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Oligosaccharins, Brassinolides, and Jasmonates: Nontraditional Regulators of Plant Growth, Development, and Gene Expression

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INTRODUCTION

Plants synthesize a diverse array of organic compounds. Several of these compounds, termed plant hormones or plant growth regulators, are biologically active within the plant and influence physiological processes such as growth, differentiation, and development at low concentrations. The effects of some plant hormones, such as auxin, gibberellins, cytokinins, abscisic acid (ABA), and ethylene, have been described and characterized for over 50 years (for a review, see Kende and Zeevaart, 1997, in this issue). In many cases, the roles of these "traditional" plant hormones were elucidated initially from experiments with exogenous hormones.

More recently, however, several other compounds that can affect plant growth and development have been described. In many cases, the roles these compounds play in modulating growth and development are being defined by the analysis of mutants or transgenic plants with either altered perception or levels of the plant hormone under study (an approach that is also shedding new light on studies of the traditional plant hormones; see Kende and Zeevaart, 1997, in this issue). This review describes recent observations on three classes of "nontraditional" plant hormones: oligosaccharins, brassinolides, and jasmonates. The roles of other nontraditional plant regulators such as salicylic acid (Raskin, 1992; Bennett and Wallsgrove, 1994; Gross and Parthier, 1994; Dempsey and Klessig, 1995; Hunt et al., 1996) and polyamines (Galston and Kaur-Sawhney, 1990; Kakkar and Rai, 1993) have been reviewed elsewhere. Far less is known about compounds such as turgorins, strigols, and other promotors/inhibitors of seed germination and plant growth (for a recent review, see Gross and Parthier, 1994).

OLIGOSACCHARINS

Oligosaccharins are complex carbohydrates that are capable of modulating plant growth and development at low concentrations. Some oligosaccharins, such as oligogalacturonids, act as elicitors and evoke pathogen defense responses. These defense responses include the accumulation of phytoalexins, proteinase inhibitors, lignin, peroxidase, lipoxygenase (LOX) and β-1,3 glucanases (Ryan, 1988; Hahn et al., 1989; Ebel and Cosio, 1994). The effect of elicitors on defense responses, which is probably mediated in part via alterations in the levels and/or sensitivities of jasmonic acid (JA; see below) and salicylic acid, is not covered in this section. Rhizobial lipo-chitin oligosaccharins (i.e., Nod factors) have also been reviewed recently (Spaink and Lugtenburg, 1994) and are not discussed here. Instead, we briefly focus on those oligosaccharins that exert dramatic effects on plant growth and development that are unrelated to disease responses. Additional information can be obtained in other excellent reviews covering oligosaccharins as elicitors and growth modulators (Aldington and Fry, 1993; Fry et al., 1993a; Côté and Hahn, 1994; Fry, 1994; Ozeretskovskaya and Romenskaya, 1996; John et al., 1997).

Pectic Oligosaccharins

Pectins are the major polysaccharide in the primary cell wall and are composed primarily of α-1,4-galacturonic acid–linked subunits. Pectins may also contain galactose, arabinose, and rhamnose. In many studies, biologically active pectic oligosaccharins are generated by chemical or enzymatic hydrolysis of cell walls, pectin, or polygalacturonic acid and purified to varying degrees. Pectic fragments (Figure 1A) generated by these procedures vary in size, with degrees of polymerization ranging from two to 20. Fragments are usually resolved by anion exchange chromatography.

Several lines of evidence from experiments utilizing excised tissues or tobacco thin-cell layer (TCL) explants suggest that exogenous pectic oligosaccharins (oligogalacturonides with a degree of polymerization ranging from 10 to 17) antagonize auxin action at levels 10- to 100-fold lower than those required for the elicitation of plant defense reponses. For example, oligogalacturonides inhibit auxin-induced growth of

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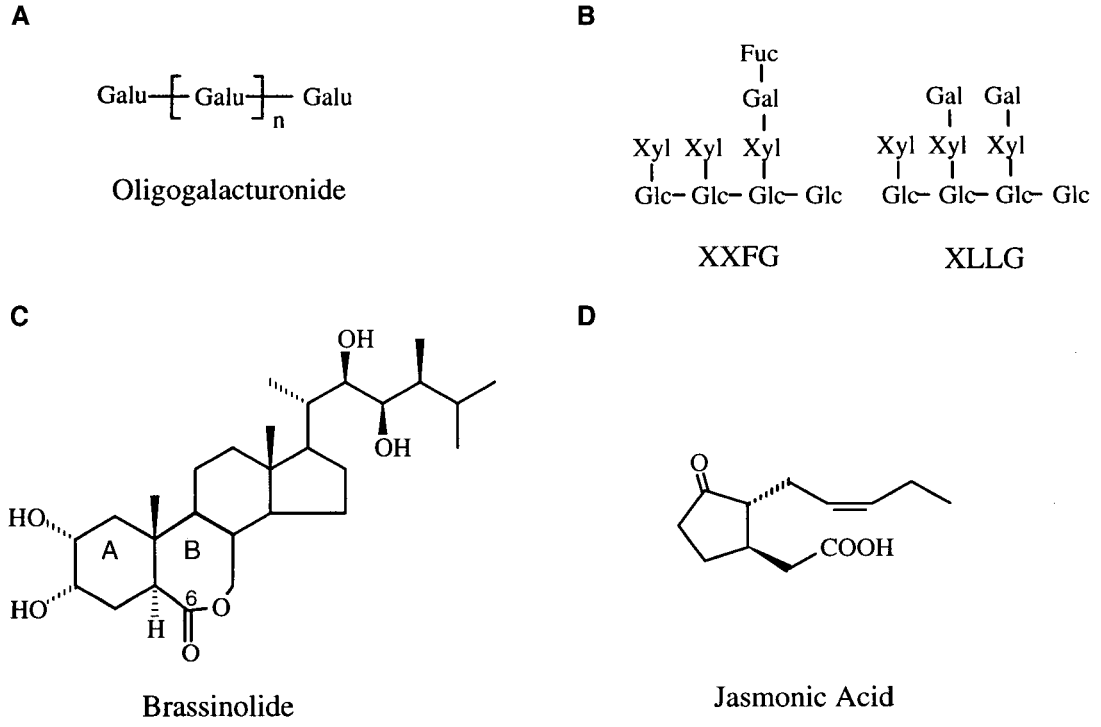

Figure 1. Chemical Structures of Nontraditional Plant Hormones.

