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Future Directions in Plant Hormone Research

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Abstract. Future directions in plant hormone research are discussed, with particular reference to the regulation of hormone biosynthesis, hormone perception, and signal transduction.

Over the past 10 years much progress has been made in plant hormone research. New techniques, in particular, those of molecular biology, have advanced our knowledge considerably. Traditionally, plant hormone research has been methods driven. Prior to the mid-1980s, these methods were based mainly on analytical chemistry. As techniques improved and routine hormone analysis became more accurate and applicable to smaller samples, considerable information about the biosynthesis and metabolism of all the hormone groups was accumulated. The identification and characterization of mutants in which hormone biosynthesis is perturbed was particularly useful. We now know much about the pathways of formation of the major classes of plant hormones. Using this information together with bioassay data on wild-type plants and biosynthetic mutants, it has become clear which are the key, biologically active compounds in each class of hormones. The gibberellins (GAs) provide a good example. The (currently) 90 naturally occurring GAs present a bewildering array of possible metabolic interconversions. However, as a result of metabolic studies utilizing single-gene mutants of pea (*le, nana*) and maize (*d1, d5*) (Reid 1990; Phinney and Spray 1990), backed up by painstaking synthetic chemistry and rigorous identification of metabolites by gas-chromatography-mass spectrometry, we now know that only a few compounds are responsible for the well-known growth and developmental effects of gibberellins. The long-standing problem of the biosynthetic origin of abscisic acid (ABA) in higher plants has also been solved using similar techniques, with the demonstration that xanthophylls are precursors of ABA (Parry and Horgan 1991). Similarly, the biosynthetic pathway of ethylene has been defined and genes coding for both of the enzymes involved have been cloned (see later).

There is still some debate on the biosynthetic pathways to indole-3-acetic acid (IAA) and cytokinins in higher plants, although the pathways in microorganisms have been established. In bacteria, and in plant cells that have been transformed by *Agrobacterium tumefaciens*, tryptophan is a precursor of IAA. Tryptophan auxotrophs would be useful to confirm this pathway to IAA in aseptic, nontransformed plants, but suitable amino-acid auxotrophs for this type of study are difficult to obtain. One available tryptophan auxotroph is the *orange pericarp* (*orp*) mutant of maize. However, instead of confirming the role of tryptophan as a precursor of IAA, studies with this mutant indicated that IAA can be produced from indole without tryptophan as an intermediate (Wright et al. 1991). Furthermore, in *Arabidopsis*, a tryptophan-deficient mutant, *trp2*, has elevated (approx tenfold) levels of IAA relative to the wild type under appropriate growing conditions (Last et al. 1991, and J. Normanly, J. Cohen, G. Fink, unpublished results cited in Estelle 1992). It is proposed that the increase in IAA levels in this mutant is a consequence of increased conversion of indole to IAA. Thus the role of tryptophan as a precursor of IAA in higher plants is in serious doubt, and it is imperative that future studies seek to define the biosynthetic route(s) to IAA in plant tissues.

Similarly, although cytokinin biosynthesis appears to be a simple condensation between the two ubiquitous molecules, 5′ adenosine-monophosphate and dimethylallyldiphosphate, an effective demonstration of the occurrence of this reaction in higher plants and purification of a plant-derived isopentenyl transferase has been difficult to obtain. Although the *Agrobacterium* gene (*ipt*) for the trans-

ferase has been cloned (Akiyoshi et al. 1984; Barry et al. 1984), an important goal is to characterize the pathway of cytokinin biosynthesis in nontransformed plants. The recent partial purification of a zeatin *cis-trans*-isomerase from *Phaseolus vulgaris* seeds (Bassil et al. 1993) suggests that the degradation of t-RNA cannot be discounted as an additional source of biologically active cytokinins.

