Gibberellin Metabolism and its Regulation

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Annu. Rev. Plant Biol. 2008.59:225-251. Downloaded from www.annualreviews.org Access provided by Chinese Academy of Sciences - Institute of Oceanology on 03/07/16. For personal use only.

Annu. Rev. Plant Biol. 2008. 59:225-51

The Annual Review of Plant Biology is online at plant.annualreviews.org

This article's doi: 10.1146/annurev.arplant.59.032607.092804

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1543-5008/08/0602-0225\$20.00

Key Words

biosynthesis, cytochrome P450 monooxygenase, deactivation, 2-oxoglutarate–dependent dioxygenase, plant hormones, terpene synthases

Abstract

Bioactive gibberellins (GAs) are diterpene plant hormones that are biosynthesized through complex pathways and control diverse aspects of growth and development. Biochemical, genetic, and genomic approaches have led to the identification of the majority of the genes that encode GA biosynthesis and deactivation enzymes. Recent studies have highlighted the occurrence of previously unrecognized deactivation mechanisms. It is now clear that both GA biosynthesis and deactivation pathways are tightly regulated by developmental, hormonal, and environmental signals, consistent with the role of GAs as key growth regulators. In some cases, the molecular mechanisms for fine-tuning the hormone levels are beginning to be uncovered. In this review, I summarize our current understanding of the GA biosynthesis and deactivation pathways in plants and fungi, and discuss how GA concentrations in plant tissues are regulated during development and in response to environmental stimuli.

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INTRODUCTION

Biologically active gibberellins (bioactive GAs) control diverse aspects of plant growth and development, including seed germination, stem elongation, leaf expansion, and flower and seed development. Among more than a hundred GAs identified from plants (http://www.plant-hormones.info/gibberellin_nomenclature.htm) (61), only a small number of them, such as GA₁ and GA₄, are thought to function as bioactive hormones. Therefore, many nonbioactive GAs exist in plants as precursors for the

bioactive forms or deactivated metabolites. The concentrations of bioactive GAs in a given plant tissue are determined by the rates of their synthesis and deactivation. In this review, I refer to biosynthesis as the production of bioactive hormones from their precursors, deactivation as the conversion of bioactive forms or their precursors into inactive (or less active) forms, and metabolism as both biosynthesis and deactivation.

The GA metabolism pathway in plants has been studied for a long time, and a number of genes encoding the metabolism enzymes have been identified. Genes encoding these enzymes have been identified through conventional enzyme purification from rich sources of GA enzymes, functional screening of a cDNA expression library, or molecular genetic approaches using dwarf mutants defective in GA biosynthesis (for reviews, see References 28, 29, 99, and 128). More recently, the availability of genomics tools in model plant species has accelerated the identification of additional genes involved in the GA metabolism pathway. However, our list of GA metabolism genes is likely still incomplete, and genes encoding enzymes with unexpected functions in GA metabolism have just recently been recognized (117, 140). In addition, genes encoding some expected GA metabolism enzymes, such as those involved in GA conjugation, have not been discovered yet. In this review, I outline our current understanding of the GA metabolism pathways, enzymes, and genes in plants, and discuss how GA concentrations are regulated during plant development under varying environmental conditions in selected systems. I also describe GA biosynthesis in fungi to discuss how the GA pathway has evolved in different kingdoms.

THE GIBBERELLIN METABOLISM PATHWAY

Bioactive Gibberellins

The major bioactive GAs, including GA₁, GA₃, GA₄, and GA₇, commonly have a

hydroxyl group on C-3 β , a carboxyl group on C-6, and a lactone between C-4 and C-10 (Figure 1). The 3β -hydroxyl group may be replaced by other functionalities at C-2 and C-3 to act as bioactive forms, as in GA5 and GA₆ (Figure 1). GA₁ has been identified frequently in a variety of plant species (61), implying that it acts as a widespread bioactive hormone. However, GA4 also exists in most species, and is thought to be the major bioactive GA in Arabidopsis thaliana and some Cucurbitaceae members. The relative roles of GA1 versus GA4 (and GA3 versus GA7) still remain to be clarified through the identification of genes encoding GA 13-oxidase (GA13ox) and mutants without this enzyme activity. The recent identification of a soluble GA receptor, GIBBERELLIN INSENSITIVE DWARF 1 (GID1), from rice (Oryza sativa) and its homologs in Arabidopsis has illustrated that these structural requirements for bioactive GAs are reflected in their affinity for receptor GID1, as well as their ability to form a complex consisting of GID1, GA, and the DELLA protein (which is a repressor of GA signaling and degraded upon interaction with the GID1-GA complex) in yeast (65, 113, 115, 137). Intriguingly, GA₄, but not GA₁, is the most favored GA for the rice GID1 in terms of the ability to replace the binding of 16,17-dihydroGA₄ to GID1 in vitro (113), the complex formation in yeast, and the degradation of the DELLA protein in seedlings and calli (114), whereas GA_1 is the major bioactive form in vegetative tissues of rice.

Gibberellin Biosynthesis

GAs are biosynthesized from geranylgeranyl diphosphate (GGDP), a common C_{20} precursor for diterpenoids (**Figure 1**). Three different classes of enzymes are required for the biosynthesis of bioactive GAs from GGDP in plants: terpene synthases (TPSs), cytochrome P450 monooxygenases (P450s), and 2-oxoglutarate–dependent dioxygenases (2ODDs) (**Figure 2**). Recent work with isotope-labeled precursors showed that the methylerythritol phosphate pathway in the plastid provides the majority of the isoprene units to GAs in *Arabidopsis* seedlings, whereas there is a minor contribution from the cytosolic mevalonate pathway (46).

Two TPSs, ent-copalyl diphosphate synthase (CPS) and ent-kaurene synthase (KS), are involved in the conversion of GGDP to the tetracyclic hydrocarbon intermediate ent-kaurene (Figure 1). Both CPS and KS are located in the plastids (1, 33, 103, 104). ent-Kaurene is then converted to GA12 by two P450s. ent-Kaurene oxidase (KO) [designated as CYP701A according to the P450 nomenclature (http://drnelson.utmem.edu/ CytochromeP450.html)] (67) catalyzes the sequential oxidation on C-19 to produce ent-kaurenoic acid, which is subsequently converted to GA₁₂ by another P450, entkaurenoic acid oxidase (KAO) (CYP88A) (67). Experiments using fusion enzymes with green fluorescent protein suggest that KO is located in the outer membrane of the plastid, whereas KAO is present in the endoplasmic reticulum (33).

