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Models of Auxin Binding

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ABSTRACT

Auxins have a universal importance for plant growth and development and have become important agrochemicals. For these reasons the identification of auxin receptors and characterization of their auxin-binding sites have been keenly sought. A number of molecular models of auxin-binding sites have been published and these are reviewed. Two of the models are based on auxin-binding protein 1 (ABP1) and comparisons between these and those based on biological activity measurements are discussed. Structural determination of ABP1 by protein crystallography is imminent and is likely to extend the pharmacological utility of this protein. In addition, new information on ABP1 sequence phylogeny suggests that it evolved with the green plants, but before the capacity for metabolism of indole-3-acetic acid and before the capacity for auxin efflux. A synthesis of data suggests that ABP1 is expressed ubiquitously throughout plant tissues and throughout their lifespan. The many physiological studies of ABP1 are not covered in this article, but all the data are consistent with ABP1 being an essential mediator of the auxin signal.

Key words: Auxin; Receptor; Molecular model; Herbicide; Ligand; Hormone; Signaling

INTRODUCTION

Auxins remain important agrochemicals. Historically their biggest market has been as dicot-selective herbicides (Grossman 2001) but the role auxin plays in so many agriculturally important plant processes extends their utility. For example, auxins are applied to promote adventitious rooting for clonal propagation and to control fruit cropping in citrus, and these applications contribute to their commercial portfolio. Nevertheless, given the diversity of responses mediated by auxin and the value of target selectivity from some synthetic auxins, it is clear that there are still many commercial opportunities to be exploited if we can learn more about the basis of auxin action. At the root of auxin action must lie auxin perception and to understand perception we must describe auxin receptors. To this end, auxin-binding sites have been sought for decades by researchers.

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There have been no unequivocal reports of auxin receptors, although we do have a strong candidate in auxin-binding protein 1 (ABP1) of which more is discussed below. A great deal has been learned about the mechanism through which auxin exerts control over transcription (del Pozo and Estelle 2000) and of genes regulated by auxin (this issue). However, it appears that none of the proteins associated with these tasks have sites for auxin perception. The characterization of the genes encoding auxin transport proteins has started to open up molecular analysis of auxin movement (Palme and Gälweiler 1999) and these transporters will have auxin-selective binding sites. Similarly, molecular genetics has identified some of the genes encoding auxin-metabolizing enzymes (Normanly and Bartel 1999) and these too must have recognition sites for their substrates. Over the coming years protein structures for some auxin-binding sites will be determined and a clear molecular picture of auxin-specific recognition will become available. It is to be expected that the architecture around the catalytic sites of en-

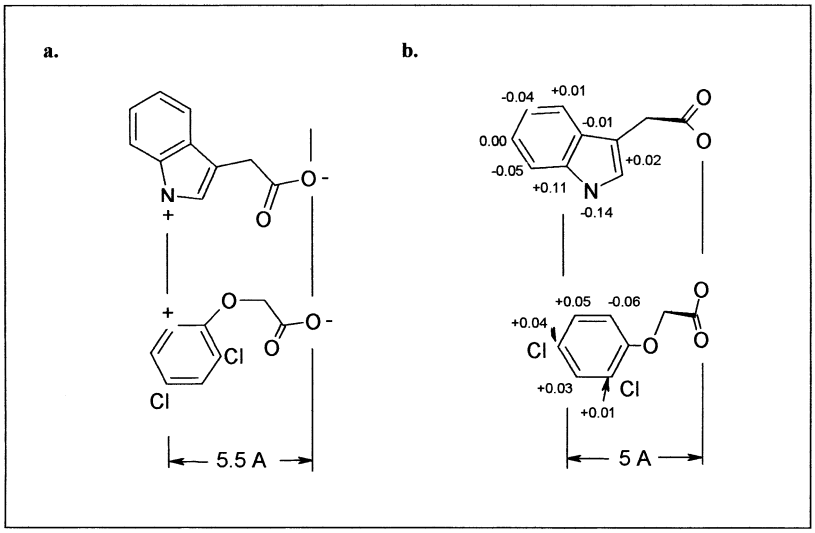

Figure 1. Charge separation models for auxin binding. **(a)** IAA and 2,4-D drawn with local charge centers separated by 5.5 Å, as described by Thimann (1963). (b) IAA and 2,4-D drawn to illustrate the charge distribution on ring atoms, as calculated by Farrimond and others (1978). Note that the indole nitrogen of IAA does not carry a net positive charge (as suggested by Thimann, Figure 1a), but there is localized positive charge on other atoms. The molecules are also represented with the carboxylate group folded out of the plane of the indole ring (see Figure 3). Both redrawn from the originals using ISIS/Draw.

zymes will differ from that around receptor sites, and transport proteins must offer both high (recognition) and low (release) affinity sites, but the ligand is small and so features of recognition are likely to be common. If we consider the selectivity of auxins applied topically as herbicides, the capacity of each of these proteins to recognize the different compounds will contribute to efficacy. Nevertheless, the most revealing advance in our knowledge of auxin action and selectivity will be the resolution of the structure of an auxin receptor. This review explores how our knowledge of auxin binding has grown prior to definitive binding site descriptions.

