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The Five "Classical" Plant Hormones

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INTRODUCTION

It is 60 years since Went and Thimann (1937) published their classic book *Phytohormones*. At that time, the term phytohormone was synonymous with auxin, although the existence of other phytohormones, such as cell division factors, was anticipated on the basis of physiological experiments. It is impressive that aside from some confusion about the structure of auxin, many of the basic phenomena of auxin physiology were already known at that time. It is equally impressive that much auxin biology, including the Cholodny-Went hypothesis (Went and Thimann, 1937) regarding the role of auxin in mediating gravi- and phototropism, the pathway of auxin biosynthesis, and the mechanism by which auxin causes cell wall loosening, remains controversial.

Since 1937, gibberellin (GA), ethylene, cytokinin, and abscisic acid (ABA) have joined auxin as phytohormones, and together, they are regarded as the "classical five" (Figure 1). This group is expected to grow as the hormonal functions of other compounds are recognized and as new hormones are discovered (see Creelman and Mullet, 1997, in this issue). As is evident from this short review, recent progress on hormone biosynthesis and on hormonal transduction pathways has been impressive. Also evident is that there are many blanks still to be filled in. With the application of the powerful new techniques of chemical analysis and molecular genetics, the rate at which new discoveries are made will continue to accelerate. It's a great time to be a plant hormonologist!

CHEMISTRY AND BIOSYNTHESIS OF HORMONES

Clearly, the amount of any compound, including hormones, in an organ of a plant is determined by the combined rates of its biosynthesis, breakdown, import, and export. The last two aspects are not considered in the following discussion of how the endogenous pool sizes of the five classical hormones are regulated. Rather, we focus on the biosynthetic pathways, the deactivation reactions, and the regulatory mechanisms involved in these processes.

During the past 25 years, the standards set for natural-product chemistry have also been applied to plant hormone research. Instead of "measuring" hormones by bioassays, unambiguous physical–chemical methods for the identification and measurement of hormones have been developed (reviewed in Hedden, 1993). The accuracy and facility of quantitative measurements have been improved by the availability of isotopically labeled versions (with ^{2}H, ^{13}C, or ^{15}N) of the hormones for use as internal standards.

Molecular genetics is another discipline that has made it possible to solve problems in hormone physiology that were hitherto intractable. Hormones are present in plants in very small amounts. Moreover, their biosynthetic and catabolic enzymes are low-abundance proteins, which, in most cases, cannot be isolated and purified by classical biochemical methods. However, when the identification of a mutant leads to the cloning of a gene, that gene can be expressed as a fusion protein with which the catalytic function can be determined (e.g., Sun and Kamiya, 1994; Xu et al., 1995; Schwartz et al., 1997b).

Auxin

The primary auxin in plants is indole-3-acetic acid (IAA; Figure 1). Although other compounds with auxin activity, such as indole-3-butyric acid, phenyl acetic acid, and 4-chloro-IAA, are also present in plants (reviewed in Normanly et al., 1995), little is known about their physiological role. For many years, it has been assumed that tryptophan is the precursor of IAA. This has recently been confirmed in seedlings of *Phaseolus vulgaris* with stable isotope labeling studies (Bialek et al., 1992). Three routes for IAA biosynthesis from tryptophan via indole-3-pyruvic acid, tryptamine, or indole-3-acetonitrile have been proposed. The latter precursor is found primarily in the Cruciferae and may be derived from

This review is dedicated to the memories of James Bonner, Anton Lang, Kenneth Thimann, and Philip Wareing, pioneers in plant hormone research, who died during the past year.

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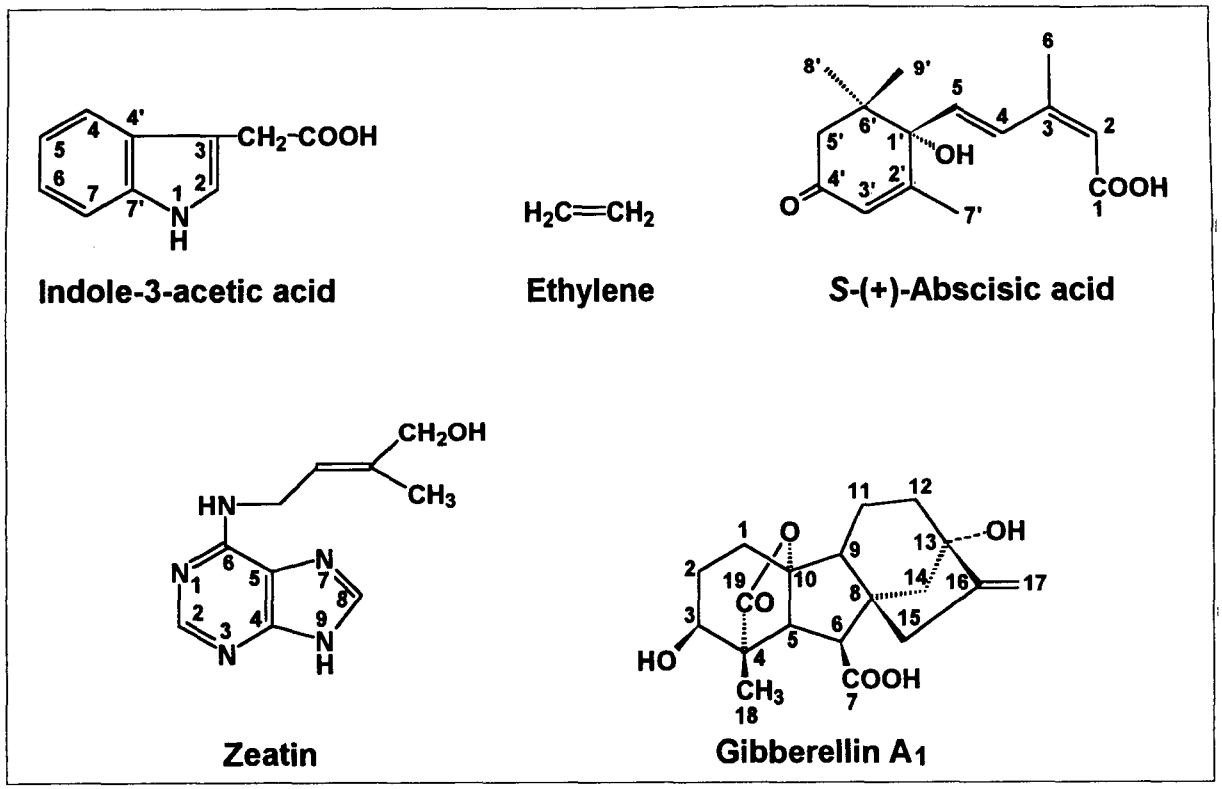

Figure 1. Structures of Representatives of the Five Classical Plant Hormones.