GA₁₂ is converted to GA₄, a bioactive form, through oxidations on C-20 and C-3 by GA 20-oxidase (GA20ox) and GA 3-oxidase (GA3ox), respectively, both of which are soluble 20DDs (Figure 1). GA20ox catalyzes the sequential oxidation of C-20, including the loss of C-20 as CO_2 and the formation of γ -lactone. Thus, GA20ox is responsible for the production of C₁₉-GAs using C₂₀-GAs as substrates. The introduction of a 3 \beta-hydroxyl group converts inactive precursors (GA_{9.20}) into bioactive GAs. Some GA3ox enzymes possess minor catalytic activity to synthesize GA₃ and GA₆ from GA₂₀ via GA₅ (6, 41, 100) (Figure 1). Although the subcellular localization of these 2ODDs has not been demonstrated experimentally, they are assumed to be cytosolic enzymes because of the lack of any apparent targeting sequence (Figure 2). GA₁₂ is also a substrate for GA13ox for the production of GA₅₃, which is a precursor for GA₁ in the 13-hydroxylated pathway. To clarify the biological relevance of 13-hydroxylation,

GIBBERELLIN INSENSITIVE DWARF 1 (GID1):

a soluble GA receptor that interacts directly with the DELLA protein in a GA-dependent manner

DELLA proteins:

members of the GRAS protein family that repress GA responses; degraded by the 26Sproteasome upon interaction with the GID1-GA complex

GGDP:

geranylgeranyl diphosphate

TPS: terpene synthase

Cytochrome P450 monooxygenase (P450):

heme-containing enzyme that catalyzes oxidation reactions of a broad range of organic compounds

2-oxoglutaratedependent dioxygenase

(2ODD): soluble enzyme that catalyzes oxidation reactions of various organic compounds using 2-oxoglutarate as a cosubstrate

CPS: *ent*-copalyl diphosphate synthase

KS: *ent*-kaurene synthase

KO: *ent*-kaurene oxidase



it will be necessary to identify genes encoding GA13ox(s). Recombinant TaGA3ox2 protein (a GA3ox from wheat) exhibits a weak GA13ox activity, in addition to GA3ox activity, in vitro, although its contribution to the overall production of 13-hydroxylated GAs in plants is likely to be small (6).

Gibberellin Deactivation

Deactivation is important for effective regulation of the concentrations of bioactive hormones in plants. GAs are metabolically deactivated in several different ways. The best-characterized deactivation reaction is 2β -hydroxylation catalyzed by a class of 20DDs, GA 2-oxidases (GA2oxs) (Figures 1 and 2). Initially identified GA2oxs use C_{19} -GAs as substrates, including bioactive GAs and their immediate precursors, GA9 and GA₂₀ (106), and they belong to class I or II on the basis of the phylogenetic relationships (58). Recently, a new type of GA2ox that accepts only C_{20} -GAs was reported (58, 93). These enzymes are categorized into class III, and are likely to play a role in depleting pools of precursor GAs (such as GA12 and GA₅₃) that are otherwise converted to bioactive forms (Figure 1).

Recent work on a recessive tall rice mutant, elongated uppermost internode (eui), revealed a new GA deactivation mechanism (140). EUI is a P450 designated as CYP714D1 (67) that epoxidizes the 16,17-double bond of non13hydroxylated GAs, including GA₄, GA₉, and GA₁₂ (**Figure 3**). In the upper internodes of rice, EUI/CYP714D1 should be the principal GA deactivation enzyme, because *eui* mu-

Figure 1

tants accumulate bioactive GAs at extremely high levels (140). 16a,17-dihydrodiols, hydrated products of the 16a,17-epoxides either in vivo or during purification (Figure 3), are detected in transgenic rice plants that overexpress the EUI gene. Thus, the discovery of this enzyme explains the occurrence of GA 16,17-dihydrodiols in many plant species (140). These results suggest that 16α , 17-epoxidation of GAs may be a general deactivation mechanism. More recent work has shown that Arabidopsis GAMT1 and GAMT2 encode enzymes (gibberellin methyltransferases) that catalyze methylation of the C-6 carboxyl group of GAs using Sadenosine-L-methionine as a methyl donor (117) (Figure 3). GAMT1 and GAMT2 utilize a variety of GAs, including bioactive GAs and their precursors, as substrates in vitro, and produce the corresponding methyl esters. Ectopic expression of GAMT1 or GAMT2 in Arabidopsis, tobacco (Nicotiana tabacum), and petunia (Petunia hybrida) results in dwarfed plants with GA-deficiency. Both GAMT1 and GAMT2 are predominantly expressed in developing and germinating seeds during Arabidopsis development. In the loss-of-function gamt1 gamt2 double mutant, the levels of bioactive GAs in developing seeds are significantly elevated, and the double mutant seeds are more resistant to a GA biosynthesis inhibitor during germination relative to wildtype seeds (117). These results suggest that the GAMT genes play a role in deactivating GAs in Arabidopsis seeds. Whether methylation of GAs is a common deactivation reaction in other plant species has yet to be investigated.

KAO: *ent*-kaurenoic acid oxidase

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The gibberellin (GA) biosynthesis pathways and deactivation by GA 2-oxidase in plants. Bioactive GAs found in a wide variety of plant species (*bigblighted grey*) are shown. As discussed in the text, GA₅ and GA₆ may also function as bioactive hormones. In each metabolic reaction, the modification is highlighted in color. GA₇ (13-nonhydroxy GA₃) is biosynthesized from GA₉ in a similar pathway to the synthesis of GA₃ from GA₂₀, but is not shown in this figure. 20x, GA 2-oxidase (Class I and II); 20x*, GA 2-oxidase (Class III); 30x, GA 3-oxidase; 130x, GA 13-oxidase; 200x, GA 20-oxidase; GGDP, geranylgeranyl diphosphate; *ent*-CDP, *ent*-copalyl diphosphate; CPS, *ent*-copalyl diphosphate synthase; KS, *ent*-kaurene synthase; KO, *ent*-kaurene oxidase; KAO, *ent*-kaurenoic acid oxidase.