Early Modeling

Indole-3-acetic acid (IAA) was first associated with promoting plant growth in 1934 (Kögl and others 1934) and an explanation of its activity has been sought ever since. Completion of numerous, careful structure-activity screens (such as those summarized by Veldstra (1953)) was necessary before models for ligand activity could be suggested. In the early 60s came the first widely accepted explanation of an active auxin molecule (reviewed in Thimann 1963), the charge separation model (Figure 1). These precise physico-chemical measurements remain valid, but more than a decade after Thimann's model, advancing chemical knowledge and the onset of computer-assisted calculation enabled correction of his interpretations on charge distribution (Farrimond and others 1978). Farrimond and coworkers pursued the idea that fractional charge separations around the auxin molecule determined activity (Figure 1b) (Farrimond and others 1980), but as they expanded the range of compounds tested they recognized that charge separation could not account for auxin activity. They also reported that there was no correlation between the magnitude of a fractional positive charge on any of the ring carbons with biological activity (Farrimond and others 1981). Although Farrimond's work made Thimann's model fall from favor, these calculations are also now outdated. The molecular dimensions and structure of IAA was established unambiguously by X-ray crystallography in 1964 (Karle and others 1964).

The first binding site models were developed in the late 1970s based on the premise that the site could be represented by inversion of a composite ligand, like a shoe can be considered the binding site of a foot (Katekar 1979; Kojić-Prodić and others 1999). The first model was that of Kaethner (1977) (Figure 2a). This binding site was presented as a cleft with a hydrophobic area to accommodate the π electrons of the benzene ring and a set of charged areas on the inside surface of the cleft to complement, for example, the carboxylic acid group. There was also an area of negative charge to complement the idea, then current, of the fractional positive charge on the indole nitrogen (shown as iv on the model). Built into this model was the concept of a conformational shift in the ligand on binding, conferring an element of ligand specificity, which will be discussed later.

At a similar time, Lehmann (1978) was presenting a three-point attachment mechanism that avoided the use of charge separation. This mechanism suggested two hydrophobic areas, either of which could complement aromatic ring systems, and a single positively charged site to accommodate the carboxylate group. Among the compounds known to be active auxins are those with single or double rings (as well as other planar, conjugated

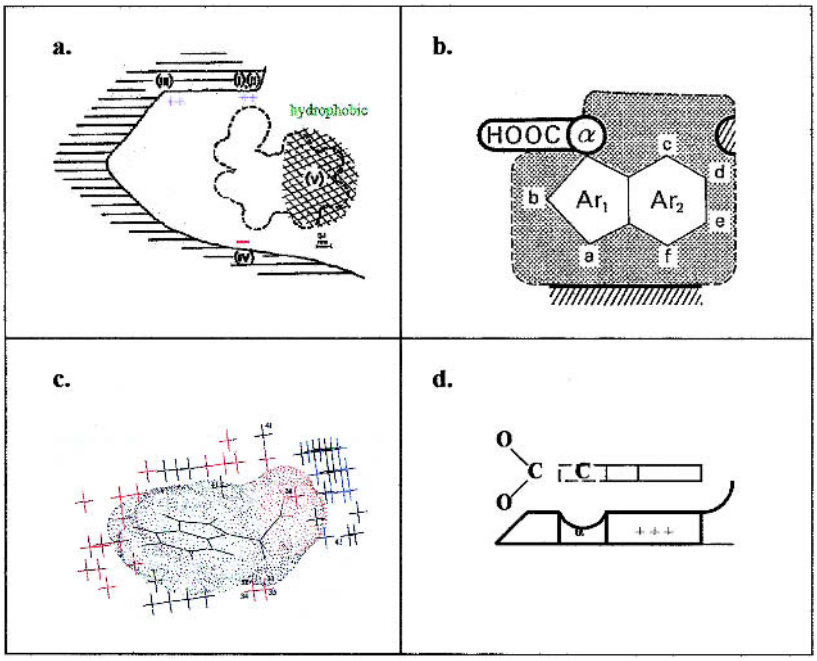

Figure 2. Auxin binding site models. **(a)** The model of Kaethner (1977), **(b)** and **(d)** show the model of Katekar (1979), **(d)** shows the site viewed in the plane of the aromatic rings; the IAA is bound in the conformation illustrated in Figure 3b. **(c)** Shows the model of Tomić and others [1998], strictly illustrating the distribution of global energy minima (+) for a set of five chemical probes.

