration could be observed on BY2 cells when ABP1 expression is completely abolished. Interestingly, an ABP1 knock-out mutant has been identified in *Arabidopsis* (Chen *et al.*, 2001). Plants heterozygous for the mutation in the single *Arabidopsis* ABP1 gene do not exhibit any phenotype whereas homozygous plants stop develop and die at the early globular stage of embryogenesis. The general architecture of *abp1* early embryos is severely altered, with a loss of symmetry, a fault in cell elongation and aberrant cell divisions affecting both the suspensor and the embryo. The identification of this null mutant for ABP1 demonstrates that the protein is essential during embryogenesis and, although difficult to work with, this resource could play an invaluable role in determining ABP1's place during auxin signalling during both elongation and cell division.

Challenges

Undoubtedly, ABP1 is an essential protein and is likely to be an auxin receptor. The challenges in the years to come will be to understand its mode of action and its relationship, if any, with already identified auxin signalling elements such as the transcription factors mediating the expression of early auxin-responsive genes or elements of the proteolytic pathway (Dharmasiri and Estelle, Hagen and Guilfoyle, and Liscum and Reed, this issue).

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