Plant Physiology, February 1999, Vol. 119, pp. 365–370, www.plantphysiol.org © 1999 American Society of Plant Physiologists

Update on Development

Genetic Analysis of Gibberellin Biosynthesis[1]

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In his studies on the nature of inheritance, Gregor Mendel (1866) examined seven pairs of traits in pea, including one he called "difference in the length of the stem" (Fig. 1). The tall character dominated the dwarf, segregating three tall to one dwarf. When Mendel's work was rediscovered at the beginning of this century, Orland White introduced the term *Le*, for Length, to represent this trait and recognized that the *Le/le* pair of alleles controlled the presence or absence of a factor for tallness (White, 1917). As described below, this factor was later identified as GA, and dwarf peas that are homozygous for *le* became extremely important in establishing GAs as natural regulators of plant growth.

The term "gibberellin" was first used in 1935 to describe a substance produced by the fungus *Gibberella fujikuroi* that caused overgrowth symptoms in rice, which was termed the bakanae disease (Yabuta, 1935). This substance proved to be a mixture of GAs, with GA_1 and GA_3 being the active factors (Takahashi et al., 1955). The early work on GAs remained undiscovered outside of Japan until after World War II, when there was an explosion of interest among plant physiologists and horticulturists. P.W. Brian, working at the Imperial Chemical Industries' Akers Laboratories in the United Kingdom, induced dwarf pea plants to elongate as normal, tall plants by applying GA_3, which he obtained from cultures of *G. fujikuroi* (Brian and Hemming, 1955). On the basis of these experiments, he proposed that GA was the natural growth factor that was deficient in the dwarf plants. This hypothesis was confirmed by Margaret Radley from the same laboratory, who reported that purified extracts from tall pea seedlings induced stem elongation in dwarf peas, thereby providing evidence that GAs were indeed naturally occurring in higher plants (Radley, 1956). Similar experiments were carried out in B.O. Phinney's laboratory at the University of California, Los Angeles, where dwarf maize was used as a bioassay to show that many plant extracts contained substances with GA-like activity (Phinney et al., 1957).

Nearly 30 years passed before the relationship between the *Le* alleles and GA content was confirmed on a firm chemical basis. During this time, the development of GC linked to MS facilitated the qualitative and quantitative analysis of GAs. Many GAs had been identified in plants, and their metabolic relationships (see below) were established using radiolabeled compounds. On the basis of the bioactivity of potential intermediates in the GA-biosynthetic pathway on GA-deficient dwarf mutants of maize, Phinney (1984) proposed that only GA_1 was active in this species and that many of the other GAs were biosynthetic precursors of GA_1 or were products of its catabolism. At approximately the same time, Ingram et al. (1984) demonstrated that the *le* pea mutant was defective in the conversion of GA_{20} to GA_1 and suggested that the *Le* gene encodes the 3β-hydroxylase responsible for this conversion (see Fig. 2). This conclusion was supported by analyses of the GA content of tall (*Le*) and dwarf (*le*) pea; shoots of the latter contain approximately 10% of the GA_1 concentration in tall plants and have a highly elevated GA_{20} content (Ross et al., 1989). In 1997, in the final chapter of this story, the *Le* gene was cloned, allowing the genetic lesion in the mutant *le* gene to be identified and thus providing the molecular basis for Mendel's observed difference in stem length (Lester et al., 1997; Martin et al., 1997).

In this *Update* we will describe this and other recent successes in isolating genes involved in GA biosynthesis. These advances are providing new insights into GA biosynthesis and its regulation.

GA BIOSYNTHESIS

GAs are products of the diterpenoid pathway and their formation is initiated by cyclization of the common C_{20} precursor GGPP. This intermediate is synthesized in plastids from isopentenyl diphosphate, shown recently to be formed in these organelles from glyceraldehyde 3-phosphate and pyruvate rather than mevalonic acid, as was previously assumed (Lichtenthaler et al., 1997). However, it has not yet been demonstrated that GAs are products of the glyceraldehyde 3-phosphate/pyruvate pathway. In growing vegetative tissues, the cyclization of GGPP occurs in proplastids and results in the formation of a hydrocarbon, *ent*-kaurene, in a two-step process requir-

[1] IACR receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the United Kingdom. This paper is Oregon Agricultural Experiment Station technical paper no. 11,412.

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Abbreviations: CPS, copalyl diphosphate synthase; GGPP, geranylgeranyl diphosphate.