Shown are indole-3-acetic acid, ethylene, abscisic acid, zeatin, and gibberellin A_1.

indoleglucosinolates (Normanly et al., 1995). Four genes encoding nitrilase, which converts indole-3-acetonitrile to IAA, have been cloned in Arabidopsis. These four genes are differentially regulated (Bartel and Fink, 1994).

Work with tryptophan auxotrophic mutants has established that IAA biosynthesis can also take place via a tryptophan-independent route. For example, the *orange pericarp* mutant in maize does not produce tryptophan but accumulates IAA to levels 50-fold higher than in the wild type (Wright et al., 1991). Tryptophan auxotrophs in Arabidopsis also accumulate more IAA than do wild-type plants. On the basis of these data, it was proposed that IAA can be synthesized through a branch point of the tryptophan biosynthetic pathway at indole or indole-glycerol phosphate (Normanly et al., 1995). Supporting this idea is the finding that in a cell-free system from immature maize endosperm, radioactive indole is converted to IAA (Rekoslavskaya and Bandurski, 1994).

Certain bacteria and plant cells transformed with *Agrobacterium tumefaciens* synthesize IAA via a unique pathway in which tryptophan is converted to IAA in two steps. The first enzyme, tryptophan monooxygenase, converts tryptophan to indole-3-acetamide, which in turn is converted to IAA by indole-3-acetamide hydrolase. The genes encoding these enzymes have been used to alter IAA levels in transgenic plants (Klee and Romano, 1994).

IAA occurs not only in the free form but also conjugated to amino acids, peptides, or carbohydrates. These IAA conjugates are biologically inactive and appear to serve functions as IAA storage forms in seeds and hormonal homeostasis. The *iaglu* gene in maize, which encodes an enzyme that esterifies IAA to glucose, has been cloned (Szerszen et al., 1994). In Arabidopsis, a gene family that encodes IAA conjugate hydrolases has been identified (Bartel, 1997).

Until recently, IAA catabolism was thought to occur via oxidative decarboxylation (i.e., through the action of an IAA oxidase). However, the major catabolic route of IAA in vivo now appears to be oxidation to oxindole-3-acetic acid and subsequent glycosylation through an added 7-hydroxyl (reviewed in Normanly et al., 1995). Another catabolic pathway is via IAA-acetylaspartate to dioxindole-3-acetylaspartate-3-*O*-glucoside.

GAs

Since the first GA from a higher plant, GA_1 (Figure 1), was identified 40 years ago, 112 GAs have been identified to date (Hisamatsu et al., 1997). Efforts to determine the physiological roles of GA and to elucidate the biosynthetic pathway have been greatly facilitated by the availability of GA-deficient (i.e., dwarf) mutants. Metabolic studies have been conducted with systems that are rich sources of GAs, such as the fungus *Gibberella fujikuroi* and immature seeds of pumpkin, pea, and bean. However, maize is the only higher plant in which the entire biosynthetic pathway has been demonstrated in vegetative tissues by feeding various intermediates (Suzuki et al., 1992; Kobayashi et al., 1996). These and other studies have shown that the GA biosynthetic pathway can be divided into three stages (Figure 2; reviewed in Graebe, 1988; Hedden and Kamiya, 1997; MacMillan, 1997). These stages are considered below.

Stage 1: From Geranylgeranyl Diphosphate to ent*-Kaurene*

The first committed step in GA biosynthesis is the cyclization of geranylgeranyl diphosphate to *ent*-copalyl diphosphate, which in turn is converted to *ent*-kaurene (Figure 2A). The enzymes that catalyze these reactions have been called *ent*-kaurene synthase A and B, respectively, but MacMillan (1997) has proposed the more appropriate terms *ent*-copalyl diphosphate synthase and *ent*-kaurene synthase. The genes encoding these enzymes have been cloned from Arabidopsis (*GA1*; Sun and Kamiya, 1994) and pumpkin endosperm (Yamaguchi et al., 1996), respectively. Biochemical evidence indicates that both enzymes are located in proplastids of meristematic shoot tissues but not in mature chloroplasts (Aach et al., 1997). Because conversions in stage 3 of the pathway can take place in mature leaves, it is likely that pathway intermediates move between different tissues and organs. In Arabidopsis, the expression of *GA1* is highly regulated during growth and development. Promoter studies with β-glucuronidase as reporter gene indicate that *GA1* expression is highest in shoot apices, root tips, and the vascular tissue of leaves (Silverstone et al., 1997).

Stage 2: From ent*-Kaurene to GA_{12}-Aldehyde*

The enzymes in the second stage of the pathway are membrane-bound P450 monooxygenases, which are thought to be located in the endoplasmic reticulum. The sequential oxidation of C-19 of *ent*-kaurene via *ent*-kaurenol and *ent*-kaurenal to *ent*-kaurenoic acid is probably catalyzed by a single enzyme, the activity of which is impaired in the *ga3* mutant of Arabidopsis (J.A.D. Zeevaart, unpublished results). *ent*-Kaurenoic acid is further oxidized to *ent*-7α-kaurenoic acid (Figure 2B). Contraction of the B-ring with extrusion of C-7 gives GA_{12}-aldehyde. The *Dwarf3* (*D3*) gene of maize encodes a P450 monooxygenase, but it is not known which step in stage 2 of the pathway is catalyzed by the D3 protein (Winkler and Helentjaris, 1995).