Arabidopsis thaliana

Enzyme Gene [Locus] AGI code (Reference)

Oryza sativa

Enzyme Gene [Locus] RAP locus (Reference)





Figure 3 Gibberellins are deactivated by ELONGATED UPPERMOST INTERNODE (EUI) through 16α,17-epoxidation and by gibberellin methyltransferases (GAMTs) through

methylation.

CDP: copalyl

diphosphate

GAs can be converted into conjugates in plants (91, 92). Conjugation of GAs to glucose occurs either through a hydroxyl group of GA to give a GA-*O*-glucosyl ether or via the 6-carboxyl group to give a GA-glucosyl ester. Although the formation of these GA conjugates may also serve to deactivate GAs, it remains unclear whether GA conjugations play any regulatory role in the control of bioactive GA levels. The identification of genes encoding GA-glycosyl transferases and the resulting reverse genetic studies will clarify their role in plants.

The identification of the majority of GA metabolism enzymes has provided a clearer view as to the mechanism by which a large variety of GAs are produced in plants. Because of the multifunctionality of several enzymes in the pathway, only six enzymes are required for the 12-step conversion of GGDP to GA₄. In addition, many GA-modifying enzymes, including the 2ODDs, 16α ,17-epoxidase (EUI/CYP714D1), and GAMTs, accept multiple GAs as substrates. The

promiscuous nature of these enzymes creates a number of branches in the pathway and contributes to the production of diverse GAs by a relatively small number of enzymes.

GIBBERELLIN METABOLISM GENES

In both *Arabidopsis* and rice, the enzymes that catalyze the early steps of GA biosynthesis are encoded by one or two genes (**Figure 2**). In *Arabidopsis*, CPS, KS, and KO are each encoded by a single gene, and the null alleles of these genes (ga1, ga2, and ga3) result in severe GA-deficient dwarves (55). Interestingly, the rice genome contains two genes that encode a functional CPS (75, 81, 88), but only one of them is likely to participate in GA biosynthesis, whereas the second one is involved in the biosynthesis of diterpene phytoalexins. Additional CPS-like enzymes (OsCyc1 and OsCyc2) function as *syn*-copalyl diphosphate (CDP) synthases in the biosynthesis of

Figure 2

Predicted subcellular localization of gibberellin (GA) metabolism enzymes and the identities of GA metabolism genes in *Arabidopsis* (*red*) and rice (*blue*) (10, 29, 31, 32, 40, 41, 58, 79, 85, 87, 88, 93, 98, 103, 106, 109, 117, 119, 130, 131, 140). For the rice genes, identifiers from the Rice Annotation Project (RAP; **http://rapdb.lab.nig.ac.jp/**) are used. AGI, *Arabidopsis* Genome Initiative; TPS, terpene synthase; CPS, *ent*-copalyl diphosphate synthase; KS, *ent*-kaurene synthase; P450, cytochrome P450 monooxygenase; KO, *ent*-kaurene oxidase; KAO, *ent*-kaurenoic acid oxidase; MT, methyltransferase; GAMT, gibberellin methyltransferase; ER, endoplasmic reticulum; GGDP, geranylgeranyl diphosphate; 2ODD, 2-oxoglutarate–dependent dioxygenase; GA2ox, GA3ox, GA16, 17ox, GA20ox, GA oxidases. Tan-Ginbozu and Waito-C are semi-dwarf cultivars carrying the *d35* and *d18* mutant alleles, respectively (40, 41).

phytoalexins (75, 125). Genetic studies indicate that KS and KO in GA biosynthesis are also encoded by single genes, although multiple KS-like and KO-like sequences exist in the genome (88).

In comparison with the early biosynthesis enzymes, the 2ODDs that catalyze the late steps in the pathway are each encoded by multigene families. Accumulating evidence indicates that members in each of these 20DD families are differentially regulated by developmental and environmental cues, and that they are the primary sites for the regulation of bioactive GA levels. For example, reverse genetic analysis indicates that, of the four genes encoding GA3ox in Arabidopsis, AtGA3ox1 and AtGA3ox2 play distinct as well as overlapping roles in vegetative development, but they are dispensable for reproductive development (64). Also, there is evidence that AtGA3ox1 and AtGA3ox2 are differentially regulated by environmental signals during seed germination (130, 134). The key role of the 2ODDs in determining bioactive GA levels is also supported by the results of overexpression studies. Increased expression of a GA200x gene in transgenic Arabidopsis plants causes an increase in GA levels and GAoverdose phenotypes (11, 36). In contrast, although overexpression of AtCPS in Arabidopsis is effective in increasing the accumulation of ent-kaurene, ent-kaurenoic acid, and GA12, it does not affect the levels of bioactive GAs or the phenotype (17). The regulation of bioactive GA levels, mainly through the 2ODDs, is summarized in the following sections.

DEVELOPMENTAL REGULATION

Sites of Gibberellin Biosynthesis

The precise sites of GA biosynthesis and response must be determined to understand how this hormone controls plant growth and development. *AtCPS* (*GA1*)-*GUS* (where *GUS* is the reporter gene, β -glucuronidase) is expressed mainly in rapidly growing tissues during *Arabidopsis* development, suggesting that its expression correlates with the sites of GA response (97). This result is consistent with the occurrence of *ent*-kaurene synthesis activity in proplastids of growing tissues in wheat (1). *Nty*, a *GA3ox* gene in tobacco, is expressed in actively dividing and elongating cells, including the rib meristem, developing anthers, and root tips (39). In rice, *OsGA20ox2* and *OsGA3ox2*, as well as *SLR1*, which encodes a DELLA protein, are expressed in rapidly elongating or dividing tissues (44). Altogether, these results suggest that, in many cases, bioactive GAs are produced at the site of their action.

