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REVIEW

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Points of regulation for auxin action

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Abstract There have been few examples of the application of our growing knowledge of hormone action to crop improvement. In this review we discuss what is known about the critical points regulating auxin action. We examine auxin metabolism, transport, perception and signalling and identify genes and proteins that might be keys to regulation, particularly the rate-limiting steps in various pathways. Certain mutants show that substrate flow in biosynthesis can be limiting. To date there is little information available on the genes and proteins of catabolism. There have been several auxin transport proteins and some elegant transport physiology described recently, and the potential for using transport proteins to manage free indole-3-acetic acid (IAA) concentrations is discussed. Free IAA is very mobile, and so while it may be more practical to control auxin action through managing the receptor and signalling pathways, the candidate genes and proteins through which this can be done remain largely unknown. From the available evidence, it is clear that the reason for so few commercial applications arising from the control of auxin action is that knowledge is still limited.

Keywords Plant hormone · Homeostasis · Auxin · Regulation · Control

Abbreviations *IAA*: Indole-3-acetic acid · *IAAld:* Indole-3-acetaldehyde · *IPA*: Indole-3-pyruvate · *1-NAA*: Naphthalene-1-acetic acid · *NPA*: 1-*N*-naphthylphthalamic acid · *TAM:* Tryptamine

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Introduction

Our knowledge about the physiological and molecular aspects of auxin is rapidly expanding. As we strive to understand how auxin works, molecular genetics is playing an increasingly important part. Great advances have been reported on the characterisation of auxin transport, the regulation of auxin-induced genes and the roles of auxin in tissue pattern formation. Yet, in no area is the picture complete. In particular, we remain ignorant of many aspects of IAA metabolism, auxin perception and the details of cross-talk between hormone pathways.

A knowledge of auxin action will increase the possibilities for controlling plant growth and development to benefit agriculture and horticulture. A fine example of such crop improvement has been the production of parthenocarpic fruit by localised auxin overproduction (Ficcadenti et al. 1999; Donzella et al. 2000). However, the introduction of the bacterial genes for auxin synthesis is not always appropriate, and more versatile options are sought.

The list of genes and proteins associated with auxin action is expanding rapidly, and yet few are likely to be viable targets for directed crop improvement, such as through transformation programmes. An attempt to improve each new genotype requires a considerable investment of time and resources. Consequently, the choice of each target for manipulation is important. This review reflects on our current knowledge of endogenous controls on the auxin pool with a view to identifying the most favourable targets for the regulation of auxin action for crop improvement programmes.

Auxin biosynthesis and homeostasis

Selections of *Arabidopsis* mutants and sophisticated analytical tools for the quantitation of hormones have contributed much new information on the pathways of IAA biosynthesis and on homeostasis. Some very good reviews on auxin biosynthetic pathways have appeared

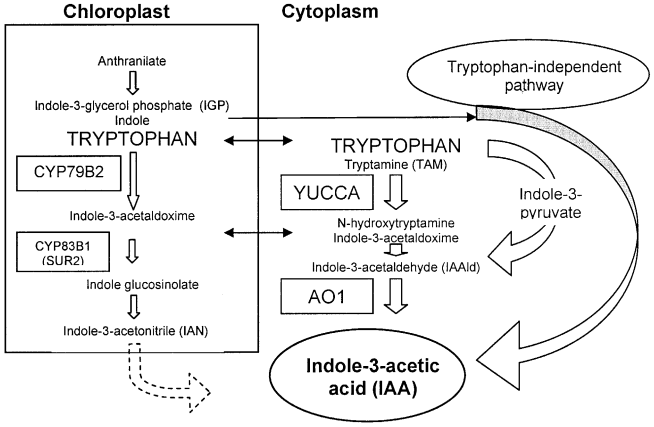

Fig. 1 Parallel pathways for auxin biosynthesis. Intermediates in the biosynthesis of IAA are named along with abbreviations *in brackets* corresponding to those used in the text. Two compartments are illustrated, the chloroplast and the cytoplasm. Where genes or mutants have been identified with particular enzymatic steps, the protein products and/or mutants are named in *boxes*. The *dashed arrow* illustrates the glucosinolate pathway restricted to few plant families. The *long arrow* on the *right* indicates the undescribed tryptophan-independent pathway

recently (Bartel 1977; Normanly and Bartel 1999; Bartel et al. 2001). However, regardless of these advances, the identities of many of the genes and proteins involved in IAA biosynthesis in plants remain unknown. In higher plants, both tryptophan-dependent and tryptophan-independent pathways have been identified, and tryptophan-dependent synthesis is split into at least three different pathways. A useful diagram is given in Bartel et al. (2001), and a summary is given here in Fig. 1. It has also become clear that IAA synthesis is not restricted to apical meristems and that all parts of young plants can synthesise IAA (Ljung et al. 2001a).