The accumulation of empirical information on the nature and relationships of hormones in wild type and mutant plants will continue, and will form the basis for interpreting results amassing from the application of the newer and rapidly moving molecular biological techniques. Over the past decade, molecular biology has become an essential part of plant hormone research. Indeed, it has brought additional plant biologists into the hormone area as more genes implicated in hormone biochemistry and physiology are identified.

The first molecular input into plant hormone research sought to identify genes that were either up- or down-regulated by plant hormones. Many of these genes were of unknown function, but others, for example, those coding for aleurone alpha-amylase, have become of prime importance in hormone research. However, it is the use of transgenic plants containing genes coding for the enzymes of hormone synthesis or for elements of the response mechanisms that holds more promise. The use of antisense technology has already been very successful, particularly for ethylene where transgenic tomato fruits with delayed ripening are not only a significant research advance but also of potential commercial importance.

The thesis that plant hormones must interact with receptors, and that this interaction causes the cascade of biochemical events that leads to the observable growth effect arises from our knowledge of animal biochemistry. The search for receptor proteins for each of the plant hormones is proceeding in a number of laboratories. Two strategies have emerged: the direct biochemical approach of searching for a hormone-binding protein of the expected specificity, and the molecular genetical approach of analysis of hormone-response mutants. Response mutants fall into two categories: those in which the response to hormone is suppressed and those in which it is constitutively expressed. Response mutants exist for all classes of plant hormones. For example, in *Arabidopsis* mutants that have altered responses to auxin (*axr1*), ethylene (*etr, ctr*), GA (*gai*), ABA (*abi*), and cytokinin (*ckr1*) have been described (see later). The use of response mutants to identify receptor proteins suffers from the disadvantage that a response mutation does not necessarily lie in the receptor gene. The direct approach to receptors, which has been very successful in the animal area, is more difficult to apply to plant tissues because, with the exception of aleurone and guard cells, there are no specialist organs or tissues where hormone receptors might be expected to be concentrated. Nevertheless, some progress has been made by the application of the new (to plant research) technology of photoaffinity labeling, particularly in the auxin field (Hicks et al. 1989; Feldwisch et al. 1992). Photoaffinity labeling is being pursued for the other plant hormones, too. For some it is more difficult to produce biologically active, radiolabeled probes but progress has been made in the GA area. Considerable effort has gone into seeking receptors in cereal aleurone cells (Beale et al. 1992a; Hooley et al. 1993).

Mechanisms by which signals, perceived by hormone receptors, are transduced to give responses are likely to be the main focus of future research. Again parallels to the animal kingdom are a possibility, and many laboratories are seeking evidence in plants of mechanisms previously identified in animal systems. For example, changes in cytosolic calcium ion concentration seem to be involved in many aspects of signal transduction, and elegant methods are available to monitor this at the single cell level. In animals, phosphoinositides are well-known mediators of calcium levels and evidence for their involvement in plants is being sought. Attention has also been focused on the possible involvement of G-proteins and protein kinases and several groups are trying to identify these in plants using homology cloning techniques.

The consequence of hormone perception and signal transduction in many instances involves changes in the levels of expression of genes controlling growth. Primary structures of some hormone-regulated genes have been thoroughly investigated, and this work is discussed in detail later. Briefly, the aim of this work is to systematically analyze components of the signal transduction chain in reverse order, by first identifying regulatory DNA-binding factors and then working 'backwards' along the chain of events. Progress here has been reported for the Em promoter where a *trans*-acting factor has been characterized and shown to be a leucine zipper protein (Guiltinan et al. 1990).

The outline above summarizes the approaches being taken in plant hormone research at the present time. Some of these approaches are new and exciting and will obviously be very fruitful in the coming years. Prediction of the future is difficult, but we wish to highlight two areas where emphasis must be given. These are (1) the regulation of hormone biosynthesis and (2) hormone perception and signal transduction. Each of these will now be

considered, highlighting recent progress, future goals and potential problems.