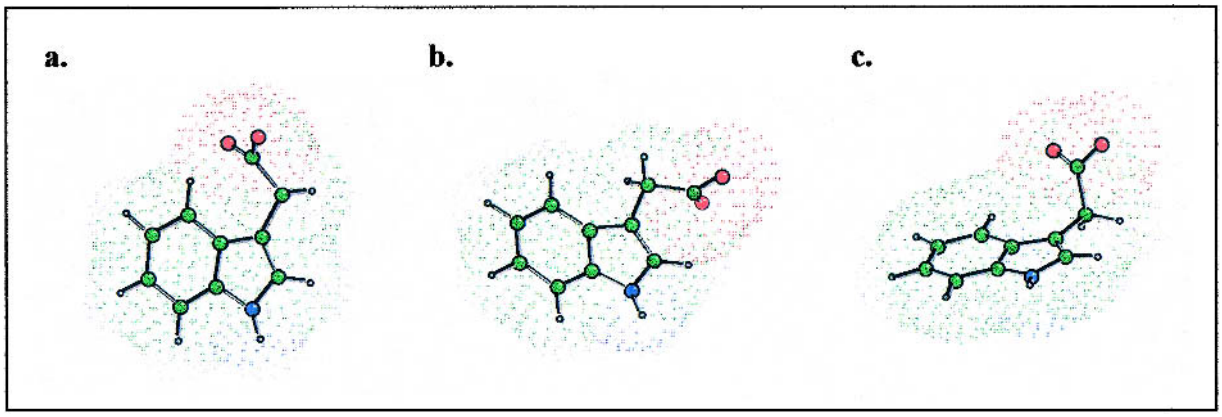

Figure 3. Conformations of IAA. The carboxylic acid group of IAA is shown in three of its possible conformations: **(a)** in the "recognition conformation" of Kaethner (1977), **(b)** in the planar conformation favored by Katekar (1979) and Edgerton and others 1994, and **(c)** in the "modulation conformation" of Kaethner. This last conformation is calculated to have the lowest energy, is the conformation of IAA determined from crystallography, and was the one used for the model of Tomić and others (1998).

structures). According to Lehmann, when the carboxylate fits into its site, different active compounds could interact with one or the other hydrophobic area.

The binding site models of both Katekar (1979) (Figures 2b,d) and Rakhaminova and others (1978) had also moved away from charge-separation. Essential features of these models were common with other representations (Figure 2). In each model a large hydrophobic platform was proposed, against which the aromatic ring system would sit adjacent to the carboxylic acid binding site. In Katekar's model (Figures 2b,d), there was also a transition site (labelled α) to accommodate the methylene carbon of IAA. All the site models gave molecular dimensions and indicated where the "fit" was tight (substitutions onto the ligand in these areas had been found to reduce auxin activity, shown by bold hatching in Figure 2b, for example). Details between the models varied, however.

The principal difference was on the conformation of the ligand. Although the aromatic ring system is planar and stable, the side chain has freedom to move (Figure 3). The energy barrier between the

orientations is low, but there are two energetically favored states in the free ligand, with the carboxyl carbon coplanar with the aromatic rings and the oxygens tilted out of plane (as seen in Figures 3a and b, Kaethner used the conformation shown in 3a), and with the carboxyl group perpendicular to the plane of the rings (Figure 3c). Kaethner built two conformations into his model. He proposed that auxins enter the binding site in a "recognition conformation", the planar conformation (Figure 3a) and active auxins then changed conformation to the "modulation conformation" (Figure 3c) inducing a matching change in the binding site. The models of both Katekar (1979) and Rakhaminova and others (1978) both imply that the receptor site is a preset template for auxin in the planar/tilted orientation (Figures 2d and 3b).

Later Models

After this early rush to model the auxin binding site there was little change for some years. Some refinement was done as computation became stronger, but reservations on the limitations of such models were also aired (Katekar and others 1986).

There has been one further detailed auxin pharmacophoric model, using calculations from contemporary molecular mechanics and molecular dynamics simulations (Tomić and others 1998) (Figure 2c). This model was developed from a series of careful analyses of auxin structures by the group of Kojić-Prodić in Zagreb and is based on the conformation found for IAA by X-ray crystallographic analysis (Figure 3c). The new model was used to assign auxins into various categories, strong agonists through to antagonists. In doing so, the surface properties of the space around the ligand were calculated and translated into a pharmacophoric binding site map.

As explained above, all these models were based on biological activity data. These data reflect not just the efficacy of a receptor to recognize each compound, but also the contributions of transporters and metabolizing enzymes to concentrate or deplete each at the sites of action. Only one model has attempted to use data of auxin binding to a single, recognized auxin-binding site, that of maize ABP1 (Edgerton and others 1994).

Edgerton and colleagues (1994) made use of the comprehensive set of binding data of Ray and others (1977), accepting that site 1 binding equated to ABP1. For modeling, Edgerton and others (1977b) used molecular mechanics to calculate low energy conformations of three ligands, IAA, 1-NAA, and 2-NAA. Superposition gave the template for their binding sites model. In essence, this is equivalent to those of Rakhaminova and others (1978) and Katekar (1979). There is a planar, electrophilic indole/naphthalene ring-binding platform adjacent to a carboxylic acid acceptor, but separated from it by a hydrophobic transition zone (similar to that seen in Figures 2b,d). Their model predicts that the ligand will be planar except for the carboxylate oxygens which are orientated perpendicular to the axis of the ring system (Figure 3b). In essence then, a model derived from the binding of auxins to ABP1 suggested a template identical to that of models developed from biological activity data.