Stage 3: From GA_{12}-Aldehyde to Various GAs

The first step in stage 3 of the pathway involves oxidation of GA_{12}-aldehyde to GA_{12}. Further metabolism of GA_{12} varies among species or organs of the same species with respect to the position and sequence of oxidative steps. The early-13 hydroxylation pathway, which involves hydroxylation at C-13 to give GA_{53}, is common in higher plants. After C-13 hydroxylation, C-20 is successively oxidized and eliminated by the multifunctional enzyme GA 20-oxidase via GA_{44} and GA_{19} to the C_{19}-GA, GA_{20} (Figure 2C). Finally, 3β-hydroxylase converts GA_{20} to the bioactive GA_1. All of these oxidative steps are catalyzed by dioxygenases that require 2-oxoglutarate and molecular oxygen as cosubstrates and Fe^{2+} and ascorbate as cofactors.

GA 20-oxidases have been cloned and expressed from a number of species (e.g., Lange et al., 1994; Phillips et al., 1995; Xu et al., 1995; Martin et al., 1996; Wu et al., 1996), although to date the gene encoding 3β-hydroxylase has been cloned only from Arabidopsis (*GA4*; Chiang et al., 1995). Expression of both the *GA4* and *GA5* (which encodes GA 20-oxidase) genes in Arabidopsis is subject to negative feedback regulation (Chiang et al., 1995; Phillips et al., 1995). In spinach, GA 20-oxidase activity is under photoperiodic control (Wu et al., 1996; see also Kreps and Kay, 1997, in this issue).

Of all the known GAs, only a few are bioactive per se, whereas the others are precursors or deactivated GAs. A 3β-hydroxyl group (as in GA_1 and GA_4) is required for activity, as was originally demonstrated with the dwarf *le* mutant of pea (Ingram et al., 1984) and *dwarf1* (*d1*) of maize (Spray et al., 1984). These mutants are impaired in 3β-hydroxylase activity, and normal growth can be restored by GA_1 but not by GA_{20}.

At the end of the pathway, bioactive GAs are generally deactivated by 2β-hydroxylation. For example, GA_1 is converted to GA_8 and GA_4 is converted to GA_{34}. Conjugation to glucosyl esters or glucosides takes place predominantly in maturing seeds. There is only one example of a deactivation mutant, the *slender* (*sln*) mutant in pea, in which 2β-hydroxylation is blocked and GA_{20} accumulates in maturing seeds (Ross et al., 1995).

Cytokinins

Naturally occurring cytokinins are N^6-substituted adenine derivatives (Figure 1). In addition to higher plants, several bacteria, including *Agrobacterium*, produce cytokinins (reviewed in Morris, 1986; Gaudin et al., 1994). The key biosynthetic

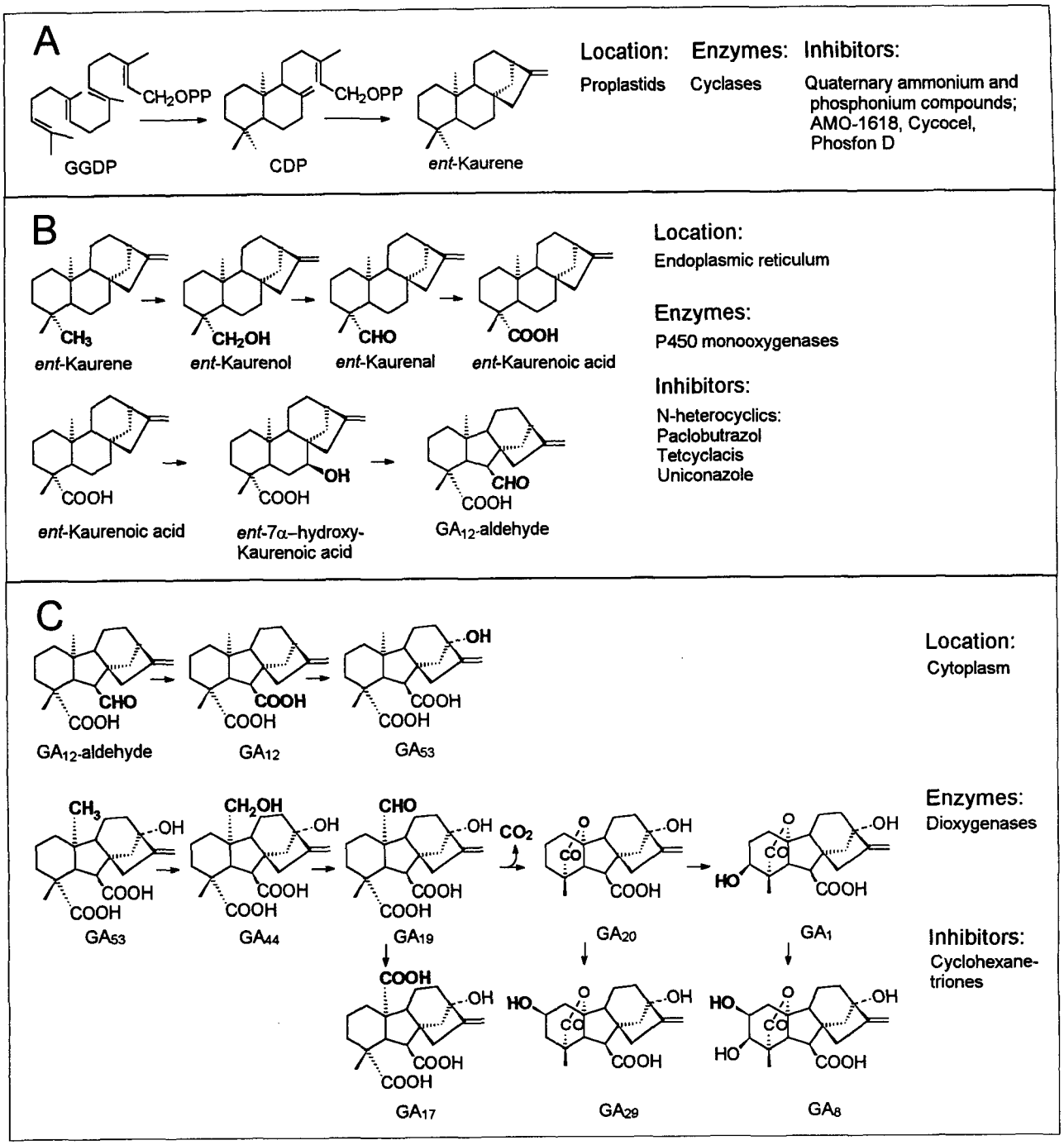

Figure 2. GA Biosynthetic Pathway.