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Figure 1. Isogenic pea seedlings (12 d old) containing wild-type (*LE*, right) or dwarfing (*le/le*, left) alleles of Mendel's height gene. They represent lines selected from an F_7 segregant from a cross between line 58 (*le*, from Prof. Ian Murfet, University of Hobart, Australia) and I3 (*Le*, a selection from Alaska).

ing the activity of two enzymes: CPS, which produces the intermediate copalyl diphosphate, and *ent*-kaurene synthase. GGPP is also the precursor of carotenoids and is incorporated into chlorophyll. These compounds are present at several orders of magnitude greater concentration than *ent*-kaurene and the GAs, so the first step on the GA pathway, catalyzed by CPS, needs to be tightly regulated.

ent-Kaurene is converted to the bioactive GAs by a series of oxidative reactions (Fig. 2) catalyzed by two types of enzyme. The early reactions, resulting in contraction of ring B from six to five C atoms to give the GA structure, occur on extraplastidic membranes. This requires the movement of *ent*-kaurene out of the plastid by an as-yet-unknown mechanism. The reactions are catalyzed by Cyt P450-dependent monooxygenases and, in the shoot tissues of most plants, give rise to GA_{12} and its 13-hydroxylated analog GA_{53}. These intermediates are metabolized further by soluble dioxygenases, which use 2-oxoglutaric acid as a cosubstrate. Two dioxygenases are required to convert GA_{12} and GA_{53} by parallel pathways to the bioactive products GA_4 and GA_1, respectively. First, GA 20-oxidase converts C-20 from a methyl group to an aldehyde and then removes the C atom to form the characteristic γ-lactone of the C_{19} GAs. Second, a hydroxyl group is introduced at the 3β position by a GA 3β-hydroxylase. A third 2-oxoglutarate-dependent dioxygenase that hydroxylates at the 2β position inactivates the GA molecule and thus ensures turnover of the active forms. The pathways (MacMillan, 1997) and biochemistry/molecular biology (Hedden and Kamiya, 1997; Lange, 1998) of GA biosynthesis have been reviewed recently elsewhere.

MUTANTS AND GENES

Approximately one-half of the genes involved in the biosynthesis of the biologically active GAs have now been isolated, using almost as many strategies as there are clones (Hedden and Kamiya, 1997; Lange, 1998). In some cases, such as the cloning of the *Le* gene (*LE* is now used) referred to above, the cloned loci are sites of known mutations that result in reduced GA content and dwarfism. Although

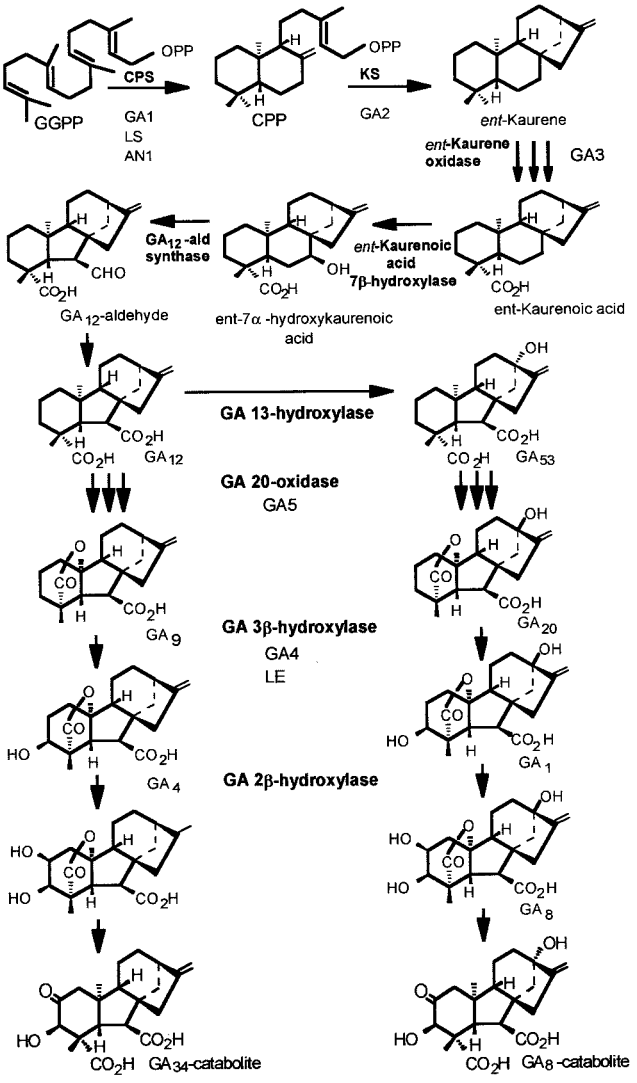

Figure 2. The GA-biosynthetic pathway from GGPP to the bioactive products GA_4 and GA_1, and their inactive catabolites formed by oxidation on C-2. Enzyme activities, with corresponding products of genes that are known sites of mutation and that have been cloned, are indicated. CPP, Copalyl diphosphate; KS, *ent*-kaurene synthase.