Regulation of Hormone Biosynthesis

We will briefly review the biosynthesis of ethylene, as remarkable progress has been made in recent years with the identification, characterization, and cloning of the enzymes involved. Regulation of ethylene biosynthesis can now be studied at the gene level. Progress made in ethylene biosynthesis will be compared with that for the other hormones, thereby pointing the way for future investigations on the biosynthesis, and its regulation, of other plant hormones.

The literature on ethylene biosynthesis has recently been expertly reviewed (Kende 1993). The pathway consists of essentially two steps: the conversion of S-adenosyl-L-methionine to 1-aminocyclopropane-1-carboxylic acid (ACC) by the enzyme ACC synthase and the breakdown of ACC to yield, among other molecules, ethylene. The latter reaction is catalyzed by ACC oxidase (ethylene-forming enzyme). Both enzymes have been cloned. ACC synthase was cloned by screening a cDNA expression library with antibodies raised against purified enzyme (Sato and Theologis 1989, Sato et al. 1991). ACC oxidase was one of a number of clones that arose from differential screening using cDNA from ripe and green tomato fruits to probe poly(A) RNA from ripening tomato. It was recognized later as an enzyme of ethylene biosynthesis (Salter et al. 1985, Smith et al. 1986, Hamilton et al. 1991). The case of ACC oxidase illustrates the importance of previously accumulated biochemical and physiological data, without which the enzyme encoded by this cDNA would not have been recognized.

Cloning the genes for the biosynthetic pathway, then opened up the area of regulation for study. Northern analysis of extracts of plants treated with ethylene, or with intermediates in, or inhibitors of the pathway or under different environmental conditions will give much information about regulatory points on the pathway. For example, feedback loops exist where both ACC synthase and ACC oxidase gene expression are induced by ethylene but blocked by norbornadiene, an inhibitor of ethylene perception (Woodson et al. 1992). Evidence is accumulating that ACC synthase is regulated by numerous factors, for example, auxin, wounding, and stress. ACC synthase exists as a family of genes that may be differentially regulated under various conditions (Yip et al. 1992). The benefits of transgenic plant technology have been elegantly demonstrated with these genes. Expression of antisense constructs to either gene results in the decrease in ethylene production and as a consequence, a delay in fruit ripening and senescence (Hamilton et al. 1991, Picton et al. 1993, Oeller et al. 1991).

How does progress with the other hormones compare with that for ethylene? The GA biosynthetic pathway is much longer and more complex than the ethylene pathway, but nevertheless substantial progress has been made. The cloning (by genomic subtraction) of the *ga1* locus of *Arabidopsis* has been reported (Sun et al. 1992). This gene is thought to code for *ent*-kaurene synthase A, one of the early enzymes of the pathway. The first report of the cloning and sequencing of a cDNA encoding one of the later enzymes of the gibberellin biosynthesis pathway will appear shortly (T. Lange, P. Hedden, J. E. Graebe, unpublished observations). This enzyme is a GA-C-20-oxidase from *Cucurbita maxima* and, like ACC oxidase, is an iron-dependent dioxygenase, but it requires 2-oxoglutarate in addition to ascorbate. Metabolic work in maize indicates that a C-20-oxidase enzyme may play an important role in regulation of the supply of active GAs for it may be the target of a feedback loop monitoring GA responses (Hedden and Croker 1992). This important breakthrough indicates the potential for gibberellin research to follow the ethylene story. Undoubtedly, the cloning of more of the enzymes of the GA pathway will follow, opening up numerous possibilities to study GA regulation of plant growth and development, in addition to investigations into enzyme mechanisms.

For ABA, the biosynthetic pathway has now been partly delineated with the demonstration that carotenoids are precursors of this hormone in higher plants. The goal must now be to isolate and clone the key biosynthetic enzymes such as the protein that catalyses the cleavage of 9′-*cis*-neoxanthin, which appears to be a rate-limiting dioxygenase (Parry and Horgan 1991).