(A) Stage 1: conversion of geranylgeranyl diphosphate (GGDP) to *ent*-kaurene. CDP, *ent*-copalyl diphosphate.
(B) Stage 2: from *ent*-kaurene to GA_{12}-aldehyde.
(C) Stage 3: represented here by the early-13 hydroxylation pathway from GA_{12}-aldehyde to GA_1 and GA_8.
For each stage, the nature of the enzymes, their location, and specific inhibitors are indicated. In **(B)** and **(C)**, the modifications taking place at each step are highlighted in boldface.

step in *Agrobacterium* is the addition of an isopentenyl group from isopentenyl diphosphate to N^6 of AMP, which is catalyzed by AMP–isopentenyl transferase (IPT). A similar enzyme activity has also been observed in extracts from plant sources (Blackwell and Horgan, 1994; Chen and Ertl, 1994), but because of its instability, the enzyme has only been partially purified (Chen and Ertl, 1994). The *IPT* gene from *Agrobacterium* has been cloned and expressed in transgenic plants (Klee and Romano, 1994). However, there are no reports of plant DNA sequences with similarity to the bacterial *IPT* genes (Binns, 1994).

Isopentenyladenosine 5′-monophosphate is the precursor of all other forms of cytokinins. Through hydroxylation of the isopentenyl side chain and reduction of the double bond, the ribotides of zeatin and dihydrozeatin are formed. It is generally thought that the free bases, such as isopentenyl adenine, zeatin, and dihydrozeatin, are the active forms of cytokinins. Cytokinins with a hydroxylated side chain can be glycosylated to form the *O*-glucoside or *O*-xyloside. These reactions are reversible, because *O*-glycosylated cytokinins have biological activity. Zeatin *O*-xylosyl transferase has been isolated from bean embryos, and antibodies have been prepared. The enzyme is predominantly localized in the endosperm (Martin et al., 1993).

Cytokinins are inactivated irreversibly by two different reactions: formation of N-conjugates with glucose at the 7- or 9-positions or with alanine at the 9-position and the oxidative cleavage of the N^6 side chain of the cytokinin substrate by cytokinin oxidase. The substrates for cytokinin oxidase are isopentenyladenine, zeatin, and their ribosides. By contrast, dihydrozeatin is resistant to cytokinin oxidase. Tobacco plants transformed with the *IPT* gene, which have an elevated cytokinin level, exhibit an increase in cytokinin oxidase activity in both leaves and roots (Motyka et al., 1996). These results indicate that cytokinin oxidase is induced by its own substrate and thus plays a role in regulating cytokinin levels in plants. Genetic manipulation of cytokinin oxidase may provide a strategy through which cytokinin levels can be modified.

ABA

Work on ABA (Figure 1) is a good example of how a combination of genetic, molecular, and biochemical approaches can lead to the elucidation of a complex biosynthetic pathway. Early on, the similarity in structure between ABA and the end groups of certain carotenoids led to the proposal that ABA may be a breakdown product of carotenoids, with xanthoxin as an intermediate. This idea was supported by the finding that plants that do not accumulate carotenoids (either because of mutation or treatment with inhibitors) also lack ABA. Furthermore, labeling studies with $^{18}O_2$ established that one ^{18}O atom is rapidly incorporated into the carboxyl group of ABA, indicating that there is a large precursor pool (i.e., carotenoids) that already contains the oxygens on the ring of the ABA molecule (Zeevaart et al., 1991). Finally, in etiolated leaves and roots, which have low levels of carotenoids, a 1:1 stoichiometry was found between the disappearance of violaxanthin and neoxanthin and the appearance of ABA and its catabolites (Li and Walton, 1990; Parry et al., 1992).

The ABA-deficient *aba1* mutant of Arabidopsis is blocked in the epoxidation of zeaxanthin to antheraxanthin and violaxanthin, indicating that the epoxycarotenoids violaxanthin and neoxanthin are essential for ABA production (Rock and Zeevaart, 1991). The *aba2* mutant of *Nicotiana plumbaginifolia* is orthologous with *aba1* of Arabidopsis and has been cloned. The corresponding fusion protein has zeaxanthin epoxidase activity (Marin et al., 1996).

The last two steps in the pathway, from xanthoxin to ABA-aldehyde to ABA, are catalyzed by constitutively expressed enzymes (Sindhu and Walton, 1988; Schwartz et al., 1997a), with the result that the level of xanthoxin in leaves is always very low relative to ABA (Parry et al., 1990). The *aba2* mutant in Arabidopsis is the only known mutant for the conversion of xanthoxin to ABA-aldehyde. By contrast, mutants for the final step, ABA-aldehyde to ABA, have been found in a number of species (reviewed in Taylor, 1991). In some cases (e.g., *nar2a* in barley, *flacca* in tomato, and *aba3* in Arabidopsis), the lesion is not in the aldehyde oxidase apoprotein but in the molybdenum cofactor that is required by the enzyme.

ABA biosynthesis increases when plant cells lose turgor (reviewed in Zeevaart and Creelman, 1988), raising the question of which step in the pathway is activated by water stress. Considering that the immediate epoxycarotenoid precursors are always present in large excess relative to ABA (Norman et al., 1990) and that the enzyme activities of the final two steps from xanthoxin to ABA are not affected by dehydration, it follows that the cleavage reaction from epoxycarotenoids to xanthoxin is the most likely regulatory step in the pathway.