(A) Oligogalacturonide. Galu represents α-galacturonic acid-(1→4).
(B) Two typical xyloglucan fragments. Fuc, α-fucopyranosyl-(1→2); Gal, α-galactopyranosyl-(1→2); Glc, β-glucosyl-(1→4) residues; Xyl, α-xylopyranosyl-(1→6).
(C) Brassinolide. The A and B rings are indicated, as is carbon 6.
(D) (−)-JA.

isolated pea stem segments (Branca et al., 1988), auxin-stimulated rooting in leaf explants (Filippini et al., 1992), and auxin-dependent somatic embryogenesis in carrot cultures (LoSchiavo et al., 1991; Filippini et al., 1992).

In some studies, TCL explants have been used to study the role of oligogalacturonides in morphogenesis. TCLs contain a limited number of cell types (epidermis, chlorenchyma, collenchyma, and parenchyma), and organogenesis occurs in TCL explants without callus formation. Hence, TCLs are an excellent system for studying the changes that occur when tissues are induced to form new organs. TCLs will form roots, flowers, or vegetative shoots, depending on the auxin/cytokinin ratio (Mohnen et al., 1990). Oligogalacturonides can induce flower formation or inhibit root formation in tobacco TCLs or leaf explants (Eberhard et al., 1989; Mohnen et al., 1990; Marfà et al., 1991); they may act by interfering with auxin binding sites (LoSchiavo et al., 1991; Filippini et al., 1992) or by altering auxin metabolism (Pressey, 1991). However, little is known about the role of these pectic oligosaccharins in whole plant growth and development.

Xyloglucan Oligosaccharins

Another group of oligosaccharins that modulate plant growth is derived from xyloglucans (York et al., 1984; McDougall and Fry, 1989). Xyloglucans are components of the hemicelluloses, which form strong noncovalent bonds with cellulose microfibrils in plant cell walls. This cross-linking between cellulose and xyloglucans has been suggested to be a major factor controlling cell elongation and growth (see Cosgrove, 1997, in this issue).

Xyloglucan fragments can be obtained following enzymatic hydrolysis with an endo-β-1,4-glucanase and subsequent purification by size exclusion chromatography and HPLC. The most common xyloglucan oligosaccharides produced by endo-β-1,4-glucanase are XXXG, XXFG, and XLFG (Figure 1B; endo-β-1,4-glucanase cleaves the xyloglucan backbone at unbranched sugars, i.e., approximately every fourth residue). Because xyloglucan oligosaccharides can be considered to be oligomers of β-1,4-glucosyl residues, the nomenclature utilizes an abbreviation defined

for different side chain substitutions in the glucan backbone (Fry et al., 1993b). For example, G and X represent β-glucosyl residues lacking a side chain or containing a xylosyl residue, respectively. The side chains need not consist of a single sugar residue; the abbreviation L is defined to be β-galactopyranosyl-(1→2)-α-xylopyranosyl, whereas F is defined as α-fucopyranosyl-(1→2)-galactopyranosyl-(1→2)-α-xylopyranosyl.

Some xyloglucan fragments can modulate plant growth. For example, XXFG inhibits auxin-, gibberellin-, proton-, and fussicoccin-induced growth as well as endogenous growth at concentrations of ~1 nM (York et al., 1984; McDougall and Fry, 1989). By contrast, at higher concentrations (~1 mM), XXFG (and also XXLG, XLLG, and XXXG) can promote pea stem elongation in the absence of auxin (McDougall and Fry, 1990).

It has been suggested that these oligosaccharins (XXFG, XXLG, XLLG, and XXXG) stimulate growth by acting as substrates for a xyloglucan endotransglycosylase (XET; Fry et al., 1992; Nishitani and Tominaga, 1992). XET acts by hydrolyzing a xyloglucan polymer and transferring the newly released xyloglucan strand to the end of another xyloglucan polymer (Fry et al., 1992; Nishitani and Tominaga, 1992). If xyloglucan polymers act to restrain cell expansion by hydrogen bonding to cellulose, transient breakage of xyloglucans by XET may cause temporary wall loosening (Fry, 1989). However, if the cut xyloglucan polymer were transferred instead to small xyloglucan oligosaccharins, the net result could be an accumulation of xyloglucan polymers, which may cause altered wall properties (Fry, 1989). XXFG probably inhibits growth by a different mechanism because the K_m of XET for xyloglucan oligomers is ~10 mM (i.e., 10,000-fold higher than is the concentration at which maximum growth inhibition is achieved).

A genetic approach has been informative in understanding the role of xyloglucan oligosaccharins in plant growth and development. Reiter et al. (1993) isolated an Arabidopsis mutant deficient in fucose (*mur1*). The mutant exhibits dwarfed stature, short petioles, reduced apical dominance, and weak stems. Because the presence of fucose on XXFG was thought to be essential for the growth inhibitory activity of this oligosaccharin, the dwarf phenotype of *mur1* plants was unexpected. However, Zablackis et al. (1996) showed that xyloglucans in *mur1* plants contain L-galactose in place of L-fucose. Thus, one possibility is that the dwarf phenotype in *mur1* arises because this new oligosaccharin (XXJG) is catabolized at a slower rate than is XXFG. This or a similar mechanism must be invoked to explain the *mur1* phenotype because the growth inhibitory properties of XXJG and XXFG are identical.