Our inability to define the biosynthetic pathway to IAA in higher plants, and uncertainty concerning the enzymes involved, make meaningful studies on its regulation difficult. However, the nature of the *iaaM* and *iaaH* genes which encode enzymes of IAA biosynthesis in *Agrobacterium tumefaciens* has been know for some time (Tomashow et al. 1986). Transgenic plants overexpressing these genes have been analyzed for auxin content and phenotype. Plants expressing these transgenes from strong promoters contain the highest levels of auxin and the most extreme phenotypes (Klee et al. 1987, Sitborn et al. 1992), though whether the aberrant phenotype is a direct consequence of elevated IAA levels has only recently been addressed. Romano et al. (1993) have recently shown that combining *iaaM*

with either genes reducing sensitivity to ethylene (*ein1-1* or *ein2-2*) or with genes inhibiting ethylene synthesis (*ACCase*) effectively uncouples IAA and ethylene overproduction. These elegant experiments demonstrated that apical dominance and leaf epinasty are primarily controlled by auxin, and that other aspects of the *iaaM* phenotype are a consequence of elevated ethylene levels.

An *Agrobacterium rhizogenes* gene, *rolB*, has been postulated to be involved in modulation of plant auxin levels by hydrolysis of indolic glycosides (Estruch et al. 1991). However, overexpression of *rolB* in tobacco has recently been shown to have no effect on IAA levels nor increase the ability of the tissue to hydrolyze indole conjugates (Nilsson et al. 1993). This work demonstrates that molecular biological techniques must be backed up by sound biochemistry and hormonal analysis.

Studies of the regulation of the biosynthesis of cytokinins in plants suffer from similar problems as auxin studies, in that the gene for biosynthesis in nontransformed plants has not been cloned. Despite the mechanistic similarity between isopentenyl transferase from *Agrobacterium* and the putative enzyme from higher plants no sequence homology to the *ipt* gene has been found in the latter. However, transgenic plants incorporating the bacterial *ipt* gene under the control of environmentally regulated or tissue-specific promoters have been used to manipulate cytokinin synthesis in situ (Medford et al. 1989). In these particular studies, greatly elevated levels of endogenous zeatin and its conjugates in heat-shocked tobacco seedlings were not accompanied by altered differentiation, and observed effects on growth (reduced apical dominance, stem growth, and leaf area) were no more marked than in nonthermally induced transgenic plants in which cytokinin levels were marginally increased. Since cytokinins can be metabolized by cytokinin oxidase, one explanation put forward by Medford et al. (1989) is that heat shock may also induce enhanced oxidase activity, so that the elevated cytokinin levels may only be transient. In other studies (Smigocki and Owens 1988), the use of a 35S-*ipt* gene construct lead to constitutive overproduction of cytokinins in tobacco seedlings and more overt effects on shoot organogenesis.

In summary, future studies on the regulation of hormone levels will yield much information on the mechanisms of growth and development. There is still much work to be done for some of the hormones, particularly in the isolation of enzymes and the cloning of the corresponding genes. Particular emphasis should be given to rate-limiting and regulatory enzymes. When this is done, transgenic plant technology coupled with biochemical and physiological assessment of the transgenes will provide much insight into how plants regulate their growth, via hormones, in response to their environment.

Hormone Perception and Signal Transduction

It is evident that the area of plant hormone research which is least understood is the mechanism(s) by which hormones are perceived and how that "signal" is transduced to activate the genome. It is an area of intense research in animal biochemistry. However, workers in the plant area have the potential to take the lead due to the relative ease of transgenic plant technology. Hormone perception begins with interaction of the hormone with a receptor molecule. During the past decade considerable effort has been expended on the search for plant hormone receptors. The techniques used vary from the classic method of isolating binding proteins with the aid of radioactive ligands to the more recent work with photoaffinity probes and anti-idiotypic antibodies.