The dimensions of the models differ little. However, the detailed analysis of more diverse compounds allowed Katekar (1979) to indicate which sides of the binding site constrained ligands most or, in other words, which parts of the site conferred greatest specificity. If these pharmacophoric models were to prove useful, they needed to be used either to help discover novel ligands or to test the structure of auxin-binding proteins.

Auxin Design

All the compounds tested by Ray and colleagues (1977) were fitted to the model of Edgerton and others (1994), including some with longer side chains like IBA. Suggestions were made about compounds not yet tested in binding assays which might bind to ABP1, or which might distinguish between binding (ABP1) and action, although no results from such tests have been published. The growing capacity for computational chemistry, used by Tomić and others (1998) for example, permits novel auxin design to be taken further, although I find no published accounts of such "designer" plant hormone discovery. Modeling of phytotropins (inhibitors of polar auxin transport) has been useful (Bures and others 1991) and some molecular orbital modeling has been used for developing auxin conjugates (Reynoso-Herrera and others 1999), although binding site models were not used.

Auxin-Bind Proteins

The number of proteins identified as having an auxin-binding activity continues to increase. These proteins have been identified by photolabeling, affinity chromatography, and auxin-binding assays. To date, no auxin-binding protein has been discovered through its cognate gene from the application of molecular genetics.

The likelihood that any of the auxin-binding pro-

teins are auxin receptors has been discussed extensively (Venis and Napier 1995; Napier and Perrot-Rechenmann 2001). Some, like the glutathione S-transferases and protein disulphide isomerase, have sites for small, hydrophobic ligands and so auxin binding properties seem reasonable. However, the sites show low specificity and are not regulatory, therefore these are not receptor proteins. Only ABP1 shows a high affinity binding site coupled with good specificity for active auxins. The remainder of this review will explain how closely ABP1 matches binding site models and will then evaluate the claim that it is a receptor.

ABP1

The discussion above indicates that ABP1 from maize equated to the auxin-binding activity originally described by Hertel and others (1972) and characterized by various groups (including Ray and others [1977]), as Site 1 auxin binding. The first purification (Löbler and Klämbt 1985) and cloning (Inohara and others 1989) led to extensive characterization at the levels of genome, protein, cell, and plant (Jones 1994; Napier and Perrot-Rechenmann 2001).

In *Arabidopsis* there is only a single ABP1 gene (Palme and others 1992) although in other plants it is represented by a small gene family (Lazarus and MacDonald 1996; Watanabe and Shimomura 1998). The organization of introns in higher plant genes is conserved and the translated sequence is also highly conserved (Figure 4). The protein is found through the plant kingdom at least as far back in phylogenetics as both Chlorophytes (green algae) and the Streptophytes (taxonomic divisions are as presented by NCBI), but appears absent from cyanobacteria. There are a number of cyanobacterial genomes sequenced and, for example, the blue-green alga *Synechocystis* has no homologue. There are no orthologues in yeast or other fungi.

It would be useful to know the sequences of ABP1 homologues in a greater number of lower plant genomes. The data for *Chlamydomonas* arises from expressed sequence tag (EST) entries and is very recent (not shown in Figure 4). There is a big taxonomic gap between this genus and the Embryophyta (the land plants). It is known that many organisms synthesize auxin, including bacteria, but many do not respond to it as a hormone. *Chlamydomonas* synthesizes plenty of IAA, although it is unclear how it responds to it as a signal. The single-celled plant *Chlorella* (also in the Chlorophytae like *Chlamydomonas*) has a capacity for facilitated uptake of auxin and the filamentous alga *Chara* (Characeae, but in the Streptophytae, not the Chlorophytae) has the capacity for both carrier-mediated uptake and efflux of auxin (Dibb-Fuller and Morris 1992). These data were discussed in terms of the appearance of efflux activity with the need for axial communication in multicellular, differentiated plants. Similarly, Sztein and others (1995) discussed auxin metabolism with respect to the appearance of vascular tissue, finding a correlation between the appearance of an enzyme capacity to metabolize free IAA and the appearance of vascular tissue in the Embryophyta.

The data available suggest that ABP1 evolved before the Embryophyta and, therefore, before the capacity to metabolize IAA or to exclude it by efflux. They suggest it arose very early in the evolution of the green plant Kingdom. However, there is insufficient genome sequence data from other branches of the Viridiplantae (green plants), or from parallel branches of the Eukaryotae (such as the red algae) to be more certain at present. Ancestry with the green plants (and prior to efflux and metabolizing activities) is relevant to the claimed function of ABP1 as an auxin receptor, but not confirmatory. Indeed, the protein encoded by the *Chlamydomonas* gene has yet to be shown to bind auxin, although key residues in both Box A (Figure 4) and elsewhere are conserved (not shown).