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GA-deficient mutants are known in many plant species, pea, Arabidopsis, and maize have been particularly well studied and, in each case, numerous loci for GA-biosynthesis genes have been identified. Several of these loci have now been cloned, confirming the nature of the encoded enzymes previously established by biochemical analysis. In Arabidopsis all five of the original mutant loci (*ga1–ga5*) that result in GA-responsive dwarfs (Koornneef and van der Veen, 1980) have now been cloned. In most cases growth of the Arabidopsis mutants was restored to normal by introducing the gene from wild-type plants, providing unequivocal evidence that the cloned gene corresponded to the mutant locus. All examples of the isolation of a previously known or suspected GA-biosynthesis gene are from pea, Arabidopsis, or maize.

Pea

Mutations in four loci, *LS*, *LH*, *LE,* and *NA,* are known to cause dwarfism and GA deficiency in pea. Biochemical evidence indicates that they encode, respectively, CPS (Ingram and Reid, 1987), *ent*-kaurene oxidase (Swain et al., 1997), GA 3β-hydroxylase (Ingram et al., 1984), and possibly GA_{12}-aldehyde synthase (Ingram and Reid, 1987). In addition, the *SLN* locus controls both GA 2β hydroxylation and further oxidation at C-2 to form the so-called GA catabolites (see Fig. 2). It is not known if *SLN* encodes an enzyme that catalyzes both steps or if it controls a regulatory factor that modifies the activity of two enzymes (Ross et al., 1995). Two of these loci have now been cloned. A cDNA encoding CPS was isolated by screening a cDNA library with a DNA sequence obtained by PCR (Ait-Ali et al., 1997). The encoded protein had high homology with CPS from Arabidopsis (*GA1*) and from maize (*An1*). When the equivalent genomic sequences from the wild-type and the *ls-1* mutant were compared, a single base change was found at a splice site, such that *ls-1* produced three incorrectly spliced mRNAs. Proteins encoded by these mutant mRNAs should all be truncated. However, *ls-1* is not a null mutation, because a more severe allele, *ls-3,* has been identified (Reid et al., 1996). The *ls-1* mutation reduces GA concentrations in shoots and in late-developing seeds to approximately 10% of those in wild-type plants, although it has a smaller effect on GA content during early seed development (Swain et al., 1995). Because the mutation does not affect flower formation or seed growth, it seems likely that other CPS genes are expressed in these organs. Although *LS* produces a transcript in young seeds (Ait-Ali et al., 1997), biochemical evidence suggests that the gene is expressed in the testa and not in the endosperm or embryo (Swain et al., 1995). However, in later seed development, *LS* is expressed in embryos. This complex pattern of gene expression during seed development has been noted with other GA-biosynthetic enzymes; different GA 20-oxidase genes are expressed in young and older seeds, corresponding to the biphasic pattern of GA production in developing seeds (Ait-Ali et al., 1997; Garcia-Martinez et al., 1997).

The *LE* locus was cloned simultaneously by two groups (Lester et al., 1997; Martin et al., 1997). After heterologous expression in *Escherichia coli*, it was shown to encode a GA 3β-hydroxylase, with a substrate preference for GA_9 over GA_{20}. Of the three mutant *le* alleles that are known, *le-1* is the mutation described by Mendel. This mutation is due to a base substitution that introduces an amino acid change (Ala to Thr) close to an amino acid motif (His-Thr-Asp) that is known to bind Fe at the enzyme active site of the related dioxygenase, isopenicillin *N* synthase (Roach et al., 1995). The result is a decrease in enzyme activity that is at least in part due to a reduced affinity for the GA substrates (Martin et al., 1997). The *le-2* mutation is caused by a base deletion that shifts the reading frame so that most of the protein is composed of a nonsense amino acid sequence. As might be anticipated, the mutant protein is completely inactive. The third mutation, *le-3*, causes an amino acid change (His to Tyr) that results in reduced enzyme activity. As expected, the *le-2* mutation is the most severe of the three, but plants containing this mutation still possess small amounts of GA_1 (Ross et al., 1989), indicating that other GA 3β-hydroxylase genes are expressed in pea. Indeed, the *le* mutations affect primarily stem extension and have no influence on flower development or seed and pod growth, which must rely on other 3β-hydroxylase genes for their source of GA.