Tryptophan-dependent synthesis

Three parallel tryptophan-dependent IAA synthesis pathways are likely in higher plants, through IPA or TAM, or via a cytochrome P450. Substitution of tryptophan's amino group by oxygen through aminotransferase activity yields IPA. Although genes for the enzymes of this pathway have been identified from bacteria and fungi, homologues have not yet been identified from plants. IPA has been shown to be an intermediate (Cooney and Nonhebel 1991), and it is likely that it is decarboxylated to yield IAAld.

Tryptophan decarboxylation yields TAM. An activation-tagged mutant of *Arabidopsis* named *yucca* displays a long list of characteristics associated with IAA overproduction. The *YUCCA* gene encodes a flavin monooxygenase-like enzyme, and assays showed that this enzyme catalyses the hydroxylation of TAM to give *N*-hydroxytryptamine which is likely to be converted further to indole-3-acetaldoxime (Zhao et al. 2001). The data also show YUCCA to represent a rate-limiting enzyme for IAA synthesis.

Tryptophan is also converted to indole-3-acetaldoxime directly by two *Arabidopsis* cytochrome P450s named CYP79B2 and B3 (Hull et al. 2000). Indole-3-acetaldoxime is converted to IAAld, but the genes or enzymes mediating this step are unknown. In *Arabidopsis*, indole-3-acetaldoxime might become the substrate for a further cytochrome P450, moving the indole nucleus into the glucosinolate pathway (Bak and Feyereisen 2001). Although indole-3-glucosinolate functions largely as a storage molecule, it can contribute to the free IAA pool through the actions of myrosinase and nitrilases. There is one well-known *Arabidopsis* mutant, *sur2* (superroot2), that has reduced levels of indole glucosinolates and elevated levels of IAA and IAA conjugates (Barlier et al. 2000). In this case it appears that the SUR2-glucosinolate pathway contributes to the regulation of IAA synthesis by modulating the availability of indole-3-acetaldoxime. In wild-type plants, SUR2 diverts indole-3-acetaldoxime into glucosinolates, while in the mutant the side-branch is blocked, leading to a flood of extra substrate and excess IAA (see Fig. 1). Although it seems likely that the glucosinolate pathway is restricted to very few plant families (Bak et al. 2001), both the *sur2* and *yucca* phenotypes illustrate that indole-3-acetaldoxime is a pathway-limiting substrate for IAA synthesis.

All three tryptophan-dependent pathways converge at IAAld, which is oxidised by IAAld oxidase to IAA. Interestingly, an aldehyde oxidase is also involved in the final step of abscisic acid biosynthesis. IAAld oxidase activity has been measured in plants, and the small aldehyde oxidase gene family in *Arabidopsis* has been described (Sekimoto et al. 1998; Seo et al. 1998). One of these aldehyde oxidase genes, named *AtAO1*, showed elevated transcription in one of the super-root mutants (*sur1*, also known as *rty*, *alf1* and *hls3*), which overproduces IAA (and IAA conjugates) and has a phenotype consistent with elevated auxin. The *SUR1* gene itself encodes a protein with similarities to aminotransferases, but it is unclear how the loss of *SUR1* gene function leads to AtAOl overproduction and high auxin activities. However, the activity of AtAO1 does appear to be rate limiting for IAA synthesis

Tryptophan-independent synthesis

The tryptophan-independent pathway is likely to have a role in IAA synthesis in all plants, including the lower land plants like mosses (Sztein et al. 2000). Much of our limited information on intermediates and precursors has come from maize and *Arabidopsis* mutants defective in tryptophan synthesis. Some accumulate high levels of indole-3-glycerol phosphate, particularly the *Arabidopsis trp3.1* mutant but, oddly, do not accumulate excessive IAA even though conjugates do increase markedly (Müller and Weiler 2000). No other mutants or metabolite pools have been identified to fill the gap between indole-3-glycerol phosphate and IAA.