But what is the nature of the enzyme that catalyzes the cleavage reaction? Recently, a viviparous mutant of maize, *vp14*, has been isolated, and the corresponding gene has been cloned. The derived amino acid sequence of VP14 shows similarity to bacterial dioxygenases. Moreover, a VP14 fusion protein catalyzes the cleavage of 9-*cis*-epoxycarotenoids (C_{40}) to form C_{25} apo-aldehydes and xanthoxin (C_{15}), but carotenoids in the all-*trans* configuration are not cleaved (Schwartz et al., 1997b). Therefore, the 9-*cis*-configuration appears to be the primary determinant of cleavage specificity. This is not surprising, because cleavage of 9-*cis*-epoxycarotenoids produces *cis*-xanthoxin, which is in turn converted to the active isomer of ABA.

The cleavage step is the first committed step in ABA biosynthesis and probably also the reaction that determines the overall rate of ABA production. This possibility can now be tested in transgenic plants in which the gene encoding the cleavage enzyme is over- or underexpressed. However, ABA is rapidly deactivated by oxidation to phaseic acid and, to a lesser extent, by conjugation to the ABA glucose ester (reviewed in Zeevaart and Creelman, 1988). Thus, overexpression of the

cleavage enzyme in transgenic plants may not necessarily result in increased ABA levels. To raise ABA levels and to make plants tolerant to stress conditions, it may be preferable to suppress the activity of ABA 8′-hydroxylase, the enzyme that converts ABA to phaseic acid, by using antisense technology. However, the experiments will have to wait until the corresponding gene has been cloned.

Ethylene

The breakthrough in unraveling the biosynthetic pathway of ethylene was the discovery in 1979 that 1-aminocyclopropane-1-carboxylic acid (ACC) is the immediate precursor of ethylene (reviewed in Yang and Hoffman, 1984). The first committed step in ethylene biosynthesis is the conversion of *S*-adenosyl-L-methionine to 5′-methylthioadenosine and ACC; this is also the key regulatory step in ethylene biosynthesis.

The enzyme that catalyzes this reaction, ACC synthase, was partially purified before the corresponding gene was cloned (reviewed in Kende, 1993; Zarembinski and Theologis, 1994). ACC synthase is encoded by a multigene family whose members are differentially expressed in response to developmental, environmental, and hormonal factors. For example, by using gene-specific probes, the differential expression of tomato ACC synthase family members has been investigated. Transcripts of one isoform increased during fruit ripening, those of another increased in response to wounding, and those of a third form increased in response to treatment with auxin (Olson et al., 1991; Yip et al., 1992). ACC synthase genes expressed in response to a particular stimulus (e.g., the application of auxin) are more similar to genes controlled by the same stimulus in other species than they are to other ACC genes in the same species (Liang et al., 1992; Trebitsh et al., 1997).

The final step in ethylene biosynthesis, the conversion of ACC to ethylene, is catalyzed by ACC oxidase. ACC oxidase was first identified by expressing the tomato cDNA pTOM13 in an antisense orientation, which resulted in greatly reduced ethylene production in tomato (Hamilton et al., 1990). The deduced amino acid sequence of pTOM13 is similar to that of dioxygenases that require Fe^{2+} and ascorbate as cofactors. When these cofactors were added to assays for ACC oxidase, enzyme activity was completely recovered (Ververidis and John, 1991). Later, it was found that CO_2 is also an essential activator of ACC oxidase (Fernández-Maculet et al., 1993).

Numerous cDNAs for ACC oxidase have been isolated from different species (see Barry et al., 1996). As is the case with ACC synthase, ACC oxidase is encoded by small multigene families. Although the initial evidence indicated that ethylene synthesis is controlled at the level of ACC synthase, there is now considerable evidence that ACC oxidase also plays a significant role in regulating ethylene biosynthesis. By using gene-specific probes for three ACC oxidase genes of tomato, distinct patterns of expression in various organs and at different stages of development have been observed (Barry et al., 1996). Moreover, the positive feedback loop in which treatment of tissue with ethylene often stimulates ethylene production by that tissue appears to take place through enhanced expression of ACC synthase and ACC oxidase (reviewed in Kende, 1993).

Besides being converted to ethylene, ACC can also be irreversibly conjugated to form *N*-malonyl-ACC (Kionka and Amrhein, 1984). Malonylation of ACC regulates the level of ACC and thus the production of ethylene. Ethylene can be metabolized by plant tissues to ethylene oxide and ethylene glycol (Sanders et al., 1989), but the physiological significance of this metabolism remains to be established. As a gas, ethylene can readily diffuse from plant tissues, so metabolism is not essential for its removal.

HORMONES AND VEGETATIVE GROWTH

Plant growth is based on the production of cells in the meristems and the ensuing elongation of these newly formed cells (see Clark, 1997; Cosgrove, 1997; Kerstetter and Hake, 1997; Schiefelbein et al., 1997, in this issue). As has been well documented, plant hormones affect both cell division and cell elongation. Here, we use selected examples to illustrate how auxin, cytokinin, and GA promote cell division.

Cell-Cycle Control

Auxin stimulates radish root pericycle cells arrested in the G2 phase of the cell cycle to enter into mitosis, thereby promoting the formation of lateral root primordia (Blakely and Evans, 1979). Similarly, the role of kinetin as the factor required for the maintenance of cell division activity in tissue cultures is well known (Miller et al., 1955). However, the role of endogenous cytokinins in controlling cell division in intact plants has not been demonstrated unequivocally. In part, this is because two tools that have been very valuable in determining the physiological functions of some plant hormones, namely, well-defined biosynthesis or perception mutants and chemical inhibitors of hormone action, are not available for cytokinins. GA induces stem growth in many rosette plants and dwarf mutants. This growth response can be quite dramatic and is the combined result of enhanced cell division activity in the subapical meristem and increased cell elongation. Sachs et al. (1959) provided a well-documented example of GA-promoted mitosis in the subapical meristem of the long-day plant *Samolus parviflorus.*

Despite these early indications that hormones regulate cell division and growth, two broad questions regarding hormonally stimulated cell division activity still require resolution: do plant hormones influence cell division directly or indirectly, and at what point of the cell cycle do plant hormones

act? Answers to the first question are mostly lacking; answers to the second are beginning to emerge.