Expression of ABP1

In the higher plants expression is generally low except in a few species like *Zea mays.* In *Zea,* ABP1 is expressed in all tissues tested (Table 1). Expressed sequence tag (EST) data also suggest that ABP1 is present in most tissues in most plants although, again, data sets are limiting. At the time of writing there is only one EST report for *Arabidopsis* (isolated from rosette leaf material), but the ABP1 knockout data of Chen and others (2001) suggest it also has a crucial role in *Arabidopsis* embryo development. It seems likely that ABP1 expression is not specific to cell or tissue, but is ubiquitous. Work on maize seedlings also suggested that the mRNA was long-lived, as indeed was the protein (Oliver and others 1995). Consequently, ABP1 may well be present throughout all higher plants (even all green plants) all the time. Some specificity of isomer expression has been reported, however (Schwob and others 1993).

Protein Analysis

There are increasing numbers of gene, cDNA, and translated protein sequences in databases (Figure 4) and to these can be added EST accessions. Collected

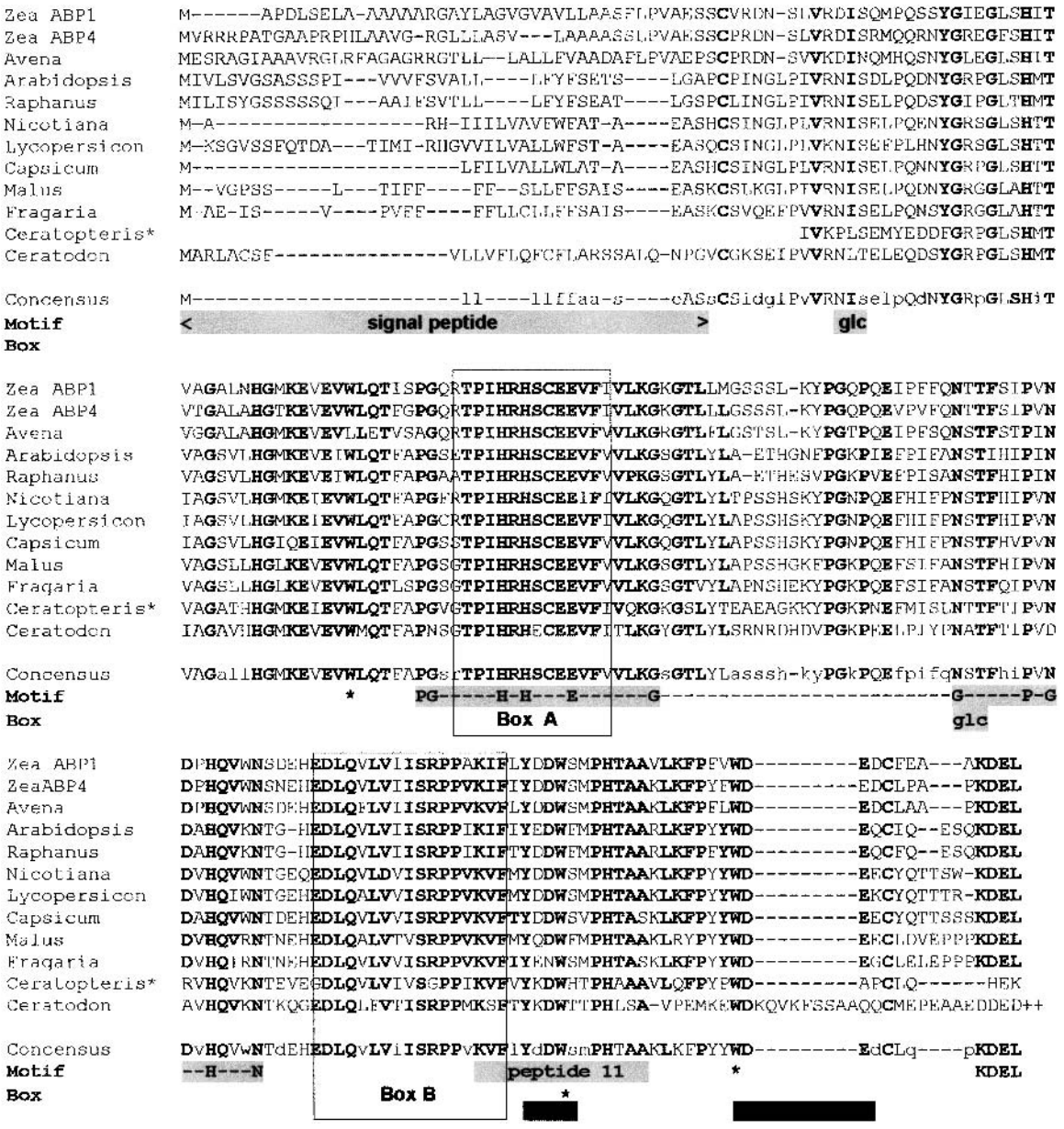

Figure 4. Sequence comparison of ABP1s. Translated sequences of ABP1s are listed against their genus names. Conserved residues are shown in bold type. A consensus sequence is shown at the foot, and lower case letters represent residues not conserved. Two boxes of completely conserved residues are outlined. Two black boxes under the data identify putative auxin mimetic sequences, discussed in the text. Several sequence motifs are listed: * represent the tryptophan residues discussed in the text, glc represents N-glycosylation motifs, and the residues in the shaded boxes represent the core cupin motif and peptide 11, the peptide reported to carry photoactivated IAA (Brown and Jones 1994).