Most successful has been the search for auxin binding proteins, of which a number have now been identified. Based on mammalian models, none of the sequenced proteins from plants show typical receptor features. Some appear to be auxin uptake/carrier proteins (Lomax and Hicks 1992), and one appears to be a β-glucosidase with a possible role in the cleavage of auxin-sugar conjugates (Campos et al. 1992; Moore et al. 1992). Another auxin binding protein recently sequenced from *Hyoscyamus muticus* appears to be a glutathione-S-transferase (Bilang et al. 1993). The most widely studied auxin-binding protein, the 22 kDa protein from maize (Lobler and Klambt 1985; Hesse et al. 1989), is an abundant protein that specifically binds auxin. However, there is still some question as to whether it is a receptor. Its KDEL sequence indicates that it is targeted to the endoplasmic reticulum, however, electrophysiological evidence indicates that it has a role in the activation of a plasma membrane H^+ ATPase (Barbier-Brygoo et al. 1992; Ruck et al. 1993). Furthermore, experiments with antibodies and impermeant analogues indicate that auxin is perceived at the plasma membrane. An explanation of this inconsistency has been put forward by Ruck et al. (1993) who suggest that auxin itself causes a migration of this protein from the endoplasmic reticulum to the plasma membrane by inducing a conformational change. The auxin binding protein story illustrates the difficulties faced by all researchers isolating hormone-binding proteins, namely, those of demonstrating that the protein is indeed a func-

tional receptor. The use of transgenic plants expressing sense or antisense genes for the binding protein which thus confer a change in hormone sensitivity offers one means to do this, provided that expression is achieved in the correct tissue and subcellular location.

For the other hormones, progress toward the identification of binding proteins has been slow. An initially exciting report of the observation of specific ABA binding proteins in guard cell protoplasts by photoaffinity labeling has not been followed up (Hornberg and Weiler 1984). Similarly, one of the first reports of plant hormone photoaffinity labeling, that of a cytokinin binding protein, has not received any further attention either (Keim and Fox 1980). A radioiodinated GA photoaffinity probe of very high specific activity has been synthesized (Beale et al. 1992a) and is being used to in vivo label aleurone protoplasts (Hooley et al. 1993). Application of photoaffinity labeling to ethylene binding proteins requires the synthesis of a suitable radiolabeled probe. It is difficult to see how this can be done, although one of the known inhibitors (e.g., norbornadiene or cyclopentadiene) might be modified to include a photoactivatable group without too much loss of biological activity. Preliminary reports of the purification, to homogeneity, by classical methods, of 10 and 12 kDa ethylene binding proteins have not been amplified (Hall et al. 1990).

In the last few years there has been much discussion in the literature concerning the use of antibodies raised against anti-ligand antibodies to identify protein receptors with which the same ligand also interacts. Most of the investigations with this so-called anti-idiotypic approach to receptors have been in animal systems that recognize peptide and protein ligands. This approach to receptors is currently controversial with papers claiming both to prove or disprove the validity of the technique. However, strong claims for the preparation of effective anti-idiotypes continue to be published, for example, the thromboxane A2 receptor (Kan and Tai 1993). In plants, there have been reports of the identification of hormone binding proteins by this approach, the most notable being that of Prasad and Jones (1991) who describe the immunoblotting of a 60 kDa auxin-binding protein from soybean. A lot more work needs to be done before confidence in this approach is regained. However, it is worth consideration providing attention is given to the purity of the anti-idiotype and that a suitable functional assay, with good controls, for hormone agonist/antagonist activity is available.