Exogenous oligosaccharins can modulate the elicitation of defense responses, growth in excised stem segments, morphogenesis in tissue culture, and induction of root nodules. However, in several studies, oligosaccharins are produced in vitro by using treatments unlikely to be encountered in vivo. In some cases, preparations of oligosaccharins may not be chemically homogeneous. Use of pure preparations is essential to determine unequivocally a role for oligosaccharins in the endogenous regulation of plant growth and development. Much more information about oligosaccharins is needed with regard to their biosynthesis, transport, binding factors, mode of action, and physiology in whole plants.

BRASSINOSTEROIDS

Brassinosteroids (BRs) are a group of naturally occurring polyhydroxy steroids. Brassinolide (Figure 1C), which was originally isolated from rape (*Brassica napus* L.) pollen in 1979, was the first BR to be characterized (Grove et al., 1979). More than 60 BRs have been identified. Of these, 31 have been fully characterized, including two conjugates (Sakurai and Fujioka, 1993). BRs have been identified in many plants, including dicots, monocots, gymnosperms, green alga, and a fern (Sakurai and Fujioka, 1993). BRs have been isolated from seeds, fruits, shoots, leaves, flower buds, and galls at levels between 0.5 pg and 30 ng/g fresh weight. BR levels are relatively high in pollen (5 to 190 ng/g fresh weight).

Exogenous BR causes cell elongation and division in excised stem segments and seedlings at micromolar to picomolar concentrations (Sakurai and Fujioka, 1993). In addition to their growth-promoting activities, exogenous BRs have been reported to inhibit root growth, enhance gravitropism, retard leaf abscission, enhance resistance to stress, and promote xylem differentiation (Sakurai and Fujioka, 1993). Several excellent reviews are available on the history, chemistry, biochemistry, and physiology of BRs (Mandava, 1988; Cutler et al., 1991; Sakurai and Fujioka, 1993; Gross and Parthier, 1994; Clouse, 1996; Yokota, 1997).

Variations in BR structure result from different substitutions in the A/B rings and side chains created in oxidation or reduction reactions that occur during biosynthesis. Of the known BRs, the most abundant are brassinolide and its biosynthetically related precursors. Biosynthesis of brassinolide begins with the reduction of the 5,6 double bond of campesterol to form campestanol (Figure 2A). The pathway to brassinolide from campestanol may occur via an early oxidation of campesteronol to form cathasterone at carbon 6 (Figure 2A) or a late oxidation at carbon 6 (reviewed in Yokota, 1997). Several BRs lacking an oxygen at carbon 6 are found in plants; however, there is no evidence for the conversion of campestanol to 6-deoxoBRs.

The ability of many naturally occurring compounds such as BRs to modulate strongly plant growth in bioassays or whole plant studies suggests that they could function as endogenous plant hormones. Unequivocal evidence that BRs are essential for some aspects of plant growth and development has been obtained with the isolation of BR-insensitive (e.g., *bri1*, brassinosteroid insensitive; *cbb2*, cabbage; *lka*) and BR-deficient (e.g., *dwf1-6/cbb1*, dwarf; *cbb3/cpd*, constitutive photomorphogenic dwarf; *det2*, deetiolated; *lkb*) Arabidobsis and pea mutants (Clouse et al., 1996;