Regulation of IAA biosynthesis

Some authors suggest that the tryptophan-independent pathway governs constitutive, maintenance levels of IAA and that the tryptophan-dependent pathways engage when higher levels are required, such as during embryogenesis and upon wounding (Bartel et al. 2001; Ribnicky et al. 2002). There are also temporal elements of regulation with the two pathways being switched on at different times, such as after germination (see below).

The distinction of pathways into the categories constitutive or responsive is interesting, but at present it is not clear how these pathways are regulated. All we know about regulation of the constitutive pathway is that it is established after germination (Ljung et al. 2001a). With respect to the responsive, tryptophan-dependent pathways, mutant plants have shown that some of the steps are rate-limiting, which offer opportunities to identify candidate genes and enzymes through which IAA accumulation can be controlled.

It is intriguing to reflect that IAA homeostasis appears to be much more acute for IAA made by the chloroplast-localised enzymes (see Fig. 1). Overactivity of these leads to accumulations of IAA conjugates (e.g. overexpression of the chloroplastic cytochrome P450s) more than of free IAA, while the overactivity of the cytoplasmic enzymes leads to elevated levels of free IAA and the associated phenotypes. This observation may not prove to be a general rule because too few steps are well-characterised and the glucosinolate pathway of *Arabidopsis* may well complicate the picture. However, current data suggest that marked changes in free IAA are more likely to arise from the manipulation of genes coding for cytoplasmic enzymes. Of these, the activities of YUCCA and AO1 are rate-limiting and so could be targets for IAA management. However, it seems abundantly clear that more information is required on each of the degenerate pathways before useful management strategies can be considered and we can progress beyond the application of the bacterial genes.

In addition, it has been shown that overexpression of biosynthetic genes and the application of exogenous auxins lead to the rapid accumulation of conjugates and increased oxidation as the plant reacts to regain homeostasis. Consequently, subtle and robust manipulation of free IAA concentrations will also require a knowledge of deactivation processes.

IAA catabolism

Irreversible removal of free IAA from the active pool proceeds by two principal routes – through oxidation to 2-oxindole-3-acetic acid and through indole-3-acetyl-*N*-aspartic acid (Chamarro et al. 2001; Kowalczyk and Sandberg 2001; Ljung et al. 2001a). It appears that oxidation is the major route for deactivation in unchallenged tissues or those under low exogenous auxin load, but that conjugation prevails at higher concentrations. Both are intermediates to further breakdown steps. No genes have been associated with either activity.

IAA conjugates

Although some of the IAA conjugated to indole-3-acetyl-*N*-aspartic acid is further catabolised, conjugates are also used as storage compounds. Indeed, most of the IAA in higher plants is stored as inactive conjugates. In non-vascular plants, conjugates seem to be less important (Sztein et al. 2000). Conjugate hydrolysis releases free IAA, and such hydrolysis is the principal source of auxin in germinating seeds. Conjugates are also transported around the plants. IAA is conjugated in a variety of ways – as amides to amino acids, peptides and proteins, and to sugars through both ester and N-linkages. Amino acid conjugates may also be glucosylated (Ljung et al. 2001a). In general terms, monocots accumulate ester-linked sugar conjugates while dicots accumulate amide conjugates, although the spectrum of conjugates appears to be both species-dependent and developmentally regulated.

The first IAA-conjugating enzyme to be identified was a glucosyltransferase from maize (*Zea mays*; Szerszen et al. 1994). Recently the UDP-glucosyltransferase multigene family has been explored in *Arabidopsis*, and one gene product, named UGT84B1, has a clear substrate preference for IAA (Jackson et al. 2001). Its intracellular location is not clear, but the pH optimum and requirement of a reducing environment are consistent with being in the cytoplasm. No details about the phenotypes arising from overexpression or gene knockdown experiments have been described as yet.

Amide conjugate synthesis may be uncharted, but some enzymes associated with conjugate hydrolysis have been described. A set of *Arabidopsis* mutants fails to respond to exogenous IAA conjugates (summarised in Bartel et al. 2001). Some mutations have been mapped to genes encoding IAA-conjugate amidohydrolases and the enzyme kinetics characterised (LeClere et al. 2002). Another enzyme, named IAR1, is thought to represent a membrane-spanning metal ion transport protein (Laswell et al. 2000). The substrate for transport and the role of this protein in conjugate hydrolysis remains unknown.