Arabidopsis

Biochemical analysis of the mutants indicated that the *GA1* and *GA2* loci of Arabidopsis were involved in the conversion of GGPP to *ent*-kaurene (Zeevaart and Talon, 1992). Nine mutant *ga1* alleles were isolated, one of which, *ga1–3*, contained a 5-kb deletion (Koornneef et al., 1983). Sun et al. (1992) took advantage of this deletion to clone the *GA1* locus by genomic subtraction and, by expressing its cDNA in *E. coli* (Sun and Kamiya, 1994), showed that it encodes CPS. The predicted 93-kD protein contains a 50-amino acid N-terminal transit sequence that targets the protein to plastids. Sun and Kamiya, (1994) demonstrated that the encoded protein is indeed imported into chloroplasts and that the transit sequence is cleaved on entry into the plastid.

Koornneef and van der Veen (1980) recognized two types of *ga1* mutants: those in which the seeds germinated to give male-sterile dwarf seedlings, and others that did not germinate unless treated with GA. The latter correspond to essentially null mutations with no active CPS produced from the *GA1* gene and would be very highly GA deficient, whereas the former may have a functional enzyme with reduced activity. Mutants containing the severe *ga1* alleles remain as rosettes unless treated with GA. When grown in a long-day photoperiod, they produce flower buds, although in the absence of GA the buds do not develop into viable flowers. In short days they do not produce flowers (Wilson et al., 1992). Despite the apparent absence of a functional *GA1* product, *ga1–3* plants contain low amounts of GAs (Talon et al., 1990), the formation of which would require another CPS-encoding gene expressed at very low levels in shoot and floral tissues, or the presence of a different terpene cyclase producing copalyl diphosphate or even *ent*-kaurene as a minor by-product.

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The *GA2* locus was cloned by heterologous screening with a cDNA from a pumpkin *ent*-kaurene synthase gene (Yamaguchi et al., 1998). The pumpkin cDNA had been obtained after purification of the enzyme from endosperm and the use of PCR with primers designed from partial amino acid sequence information (Yamaguchi et al., 1996). The function of the proteins encoded by the pumpkin cDNA and by cDNA corresponding to *GA2* was confirmed by heterologous expression in *E. coli*, demonstrating that the expression products converted [^{3}H]copalyl diphosphate to *ent*-[^{3}H]kaurene (Yamaguchi et al., 1996, 1998). The *ga2–1* mutant, which has a severe (nongerminating) GA-deficient phenotype, contains a base substitution at the *GA2* locus that results in the introduction of a premature stop codon and a highly truncated gene product (Yamaguchi et al., 1998). The full-length *GA2* cDNA encodes a 90-kD protein with 70% similarity (52% identity) to the pumpkin *ent*-kaurene synthase. Both proteins contain a potential N-terminal transit sequence for import into plastids, although such import has not been demonstrated.

On the basis of northern-blot analysis, *GA2* is expressed in all tissues at relatively high levels (Yamaguchi et al., 1998). In contrast, expression of *GA1* is at a much lower level and could be detected only by reverse-transcriptase PCR or the use of a GUS reporter gene driven by the *GA1* promoter (Silverstone et al., 1997). Examination of transgenic Arabidopsis plants containing this reporter gene construct revealed that *GA1* promoter activity was highest in rapidly growing tissues or in the vascular tissues of nongrowing organs, such as leaves. Thus, *GA1* (*CPS*) is a tightly regulated gene, which is consistent with the role of CPS as the first enzyme in the GA-biosynthetic pathway. The cell types that express *GA1*, i.e. dividing cells or cells of the vascular system, indicate that CPS is active in proplastids rather than in mature chloroplasts, as also shown by biochemical studies (Aach et al., 1997).

Helliwell et al. (1998) have recently used a combination of positional cloning and random sequencing of a bacterial artificial chromosome to identify a putative Cyt P450 gene that maps to the Arabidopsis *GA3* locus. They also provided convincing evidence, based on the accumulation of *ent*-kaurene and the lack of a growth response of the *ga3* mutant to applied *ent*-kaurene, that the mutant lacks *ent*-kaurene oxidase activity. Thus, *GA3* encodes *ent*-kaurene oxidase, which is a Cyt P450-dependent monooxygenase. *GA3* contains six introns and an open reading frame of 1678 bp, encoding a protein of 58.1 kD. Two transcripts were detected due to alternative splicing sites at the downstream intron 6-exon boundary. The enzyme belongs to a new class of Cyt P450 and thus differs from the previously cloned *Dwarf-3* gene of maize, which is also a Cyt P450 (Winkler and Helentjaris, 1995). Although *Dwarf-3* is assumed to be involved in GA biosynthesis (Phinney, 1984), its function is unknown. Two mutant *ga3* alleles were sequenced by Helliwell et al. (1998); both alleles contain single base substitutions that introduce in-frame stop codons.