together there are a number of clear messages. The signal peptide is the only highly variable and divergent part of translated sequences. The processed polypeptide is very highly conserved throughout most of its length. There are two boxes showing extremely high residue conservation, labeled A (also known as the D 16 box) and B. Between these two boxes lies a conserved N-glycosylation site and all dicot sequences carry a second N-glycosylation site towards the N-terminus. Some sequences contain other, additional N-glycosylation sites and there is evidence that all are used (Lazarus and MacDonald 1996). Considering the number of residues conserved throughout the phylogeny it is difficult to single out many of special merit. Three cysteines (C), one at the N-terminus, one close to C-terminus and

Table 1. A summary of plants and tissues for which ABP1 expression has been reported.

Plant	Tissue	Method
Zea	Immature embryo	EST
	Tassel and tassel primordium	
	Root	Antibody
	Coleoptile, mesocotyl, first leaf	Northern
	Seedling root	
	Tassel, silk, leaves	
Hordeum	Seedling shoot	EST
	Seedling root	
	Anther	
	Developing caryopsis	
	Pre-anthesis spike	
Triticum	Seedling shoot	EST
	Etiolated seedling root	
	Salt-stressed crown	
	Embryo	
Arabidopsis	Rosette	EST
Lycopersicon	Flower buds	EST
	Seed	
	ovary	
Ceratodon	Not specified	EST
Glycine	Not specified	EST
Ceratopteris	Spore	EST
Chlamydomonas		EST

one in Box A, have been mentioned by others (Venis and Napier 1995; Jones 1994; Venis and others 1992) and such conservation points to a functional significance. Two of the three are likely to be disulfide-bonded together within the ABP1 monomer (Tillmann and others 1989; Napier unpublished). Brown and Jones (1994) noted three conserved tryptophans (W). With additional sequences to pile-up (Figure 4) this remains true, although there is one inconsistent sequence (*Avena,* just upstream from Box A). This set of data needs checking and confirming.

Two additional sequence motifs are conserved: one is the C-terminal endoplasmic reticulum (ER) retention sequence — KDEL, the other is the cupin motif.

The KDEL Motif

The KDEL sequence identifies ABP1 as a reticuloplasmin, a protein targeted to and retained in the lumen of the ER. This targeting motif has been shown to be functional and most cellular ABP1 does lie in the ER (Napier 1997). Nevertheless, most of the data suggesting that ABP1 is an auxin receptor also require that active ABP1 is present on the outer face of the plasma membrane. To this end, several groups have used a variety of techniques to verify that some, perhaps less than 2%, escapes ER retention and arrive at the plasma membrane through the constitutive secretory pathway (Löbler and Klämbt 1985; Jones and Herman 1993; Diekmann and others 1995; Henderson and others 1997). Such escape from ER targeting has been detected in other reticuloplasmins (Napier 1997; Napier and Perrot-Rechenmann 2001). It is also interesting to note that the ABP1 sequence from the moss *Ceratodon* does not have a recognizable ER retention sequence (Figure 4). The ESTs for the fern *Ceratopteris* do not have sufficient data for comment at present. However, efforts to demonstrate that some ABP1 does arrive at the plasma membrane distracted attention from the question of why it was targeted elsewhere.

Experiments to seek the function of ABP1 inside the ER have been unsuccessful so far. Efforts to co-precipitate ABP1 with substrates or complexes of functional proteins suggested associations, but could identify none of the possible partners (Oliver and others 1995). One early model (Cross 1991) suggested that ABP1 chaperoned cell wall building blocks along the secretory pathway in an auxin-dependent manner. However, the low abundance of ABP1 and its very slow turnover time (Oliver and others 1995) did not favor the model and no binding to wall material has ever been reported. This model would also have required auxin binding in the ER, not at the plasma membrane.

A family of IAA-amide conjugate hydrolases have been found with C-terminal HDEL and KDEL motifs (Davies and others 1999). This suggests that the ER is a compartment active in synthesizing or hydrolyzing auxin conjugates, making the ER important for auxin homeostasis. Observers have often asked whether or not ABP1 might be a store of free IAA in the ER; others have argued that this cannot be the case (Napier 1995). The pH of the lumen of the ER is likely to be around neutrality, like the cytoplasm and as measured in animal cells. If so, ABP1 would bind little auxin there because of the steep pH-dependence of auxin binding. Binding is maximal at around pH 5.0, little binding activity remains at pH 7.0 (Ray and others 1977). Imposing pH 7.0 on plant ER confirmed that little auxin would be bound under these conditions (Tian and others 1995). However, the pH of plant ER has never been measured *in situ;* it remains possible that it is acidic and under these conditions ABP1 would bind free auxin. Either way, ABP1 compartmentalization with the conju-

gate hydrolases is an intriguing coincidence and warrants further investigation.