IAA-conjugate amidohydrolases are targeted to the endoplasmic reticulum (ER), although the significance of such compartmentation is unclear because there is no clear evidence on the storage location of any conjugate. The vacuole has always seemed a likely storage depot. However, for amide conjugates the ER now seems the logical target compartment. It is also the principal compartment of the auxin-binding protein ABP1.

Regulation of catabolism

With the possible exception of conjugate hydrolysis during germination, conjugate synthesis, hydrolysis and ir-

reversible catabolism are under careful developmental regulation. Recent descriptions of IAA pool sizes from *Arabidopsis* (Ljung et al. 2001a), *Pinus sylvestris* (Ljung et al. 2001b) and *Citrus sinensis* (Chamarro et al. 2001) all give examples of this. For example, *Pinus* seeds hydrolyse ester-linked conjugates for 3–4 days after imbibition, giving a pulse of free IAA between days 2 and 3. Root growth and germination start between days 3 and 4. After this, around day 4, de novo synthesis starts on the tryptophan-dependent pathway, along with oxidation and amide conjugate synthesis. Around day 7 synthesis via the tryptophan-independent pathway starts.

These exquisite reports all illustrate developmental regulation. However, for the present we have no idea how this regulation is manifested or how we might intervene to manage it. Only a few genes and enzymes from the catabolic pathways have been identified, and almost all the information available is kinetic. This will be of great value as we start to understand homeostasis, but we remain poorly informed about most of the key steps in these processes. Using the developmental timelines and the responses to exogenous auxins referred to above, well-designed microarray experiments should soon identify steps at which transcriptional regulation is critical. These will become targets for intervention, and if used in conjunction with the regulation of synthesis, there will be considerable scope for subtle IAA management.

Auxin transport and plant development

It is likely that local and transient changes in the concentrations of free IAA brought about by IAA transport are at least as important as changes induced from synthesis or conjugate hydrolysis. Indeed, only transport fluxes possess vectorial (directional) information. Consequently, transport should offer amenable regulatory targets for auxin action.

The role of auxin transport in developmental responses has been demonstrated for both lateral root development (Reed et al. 1998; Casimiro et al. 2001; Rashotte et al. 2001; Bhalerao et al. 2002; Marchant et al. 2002) and the development of the shoot apical meristem (Vernoux et al. 2000; Benjamins et al. 2001; Muday and DeLong 2001). An example of root development is described briefly as an illustration.

Lateral root primordium development proceeds through three stages of dependence on auxin transport: (1) initiation, during which discrete pericycle cells undergo a set of defined divisions (Casimiro et al. 2001); (2) emergence, which requires a second and independent source of auxin; (3) independence, the point at which the lateral root apex governs its own auxin balance and can synthesise its own IAA.

The first phase, initiation, is dependent on IAA from the primary root apex. The auxin uptake carrier mutant *aux1* gives a phenotype with a 50% loss of lateral root primordia. The initial loss is attributed to a deficiency in loading IAA into the phloem in the cotyledons, a further loss occurs in unloading it from the phloem into the root and a third loss occurs as the transit of IAA back from the apex to the elongation and lateral root zones is impaired (Marchant et al. 2002).

Between days 3 and 10 lateral roots begin to emerge, and this emergence is coincident with a new supply of IAA from apical tissues in the form of a pulse of IAA produced in young leaves (Ljung et al. 2001a) that flows slowly down to the roots (Bhalerao et al. 2002). The peak of the pulse in the roots is between days 6 and 7 following germination and represents approximately double the free IAA concentration (of whole roots) measured before and after the pulse. The dependence of lateral *emergence* on this pulse was indicated by the observation that decapitation prevents emergence but not the *initiation*, of lateral root meristems. NPA, a polar auxin transport inhibitor, applied to the root-shoot junction also prevented lateral emergence. Ten days after germination, IAA synthesised by the primary root itself appears to be sufficient to reduce the dependence of lateral emergence on foliar auxin and, once emerged, the laterals start to control their own auxin balance.

These observations show that discrete parts of the root regulate their own internal auxin concentration precisely and that discrete signals from transported auxin are required to trigger the progression through phases of development. Consequently, the genes and proteins of auxin transport are key targets for the regulation of growth and development.

Genes and proteins of auxin transport

A number of auxin transport proteins have been described recently, supplementing AUX1, which was characterised by Bennett et al. in 1996. In 1998 a set of reports appeared in which alleles of *PIN1* and *PIN2* (also known as *AGR1* and *EIR1*; Chen et al. 1998; Gälweiler et al. 1998; Luschnig et al. 1998, respectively) were characterised. The PIN proteins, which are transmembrane transporters, were found to be the auxin efflux carriers and, as such, they represent a central part of the polar auxin transport complex (recently reviewed by Friml and Palme 2002; see also Fig. 2).