Analysis of the GA content of the *ga4* and *ga5* mutants indicated that they were defective in GA 3β-hydroxylase and GA 20-oxidase activity, respectively (Talon et al., 1990). This was confirmed by the cloning of these loci. The *GA4* locus was isolated after it was tagged by a chance T-DNA insertion (Chiang et al., 1995). The gene contained an open reading frame with a single intron and encoded an enzyme of 40.2 kD. Based on its amino acid sequence, this enzyme is believed to belong to a group of dioxygenases, most of which use 2-oxoglutaric acid as a cosubstrate. By obtaining expression of its cDNA in *E. coli* and demonstrating that the recombinant protein converted GA_9 to GA_4 and GA_{20} to GA_1, Williams et al. (1998) confirmed that *GA4* encodes a 2-oxoglutarate-dependent dioxygenase with GA 3β-hydroxylase activity. GA_9 was the preferred substrate, with a K_m value approximately 10-fold lower than that for GA_{20}.

The *GA5* locus was cloned by a strategy similar to that used for the isolation of *GA2* described above. Xu et al. (1995) obtained it from a genomic library by screening with a pumpkin GA 20-oxidase cDNA that had been isolated using antibodies raised against the purified enzyme. The Arabidopsis gene contains two introns and an open reading frame of 1131 bp, encoding a 377-amino acid protein of 43.4 kD. Its function as a GA 20-oxidase was confirmed by demonstrating the ability of a fusion protein, obtained by expressing a cDNA clone in *E. coli*, to convert GA_{53} to GA_{44} and GA_{19}, and GA_{19} to GA_{20}. Xu et al. (1995) obtained proof of the identity of the genomic clone by mapping it to the *GA5* locus and establishing that the homologous gene from the *ga5* mutant contained a base substitution, relative to the wild-type Arabidopsis ecotype (*Landsberg erecta*) gene, that resulted in the introduction of a premature stop codon. At approximately the same time that *GA5* was cloned, Phillips et al. (1995) isolated two GA 20-oxidase cDNA clones from the *ga1–2* mutant of Arabidopsis and discovered a third 20-oxidase sequence in a database of cDNA sequences. The three cDNAs encoded functionally similar enzymes, which converted GA_{12} to GA_9 and GA_{53} to GA_{20}, with GA_{12} being the preferred substrate. Phillips et al. (1995) showed that the cDNAs corresponded to genes that exhibited different patterns of expression; one gene, identical to *GA5*, was expressed in stems, another in the inflorescence and silique (fruit), and the third only in siliques.

In contrast to *GA1*, *GA2*, and *GA3*, there are no known mutant alleles of *GA4* and *GA5* with the severe (nongerminating) phenotype. The *ga4* and *ga5* mutations are "leaky" semidwarfs that produce fertile flowers and normal siliques. The original *ga4–1* allele (Koornneef and van der Veen, 1980) contains a base substitution that results in a change of Cys to Tyr (Chiang et al., 1995), which may not abolish enzyme activity completely. However, the *ga4–2* mutant with the T-DNA insertion is unlikely to contain an active GA4 protein, although it is also a semidwarf. Furthermore, the *ga5* mutant contains 10% to 30% of the C_{19}-GA content of wild-type plants (Talon et al., 1990), despite apparently producing a truncated enzyme. It is now clear that several GA 20-oxidase genes are expressed in Arabidopsis and other species (Hedden and Kamiya, 1997); therefore, the loss of one enzyme can be partially compensated for by the activity of the others, perhaps via movement of GAs or their precursors between tissues. It seems likely that the GA 3β-hydroxylases are also encoded

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by multiple genes, whereas CPS, *ent*-kaurene synthase, and *ent*-kaurene oxidase are predominantly the products of single genes in Arabidopsis, namely *GA1*, *GA2*, and *GA3*, respectively.

REGULATION OF GA BIOSYNTHESIS

GAs mediate many developmental and environmental responses in plants. Consequently, regulation of GA biosynthesis is necessarily complex. The isolation of GA-biosynthetic genes has enabled direct analysis of their expression in terms of transcript abundance, allowing new insights into regulatory mechanisms.