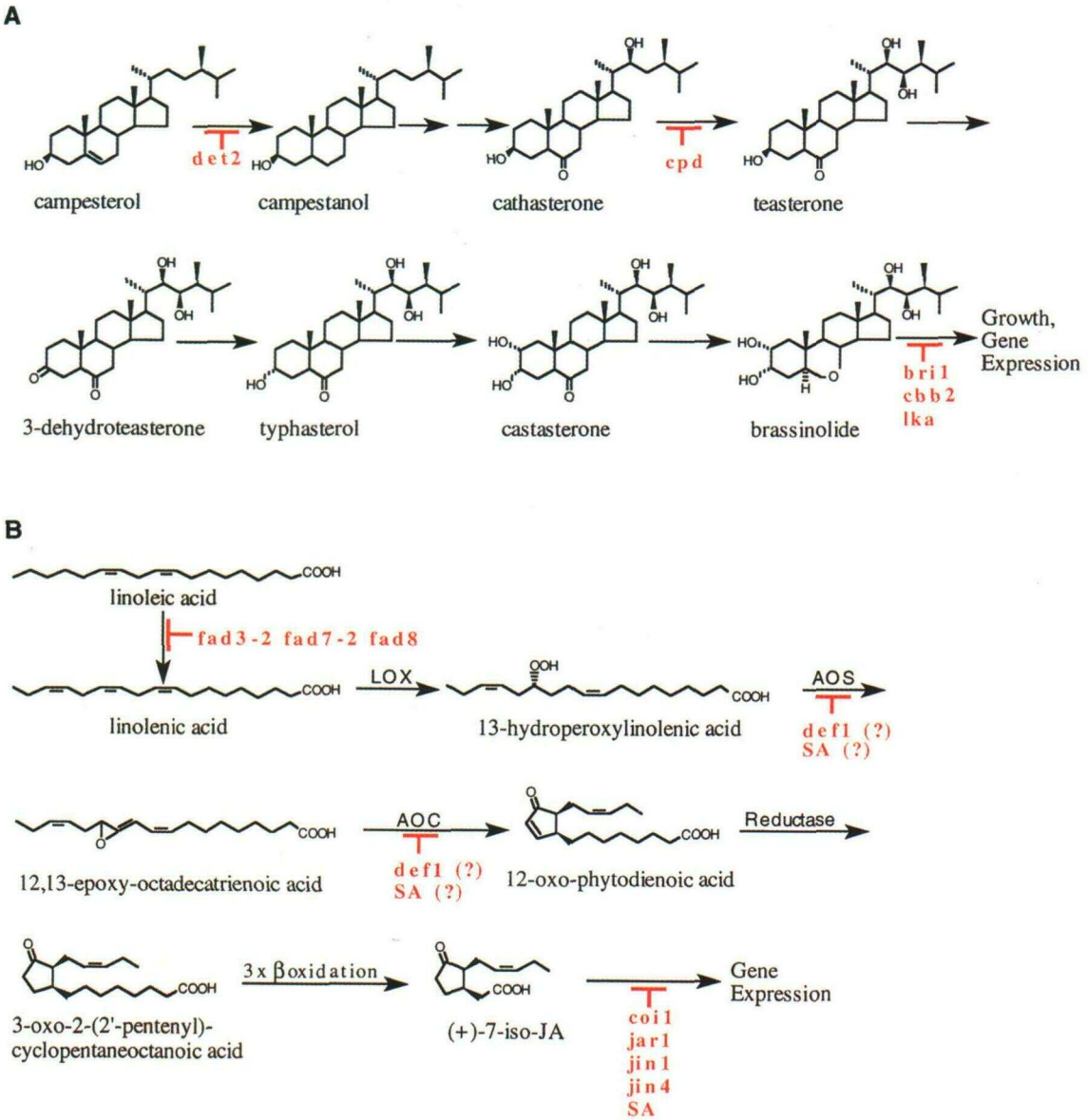

Figure 2. Biosynthetic Pathways for Brassinolides and JA.

(A) The biosynthetic pathway of brassinolides. This pathway begins with the reduction of the 5,6 double bond of campesterol to form campestanol. Illustrated here is the pathway that includes the early oxidation at carbon 6 to form cathasterone (Fujioka et al., 1995). Another possible pathway (not shown) utilizes 6-deoxy intermediates derived from campesterol (Yokota, 1997) with a final oxidation at carbon 6 to give castasterone. Castasterone is then converted to brassinolide. Abbreviations in red indicate biosynthetic lesions or sensitivity mutants of Arabidopsis and pea (*lkb* is not shown, but its lesion is before teasterone). See text for details.

(B) The biosynthetic pathway of JA. This pathway begins with the release of linolenic acid from lipids and its hydroperoxidation by LOX. After reactions catalyzed by allene oxide synthase (AOS) and allene oxide cyclase (AOC), 12-oxo-phytodienoic acid is formed. After a reduction and three rounds of β oxidation, (+)-7-iso-JA is formed. Abbreviations in red indicate biosynthetic lesions, inhibitor blocks, or sensitivity mutants of Arabidopsis and tomato. SA, salicylic acid.

Kauschmann et al., 1996; Li et al., 1996; Szekeres et al., 1996; Nomura et al., 1997).

These mutants were isolated by using a variety of screening methods: *bri1* by screening for a long root phenotype when grown in the presence of 24-epibrassinolide; *dwf1-6/cbb1*, *cbb2*, and *cbb3* by visual identification; and *det2* and *cpd* by screening for characteristics of light-grown plants when grown in the dark. Based on mapping and complementation tests, *dwf1-6* and *cbb1* are allelic to each other, as are *cbb3* and *cpd.* All of the Arabidopsis mutants exhibit male sterility or reduced male fertility, severe dwarfism, and reduced apical dominance. When they are grown in darkness, they show reduced hypocotyl elongation, unregulated opening of cotyledons, and emergence of primary leaves. The pea mutants have similar phenotypes showing a reduction in internode and penducle length, diminished basal branching, and a ridged surface (Reid and Ross, 1988). Interestingly, dark-grown *lka* and *lkb* pea seedlings do not exhibit a deetiolated phenotype (Nomura et al., 1997), suggesting that in peas, BRs may not play as strong a role in photomorphogenesis as they do in Arabidopsis.

Four of the mutants, *dwf1-6/cbb1*, *cbb3/cpd*, *det2*, and *lkb,* appear to have blocks in the BR biosynthetic pathway (Figure 2A; Kauschmann et al., 1996; Li et al., 1996; Nomura et al., 1997) because exogenous BRs restore the phenotype of these mutants to the wild type. Moreover, the deduced amino acid of the *DET2* gene product shares significant homology to mammalian steroid 5α-reductases (Li et al., 1996). Hence, DET2 may catalyze the removal of the 5,6 double bond of campesterol during its conversion to campestanol. Similarly, the *CBB3/CPD* gene product shows a significant degree of homology with mammalian cytochrome P450 monooxygenases, a large family of enzymes that includes steroid hydroxylases (Szekeres et al., 1996). Thus, CBB3/CPD may catalyze one or several of the hydroxylations occurring during the conversion of campesterol to brassinolide (Figure 2A). Based on BR feeding studies, the block in *lkb* appears to be before teasterone (Nomura et al., 1997), but the exact position is not known.

The role of the *DWF1* gene product is less clear. The *dwf1* phenotype is less severe than that of *cbb3* and *det2.* This implies that a *dwf1*-mediated block in the biosynthestic pathway leads to a moderate but not complete reduction in BR levels. Similarly, the ability to suppress the *dwf1-6* phenotype with exogenous BRs may suggest that a partial insensitivity to BRs is being overcome. The amino acid sequence of DWF1 indicates the presence of a FAD binding domain (Mushegian and Koonin, 1995) and a nuclear targeting signal (Takahashi et al., 1995). Consequently, DWF1 may act as a FAD-dependent oxidase or have a regulatory role in the nucleus.