In the intercalary meristem of deepwater rice, GA promotes cell division and cell elongation (Sauter and Kende, 1992). This leads to internodal growth rates of up to 5 mm/hr. Measurements of cell length and determinations of the progress of cells through the cell cycle by flow cytometry and ^{3}H-thymidine incorporation indicated that GA-induced cell elongation preceded the promotion of cell division. Therefore, it has been proposed that the primary action of GA in the intercalary meristem of rice is on cell elongation and that entry into the cell cycle is a function of cell size, a phenomenon that has been well documented in yeast (e.g., Nurse, 1991).

Passage of cells through phases of the mitotic cycle is controlled by a family of serine/threonine protein kinases and their regulatory subunits, the cyclins (see Jacobs, 1995, 1997, in this issue). The promotion of cell division activity by plant hormones is reflected in hormonally induced activities of cyclin-dependent p34^{cdc2}-like protein kinases, in the level of their mRNAs, and also in the level of cyclin transcripts.

For example, John et al. (1993) showed that auxin alone increased the level of a p34^{cdc2}-like protein in cultured tobacco cells, but addition of a cytokinin was required for activation of this kinase. Because cytokinin increased the abundance of cyclin mRNA in suspension-cultured Arabidopsis cells (Soni et al., 1995), it is conceivable that the observed activation of the p34^{cdc2}-like protein kinase in tobacco cells was based on the synthesis of a cyclin. Similarly, auxin has been reported to increase both p34^{cdc2} and cyclin mRNA levels in roots in conjunction with the induction of cell divisions (e.g., Miao et al., 1993; Ferreira et al., 1994). Moreover, GA promotes the activity of a p34^{cdc2}-like protein kinase and the expression of genes encoding a p34^{cdc}-like protein kinase and cyclin homologs in the intercalary meristem of deepwater rice (Sauter et al., 1995). However, in no case has it been shown that plant hormones regulate directly the expression of genes that code for regulatory proteins of the cell cycle.

A completely different aspect of the hormonal control of the cell division cycle has been described by Houssa et al. (1994). These authors observed that exogenous cytokinin reduced the size of chromosomal DNA replication units in the shoot meristems of *Sinapis alba* and *Lolium temulentum* and in ovules of tomato. On the basis of these data, Houssa et al. (1994) proposed that the activation of latent replication origins is a universal effect of cytokinins in the promotion of cell division.

Hormones and Cell Expansion

Auxin and GA are viewed as hormones that promote cell elongation. Growth of plant cells is driven by water uptake, which in turn results from stress relaxation of the cell wall (Cosgrove, 1993, 1997, in this issue). To promote growth, plant hormones are expected to cause loosening of the cell wall, but how is this achieved? The acid-growth theory postulates that secretion of hydrogen ions into the cell wall is stimulated by auxin and that the lowered pH in the apoplast activates wall-loosening processes (Rayle and Cleland, 1970; Hager et al., 1971). Indeed, there are several lines of evidence that support this hypothesis, in particular, the facts that auxin causes acidification of the cell wall and that acidic buffers induce growth in auxin-sensitive tissues (Rayle and Cleland, 1992).

However, the acid-growth theory of auxin action is not universally accepted. For example, critics point out that the wall pH in auxin-treated tissues is not low enough to elicit the growth rates observed. Nevertheless, all postulates of the acid-growth hypothesis have been shown to hold for growth induced by the fungal toxin fusicoccin (Kutschera, 1994). Technical difficulties, such as determining the pH of the cell wall, appear to have precluded a resolution of this controversy. The discovery of the expansins, a family of proteins that exhibit wall-loosening activity at pH ~4.5 (Cosgrove, 1996, 1997, in this issue), may open new approaches to the problem of hormonally induced stress relaxation of the cell wall. It will be necessary to establish the role of the expansins in growth by, for example, genetic manipulation of their expression and to examine the connection, if any, between hormone levels and expansin action.

The relationship between auxin and GA action also awaits resolution. It was thought that auxin-stimulated growth could only be observed in excised, auxin-depleted tissue, whereas GA could induce the growth of intact plants. However, exceptions to this generalization have recently been reported. For example, it has been shown that the continuous supply of auxin via a cotton wick stimulates growth of two dwarf mutants of pea, *le* and *lkb* (Yang et al., 1996). The first is a GA-deficient mutant whose growth can be fully restored by treatment with GA_1 (Ingram et al., 1983). The latter is a dwarf mutant with a two- to threefold reduction in IAA level (McKay et al., 1994) whose growth is not promoted by applied GA (Yang et al., 1996). On the basis of these data, it was concluded that auxin and GA control separate processes in stem elongation. GA may act preferentially in younger cells compared with auxin and induce cell division and cell elongation, whereas auxin may act by promoting cell extension (Yang et al., 1996).

How could these observations be explained? For a cell to elongate, its load-bearing cellulose microfibrils must be oriented perpendicular to the direction of growth (Green, 1980). Induction of cell elongation by GA may be confined to meristematic and young cells because their cellulose microfibrils are orientated transversely. Under the influence of GA, this transverse orientation of the cellulose microfibrils is maintained over a longer distance, thus extending the elongation zone of the organ (Sauter et al., 1993). By contrast, auxin is known to cause a reorientation of cellulose microfibril deposition from the oblique/longitudinal to the transverse and thereby promote the elongation of cells that have

stopped growing (Bergfeld et al., 1988). This may explain why GA action usually requires the presence of a meristem, where GA promotes cell elongation and, perhaps indirectly, cell division (Sauter and Kende, 1992), whereas auxin can promote elongation of older cells in the absence of a meristem (see Jacobs, 1997, in this issue).

Ethylene inhibits the elongation of terrestrial plants and causes thickening of their stems. This effect has been ascribed to a reorientation of both the cortical microtubules and the newly deposited cellulose microfibrils from mostly transverse to mostly oblique/longitudinal (Lang et al., 1982; Roberts et al., 1985). By contrast, the rapid elongation of many semiaquatic plants upon submergence is mediated by ethylene, which accumulates in the submerged tissue (Voesenek et al., 1992). It has been shown for two semiaquatic plants, *Callitriche platycarpa* (Musgrave et al., 1972) and deepwater rice (Raskin and Kende, 1984), that ethylene acts by increasing the tissue's responsiveness to GA and that GA is the immediate growth-promoting hormone.