During the postgerminative growth of cereal grains, GAs are synthesized in the embryo and then transported to the aleurone cells, where α -amylase gene expression is induced for the hydrolysis of endosperm starch. Kaneko and coworkers (44, 45) found two distinct spatial expression patterns of GA biosynthesis genes among OsGA20ox1, OsGA20ox2, OsGA3ox1, and OsGA30x2 in rice. Transcripts of OsGA200x1 and OsGA30x1 are detectable only in the epithelium of the scutellum, whereas OsGA200x2 and OsGA30x2 are also expressed in the growing shoots, as well as in the epithelium. Genetic evidence from the d18 mutant (defective in OsGA3ox2) (Figure 2) proves that OsGA30x2 is essential, whereas OsGA3ox1 is not sufficient, for the production of bioactive GAs for α -amylase induction (45). None of these GA biosynthesis genes are expressed in the aleurone, whereas expression of the SLR1 transcript is detectable (44). These results support the premise that the cereal aleurone is a non-GA producing tissue that responds to GAs transported from the embryo. Therefore, in these cases, GAs likely function as a paracrine signal. In germinating Arabidopsis seeds, transcripts of AtGA3ox1 and AtGA30x2 are present predominantly in the cortex and endodermis of embryonic axes, illustrating that these are the major sites of GA biosynthesis (129). However, in situ hybridization analysis of three GA-upregulated transcripts indicates that GA-dependent transcriptional events are not restricted to the sites of bioactive GA synthesis (69). These results suggest the movement of bioactive GAs, or a GA signal, during the induction of *Arabidopsis* seed germination.

Studies in several plant species suggest that the tapetum of anthers is one of the major sites of bioactive GA synthesis during flower development (39, 44). Weiss and colleagues (120) proposed that the anthers might be a source of GAs for other flower organs in petunia, because emasculation of anthers causes reduced growth of the corolla, which can be rescued by GA treatment. In rice, expression of GA biosynthesis genes in flowers is restricted to the tapetum cells in anthers, whereas GAsignaling genes are expressed in additional organs, supporting the potential role of anthers in providing GAs to other floral organs (44). Interestingly, the tapetum is another major site of expression of the epithelium-specific genes, OsGA20ox1 and OsGA3ox1, implying the role of these genes in the production of GAs that function as a paracrine (or an endocrine) signal (44). In Arabidopsis flowers, the expression of all four AtGA30x genes (Figure 2) is restricted to stamens and flower receptacles, whereas bioactive GAs are required for petal growth as well (55), suggesting that GAs originating from other floral organs (possibly stamens) are responsible for petal growth (J. Hu, M.G. Mitchum & T-p. Sun, unpublished data).

In most *Arabidopsis* tissues, expression of an early GA biosynthesis gene, *AtCPS*, overlaps with expression of at least one of the *AtGA3ox* genes (64). However, the early and late stages of the GA biosynthesis pathway may occur in separate cell types. In germinating *Arabidopsis* seeds, expression of the early GA biosynthesis gene, *AtCPS*, is localized to the provasculature, whereas transcripts of *AtKO*, *AtGA3ox1*, and *AtGA3ox2* are mainly present in the cortical and endodermal cell layers (129). Thus, the synthesis of bioactive GAs in this system would require an intercellular movement of a pathway intermedi-

ate, possibly ent-kaurene, within the embryonic axis. A recent finding that ent-kaurene $(C_{20}$ hydrocarbon) is readily released into the atmosphere from plants suggests the facility of transporting this compound from the plastid to other sites of the plant (76). In addition, Arabidopsis ent-kaurene-deficient mutants efficiently take up and metabolize ent-kaurene from the headspace into GAs, implying that this hydrocarbon intermediate may be transported to the site of metabolism efficiently. A possible physical separation of early and late GA biosynthetic steps is also seen in Arabidopsis roots: The AtGA30x1 and AtGA30x2 genes are expressed in similar cell types along the vasculature of nondividing, nonelongating regions of roots (64), but AtCPS expression is absent in these cells (97). Determining the biological significance of the possible separations of early and late steps requires further studies.

Homeodomain Proteins in Meristem Function

Recent studies have linked the homeodomain proteins to GA metabolism and highlighted the role of bioactive GAs in promoting the shift from meristem identity to organ differentiation. KNOTTED1-like homeobox (KNOX) proteins are key regulators in the establishment of meristem identity and leaf morphology. Ectopic expression of KNOX genes from several plant species results in dwarfism and a reduction in endogenous GA levels (56, 105). Sakamoto and coworkers (86) discovered that a tobacco KNOX protein, NTH15, represses GA biosynthesis by suppressing GA20ox expression through direct binding to its first intron. Importantly, KNOX genes are expressed in the corpus of the shoot apical meristem (SAM) in tobacco and Arabidopsis, thereby GA20ox expression is excluded in this region (Figure 4a). Conversely, the absence of KNOX expression at the flanks of the SAM and the subapical region permits GA biosynthesis in these tissues (26, 86). These results suggest that

KNOTTED1-like homeobox (KNOX) proteins: key regulators in the establishment of meristem identity and leaf morphology



Figure 4

Developmental, hormonal, and environmental regulation of the gibberellin (GA) metabolism pathway. (a) Summary of the roles of KNOTTED1-like homeobox (KNOX) proteins in the control of hormonal balance in shoot apices (26, 43, 86, 89). KNOX proteins suppress GA biosynthesis (GA200x expression) and activate cytokinin (CK) synthesis in the corpus of the shoot apical meristem (SAM) (orange). Without KNOX expression, suppression of GA200x expression is alleviated in leaf primodia (green). The expression of GA deactivation genes (GA2ox) at the base of the meristem (blue) may assist the establishment of a low-GA regime in the SAM. (b) Auxin regulation of bioactive GA levels (73, 84). In pea internodes, indole-3-acetic acid (IAA) upregulates PsGA30x1 and downregulates PsGA20x1. PsGA20x1 also catalyzes 2-oxidation of GA20 to produce GA29, but for simplification this reaction is not shown here. (c) Phytochrome-regulation of GA metabolism in Arabidopsis seeds (71, 94, 130, 135). In the absence of active phytochrome, PHYTOCHROME-INTERACTING FACTOR 3-LIKE 5 (PIL5) suppresses GA3ox expression and activates GA2ox expression. Once phytochrome is activated by red (R) light (Pfr), the PIL5 protein is degraded. This allows for the upregulation of GA3ox genes and causes the downregulation of GA20x expression. As a consequence, GA4 levels are elevated and germination is stimulated. GA3ox signifies AtGA3ox1 and AtGA3ox2; GA2ox signifies AtGA2ox2. AtGA2ox2 also catalyzes 2-oxidation of GA₉ to produce GA₅₁, but for simplification this reaction is not shown here. FR. far-red; Phy, phytochrome.