Two BR-insensitive mutants, *bri1* (Clouse et al., 1996) and *cbb2* (Kauschmann et al., 1996), have been isolated from Arabidopsis, and one, *lka*, has been isolated from pea (Nomura et al., 1997). In Arabidopsis, the mutant loci map to the same region on the lower half of chromosome 4, and preliminary data (results from one successful cross) indicate that they are allelic (T. Altmann and S. Clouse, personal communication). The inability of exogenous BR to rescue the *bri1/cbb2* and *lka* phenotypes suggests that the BRI1/CBB2 and LKA gene product(s) may be either a BR receptor or a component of the BR signal transduction pathway.

BR-promoted pea epicotyl elongation exhibits a longer lag time than does auxin-induced elongation. Auxin typically causes elongation within 10 to 15 min, whereas the effects of exogenous BRs are not apparent for 40 to 50 min (Zurek et al., 1994). BRs appear to cause elongation by affecting wall extensibility, increasing wall relaxation properties in *Brassica chinensis* (Wang et al., 1993) and plastic extensibility in soybean (Zurek et al., 1994; for a review of wall extensibility, see Cosgrove, 1997, in this issue).

Some insight into the molecular mechanisms underlying these changes in wall extensibility comes from analyses of the expression of *BRU1*, a BR-responsive gene, which is high in apical regions of soybean hypocotyls and epicotyls (Clouse et al., 1993). In addition to sequence similarity (Clouse et al., 1993; Zurek and Clouse, 1994), recent evidence suggests that the recombinant BRU1 protein possesses XET activity and that XET activity in soybean epicotyls is increased by BR treatment (Romanov et al., unpublished data, cited in Clouse et al., 1996, and Clouse, 1996). That XET activity is induced by exogenous BRs suggests that the dwarf phenotype of *det2*, *dwf1*, *cbb2/bri1*, and *cbb3* and the diminished internode length in *lka* and *lkb* could result from reductions in BR levels or sensitivity, which in turn decrease XET activity, and a reduction in cell wall extensibility (see above). The *TCH4* (touch; Xu et al., 1995) and *MERI5* (meristem; Medford et al., 1991) genes also show a high degree of similarity to BRU1 and other XETs. That *TCH4* and *MERI5* show reduced expression in the *dwf1*, *cbb2/bri1*, and *cbb3* mutants (Kauschmann et al., 1996) provides further evidence in support of a role for XETs and BRs in modulating plant growth. Experiments designed to alter the activity of XET-encoding genes via sense and antisense technology should shed light on the roles of BRs and XETs in plant growth.

Measurements of changes in BR levels in response to mechanical perturbations such as touch and other environmental changes need to be performed, although the extremely low levels of BRs in plants may make such analyses difficult. In addition, in vitro demonstrations of enzymatic activity for DET2, CBB3, and DWF1 are needed to define the role of the corresponding genes in the BR biosynthetic pathway. Nevertheless, the pleiotropic effects of the *det2*, *dwf1-6/cbb1*, *cbb2*, *cbb3/cpd*, *bri1*, *lka*, and *lkb* mutations on leaf morphology, pollen fertility, light development, and cell elongation suggest that BRs are an important class of plant hormones.

JASMONATES

JA (Figure 1D) and its methyl ester (methyl jasmonate [MeJA]) are linolenic acid (LA)–derived, cyclopentanone-

based compounds. Early studies showed that exogenous JA or MeJA can promote senescence and regulate growth. Subsequent research has revealed that JA specifically alters gene expression and that wounding and elicitors can cause JA and MeJA accumulation in plants. This section summarizes our rapidly expanding knowledge of JA function, biosynthesis, distribution, and signal transduction in plants. Additional information on this topic can be found in recent reviews (Sembdner and Parthier, 1993; Creelman and Mullet, 1997).

Distribution of JA

JA levels in plants vary as a function of tissue and cell type, developmental stage, and in response to several different environmental stimuli. For example, levels of JA in soybean seedlings are higher in the hypocotyl hook, a zone of cell division, and in young plumules than they are in the zone of cell elongation and more mature regions of the stem, older leaves, and roots. High levels of JA are also found in flowers and in the pericarp tissues of developing reproductive structures (Lopez et al., 1987; Creelman and Mullet, 1995).

Jasmonate levels are rapidly and transiently increased by mechanical perturbations, such as those causing tendril coiling (Falkenstein et al., 1991; Weiler et al., 1993), turgor reduction induced by water deficit (Creelman and Mullet, 1995), and wounding (Creelman et al., 1992). In addition, JA accumulation can be induced in cell cultures and plants by oligosaccharides derived from plant cell walls, by elicitors such as chitosans derived from fungal cell walls, and by peptide inducers (Gundlach et al., 1992; Doares et al., 1995b; Nojiri et al., 1996). These compounds may stimulate JA biosynthesis via receptor-mediated processes.

Transcripts of JA and JA-responsive genes accumulate systemically in plants in response to localized wounding (Pearce et al., 1991). The systemic signal is apparently released at the wound site and migrates through the phloem to other parts of the plant. Systemin, an 18–amino acid peptide, has been shown to move in the phloem and to induce the synthesis of JA and the expression of JA-responsive genes throughout the apical portion of plants (Pearce et al., 1991). Systemin may be released from the wound site upon hydrolysis of a precursor polypeptide (McGurl and Ryan, 1992). The tomato mutant JL1 may be impaired in the ability to make or release systemin at or near the site of wounding (Lightner et al., 1993).