Prior to the identification of the PINs, many experiments exploring polar auxin transport used NPA as a tool for inhibiting auxin flow. Some elegant physiological work had suggested that the regulatory NPA binding site was a protein distinct from the auxin efflux catalyst itself and that both these proteins might be connected by a third, labile regulatory element (Morris et al. 1991). It has also been shown that this complex, or part of it, cycles between the plasma membrane and an endomembrane compartment, thereby making the processes of secretion and endocytosis integral to the regulation of auxin efflux (Morris and Robinson 1998; Robinson et al. 1999) and influx (Grebe et al. 2002). It has recently been suggested that the traffic of auxin transport protein(s) is directed along actin microfilaments (Butler et al. 1998;

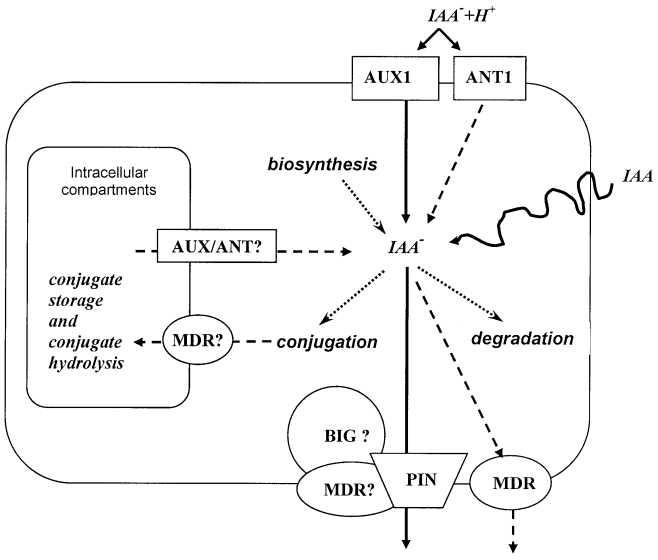

Fig. 2 A simplified scheme describing auxin homeostasis and transport in plant cells. Proteins possibly involved in auxin transport are named in *boxes*. *Full arrows* show the auxin flow through a cell and its compartments, *dotted arrows* illustrate metabolic processes, *dashed arrows* represent additional actions of tentative transporters, and a *zigzag arrow* depicts passive diffusion of undissociated IAA molecules through the plasma membrane into the cell

Hu et al. 2000; Geldner et al. 2001; Gil et al. 2001; reviewed by Muday 2000; Muday and Murphy 2002). Geldner et al. (2001) suggested that inhibitors of polar auxin transport (like NPA) act by impairing actin-dependent membrane movements.

It seems likely that the activity of the efflux complex is regulated by phosphorylation (Delbarre et al. 1998). Auxin efflux was found to be more sensitive to NPA in seedlings of an *Arabidopsis* mutant named *rcn1* ("root curl in NPA") than in the wild type. The *RCN1* gene was found to encode a subunit of protein phosphatase 2A (Garbers et al. 1996), and the mutant can be phenocopied with a phosphatase inhibitor (Deruere et al. 1999). A partnering kinase has not been identified unequivocally, but PINOID is a protein kinase, and it does enhance polar auxin transport (Benjamins et al. 2001). Both these enzymes may be candidates for the hypothetical third labile component of auxin efflux complex.

Two additional *Arabidospsis* mutants, *tir3* (Ruegger et al. 1997) and *doc1* (Li et al. 1994), have shed light on the composition of the polar auxin efflux complex. These two mutants are allelic but were identified from distinct phenotype screens, altered expression to light-regulated genes (*doc1*) or reduced auxin transport (*tir3*). The wild-type gene has been renamed *BIG* because of the extraordinary size of the protein product (Gil et al. 2001). This protein has some similarity to a mammalian protein called calossin that contributes to vesicle traffic during synaptic signalling. The mutants are defective in NPA binding activity, and the hypothesis being presented is that BIG, or a closely associated protein, stops vesicle flow when binding polar auxin transport inhibitors. This in turn reduces the activity of the auxin efflux complex. Further, BIG binds to the actin cytoskeleton and might regulate asymmetric deposition of active efflux units (Lusching 2001; Muday and DeLong 2001; Muday and Murphy 2002). Auxin gradients arise through such an asymmetric distribution of carriers to mediate characteristic responses such as gravitropism and organ identity/cell fate adjacent to meristems (e.g. Moctezuma 1999; Friml et al. 2002; Vernoux et al. 2000).