KNOX proteins maintain the indeterminate state of corpus cells in part by suppressing GA biosynthesis. In Arabidopsis, the KNOX protein SHOOTMERISTEMLESS (STM) upregulates expression of the GA deactivation genes, AtGA20x4 and AtGA20x6, at the base of the meristem, similar to the ring-shaped localization pattern of the OsGA2ox1 transcript that accumulates around the vegetative shoot apex (87). This expression would also contribute to the establishment of a low-GA regime by KNOX proteins (43) (Figure 4a). Recent studies have demonstrated that KNOX proteins also activate cytokinin (CK) biosynthesis through the upregulation of IPT genes (encoding isopentenyltransferase) and that CK plays a role in the induction of GA2ox gene expression (43, 89, 136) (Figure 4a). Taken together, these results suggest that KNOX proteins act as orchestrators to control the balance of CK and GA in the SAM and promote meristem identity.

The homeotic gene *AGAMOUS* (*AG*) is expressed after flower induction, terminates meristem activity, and promotes development of floral organs. Microarray analysis has shown that AG elevates expression of *AtGA30x1*, which may cause an increase in GA levels in the floral meristem and promote the shift from meristem identity to differentiation (24). AG binds to the promoter of *AtGA30x1* both in vitro and in vivo, indicating that this gene is directly regulated by AG.

Regulators of Seed Development

Bioactive GAs and abscisic acid (ABA) act antagonistically to control seed development and germination. The levels of GA and ABA are negatively correlated during seed development (7, 121), thus there should be a mechanism that tightly regulates the balance between these hormones. LEAFY COTYLEDON 2 (LEC2) and FUSCA 3 (FUS3) are *Arabidopsis* B3 transcription factors, and both are essential for seed maturation processes. Curaba and colleagues (13) found that the levels of bioactive GAs are elevated in immature seeds of the fus3 and lec2 mutants, in part through ectopic activation of AtGA30x2 gene expression. Furthermore, FUS3 binds directly to two RY elements in the promoter of AtGA3ox2 in vitro. Studies by Gazzarrini and coworkers (22) show that a transient induction of FUS3 expression results in an increase in ABA content and repression of the GA biosynthesis genes, AtGA3ox1 and AtGA200x1. These results indicate that FUS3 functions as a positive and negative regulator of ABA and GA levels, respectively. Moreover, FUS3 protein levels are regulated positively by ABA and negatively by GA, suggesting that these regulatory loops may play a role in the establishment of GA-ABA balance in the seed (22).

AGAMOUS-LIKE 15 (AGL15) is a member of the MADS [named for MCM1, AGAMOUS, DEFICIENS, and serum response factor (SRF)] domain family and accumulates during embryo development in *Arabidopsis. AtGA20x6*, involved in GA deactivation, has been identified as a direct target of AGL15 in vivo by chromatin immunoprecipitation (ChIP) analysis, and this gene is activated in seeds that overexpress *AGL15* (119). These results indicate that AGL15 plays a role in lowering GA content during embryogenesis through upregulation of a GA-deactivation gene.

HORMONAL REGULATION

Gibberellin Homeostasis

The levels of bioactive GAs in plants are maintained via feedback and feedforward regulation of GA metabolism (29, 72). Transcript analysis shows that GA signaling is mainly targeted to 2ODDs in the GA metabolism pathway to establish homeostasis; e.g., expression of the *Arabidopsis* GA biosynthesis genes, *AtGA20ox1* and *AtGA3ox1*, is highly elevated in a GA-deficient background, whereas these genes are downregulated after application of bioactive GAs (10, 63, 79, 127, 130). In contrast, expression of the GA deactivation Chromatin immunoprecipitation (ChIP): a useful method to detect direct binding of a transcription factor to DNA in vivo IAA: indole-3-acetic acid

genes, AtGA2ox1 and AtGA2ox2, is upregulated upon GA treatment (106). Although the molecular mechanisms underlying this homeostatic regulation have yet to be elucidated, the central GA signaling components are clearly required for this response, including the soluble GA receptor GID1, the DELLA proteins, and the F-box proteins SLEEPY 1 (SLY1) (Arabidopsis)/GID2 (rice) (for reviews, see References 102 and 115). For example, in the rice gid1 and gid2 mutants, expression of the OsGA20ox2 (SD1) gene is upregulated and the levels of bioactive GA1 are highly elevated (90, 113). Conversely, a DELLA lossof-function mutant of Arabidopsis has reduced levels of AtGA30x1 transcripts even in a GAdeficient mutant background (15, 50).

REPRESSION OF SHOOT GROWTH (RSG) is a tobacco transcriptional activator that contains a basic leucine zipper domain and binds to the promoter of the Arabidopsis AtKO gene (20). A dominant negative version of RSG thus represses AtKO expression and causes dwarfism in tobacco. In addition, the feedback regulation of GA20ox expression is impaired in transgenic plants expressing the dominant negative RSG (38). 14-3-3 proteins interact with RSG and suppress its function by sequestering it in the cytoplasm (37). Importantly, RSG is translocated into the nucleus in response to a reduction in GA levels (38). These results suggest that RSG is negatively modulated by GAs via binding to 14-3-3 proteins and might be involved in the feedback regulation of GA20ox expression. The possible role of RSG in GA homeostasis appears to be restricted to GA200x, because it does not affect the feedback regulation of the GA3ox gene (38).