One such mechanism that plants use to maintain GA homeostasis (i.e. keeping the concentrations of bioactive GAs within certain limits) involves feedback regulation of GA biosynthesis, as illustrated in Figure 3. This effect was first observed in GA-response mutants, such as *Rht3* in wheat, *Dwarf8* in maize, and *gai* in Arabidopsis. Although they are dwarf mutants, they contain large amounts of bioactive GAs (for review, see Scott, 1990; Hedden and Kamiya, 1997). In contrast, slender mutants such as the *la cry*^{s} mutant of pea grow as if they were treated with large quantities of GA; however, they actually contain reduced amounts of active GAs (Martin et al., 1996). Although these relationships initially seemed paradoxical (Scott, 1990) because stem elongation is normally controlled by the content of bioactive GA, they revealed a link between GA response and biosynthesis. Dwarf mutants unable to respond to GA were also unable to down-regulate GA biosynthesis, whereas slender mutants down-regulated GA biosynthesis strongly. Current evidence suggests that feedback regulation modifies expression of GA 20-oxidase and 3β-hydroxylase genes. Thus, transcript levels of the Arabidopsis *GA5* and *GA4* genes were highly elevated in GA-deficient plants and reduced when such plants were treated with GA (Chiang et al., 1995; Phillips et al., 1995; Xu et al., 1995). In addition, the *gai* response mutant, which accumulates GAs, also contained increased 20-oxidase transcript levels (Xu et al., 1995). Conversely, leaves of the slender *la cry*^{s} mutant of pea contain reduced amounts of GA 20-oxidase mRNA relative to wild-type plants, which is consistent with a reduced GA_1 content (Martin et al., 1996). Treatment of wild-type peas with bioactive GA also down-regulated the GA 20-oxidase message and GA_1 content, thereby mimicking the effect of *la cry*^{s} (Martin et al., 1996). By comparing the activities of GA analogs, Cowling et al. (1998) demonstrated a close link between GA-induced growth and the down-regulation of *GA4* (GA 3β-hydroxylase) expression in Arabidopsis.

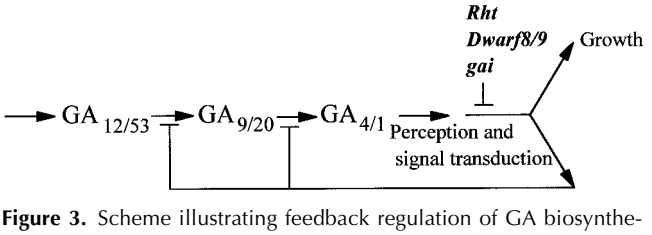

Figure 3. Scheme illustrating feedback regulation of GA biosynthesis. Expression of genes encoding GA 20-oxidase and 3β-hydroxylase is down-regulated by bioactive GAs. The semidominant mutations, *Rht* (wheat), *Dwarf8* and *Dwarf9* (maize), and *gai* (Arabidopsis) that cause a reduction in GA responsiveness also reduce feedback regulation of GA biosynthesis.

Induction by photoperiod of the rapid stem elongation (bolting) that accompanies flowering in many rosette plants is mediated by GAs. Several studies indicate that 20-oxidation is the main step controlled by photoperiod in such plants (for review, see Hedden and Kamiya, 1997). Regulation of GA 20-oxidase gene expression by photoperiod was directly demonstrated in spinach, which is an obligate long-day plant (Wu et al., 1996). Higher expression of this gene in long days correlated with increased stem elongation, whereas expression was lower in short days, in which plants maintained rosettes. This pattern of 20-oxidase expression was established within 2 d after a change in photoperiod. In Arabidopsis, a facultative long-day plant, bolting is accelerated in long days and correlated with a slightly higher GA content and increased sensitivity to GAs (Xu et al., 1997). Furthermore, Xu et al. (1997) found that exposure to long days resulted in an accumulation of *GA5* (20-oxidase) transcript but did not affect expression of *GA4* (3β-hydroxylase).

CONCLUSIONS

Cloning the genes of the GA-biosynthetic pathway is opening a wide range of research opportunities in plant biology. This *Update* highlights the rapid progress in exciting but preliminary studies of how GA mediates developmental and environmental effects on growth. This progress is also providing new information on the sites of GA biosynthesis and on the function and structures of the biosynthetic enzymes, allowing genetic manipulation of specific steps in the pathways of transgenic plants. Furthermore, it has finally been possible to establish the molecular basis for many of the mutations that affect plant stature, some of which have been known for many years and are an important component of several crop varieties.

Received September 29, 1998; accepted October 28, 1998.