JA Biosynthesis

The biosynthesis of jasmonates begins with LA (Figure 2B). This fatty acid is converted to 13-hydroperoxyLA by LOX. 13-HydroperoxyLA is a substrate for allene oxide synthase (AOS), the product of which is a substrate for allene oxide cyclase (AOC). AOC catalyzes reactions that result in the formation of 12-oxo-phytodienoic acid. Following reduction and three steps of β-oxidation, (+)-7-iso-JA is formed (Figure 2B). However, two isomers of jasmonate are found in plants, (+)-7-iso-JA and (−)-JA, which occur in a ratio of ~1:9. Due to increased steric hindrance, the *cis* orientation of the side chains is less stable in (+)-7-iso-JA, and they epimerize to the more stable *trans* configuration. This occurs via a *keto–enol* tautomerization involving the C6 ketone and the C7 proton to form the corresponding diastereomers.

Increases in JA levels could result from the activation of phospholipases that release LA from membranes (Farmer and Ryan, 1992). However, only indirect evidence for this possibility is currently available. For example, in one study, free LA levels doubled within 1 hr after wounding, whereas JA levels rose 10-fold (Conconi et al., 1996a). Moreover, treatment of tobacco cells with elicitors derived from the fungus *Phytophthora parasitica* var *nicotianae* (Roy et al., 1995) caused the amount of phosphatidylcholine to decrease concomitant with an increase in phospholipase A activity. Similarly, in soybean cell culture, harpin and an extract from the pathogenic fungus *Verticillium dahliae* promoted rapid increases in phospholipase A activity (Chandra et al., 1996). Upon wounding of potato tuber tissue, JA levels rose ~100-fold in 4 hr, but two inhibitors of animal phospholipase A_2 (manoalide and quinacrine) did not prevent the accumulation of JA (Koda and Kikuta, 1994). The close similarities in the signaling pathways of defense reactions in plants and animals (Bergey et al., 1996) suggest that a phospholipase is involved in the production of free LA.

Many of the enzymes involved in JA biosynthesis are localized in chloroplasts. This raises interesting questions regarding wound signal transduction in light of the presumed spatial separation of wound/elicitor signal perception on the plasma membrane and JA synthesis in the plastid. The physiological role of a chloroplast-targeted LOX was analyzed by reducing AtLOX2 accumulation in transgenic Arabidopsis plants (Bell et al., 1995). The reduction of *AtLox2* expression by using sense cosuppression caused no obvious changes in plant growth. However, wound-induced accumulation of JA, which was observed in control plants, was significantly reduced in leaves of transgenic plants lacking AtLOX2. Therefore, this plastid-localized LOX is required for wound-induced synthesis of jasmonates in Arabidopsis leaves.

Flax and Arabidopsis AOS have been cloned and characterized (Song and Brash, 1991; Song et al., 1993; Brash and Song, 1995). Flax AOS is a 55-kD hemoprotein with the spectral characteristics of a cytochrome P-450. The flax cDNA encodes an N-terminal sequence characteristic of chloroplast transit peptides, consistent with localization of AOS activity in chloroplasts (Blée and Joyard, 1996). The deduced amino acid sequence of the Arabidopsis AOS (expressed sequence tag 94J16T7), which shares a high degree of identity with that of flax AOS, also contains a putative N-terminal plastid targeting sequence (R.A. Creelman, E. Bell, and J.E. Mullet, unpublished observations). Overexpression of flax AOS in transgenic potato plants increases JA levels (Harms et al., 1995), indicating that the amount of

AOS protein is a rate-determining step for JA biosynthesis. The tomato mutant *def1* (defenseless) is inhibited in the conversion of 13-hydroperoxyLA to 12-oxo-phytodienoic acid (Figure 2B; Howe et al., 1996).

JA Function in Plants

JA and MeJA inhibit the germination of nondormant seeds and stimulate the germination of dormant seeds (see Bewley, 1997, in this issue for a review of germination and dormancy). JA, MeJA, ABA, and ethylene inhibit the germination of the recalcitrant seeds of *Quercus robur* (Finch-Savage et al., 1996). When these desiccation-sensitive seeds were dried, the concentrations of MeJA and JA increased prior to the loss in seed viability. The increase in jasmonate was correlated with lipid peroxidation, suggesting that the production of jasmonate may not regulate germination but rather is a consequence of membrane damage. The level of jasmonate in soybean seeds 12 days after anthesis is low (~0.1 ng/g fresh weight), whereas in older seeds, JA levels are higher (0.5 ng/g fresh weight; Creelman and Mullet, 1995). Twelve hours after imbibition, the level of JA increased fourfold to 2 mg/g fresh weight in seed axes. The observed increase in JA levels following imbibition is correlated with seed reserve mobilization and may be a consequence of rather than a trigger for germination. The seeds of the Arabidopsis *jin4* (jasmonate insensitive) and *jar1* (jasmonate response) mutants show increased sensitivity to ABA (Staswick et al., 1992; Berger et al., 1996), suggesting that JA may stimulate seed germination by decreasing sensitivity to ABA.

In addition to its effects on seed germination, JA also strongly inhibits root growth by a mechanism that is not mediated by ethylene (Berger et al., 1996). JA also inhibits indoleacetic acid–stimulated coleoptile elongation, possibly by blocking the incorporation of glucose into cell wall polysaccharides (Ueda et al., 1995). Furthermore, JA activates the differential growth involved in tendril coiling in pea, a response that does not directly involve ethylene or indoleacetic acid (Falkenstein et al., 1991).