Conjugate transport

For a while it seemed as if the list of components of auxin transport was close to completion. Recently, however, there have been two independent reports of multi-drug resistance-like proteins (MRPs; ABC transporters) affecting auxin movement. As in animal genomes, there is a family of *MRP* genes in the *Arabidopsis* genome (Gaedeke et al. 2001). Examination of the knockout of one known as *AtMRP5* gave a seedling phenotype similar to that of plants grown on NPA and to seedlings having elevated internal auxin levels. Indeed, the roots contained double the free IAA level of wild-type seedlings. Interestingly, the phenotype was light-regulated, like the phenotypes of *big* mutants. However, the authors suggest that *AtMRP5* moves conjugates of auxin between compartments, not between cells.

In another report, Noh et al. (2001) showed NPA binding by two other members of the MRP family in *Arabidopsis,* AtMRP5 and AtPGP1, and mutations in the two corresponding genes led to impaired polar auxin transport and reduced apical dominance. Given the mutant phenotypes and the binding data, these proteins could fill the gap between BIG and the PINs (Luschnig 2001). However, the data suggest they could also work in (polar) auxin transport in addition to and separately from the PINs. Although these two MRP proteins are present in plasma membrane fractions, their distribution need not necessarily be restricted to the plasma membrane. While advanced fluorescence microscopy used for the PINs has yet to be applied to MRPs'. it seems likely that MRPs are responsible for auxin conjugate movement to and from storage compartments and also contribute to complexes for polar auxin efflux.

Auxin uptake

Considerable physiological data suggest that AUX1 is an uptake carrier and that uptake is necessary for root gravitropic responses (Parry et al. 2001). A new addition to the family of uptake carriers is ANT1, an aromatic and neutral amino acid transporter that can also transport IAA and 2,4-dichlorophenoxyacetic acid (L. Chen et al. 2001). Both AUX1 and ANT1 are members of the amino acid-proton co-transporter superfamily, but

similarity between the two proteins is low (14%). Expression of *ANT1* in yeast conferred uptake; expression of *AUX1* in heterologous systems has not yielded an active transporter.

Regulation of auxin transport

Clearly, there are many transport proteins through which auxin action might be regulated (a schematic summary is given in Fig. 2), yet we know very little about how any of these proteins are regulated. The auxin efflux complex can be inhibited by synthetic compounds like NPA, but such imprecise tools are unlikely to prove useful in agriculture. Flavonoids have been reported to be endogenous regulators of polar transport (Brown et al. 2001), but tissue and temporal specificity will be needed for biotechnological applications. It seems unlikely that control of flavonoid concentrations will be feasible in the near future. Consequently, the transport complexes themselves and their genes remain more attractive targets. Members of the PIN family are tissue-specific and, as they become described, particular phenotypes will be attributed to each. From these details it may become possible to influence specific aspects of auxin action, such as reducing apical dominance or increasing parthenocarpic fruit set. However, we still need a greater knowledge of the protein complexes conferring influx, efflux and intracellular conjugate fluxes before any can be deployed wisely for crop improvement.

Auxin perception

A long list of proteins has been found to bind auxin with some specificity, but only one (ABP1) has been linked to auxin-specific and auxin-dependent responses, the characteristics necessary for a receptor (Venis and Napier 1995; Napier et al. 2002). Auxin binding activity by ABP1 has also been related to models of auxin binding (Napier 2001). The crystal structure of ABP1 with bound auxin has been published recently (Woo et al. 2002).

Overexpression and antisense experiments on ABP1 have been expected to illustrate a receptor function, but ABP1-dependent phenotypes have proved elusive until recently. These functions are subtle, but ectopic and inducible expression of ABP1 confers auxin-dependent cell expansion (Jones et al. 1998) and alters ion transport responses to auxin in guard cells (Bauly et al. 2000). Antisense suppression prevents auxin-induced cell elongation and reduces cell division and, most notably, a homozygous null mutation (knockout) in ABP1 results in embryo lethality (J.G. Chen et al. 2001). Together, these and previous data indicate that ABP1 is an essential regulator for auxin-related responses, although the bulk of ABP1-regulated responses relate to actions at the plasma membrane and not to cell division or the control of transcription. Other auxin receptors might well lie undiscovered in the plant genome.