Recently, additional components that might act in GA homeostasis have been identified. AT-hook protein of GA feedback regulation (AGF1) is an AT-hook protein that binds to the 43-bp *cis*-element for GA negative feedback response in the *AtGA3ox1* promoter (63). How AGF1 mediates the GA signal remains unclear, because GA does not affect the AGF1 transcript levels or the nuclear localization of AGF1-GFP. Interestingly, this ciselement appears to be absent in the promoter of AtGA200x1, suggesting that the GA signal may regulate AtGA3ox1 and AtGA20ox1 transcription by different mechanisms. YABBY1 (OsYAB1) may be a mediator of GA homeostasis downstream of the DELLA protein in rice, because its expression is dependent on the DELLA protein and overexpression of OsYAB1 results in a semidwarf phenotype and a decrease in GA₁ levels, which might be caused by downregulation of the OsGA3ox2 gene and upregulation of the OsGA2ox3 gene (14). In OsYAB1-cosuppression plants, the GA-dependent repression of OsGA3ox2 expression is impaired, suggesting a role for OsYAB1 as a mediator of feedback inhibition. In addition, OsYAB1 binds to a GAresponsive element in the OsGA3ox2 promoter in vitro (14).

Regulation by Other Hormones

Multiple hormones are often involved in the regulation of a given biological process. Therefore, how different hormones cooperatively regulate a common developmental process has been an important question. Here, our recent understanding on the regulation of GA metabolism by other hormones is summarized.

Both bioactive GAs and auxin positively regulate stem elongation. In pea (*Pisum sativum*), the auxin indole-3-acetic acid (IAA) is essential for the maintenance of GA₁ levels in elongating internodes (84). Decapitation (removal of the apical bud, the source of auxin) dramatically reduces the level of endogenous GA₁ in elongating internodes, and application of IAA to the decapitated plants completely reverses these effects. The IAA induction of GA₁ levels is correlated with an increase in *PsGA30x1* transcript abundance and a decrease in *PsGA30x1* transcript levels (73) (**Figure 4b**). A similar auxin-GA interaction occurs in stems of tobacco plants as well (123).

In barley, auxin from the developing inflorescence plays a role in stem elongation by upregulating GA 3-oxidation and downregulating GA 2-oxidation (122). Collectively, these results suggest that auxin regulation of GA metabolism in stems is a general mechanism. In pea, auxin-regulation of GA biosynthesis is also evident during fruit development (68). Growth of young pea fruit (pericarp) is dependent on the presence of developing seeds. In the absence of seeds, treatment with GA or the auxin 4-chloroindole-3-acetic acid (4-chloro-IAA) is effective in promoting the growth of pericarps (77, 116). In developing pericarps, expression of PsGA3ox1 and PsGA20ox1 is induced by 4-chloro-IAA, which is provided by the seeds. Taken together, these results support the hypothesis that auxin regulation of GA biosynthesis plays a role in the coordination of growth control between different organs/tissues. In these cases, auxin functions as a mobile signal that modulates the synthesis of other growth hormones, GAs. Frigerio and colleagues (19) found that auxin treatment upregulates expression of several AtGA20ox and AtGA2ox genes in Arabidopsis seedlings. Studies using various auxin-response mutants revealed that the Aux/IAA- and ARF-dependent signaling pathways are involved in these transcriptional changes, whereas they are independent of the feedback regulation mediated by DELLA proteins. Partial alleviation of the phenotype of several gain-of-function mutants of Aux/IAA genes by GA application suggests that changes in GA metabolism (GAdeficiency) mediate part of auxin action during Arabidopsis seedling development (19).

The role of brassinosteroid (BR) as a regulator of GA metabolism is less clear. In an *Arabidopsis* BR-deficient mutant, *AtGA200x1*, mRNA accumulation is elevated after application of 24-*epi*-brassinolide, an active BR (9). However, how the induction of *AtGA200x1* expression by BR affects endogenous GA metabolism is unclear. On the basis of the effect of BR on bioactive GA levels, Jager and coworkers (42) concluded that the BR growth response in pea is not mediated by changes in bioactive GA content.

Previous studies have suggested that ethylene may increase the level of GAs during internode elongation of deepwater rice upon submergence (35). Recently, ethylene was shown to delay Arabidopsis flowering by reducing bioactive GA levels (2). Arabidopsis plants grown in the presence of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid, or in an ethylene-rich atmosphere, flower late. In addition, the ctr1-1 loss-of-function mutation, which confers constitutive ethylene responses, causes later flowering, especially in short-day photoperiods. All these ethylenestimulated late flowering phenotypes are rescued by exogenous GA treatment. The levels of GA₁ and GA₄ are substantially reduced in the ctr1-1 mutant, suggesting that the ethylene signal is initially targeted to the GA metabolism pathway, and then to DELLA proteins as a consequence of altered GA content. In the ctr1-1 mutant, transcript abundance of AtGA3ox1 and AtGA20ox1 genes is elevated, presumably through the negative feedback mechanism caused by the decreased bioactive GA levels. Further studies are required to clarify how the ctr1-1 mutation alters bioactive GA levels.

Antagonistic effects of ABA and GA on seed germination have been well documented. However, evidence as to whether the endogenous GA levels are regulated by ABA, or vice versa, remains inconclusive. Recently, Seo and coworkers (94) found that GA₄ levels in dark-imbibed Arabidopsis seeds (after inactivation of phytochrome by a far-red light pulse) are elevated in the ABA-deficient aba2-2 mutant. This is correlated with an increase in AtGA3ox1 and AtGA3ox2 transcript abundance in aba2-2 mutant seeds relative to wildtype seeds. Furthermore, activation of GA biosynthesis genes in the aba2-2 mutant is also observed during seed development. These results indicate that ABA plays a role in the suppression of GA biosynthesis in both imbibed and developing Arabidopsis seeds (94).

ENVIRONMENTAL REGULATION Light Regulation

As detailed below, bioactive GAs function as key mediators between the perception of environmental signals and the resulting growth responses. Light is one of the major environmental factors that affects plant growth and development. Among light-dependent processes, current evidence indicates that changes in GA concentrations are, at least in part, responsible for light-regulated seed germination, photomorphogenesis during deetiolation, and photoperiod regulation of stem elongation and flowering.