LITERATURE CITED

Aach H, Bode H, Robinson DG, Graebe JE (1997) *ent*-Kaurene synthase is located in proplastids of meristematic shoot tissues. Planta **202**: 211–219

Ait-Ali T, Swain SM, Reid JB, Sun T-p, Kamiya Y (1997) The *LS* locus of pea encodes the gibberellin biosynthesis enzyme *ent*-kaurene synthase A. Plant J **11**: 442–454

Brian PW, Hemming HG (1955) The effect of gibberellic acid on shoot growth and development. Physiol Plant **8**: 669–681

Chiang H-H, Hwang I, Goodman HM (1995) Isolation of the Arabidopsis *GA4* locus. Plant Cell **7**: 195–201

Cowling RJ, Kamiya Y, Seto H, Harberd NP (1998) Gibberellin dose-response regulation of *GA4* gene transcript levels in Arabidopsis. Plant Physiol **117**: 1195–1203

García-Martínez JL, López-Diaz I, Sánchez-Beltrán MJ, Phillips AL, Ward DA, Gaskin P, Hedden P (1997) Isolation and transcript analysis of gibberellin 20-oxidase genes in pea and bean in relation to fruit development. Plant Mol Biol **33**: 1073–1084

Hedden P, Kamiya Y (1997) Gibberellin biosynthesis: enzymes, genes and their regulation. Annu Rev Plant Physiol Plant Mol Biol **48**: 431–460

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Helliwell CA, Sheldon CC, Olive MR, Walker AR, Zeevaart JAD, Peacock WJ, Dennis ES (1998) Cloning of the *Arabidopsis ent*-kaurene oxidase gene *GA3*. Proc Natl Acad Sci USA **95**: 9019–9024

Ingram TJ, Reid JB (1987) Internode length in *Pisum*. Gene *na* may block gibberellin synthesis between *ent*-7α-hydroxykaurenoic acid and gibberellin A_{12}-aldehyde. Plant Physiol **83**: 1048–1053

Ingram TJ, Reid JB, Murfet IC, Gaskin P, Willis CL, MacMillan J (1984) Internode length in *Pisum.* The *Le* gene controls the 3β-hydroxylation of GA_{20} to GA_1. Planta **160**: 455–463

Koornneef M, van der Veen JH (1980) Induction and analysis of gibberellin-sensitive mutants in *Arabidopsis thaliana*. (L.) Heynh. Theor Appl Genet **58**: 257–263

Koornneef M, van Eden J, Hanhart DJ, de Jongh AMM (1983) Genetic fine-structure of the GA-1 locus in the higher plant *Arabidopsis thaliana* (L.) Heynh. Genet Res **41**: 57–68

Lange T (1998) Molecular biology of gibberellin synthesis. Planta **204**: 409–419

Lester DR, Ross JJ, Davies PJ, Reid JB (1997) Mendel's stem length gene (*Le*) encodes a gibberellin 3β-hydroxylase. Plant Cell **9**: 1435–1443

Lichtenthaler HK, Rohmer M, Schwender J (1997) Two independent biochemical pathways for isopentenyl diphosphate biosynthesis in higher plants. Physiol Plant **101**: 643–652

MacMillan J (1997) Biosynthesis of the gibberellin plant hormones. Nat Prod Rep **14**: 221–243

Martin DN, Proebsting WM, Hedden P (1997) Mendel's dwarfing gene: cDNAs from the *Le* alleles and the function of the expressed proteins. Proc Natl Acad Sci USA **94**: 8907–8911

Martin DN, Proebsting WM, Parks TD, Dougherty WG, Lange T, Lewis MJ, Gaskin P, Hedden P (1996) Feedback regulation of gibberellin biosynthesis and gene expression in *Pisum sativum* L. Planta **200**: 159–166

Mendel G (1866) Experiments in Plant Hybridization. Royal Horticultural Society of London, translation (1938), Harvard University Press, Cambridge, MA

Phillips AL, Ward DA, Uknes S, Appleford NEJ, Lange T, Huttly AK, Gaskin P, Graebe JE, Hedden P (1995) Isolation and expression of three gibberellin 20-oxidase cDNA clones from Arabidopsis. Plant Physiol **108**: 1049–1057

Phinney BO (1984) Gibberellin A_1, dwarfism and the control of shoot elongation in higher plants. *In* A Crozier, JR Hillman, eds, The Biosynthesis and Metabolism of Plant Hormones. Society for Experimental Biology Seminar Series 23. Cambridge University Press, Cambridge, UK, pp 17–41

Phinney BO, West CA, Ritzel MB, Neely PM (1957) Evidence for gibberellin-like substances from flowering plants. Proc Natl Acad Sci USA **43**: 398–404

Radley M (1956) Occurrences of substances similar to gibberellic acid in higher plants. Nature **178**: 1070–1071

Reid JB, Weller JL, Sherriff LJ (1996) A more severe mutant allele at the *ls* locus. Pisum Genet J **28**: 15–17

Roach PL, Clifton IJ, Fulop V, Harlos K, Burton GJ, Hajdu J, Anderson I, Schofield CI, Baldwin JE (1995) Crystal structure of isopenicillin *N* synthase is the first from a new structural family of enzymes. Nature **375**: 700–704