Considering all the effort that has been put into the identification of plant hormone receptors, it is surprising that no totally convincing candidate protein has yet emerged. One could speculate that plants perceive hormone ligands by an (as yet) unknown mechanism or that the levels of receptors are very low and, therefore, difficult to detect. In animals, the G-protein-coupled receptors play an important role in ligand recognition and initiation of intracellular events via GTP-binding proteins. GTP-binding proteins have been identified in plants (for example, Jacobs et al. 1987) and a future area of research will be the search for further analogs by homology cloning (for example, Ma et al. 1990). However, GTP-binding proteins are involved in a number of cellular functions, such as protein translocation and differentiation, as well as signal transduction. Therefore, demonstration of the involvement of characterized GTP-binding proteins in hormone signal transduction will be a necessity before we can say that G-protein-coupled receptors operate in plants. In addition to G-protein-coupled receptors, receptor protein kinases are also important in ligand recognition at the membrane. Examples of these proteins have recently been cloned from plants (Chang et al. 1992; Walker 1993) and use of transgenic plant technology will aid in ascribing to them any possible hormone-response functions. Generally, phosphorylation by kinases, of tyrosine, serine, and threonine residues in proteins, is a common way to modulate their function. Indeed, the ethylene response has recently been shown to be transduced via phosphorylation events (Raz and Fluhr 1993). Therefore, the search for homologous kinases in plants may also be rewarding.

There are indications that some sort of hormone recognition system on the plasma membrane may exist for some of the hormones. This is particularly true of the cereal aleurone system which responds to low levels of GAs with increased transcription of, *inter alia,* alpha amylase encoding genes. This increased rate of transcription is abolished by either ABA or the withdrawal of GA. Incubation of aleurone protoplasts of *Avena fatua* with GA covalently linked to Sepharose causes transcription of alpha amylase, indicating that the primary site of perception is on the outside of the membrane (Hooley et al. 1991). This result has recently been confirmed using membrane-impermeant GA sulphonic acid derivatives (Beale et al. 1992b) and independently by Gilroy et al. (1993) who demonstrated that aleurone protoplasts, microinjected with GA, do not respond but retain their ability to react to externally applied hormones. Microinjection techniques can be used to test the response of cells to all sorts of putative intracellular messengers and will undoubtedly yield many meaningful results on the nature of these signals in the future.

Fluctuations in the cytoplasmic calcium ion con-

centration in plant cells seem to play an important role in intracellular signaling. The use of fluorescent Ca^{2+} indicating dyes such as Indo-1 and fluorescence ratio imaging allows intracellular Ca^{2+} concentrations to be monitored in single cells impaled on micropipettes. This technique has been applied with good effect to both aleurone and guard cells, demonstrating that GA and ABA affect calcium ion concentration (Bush 1992; Gilroy et al. 1992). The components of calcium-based signaling pathways, for example, calmodulin, membrane Ca^{2+} pumping ATPases, and Ca^{2+} ion channels, have all been shown to be present in plant cells. In animal cells $[Ca^{2+}]$ is modulated by the eternal stimulus causing activation of phospholipase C which releases inositol triphosphate (IP_3) and diacylglycerol (DAG) from phosphatidylinositol diphosphate (PIP_2). IP_3 then releases Ca^{2+} from intracellular stores by action at a receptor while DAG activates a protein kinase C. Many of the molecules involved in this cascade have been identified in various plant cells (Boss 1989), although the levels of PIP_2 are low and there may be some differences in the way that it is metabolized. There is, doubtless, a great deal of work to do in this area and it will be the subject of intense research in the future. The new technologies of fluorescence imaging, microinjection of putative transducing molecules (ranging from putative second messengers to peptide mimics and reporter genes), and the use of caged molecules, including caged plant hormones (M. Beale and A. Trewavas, unpublished observations) will help in pushing forward this area.

The final outcome of hormone perception and signal transduction is the regulation of gene expression. Study of the primary structure of hormonally regulated genes has been a fruitful area in the past 10 years. Some of these genes, for which considerable information is now available, are auxin-inducible SAUR and GH genes (Guilfoyle et al. 1992) and their analogues GmAux22 and GmAux28 (Nagao et al. 1993), ABA-inducible genes *Em* (Guiltinan et al. 1990) and *Rab* (Mundy et al. 1990), and the GA/ABA-responsive alpha amylase genes (Skriver et al. 1991; Huttly et al. 1992; Rushton et al. 1992; Rogers and Rogers 1992). The alpha amylase gene is the most thoroughly investigated. With these genes, one focus of research has been to identify the hormone-responsive elements of the promoter sequences, using a number of techniques such as transient expression analysis and DNase footprinting. The regions of the promoter (*cis* elements) involved in binding to putative regulatory proteins (*trans*-acting factors) have been delineated but no such protein has yet been isolated for these genes. However, a *trans*-acting factor that binds to the Em promoter has been described (Guiltinan et al. 1990).