That JA may play a role in the formation of flowers, fruit, and seed is suggested by the relatively high levels of this compound in developing plant reproductive tissues. The presence of jasmonate and related volatile fatty acid derivatives in the flower may indicate a function in insect attraction and thus pollen dispersal. Other aspects of flower, fruit, and seed development that can be modulated by jasmonate include fruit ripening, fruit carotenoid composition (Czapski and Saniewski, 1992), and the expression of genes encoding seed and vegetative storage proteins (VSPs). By contrast to the effects of JA on root growth, jasmonate-stimulated tomato and apple fruit ripening most likely occurs through the activation of ethylene-forming enzyme and the production of ethylene (Czapski and Saniewski, 1992).

A role for JA in mediating the accumulation of secondary plant products has also been proposed (Gundlach et al., 1992). Exogenous JA causes the accumulation of paclitaxel and related taxanes in *Taxus* (Yukimune et al., 1966), alkaloids in *Cantharanthus* and *Cinchona* (Aerts et al., 1994), anthocyanins in soybean (Franceschi and Grimes, 1991), and rosmarinic acid in *Lithospermum* (Mizukami et al., 1993).

JA levels are high in vegetative sink tissues, such as soybean axes, plumules, and the hypocotyl hook, suggesting that JA may be involved in the regulation of protein storage in plants (Creelman and Mullet, 1995). In six-week-old soybean seedlings, JA levels are higher in young growing leaves that are importing carbon and nitrogen than they are in older, fully expanded leaves (Creelman and Mullet, 1995). High levels of JA are also present in developing reproductive structures, especially pods, with lower levels in seeds. JA or a derivative, tuberonic acid, has been proposed to play a role in the formation of tubers, a specialized vegetative sink (Pelacho and Mingo-Castel, 1991; Koda, 1992; Ravnikar et al., 1992).

A second reason to suggest that jasmonates play an important role in protein storage during plant development derives from the discovery that genes encoding VSPs (Staswick, 1994) are regulated by JA (Anderson, 1988). VSPs accumulate in the vacuoles of paraveinal mesophyll and bundle sheath cells in soybean leaves (Franceschi and Grimes, 1991). If pods are continuously removed from plants, VSPs accumulate and can account for as much as 45% of the soluble protein in leaves (Wittenbach, 1983). Other studies showed that VSPs accumulate in pods and other parts of the developing reproductive structure but not in seeds (Staswick, 1989a).

VSP accumulation in soybean axes, hypocotyl hooks, and young developing leaves is correlated with *Vsp* expression and endogenous levels of JA (Mason et al., 1992, 1993; Creelman and Mullet, 1995). Included among the soybean VSPs are two proteins with low acid phosphatase activity (VSPa and VSPb; DeWald et al., 1992) and LOX (Tranbarger et al., 1991; Kato et al., 1993). The genes encoding these proteins are regulated in a complex way by JA, sugars, phosphate, nitrogen, and auxin (Staswick et al., 1991; Mason et al., 1992; DeWald et al., 1994; Sadka et al., 1994).

Soybean *Vsp* genes and the Arabidopsis *AtVsp* genes show high expression in flowers and developing fruit (Staswick, 1989a, 1989b; Berger et al., 1995). VSPs in these tissues may provide temporary storage of carbon and nitrogen arriving at the reproductive apex for use during rapid synthesis of seed storage proteins. The AtVSP proteins are barely detectable in flowers of *coi1* (coronatine insensitive) mutants, which are also JA insensitive, and expression of *AtVsp* could be induced by exogenous JA in wild-type plants (Benedetti et al., 1995). Moreover, the JA- and LA-deficient triple mutant of Arabidopsis, *fad3-2 fad7-2 fad8* (for fatty acid desaturase deficient), does not express *AtVsp* unless the plants are provided with exogenous JA (McConn et al., 1997). Ovules of the LA-deficient mutant were viable, indicating that JA and expression of the *AtVsp* genes are not essential for seed formation. Therefore, although JA may modulate expression of genes encoding seed storage proteins (Wilen et al., 1991),

JA is not essential for the production of viable ovules in Arabidopsis. However, the *fad3-2 fad7-2 fad8* and *coi1* mutants fail to produce viable pollen unless supplied with JA (McConn and Browse, 1996).

With regard to effects of JA on gene expression, application of JA to leaves decreases expression of nuclear and chloroplast genes involved in photosynthesis (Weidhase et al., 1987; Bunker et al., 1995). JA treatments also cause a loss of chlorophyll from leaves and cell cultures (Weidhase et al., 1987). The ability of JA to cause chlorosis led to the suggestion that this compound plays a role in plant senescence (Ueda et al., 1981). However, this suggestion is difficult to reconcile with the high levels of JA that are found in zones of cell division, young leaves, and reproductive structures. Unfortunately, a complete analysis of JA levels in senescing leaves has not been performed, although a limited study of this question in soybean revealed only small changes in JA amounts in leaves during pod fill (Bunker et al., 1995). Thus, although JA can induce senescence-like symptoms, the role of this hormone in mediating senescence is at present unclear (see Bleecker and Patterson, 1997, in this issue, for a review).

The ability of JA to inhibit expression of genes involved in photosynthesis suggests that jasmonates could help reduce the plant's capacity for carbon assimilation under conditions of excess light or carbon. Inhibition of genes encoding the photosynthetic apparatus under these conditions would eventually balance the absorption and utilization of light energy. In the short term, JA-mediated induction of VSP synthesis under conditions of high sugar accumulation creates a sink for carbon and nitrogen and releases phosphate from sugar phosphate pools for further carbon fixation.