Seed germination is under phytochrome control in some small-seeded plants, such as lettuce (Lactuca sativa), tomato (Solanum lycopersicum), and Arabidopsis (95). In Arabidopsis, phytochrome B (phyB) is responsible for the low fluence response shortly after the onset of imbibition and stimulates germination in response to red (R) light in a far-red (FR) light-reversible manner (96). Unlike phyB, phyA accumulates during dark-imbibition and promotes germination by sensing very low fluence light in a wide range of wavelengths. In both phyA- and phyB-dependent germination conditions, light treatments that activate phytochromes elevate expression of AtGA3ox1 and AtGA3ox2, whereas AtGA2ox2 expression is suppressed; these changes in gene expression result in an increase in GA₄ levels in the seeds (71, 94, 130, 135) (Figure 4c). PHYTOCHROME-INTERACTING FACTOR 3-LIKE 5 (PIL5), a light-liable helix-loop-helix (bHLH) protein, basic is a negative regulator of phytochromedependent seed germination, and is involved in both phyA- and phyB-mediated regulation of GA metabolism genes (71) (Figure 4c). ChIP analysis showed that PIL5 does not bind to the promoters of AtGA3ox1, AtGA30x2, or AtGA20x2 genes, suggesting that an additional component or components are required for phytochrome regulation of GA metabolism genes in Arabidopsis seeds (70). In contrast, the same study showed

that PIL5 binds directly to the promoters of REPRESSOR OF GA1-3 (RGA) and GIBBERELLIC ACID INSENSITIVE (GAI), both of which encode DELLA proteins, and regulates GA responsiveness. Besides regulating GA metabolism, PIL5 is involved in phytochrome regulation of ABA metabolism (70), which illustrates a role of PIL5 in balancing GA and ABA levels during light-dependent seed germination. In lettuce seeds, R light induces expression of the LsGA3ox1 gene and suppresses LsGA2ox2 expression, whereas a subsequent FR light treatment cancels the effect of R light, under a light regime similar to the phyB-dependent germination condition (66, 108). Thus, the phytochrome signal is commonly targeted to GA3ox and GA2ox genes to alter bioactive GA content in lettuce and Arabidopsis seeds.

Although active phytochrome positively regulates GA biosynthesis and response during seed germination, this relationship is reversed during photomorphogenesis of etiolated seedlings after irradiation. In darkgrown pea seedlings, GA1 levels decrease significantly after exposure to white light (4, 23, 74). The reduction of GA_1 levels occurs within 4 h of exposure to R, blue, or FR light, and is mediated redundantly by phyA and cryptochrome 1 (cry1), but not phyB (18, 82). The reduction in GA₁ levels is correlated with downregulation of PsGA3ox1 gene expression and upregulation of PsGA2ox2 expression (18, 82). A similar role of cryptochromes (cry1 and cry2) in response to blue light was recently shown during de-etiolation of Arabidopsis seedlings; blue light downregulates expression of AtGA20ox1 and AtGA3ox1 genes, whereas it induces AtGA2ox1 expression (139). These transcriptional changes correlate with a cry-dependent transient decrease in GA₄ levels after exposure to blue light. These studies in pea and Arabidopsis suggest a general role of blue light in regulating bioactive GA levels during de-etiolation. This hormonal change is likely to contribute, at least in part, to a rapid inhibition of stem elongation (and maybe other processes as well) during the establishment of photomorphogenesis. This

hypothesis is supported by the essential role of GA in the repression of photomorphogenesis, as revealed by studies using GA-deficient mutants of Arabidopsis and pea (5). CPS activity is inhibited by the substrate GGDP and Mg²⁺ synergistically in vitro (49, 80). Thus, GGDP and Mg²⁺ may inhibit CPS activity to limit GA biosynthesis during de-etiolation, because the levels of GGDP in plastids presumably increase for the production of photosynthetic pigments and light also induces increases in plastid Mg²⁺ levels (80). However, the physiological significance of this posttranslational mechanism requires further study, because the actual GGDP concentrations in plastids are unknown.

Extensive studies have established that photoperiodic control of stem elongation in long day (LD) rosette plants is mediated by GAs (for a review, see Reference 21). Spinach (Spinacia oleracea) is an LD rosette plant in which exposure of short day (SD)-grown plants to FR-rich LD triggers stem elongation and subsequent floral development. This LD-induced stem elongation is inhibited by the application of GA biosynthesis inhibitors and this effect is reversed by GA₃ treatment (138). Transfer of spinach plants from SD to LD results in elevated levels of C₁₉-GAs, which is primarily attributable to upregulation of SoGA200x1 transcript levels (57, 59, 124). Recent work has shown that this transcriptional change is reflected in elevated accumulation of SoGA20ox1 protein in LD (59). When ¹⁴C-GA₅₃ is fed to spinach plants, more ¹⁴C-GA₅₃ is metabolized to ¹⁴C-GA₉₇ by 2β -hydroxylation (Figure 1) in SD than in LD, and more ¹⁴C-GA₂₀ is formed in LD than SD (58). These observations are consistent with strong upregulation of GA20ox activity in LD, although there is little change in the transcript levels of SoGA20x3, which controls the conversion of GA53 to GA97 (58). Likewise, in Arabidopsis, an increase in the levels of bioactive GAs by LD treatments is, at least in part, attributed to increased expression of AtGA200x1, whereas expression of AtGA30x1 is not under photoperiodic control (126). Petiole elongation of SD-grown *Arabidopsis* plants is enhanced by FR-rich LD treatment or a brief end-of-day FR exposure in SD. This response involves regulation of *AtGA20ox2* expression and, to a lesser extent, *AtGA20ox1* expression via phyB or related phytochrome(s) (34). Accordingly, stimulation of petiole elongation by end-of-day FR treatment is reduced in *AtGA20ox2* knockdown transgenic plants.