Where does this type of work go from here? The binding of *trans*-acting factors to DNA is not simple and may involve formation and binding of protein heterodimers whose composition may be changed according to whether or not hormone induction has occurred. It is these changes in the DNA-protein complex structure that regulate transcription. Thus, after the identification of *trans*-acting proteins, research towards the previous step in the hormone transduction pathway must be to find out what hormone induction does to these factors. There are many possibilities. Is it simply a change in concentration of these proteins? Does it involve phosphorylation/dephosphorylation events altering the protein–protein or DNA–protein binding characteristics? Perhaps these factors themselves are the elusive hormone receptors?

All of the above represent "direct" approaches to the elements of hormone perception and signal transduction. In theory, mutation in a gene coding for an element in a signal transduction pathway will render the plant insensitive to that hormone. Thus a molecular genetical approach, generating and characterizing sensitivity mutants has the potential to quickly identify components of the pathways. Sensitivity mutants are known for auxin, GA, ABA, and ethylene, and there are preliminary reports on cytokinin resistance mutants in *Arabidopsis* (Su and Howell 1992). The *viviparous-1* (*vp1*) mutant of maize is associated with reduced sensitivity of embryos to ABA. The seed-specific *VP1* gene has been isolated by transposon tagging (McCarty et al. 1989) and subsequently has been shown to encode a transcriptional activator, believed to be involved, with other factors, in the ABA-controlled seed maturation process (McCarty et al. 1991). In *Arabidopsis* the *abi3* mutant also shows a reduced sensitivity to ABA. The *ABI3* gene has been recently cloned (Giraudat et al. 1992). It encodes a protein that has some homologies with the *VP1* protein and thus it also appears to be a transcriptional activator. However, it is unclear whether the *ABI3* protein is the exact functional *Arabidopsis* counterpart of *VP1*.

The auxin-resistant *Arabidopsis* mutant *axr1* displays a number of morphologies consistent with a reduction in auxin sensitivity. The gene involved has been cloned recently by chromosome walking (Leyser et al. 1993). The gene encodes a protein that has sequence homology with the ubiquitin-activating enzyme E1, but lacks an important catalytic cysteine residue. Thus, although the exact function of this protein is unclear the results suggest that the ubiquitin pathway may be involved in auxin action.

Cloning of an ethylene response gene has also been reported recently (Kieber et al. 1993). The *CTR1* gene of *Arabidopsis* encodes a protein kinase of the *raf* family and there is good evidence that the enzyme is involved in ethylene signal transduction. Thus, mutagenesis programs are turning up some very interesting hormone-response mutants. Although the isolation of these genes is very labor intensive, the approach can be rewarding. There are other sensitivity mutants that have not yet yielded to the molecular biologist, the most notable being the GA-insensitive (gai) mutant of *Arabidopsis*.

Conclusion

The vast problem of hormonal control of plant growth is being attacked from many sides by a variety of modern techniques in numerous laboratories around the world. The delineation of the numerous interlocking pathways will certainly take years to accomplish. A good start has been made and some elegant work is being done. We have tried to highlight recent papers that we think are exciting and will form the basis of future avenues of research. We have restricted ourselves to the five "usual" classes of plant hormones. Other interesting signal molecules that have not yet earned the classification of "plant hormone" include salicylic and jasmonic acids. Investigation into the roles of these compounds in responses to such stimuli as wounding, pathogen attack, and mechanical stimulation are expected to be particularly rewarding (Raskin 1992; Staswick 1992).

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