Cupins

The cupin superfamily of proteins was described from a conserved double motif of disparate residues (Dunwell and others 2000). Cupins have been identified in all kingdoms and the diversity of functions attributed to them is considerable. The cupin motif in APB1 commences in Box A and includes key, conserved residues of this domain (Figure 4).

One of the features of protein superfamilies is that the polypeptide folding pattern is often conserved despite high sequence digression outside the core motifs. The structures of two cupins have been determined and these have been used as the scaffold on which to model the structure of ABP1. (Warwicker 2001).

ABP1 as a Modeled Auxin Binding Site

Among the cupins are the vicilins, and among the vicilins are the lectin concanavalin and the storage protein phaseolin. Sequence alignment using the cupin motif suggested that homologies with ABP1 were strong enough to permit molecular modeling for the central part of the sequence. Homology was too weak to include either N- or C-termini in the exercise. Nevertheless, it was suggested that ABP1 folded into an antiparallel β barrel, a common structural motif.

The protein germin (also known as oxalate oxidase), another cupin in the vicilin group, was included in the pile-up. Similar molecular modelling on germin indicated that it would fold into an antiparallel β barrel and coordinate a metal ion composed of three histidine residues and one glutamic acid. The structure of germin is now determined (Woo and others 2000a) and has confirmed these predictions. The model for ABP1 suggested a similar histidine cluster within the β barrel and therefore, a metal ion binding site. This metal ion would then form the coordination site for the carboxylic acid group of bound auxin, and in doing so would represent the strong electrophilic site of all previous models. X-ray crystallography of ABP1 has shown that this part of the model is correct and that ABP1 does indeed contain a divalent metal ion in this histidine cluster. The core structure of ABP1 is also confirmed as an antiparallel β barrel (Napier and Pickersgill unpublished). It seems extraordinary that no biochemistry or physiology had suggested such metal ion coordination in the long history of the study of this protein or of auxin binding.

In germin, the metal ion is manganese (Woo and others 2000a) and it is active in coordinating the binding of the substrate oxalic acid. In this respect the similarity of the carboxylate binding sites is, perhaps, to be expected. However, the manganese of germin is also a redox-active center conferring both oxidase activity and a measurable superoxide dismutase activity. ABP1 does not bind oxalate, does not have oxalate oxidase activity or superoxide dismutase activity (Napier and Marshall unpublished), and there is evidence that the metal ion in ABP1 is not manganese (Napier and Marshall unpublished). Without a redox center in the protein, the metal ion may contribute structural rigidity and an electrophilic binding center, but not an enzyme oxidase activity. Even so, the possibility that ABP1 might have some enzymic activity, at least under the conditions prevailing in the ER, should not be ruled out.

All previous models for an auxin-binding site included a hydrophobic platform for the indole ring system (see sections on Modeling; Figure 2). Warwicker's model suggests that one of the trytophan residues of ABP1, W44 in his alignment, formed this platform. This tryptophan sits across the center of the β barrel from the histidine cluster and the model predicts that the indoles stack against each other. In contrast, earlier photolabelling experiments using tritiated azido-IAA (Brown and Jones 1994) found that the label attached to peptide 11 (see Figure 4) and suggested that it attached specifically to aspartate 134 (D134). This led the authors to suggest that the adjacent tryptophan (W136) would form the hydrophobic platform. This tryptophan is found to be conserved (Figure 4), whereas W44 appears not to be (although the *Avena* sequence needs to be confirmed). Only when there is a published crystal structure, with the ligand bound, will either of these predictions be found to be correct or incorrect.

It was mentioned in the Introduction, that some enzymes capable of metabolizing IAA might share features with receptor sites. It has long been recognized that peroxidases oxidize auxins, although there are no reports of auxin specificity. Some sequence homology between a plant heme peroxidase and ABP1 has been reported (Savitsky and others 1999). However, the principal region of similarity was over the histidine-rich part of Box A (Figure 4), which corresponds to residues in peroxidase that coordinate the heme group. As seen in Warwicker's model (Warwicker 2001) and in germin (Woo and others 2000a) the box A peptide histidines coordinate a metal ion in ABP1. Consequently, the sequence homology identifies a metal-binding domain, not an auxin-specific binding motif. The protein structure of peroxidase has been solved and is

primarily an α helix, in contrast to the β barrel of cupins (see Savitsky and others 1999). Nevertheless, one of the residues of peroxidase found to be important for electron transfer to the substrate is a tryptophan, a residue considered likely to form the hydrophobic platform for auxin in ABP1. It will be interesting to explore similarities between the active sites of disparate auxin-active proteins, especially when presented on such divergent scaffolds.

Conformational Change on Ligand Binding

Warwicker goes further in his predictions, suggesting that either one of two tripeptides in the unmodeled C-terminal end of ABP1 could be a pseudosubstrate for the auxin-binding site in the absence of free auxin. The proximity of an indole side chain (on a tryptophan) and a carboxylic acid side chain (on either glutamic or aspartic acids) within two residues suggests the possibility of auxin mimicry. The tripeptides identified are glutamate-glutamate-tryptophan (DDW) and tryptophan-glutamate-aspartate (WDE), Figure 4, black boxes. The model cannot predict which might be most likely to fold and occupy the auxin site, but it can be seen (Figure 4) that neither is fully conserved. Whichever tripeptide it might be, free auxin would displace the peptide to induce a conformational change as the C-terminus moved out of the site. However appealing this modeling might be, it should be remembered that it is only a hypothesis.