Further support for this hypothesis comes from experiments with deepwater rice in which ethylene treatment led to a rapid decline of endogenous ABA levels (Hoffmann-Benning and Kende, 1992). Because ABA is a potent antagonist of GA action in rice, it has been suggested that the increased responsiveness to GA is based on reduced ABA content. In other words, the growth rate of the plant would be determined by the balance of an inhibitor (i.e., ABA) and a promoter (i.e., GA) of growth. In support of this hypothesis, it was shown that elongation of rice coleoptiles, whose growth is also promoted by ethylene, is stimulated by fluridone, an inhibitor of carotenoid and ABA biosynthesis (Hoffmann-Benning and Kende, 1992).

HORMONAL TRANSDUCTION PATHWAYS IN GROWTH

The first attempts to identify plant hormone receptors were based on hormone binding experiments. More recently, isolation of Arabidopsis hormone response mutants led to the identification of putative hormone receptors and elements of hormonal transduction pathways. In some instances, these elements fit to existing paradigms of signal transduction pathways. In other instances, they do not, or the function of the respective gene products is still unknown. We limit our discussion to components of hormonal transduction pathways whose protein sequences have been elucidated and for which some function can be suggested.

Identification of the first putative hormone receptor in plants, an auxin binding protein (now called ABP1), was based on the binding experiments of Hertel et al. (1972); purification of the protein and cloning of the corresponding cDNA showed the presence of an endoplasmic reticulum localization signal (ABP1 and other auxin binding proteins are reviewed in Jones, 1994). An ABP1 homolog or an immunologically related protein is also localized at the plasma membrane. Evidence for a receptor function of the plasma membrane–bound ABP comes from experiments showing that auxin-induced hyperpolarization is inhibited by antibodies against ABP1 (Barbier-Brygoo et al., 1989). It is not known, however, whether ABP1 plays any role in mediating growth.

A number of "auxin-resistant" mutants of Arabidopsis have been isolated for the genetic dissection of the auxin transduction pathway (Walden and Lubenow, 1996). These mutants were selected for their ability to grow on high concentrations of auxin. By using this strategy, Leyser et al. (1993) showed that the product of one such auxin resistance gene, *AXR1*, has similarity to the ubiquitin-activating enzyme E1. Mutants in the *AUX1* gene of Arabidopsis show auxin resistance with respect to root growth and do not exhibit root gravitropism. The product of the *AUX1* gene has sequence similarities to plant and fungal amino acid permeases, indicating a role for this protein in auxin transport (Bennett et al., 1996).

Several genes rapidly upregulated by auxin application have been identified (Abel and Theologis, 1996), of which two classes are described briefly. McClure et al. (1989) characterized the so-called small auxin up RNA (*SAUR*) genes from soybean, some of which are auxin regulated. When soybean seedlings were oriented horizontally, expression of *SAUR* genes became asymmetrical, that is, the level of *SAUR* mRNA on the lower side of the hypocotyl was much higher than on the upper side (McClure and Guilfoyle, 1989). This may indicate that asymmetrical expression of *SAUR* genes results from lateral redistribution of auxin in gravitropically stimulated stems, as postulated by the Cholodny-Went hypothesis. Another family of early auxin-induced genes has been described by Abel et al. (1994). They encode short-lived nuclear proteins that may act as activators or repressors of genes whose products mediate auxin responses.

Progress has also been made in identifying components of the signal transduction pathway in GA-regulated growth by using Arabidopsis response mutants (Swain and Olszewski, 1996). Such mutants fall into two categories: those that have a dwarf phenotype but do not grow in response to GA, and those that grow tall and slender, mimicking the effects of GA treatment. The *spindly* (*spy*) mutant falls into the latter category and is defective in a tetratricopeptide repeat–containing protein that may be involved in protein–protein interactions (Jacobsen et al., 1996). A similar approach led to the identification of the Arabidopsis gene *ABA-INSENSITIVE1* (*ABI1*) (Leung et al., 1994; Meyer et al., 1994). A mutation in this gene renders plants insensitive to ABA. *ABI1* was found to encode a protein with high similarity to a 2C-class serine/threonine protein phosphatase with an N-terminal calcium binding site. This indicates that the ABI1 protein functions as a calcium-regulated protein phosphatase and is thus part of a phosphorylation-dependent transduction pathway that mediates a broad spectrum of ABA responses.

Substantial progress has been made in elucidating the ethylene transduction pathway by screening for ethylene response mutants in Arabidopsis (Ecker, 1995). In the pres-

ence of ethylene, dark-grown seedlings of such mutants either do not exhibit the "triple response" (i.e., stunted growth, swelling of the root and hypocotyl, and exaggerated apical hook formation) or show the triple response phenotype even in the absence of ethylene. We limit our discussion of ethylene signal transduction to four components of the pathway whose functions have been derived from sequence analysis of the corresponding genes and from direct ethylene binding experiments.

The mutation *ethylene-resistant1* (*etr1*) is dominant, and the mutant lacks a number of responses to ethylene, including inhibition of cell elongation, promotion of seed germination, enhancement of peroxidase activity, acceleration of leaf senescence, and feedback inhibition of ethylene biosynthesis (Bleecker et al., 1988). The capacity of *etr1* to bind ethylene in vivo was one-fifth that of the wild type, indicating that the mutant is impaired in receptor function.

The *ETR1* gene was isolated by map-based cloning and was found to encode a protein with sequence similarity to bacterial two-component regulators (Chang et al., 1993). In its N-terminal portion, it contains a sensor domain with a putative input and histidine kinase region (Figure 3); fused to this is a receiver domain, but the equivalent of an output domain is missing. Using protein expressed in yeast, Schaller and Bleecker (1995) showed that the hydrophobic N-terminal region of ETR1 binds ethylene and that the *etr1-1* mutation, which is localized in this region and which leads to ethylene insensitivity, abolishes ethylene binding. These results constitute compelling evidence that ETR1 is an ethylene receptor and that the ethylene binding site is located in a membrane-spanning region of the N-terminal input domain. The *ETR2* and *ETHYLENE-INSENSITIVE4* (*EIN4*) genes encode homologs of *ETR1*, and mutations in these genes confer dominant ethylene insensitivity onto Arabidopsis seedlings (Roman et al., 1995; Hua et al., 1997).