Application of jasmonate to plants causes large changes in translation, transcription, and mRNA populations (Sembdner and Parthier, 1993). For example, decreased translation of the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (*rbcL*) in plastids was correlated with a site-specific cleavage in the 5′ untranslated portion of the *rbcL* mRNA (Reinbothe et al., 1993a, 1993b, 1993c). The modified *rbcL* mRNA 5′ end presumably reduces access to the ribosome binding site located near the site of translation initiation. Reduced synthesis of the small subunit of ribulose-1, 5-bisphosphate carboxylase/oxygenase and other cytoplasmic proteins occurred through the suppression of translation initiation and the reduction of mRNA levels (Reinbothe et al., 1993c).

Inducible plant defenses to UV light may be mediated in part by alterations in JA levels (Conconi et al., 1996b). Treatment of tomato leaves with UV-B or UV-C irradiation induced the accumulation of defense gene transcripts encoding the protease inhibitors Pin1 and Pin2. However, no accumulation of *Pin1* or *Pin2* mRNA was observed in response to UV-B/UV-C when leaves were treated with salicylic acid, a strong inhibitor of JA biosynthesis and action (Doares et al., 1995a). The UV-mediated induction of these genes was also blocked in *def1*, a tomato mutant with a defect in the JA biosynthetic pathway (Conconi et al., 1996b; see above). This result suggests that some defense responses to UV irradiation are mediated in part by alterations in JA and its signaling pathway.

JA also plays an important role in plant insect and disease resistance. Several lines of evidence support this conclusion. First, JA accumulates in wounded plants (Creelman et al., 1992) and in plants or cell cultures treated with elicitors of pathogen defense (Gundlach et al., 1992). Second, JA activates genes encoding protease inhibitors that help protect plants from insect damage (Johnson et al., 1989), and JA activates the expression of genes encoding antifungal proteins such as thionin (Johnson et al., 1989), osmotin (Xu et al., 1994), and the ribosome-inactivating protein RIP60 (Chaudhry et al., 1994; Reinbothe et al., 1994). JA modulates the expression of genes encoding cell wall proteins, such as hydroxyproline-rich glycoproteins and proline-rich proteins (Creelman et al., 1992), which may be involved in the synthesis of barriers to infection. Furthermore, JA induces the expression of genes involved in phytoalexin (Creelman et al., 1992; Choi et al., 1994) and phenolic biosynthesis (Doares et al., 1995b).

Another line of evidence for JA's role in pest resistance comes from analyses of plants having modified levels of JA. For example, treatment of potato with JA increases resistance to the fungus *Phytophthora infestans* (Cohen et al., 1993), and the tomato mutant *def1* is more susceptible to damage by larvae of the tobacco hornworm (*Manduca sexta*; Howe et al., 1996). Furthermore, the Arabidopsis *fad3-2 fad7-2 fad8* triple mutant has very low levels of LA and is unable to accumulate JA and to induce JA-responsive genes after wounding (McConn and Browse, 1996; McConn et al., 1997). These mutant plants are also very susceptible to the larvae of a common sacrophagous fungal gnat, *Bradysia impatiens*. Treatment of the mutants with JA restores fungal gnat resistance, demonstrating an essential role for JA in resistance to this pest in Arabidopsis.

JA Signal Transduction

It is presumed that jasmonate interacts with receptors in the cell to activate a signaling pathway that ultimately triggers changes in the transcription and/or translation of genes participating in the responses mediated by JA. The JA signal transduction pathway is being elucidated through analyses of promoter elements that direct the JA-mediated activation of gene transcription and of JA-insensitive mutants.

To date, up to four different classes of JA-insensitive mutants have been identified: *jar1*, *coi1*, *jin1*, and *jin4* (Staswick et al., 1992; Benedetti et al., 1995; Berger et al., 1996). Genetic studies have not been able to determine whether or not *jin4* and *jar1* are allelic (Berger et al., 1996). The *jar1*, *jin1*, and *jin4* mutants were recovered by screening for plants capable of growing on concentrations of JA (~10 mM) that inhibit wild-type root growth. By contrast, the *coi1* mutant was identified by virtue of its resistance to coronatine (Feys et al., 1994), a chlorosis-inducing toxin that has a chemical struc-

ture and biological activity similar to those of JA. Root growth in *coi1* plants is also insensitive to MeJA.

The promoters of two jasmonate-inducible genes, *Pin2* and *VspB*, have been analyzed in detail (Kim et al., 1992; Mason et al., 1993). A 50-bp region that was identified in the promoters of both genes confers JA responsiveness on truncated reporter gene constructs. Each of these JA-responsive regions contains a G-box sequence (CACGTG), which is a potential binding site for bZIP transcription factors (Williams et al., 1992). However, mutagenesis of the G-box in the *Pin2* promoter did not prevent JA-mediated induction, demonstrating that this element is not essential for JA modulation of *Pin2* transcription (Lorbeth et al., 1992). Interestingly, bestatin, an inhibitor of aminopeptidases in plants and animals, induces *Pin2* expression in the absence of JA (Schaller et al., 1995). This observation suggests that the expression of *Pin2*, and perhaps that of other jasmonate-modulated plant genes, is normally prevented by the action of an aminopeptidase. Therefore, the induction of JA-responsive genes could be mediated via the inactivation of this hypothetical protease or via stabilization of its target protein.

SUMMARY

Each of the nontraditional plant hormones reviewed in this article, oligosaccharins, brassinolides, and JA, can exert major effects on plant growth and development. However, in many cases, the mechanisms by which these compounds are involved in the endogenous regulation of morphogenesis remain to be established. Nevertheless, the use of mutant or transgenic plants with altered levels or perception of these hormones is leading to phenomenal increases in our understanding of the roles they play in the life cycle of plants. It is likely that in the future, novel modulators of plant growth and development will be identified; some will perhaps be related to the peptide encoded by *ENOD40* (Van de Sande et al., 1996), which modifies the action of auxin.

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