There is additional evidence for a ligand-induced conformational change in ABP1. One of the earliest reports on purified protein showed that there was a small change in circular dichroism when 1-NAA bound, a slightly different change when the anti-auxin 2-NAA bound (Shimomura and others 1986). These changes were interpreted as evidence for movement in the protein, although they can also be interpreted as changes in solvation of certain tryptophan residues induced by the proximity of bound ligand.

A monoclonal antibody raised against ABP1 was used to assess epitope accessibility in the presence of a wide range of auxins using ELISAs (Napier and Venis 1990). The antibody was competed off ABP1 by rising concentrations of active auxins, but not by inactive analogues. Again, this was interpreted as evidence of ligand-induced conformational change, with the epitope becoming inaccessible when auxin was bound. The relatively high concentrations of auxin needed to displace the antibody were attributed to the assay conditions (pH 7.4 in saline buffer) and antibody binding kinetics. However, other proteins have domains at which small ligands bind at low affinity, such as glutathione *S*-transferases and protein disulfide-isomerase — another reticuloplasmin (Primm and Gilbert 2001). It is possible that auxins could bind to such a site on the surface of ABP1 (rather than to the internal, high affinity site) leading to reduced antibody binding.

Very recently, surface plasmon resonance spectrometry has been used with a panel of monoclonal antibodies (David and others 2001). Using bacterially expressed ABP1 (and so post-translational glycosylation will be absent), the binding kinetics of the antibodies were monitored in the presence and absence of auxin. It is a shame that we do not know the kinetics of auxin binding for this bacterially expressed protein but again, the data suggest that auxins induced conformational rearrangements in ABP1, or changed the flexibility of epitopes.

Only structural data from crystallography, or nuclear magnetic resonance spectroscopy, can give direct and unequivocal evidence of conformational changes induced by ligand binding. It can be hoped that such data will also help determine how ABP1 might interact with other components of action. Crystallography of ABP1 is in progress (Woo and others 2000b) and such data will be available shortly.

Models, Function, and Herbicides

Models for auxin binding have evolved with increasing knowledge of chemistry and increasing computational power. Early ideas involving charge separation were later shown to be unlikely explanations of auxin recognition. All subsequent models have suggested that the binding site is likely to comprise a planar, hydrophobic platform separated from a carboxylic acid acceptor site by a short electrophilic transition region. Most models suggest that auxin is bound as a totally flat molecule except that the carboxylate oxygens lie in a plane perpendicular to the rest (Figure 3b), the straddle position of Edgerton and others (1994). Two models disagree. Kaethner (1977) suggested a straddle-like "recognition conformation" for the initial contact which, for active auxins, changed with movement in the receptor to a tilted "modulation conformation" (Figure 3c). Tomić´ and others (1998) have used their extensive physical chemical analysis to derive a model in which bound auxin is in the tilted conformation, equivalent to Kaethner's "modulation conformation". Much careful experimentation has also suggested that specific residues of ABP1 are involved in

recognition. With the arrival of a crystal structure there will be a chance to test binding site coordinates against all the models as well as to determine the consequences of auxin binding. However, the most critical question of all the data is whether or not we are better placed to explain the function of ABP1.

Binding site models generated from ABP1 data and from biological activity data match closely. Examinations of sequence and expression data are consistent with the suggestion that ABP1 is ubiquitous in the green plant kingdom, predating evolution of specific auxin metabolizing and carrier mechanisms. Physiological data, not reviewed here, are consistent with the action of ABP1 as a receptor when at the plasma membrane and the gene knockout data confirm that ABP1 has a function essential in early embryogenesis (Chen and others 2001). The structure (Woo and others 2000b) will illustrate how ABP1 binds auxins. The true value of such an advance will only be realized if it extends our understanding of how the protein works or if it can be used to increase the utility of auxins in industry, primarily in agriculture. There have been suggestions in the literature that resistance of some races of *Sinapis* to auxinic herbicides might be attributed to biotype variation in ABP1s (Chibbar and Chen 1999) and herbicide resistance in *Sinapis* has been correlated with impaired binding of auxinic herbicides to auxin-binding sites (Deshpande and Hall 1999). Binding of auxins to a dicot version of ABP1 has never been tested thoroughly because of the very low abundance of the protein in these species. These are the first reports suggesting a link between ABP1 and herbicidal action. However, with the availability of a structure for the *Zea* protein, homology modeling of a dicot ABP1 (dicots being generally susceptible to auxinic herbicides) will be possible. It is hoped that a crystallographic structure for one of the dicot ABPs will follow, along with accurate data for binding specificity against a defined and purified protein. With all these resources, ligand design should have a new lease on life and new ways of using auxins and their recognition sites should add new commercial opportunities to the repertoire of this family of long-serving agrochemicals.

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