Hua et al. (1995) have cloned an Arabidopsis gene, *ETHYLENE RESPONSE SENSOR* (*ERS*), that encodes a second type of putative ethylene receptor. The sensor domain of the ERS protein shows high similarity to ETR1, but it lacks a receiver domain (Figure 3). When, by site-directed mutagenesis, the same amino acid change was introduced into ERS, as was found in the mutant protein ETR1-4, the corresponding transgenic plants showed dominant ethylene insensitivity. Thus, Arabidopsis contains at least four genes that encode putative ethylene receptors, *ETR1*, *ETR2*, *EIN4*, and *ERS*. This potential redundancy could explain why no loss-of-function mutations have been found for any of these genes. Redundancy in ethylene perception is also evident from the fact that intragenic suppressor mutations in *etr1* resulted in recovery of ethylene sensitivity (Hua et al., 1997). Homologs of *ETR1* and *ERS* have also been isolated from tomato (Wilkinson et al., 1995; Zhou et al., 1996).

Genes acting downstream of ethylene reception in Arabidopsis include *CONSTITUTIVE TRIPLE RESPONSE* (*CTR1*). *ctr1* mutants express the triple-response phenotype constitutively, even in the absence of ethylene (Kieber et al., 1993). Genetic analyses have shown that *CTR1* acts downstream of *ETR1*, *ETR2*, *EIN4*, and *ERS* and that it is a negative regulator of ethylene responses. *CTR1* encodes a putative serine/threonine protein kinase that is related to Raf protein kinases. This relationship indicates that the ethylene transduction pathway may be similar to a mitogen-activated protein

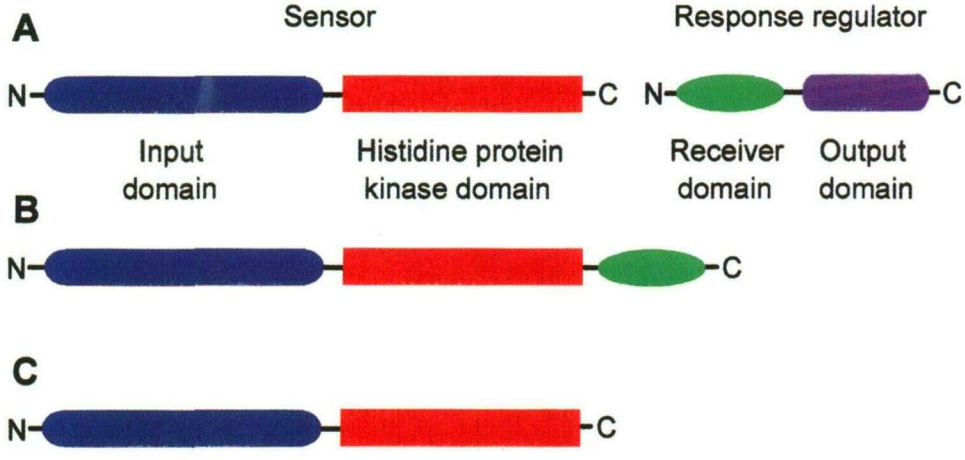

Figure 3. Two-Component Signaling Systems.

(A) The bacterial two-component system composed of sensor and response regulator proteins.

(B) The ETR-type two-component system. The ethylene binding site is located in the *trans*-membrane region of the input domain. The tomato eTAE1 homolog implicated in ethylene signal transduction (Zhou et al., 1996) and the CKI1 homolog implicated in cytokinin signaling (Kakimoto, 1996) are of this type.

(C) The ERS-type protein. ERS lacks the receiver domain; the tomato NR protein is a homolog of ERS (Wilkinson et al., 1995).

kinase–mediated phosphorylation cascade, albeit one linked to a receptor related to those typically associated with prokaryotic sensing systems.

A second downstream gene is *HOOKLESS1* (*HLS1*) of Arabidopsis, which was identified as an ethylene-responsive gene whose expression is required for the formation of the apical hook (Lehman et al., 1996). It has been suggested that the *N*-acetyltransferase encoded by *HLS1* affects the distribution of auxin in seedlings and as such could constitute a link between ethylene and auxin action in asymmetric growth.

Arabidopsis mutants that form calli and shoots in tissue culture without added cytokinin were isolated by activation T-DNA tagging (Kakimoto, 1996). The gene *CYTOKININ-INDEPENDENT1* (*CKI1*), which was tagged in four of these mutants, was found to encode a protein similar to two-component regulators. It has a putative histidine kinase and receiver domain and resembles ETR1. Thus, in all likelihood, it functions in the transduction of cytokinin responses and may indeed be a cytokinin receptor.

CONCLUSIONS AND PROSPECTS

Although combining the disciplines of biochemistry, molecular genetics, and physiology has led to major advances in our understanding of the role of hormones in plants, much more remains to be learned. The biosynthesis of IAA and cytokinins in higher plants is still poorly understood, and only in the case of ethylene has a receptor been identified. As the entire genome of Arabidopsis is sequenced over the next few years, a plethora of genes, including those involved in hormone metabolism and signal transduction, will become available; the challenge will be to determine their functions.

The levels of IAA and cytokinin have been altered in transgenic plants, mostly with constitutively expressed promoters (reviewed in Klee and Romano, 1994). Similar experiments with ABA and GA biosynthetic genes can be anticipated. In the future, these analyses should be refined by using specific promoters so that manipulation of hormone levels in certain cells, organs, and tissues and at specific times can be achieved (e.g., Gan and Amasino, 1995). Transgenic plants with modified hormone levels or altered hormone responses may offer an alternative to the practice of spraying plants with hormones to manipulate their growth and development.

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