Regulation through perception

A knowledge of the amino acids lining the binding pocket and contributing to auxin binding specificity (Woo et al. 2002) could become a useful tool by which to alter binding specificity and to specify herbicidal tolerances, although the link between ABP1 and herbicidal action has not yet been made. On the other hand, the subtle phenotypes of ABP1 transgenics do not suggest obvious applications in agriculture, and we still know little about how the activity of ABP1 can be regulated, or if this is possible at all. The lethality of the knockout suggests that to become useful, conditional phenotypes will be required, although accurate and robust targeting of defective proteins might also prove effective, as in the case of the ethylene receptor (Ciardi and Klee 2001).

Auxin signalling

Data on candidate signalling intermediates is scarce. We are aware of auxin responses occurring within seconds of auxin addition, such as plasma membrane hyperpolarisation (Ruck et al. 1993), and of transcriptional activation within minutes (Abel and Theologis 1996). The most recent review of auxin-controlled gene activation concedes that the receptor and signalling cascade for transcriptional control are upstream of the Aux/IAA-ARF complex, but candidates are not identified (Hellmann and Estelle 2002). The gap between receptor and H^+-ATPase at the plasma membrane might include a phospholipase A2 (Scherer et al. 2000), and fluxes of potassium ions are evoked as both signal and osmoticum for cell expansion (Bauly et al. 2000). Changes in intracellular calcium and pH have been reported, but no specific intermediate has been identified. Overall, the prospects of regulating auxin action through the control of signalling intermediates are remote at present.

On the other hand, there have been impressive advances in our understanding of the elements of auxin-mediated transcriptional control. These advances have been thoroughly reviewed. Auxin response factors (ARFs) are a family of transcription factors that bind to auxin response elements (AuxREs), some acting as repressors, others as activators (Guilfoyle and Hagen 2001). The proteins contain a set of domains for DNA binding and another for oligomerisation. The C-terminal oligomerisation domains also confer auxin regulation and share considerable domain homology with the Aux/IAA proteins, also transcription factors, with which mixed dimers can form. Amongst the functional features that differ between *ARF*s and *Aux/IAA*s is that expression of the *Aux/IAA* genes is auxin-regulated while that of the ARFs is not (Abel et al. 1995; Ulmassov et al. 1999).

The current model for the mechanism by which ARFs and Aux/IAAs control transcription is through ubiquitin-mediated proteolysis [Guilfoyle and Hagen (2001) and Ward and Estelle (2001); both of which give diagrams summarising the model]. In brief, at low auxin levels

ARF activators combined with Aux/IAA repressors confer repression. A rise in auxin concentration, which gives a signalling stimulus from a receptor, induces the complexes to dissociate, leading to derepression. Another consequence of dissociation is the phosphorylation of ARFs and Aux/IAAs (Colon-Carmona et al. 2000), marking them as substrates for ubiquitination and proteolysis (Gray et al. 2001). A subsequent fall in auxin stimulus reduces the rate of proteolysis (Zenser et al. 2001), and the ARF-Aux/IAA complexes reform as new proteins are synthesised. A similar mechanism of ubiquitin-mediated proteolysis has been suggested for the auxin efflux carrier proteins of the PIN family (Sieberer et al. 2000; summarised in Leyser 2001), and so there is little doubt that protein breakdown and synthesis are at the centre of auxin-regulated control.

Regulation by transcriptional control

Many of the well-known auxin-sensitivity mutants relate to the *Aux/IAA* gene family of transcriptional regulators (e.g. *axr3*; Ouellet et al. 2001) or to the genes coding for proteins forming the proteasome (e.g. *axr1*, Leyser et al. 1993; *tir1*, Ruegger et al. 1997). Mutations in the *Aux/IAA* gene family also give rise to auxin morphogenic mutants, such as *monopteros* (Hardtke and Berleth 1998). These mutants illustrate that transcription factors, and the processing machinery controlling their activities, offer tangible targets for regulating plant development. What seems galling is that where in other areas there is a paucity of targets, for these proteins the field is so large and the number of possible combinations of ARFs and Aux/IAAs is so great that it will still take considerable time to decipher the details of each regulatory unit. This multiplicity of transcriptional regulators accounts for the pleiotropic character of auxin action; it also offers a set of potential handles for manipulating discrete parts of the auxin response network.