Ross JJ, Reid JB, Gaskin P, MacMillan J (1989) Internode length in *Pisum*: estimation of GA_1 levels in genotypes *Le*, *le* and le^d. Physiol Plant **76**: 173–176

Ross JJ, Reid JB, Swain SM, Hasan O, Poole AT, Hedden P, Willis CL (1995) Genetic regulation of gibberellin deactivation in *Pisum*. Plant J **7**: 513–523

Scott IM (1990) Plant hormone response mutants. Physiol Plant **78**: 147–152

Silverstone AL, Chang C-w, Krol E, Sun T-p (1997) Developmental regulation of the gibberellin biosynthetic gene *GA1* in *Arabidopsis thaliana*. Plant J **12**: 9–19

Sun T-p, Goodman HM, Ausubel FM (1992) Cloning the Arabidopsis *GA1* locus by genomic subtraction. Plant Cell **4**: 119–128

Sun T-p, Kamiya Y (1994) The Arabidopsis *GA1* locus encodes the cyclase *ent*-kaurene synthetase A of gibberellin biosynthesis. Plant Cell **6**: 1509–1518

Swain SM, Reid JB, Kamiya Y (1997) Gibberellins are required for embryo growth and seed development in pea. Plant J **12**: 1329–1338

Swain SM, Ross JJ, Reid JB, Kamiya Y (1995) Gibberellins and pea seed development: expression of the lh^i, *ls* and le^{5839} mutations. Planta **195**: 426–433

Takahashi N, Kitamura H, Kawarada A, Seta Y, Takai M, Tamura S, Sumiki Y (1955) Biochemical studies on "bakanae" fungus. Part XXXIV. Isolation of gibberellins and their properties. Bull Agric Chem Soc Jpn **19**: 267–277

Talon M, Koornneef M, Zeevaart JAD (1990) Endogenous gibberellins in *Arabidopsis thaliana* and possible steps blocked in the biosynthetic pathways of the semidwarf *ga4* and *ga5* mutants. Proc Natl Acad Sci USA **87**: 7983–7987

White OE (1917) Studies of inheritance in *Pisum*. II. The present state of knowledge of heredity and variation in peas. Proc Am Philos Soc **56**: 487–588

Williams J, Phillips AL, Gaskin P, Hedden P (1998) Function and substrate specificity of the gibberellin 3β-hydroxylase encoded by the Arabidopsis *GA4* gene. Plant Physiol **117**: 559–563

Wilson RN, Heckman JW, Somerville CR (1992) Gibberellin is required for flowering in *Arabidopsis thaliana* under short days. Plant Physiol **100**: 403–408

Winkler RG, Helentjaris T (1995) The maize *Dwarf3* gene encodes a cytochrome P450-mediated early step in gibberellin biosynthesis. Plant Cell **7**: 1307–1317

Wu K, Li L, Gage DA, Zeevaart JAD (1996) Molecular cloning and photoperiod-regulated expression of gibberellin 20-oxidase from the long-day plant spinach. Plant Physiol **110**: 547–554

Xu Y-L, Gage DA, Zeevaart JAD (1997) Gibberellins and stem growth in *Arabidopsis thaliana*. Effects of photoperiod on expression of the *GA4* and *GA5* loci. Plant Physiol **114**: 1471–1476

Xu Y-L, Li L, Wu K, Peeters AJM, Gage DA, Zeevaart JAD (1995) The *GA5* locus of *Arabidopsis thaliana* encodes a multifunctional gibberellin 20-oxidase: molecular cloning and functional expression. Proc Natl Acad Sci USA **92**: 6640–6644

Yabuta T (1935) Biochemistry of the "bakanae" fungus of rice. Agriculture and Horticulture **10**: 17–22

Yamaguchi S, Saito T, Abe H, Yamane H, Murofushi N, Kamiya Y (1996) Molecular cloning and characterization of a cDNA encoding the gibberellin biosynthetic enzyme *ent*-kaurene synthase B from pumpkin (*Cucurbita maxima* L.). Plant J **10**: 203–213

Yamaguchi S, Sun T-p, Kawaide H, Kamiya Y (1998) The *GA2* locus of *Arabidopsis thaliana* encodes *ent*-kaurene synthase of gibberellin biosynthesis. Plant Physiol **116**: 1271–1278

Zeevaart JAD, Talon M (1992) Gibberellin mutants in *Arabidopsis thaliana. In* CM Karssen, LC Van Loon, D Vreugdenhill, eds, Progress in Plant Growth Regulation. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 34–42

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