Regulating auxin action, an overview

In each area reviewed above, analyses of mutants have helped to identify genes and proteins that confer distinct and auxin-related phenotypes when defective or overexpressed. However, little of the information is complete, pathways have gaps and descriptions of genetic and biochemical regulation are fragmentary. Even for well-studied genes and proteins, the modes of endogenous regulation are not explicit and so their use in crop improvement programmes is likely to be a long way off.

Each deployment of new genes or proteins for crop improvement is likely to have different specifications, and so it is, perhaps, unnecessary to try and identify a single, key target. To illustrate the point we can again refer to the examples of expressing bacterial genes in tomato and eggplant (*Solanum melanogena* L.) fruits (Ficcadenti et al. 1999; Donzella et al. 2000). The strategy was successful, parthenocarpy and winter cropping were achieved, and the technique seems likely to be transferred to other fruiting crops. In fruiting, the developmental process is terminal, and so overproduction of IAA may not be detrimental. However, there have been other cases in which the overproduction of hormone has led to undesirable development elsewhere in the plant, such as when using fruit expression of the bacterial cytokinin biosynthetic gene *ipt* (Martineau et al. 1994). It is surprising that the same has not been reported for the tomato and eggplant for auxin, and for some traits it might be an advantage, such as enhanced apical dominance in forestry.

The opposite of elevating auxin concentrations is to engineer crops for reduced IAA. Pathway degeneracy is likely to make reduced synthesis unattractive. Additionally, IAA is made at several sites in the plant and so such a strategy will lead to pleiotropic phenotypes that are unlikely to be useful. However, at the local, target-tissue level, reduced IAA concentrations might be managed by the overexpression of conjugating enzymes or transporters, thereby removing free IAA. Plants overexpressing glucosyltransferases should soon be able to test this strategy (Jackson et al. 2001).

The manipulation of transporters seems attractive not only for depleting endogenous auxin levels but also for concentrating free IAA into target areas. Recent data shows how local auxin gradients are likely to be generated by the distribution of PIN proteins (Friml et al. 2002). When targeting for these proteins is understood, directed expression of PINs (and other transporters) could offer an attractive mechanism for concentrating free IAA in certain cells or tissues. Such a system mimics endogenous arrival of the hormonal stimulus and, because it channels auxin into the specified cells, it is less likely to cause leakage of excess IAA into non-target tissues. These characteristics make transporters important candidates for regulating auxin action. Of the different classes of auxin transporter mentioned above, the PINs are both specific and targetable, making them the most attractive proposition for auxin manipulation; AUX1 is another possibility. However, no examples of exploitation of auxin transporters for agriculture have been reported so far.

The mobility of free IAA in plants is no longer an issue if receptor or signalling elements are deployed for crop improvement. This makes their identification and characterisation a priority. No uses for ABP1 overexpression or suppression have been identified yet, but detailed analysis of transgenics is still in progress. Moving downstream in signalling to transcriptional control, of all the plants characterised from ARF and Aux/IAA mutants, none has yet been found to confer a single, discrete phenotype. As discussed above, the heterologous combination of these transcriptional regulators is always likely to lead to multiple responses if expressed constitutively. However, if their expression was to be targeted to specific cells, appropriate control might be conferred.

In conclusion, control steps in synthesis, transport and response to auxin have been discussed and the merits of

each considered with reference to requirements for crop improvement. Some genes and proteins are more likely to prove useful than others, but no single target is likely to meet the needs of all applications. It seems unlikely that the biosynthetic pathways will prove to be more useful than the application of bacterial *IAAM* and *IAAH* genes until more steps are characterised. Of all the contributors to auxin homeostasis reviewed, the auxin transport proteins have been found to offer the most favourable targets for agricultural exploitation, the PINs in particular. However, this is set against the current lack of information on auxin perception and signalling. As new signal transduction elements are identified, they are likely to make useful tools for agriculture. Further down the signalling process, the mechanisms of transcriptional control by auxin are well understood. However, the multiplicity of heterologous interactions possible between ARFs and Aux/IAA proteins will make specific control difficult to manage.

Future research will fill in knowledge gaps and identify new genes and proteins relevant to auxin action. All these will help make auxin manipulation more amenable. If one area is to be highlighted for progress, the mechanisms that target transport proteins to particular domains of the cell deserve acute attention. From this targeting come the possibilities of polar transport and compartmentalisation of the signal. Overall, there is some way to go before auxin management is used widely in agriculture.

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