The grass Lolium temulentum flowers in response to a single LD treatment and has been utilized for studies on GA-induced flowering (51). Interestingly, GA₅ and GA₆ (Figure 1) are more effective in inducing flowering than GA₁ or GA₄, both of which are active in inducing stem elongation in L. temulentum. Evidence suggests that GA5 and GA₆ are synthesized in the leaf upon exposure to LD and transported to the shoot apex to induce floral initiation (52-54). A twofold increase in the levels of GA5 and GA6 occurs in the shoot apex following an LD exposure, and this increase is preceded by a rapid increase in GA₅ content and a drastic increase in the levels of LtGA20ox1 mRNA in the leaf after the LD treatment. GA1 and GA4 do not reach the shoot apex, presumably because of deactivation by GA2ox; the corresponding gene in rice (OsGA2ox1) is strongly expressed at the base of the vegetative shoot apex (Figure 4a), but the expression disappears after transition to the reproductive phase (87). In contrast, GA5 and GA6 are resistant to deactivation by GA2ox, so logically, these compounds may act as floral stimuli. In a related species, Lolium perenne, both vernalization and LD are required for flower initiation. In this species, exposure to two LDs upregulates expression of the LpGA20ox1 gene and causes an increase in some GAs in the leaf and young shoot tissues, regardless of the vernalization status (60). Thus, GA biosynthesis is regulated by the photoperiodic signal, whereas it is independent of vernalization in L. perenne.

In *Arabidopsis*, flower initiation under SD conditions requires GA biosynthesis. The flower meristem identity gene *LEAFY*(*LFY*)

is a key component downstream of the GA signal (8). Unlike in L. temulentum, in Arabidopsis GA4 increases drastically in abundance shortly before floral transition in the shoot apex, and functions as the major bioactive GA for the induction of LFY expression in the shoot apex (16). This GA₄ increase might be due to transport of GAs from other tissues, because the expression of the known GA metabolism genes in the shoot apex does not correlate with the acute increase in GA₄ content in this tissue. After application of deuterium-labeled GA₄ to a single leaf, the labeled GA₄ is detectable at the shoot apex (16). This suggests that endogenous GA4 made in the leaf may be transported to the shoot apex to induce flowering, although further studies are necessary to verify this hypothesis.

Temperature Regulation

Exposure of imbibed seeds to cold temperature (cold stratification) accelerates the release from seed dormancy and induces germination in many plant species. In dark-imbibed afterripened Arabidopsis seeds, cold treatment stimulates the expression of the GA biosynthesis genes, AtGA3ox1 and AtGA20ox2, whereas the GA deactivation gene AtGA2ox2 is downregulated (134). Consistent with these results, the levels of bioactive GAs are significantly increased by cold treatment. Microarray analysis showed that approximately a quarter of cold-responsive genes correspond to GAregulated genes, suggesting an important role for GA in mediating the cold temperature signal in Arabidopsis seeds (134). Recent work showed that SPATULA (SPT), a bHLH transcription factor closely related to PIL5, acts as a light-stable repressor of seed germination and controls responses to cold stratification in part through regulating the AtGA30x genes (78). To a lesser extent, SPT also plays a role in phytochrome-dependent responses to light in seeds.

Plants monitor day and night temperatures and alter their growth and development accordingly. The ability of plants to detect the diurnal temperature change is referred to as thermoperiodism. In pea, a day/night temperature combination of 13°C/21°C significantly reduces stem elongation as compared with 21°C/13°C (and 17°C/17°C), and this response is correlated with a decrease in GA₁ levels (101). In addition, PsGA2ox2 transcript abundance increases significantly in 13°C/21°C conditions compared with 21°C/13°C, suggesting that a higher rate of GA deactivation is involved in the thermoperiodic response in pea. Furthermore, a constitutive GA-response mutant, la crys, shows no or very poor thermoperiodic response in stem elongation (25, 101). This supports the interpretation that GA₁ acts as a mediator of the thermoperiodic response.

Stress Responses

Upon exposure to stresses, plants reduce their growth rate. An intimate relationship has been suggested to exist between GA levels and the acquisition of stress protection in barley (Hordeum vulgare) (118). Evidence has emerged that the GA metabolism pathway is altered in response to abiotic stresses. Overexpression of DWARF AND DELAYED FLOWERING 1 (DDF1) causes a reduction in GA₄ content and dwarfism in *Arabidopsis* (62). DDF1 encodes an AP2 transcription factor that is closely related to the dehydration responsive element-binding proteins (DREBs) involved in stress responses, and DDF1 expression is strongly induced by high-salinity stress. In addition, transgenic plants overexpressing DDF1, as well as a GA-deficient ga1-3 mutant, exhibit a higher survival rate under high-salinity conditions, whereas exogenous GA treatment reduces the survival rate (62). Similarly, there is a correlation between the survival of salt toxicity and the function of DELLA proteins (3). These results suggest that the salt-inducible DDF1 gene is involved in growth responses under high salinity conditions in part through altering GA levels. In fact, salt-treated Arabidopsis plants contain reduced levels of bioactive GAs (3), supporting the idea that salt slows growth by modulating the GA metabolism pathway. Recent work has shown that AtGA2ox7, which encodes a GA2ox that specifically deactivates C_{20} -GAs (**Figures 1** and **2**), is a target of DDF1, suggesting that salt decreases bioactive GA levels through elevated deactivation (H. Magome, S. Yamaguchi, K. Oda, unpublished data).

EVOLUTION OF GIBBERELLIN BIOSYNTHESIS: FUNGI AND LOWER PLANTS

Gibberellin Biosynthesis in Fungi

GAs were first isolated as metabolites of a fungal rice pathogen, *Gibberella fujikuroi*. GAs also occur in other fungi and bacteria (61). Recent identifications of genes encoding GA biosynthesis enzymes from *G. fujikuroi* and a species of *Phaeosphaeria* revealed remarkable differences in GA biosynthesis pathways and enzymes between plants and fungi.

In plants, two separate terpene cyclases (CPS and KS) are involved in the synthesis of *ent*-kaurene from GGDP (**Figure 1**), whereas in fungi these two reactions are catalyzed by a single bifunctional enzyme (CPS/KS) (48, 107, 110) (**Figure 5**). In *G. fujikuroi*, multifunctional P450s, P450-4 and P450-1, play a similar role to that of KOs (CYP701As) and KAOs (CYP88As) in plants, respectively (83). However, despite their similar catalytic activities, the fungal P450s are not closely related to



Figure 5

Comparison of the major gibberellin (GA) biosynthesis pathways in the fungus Gibberella fujikuroi and plants. The fungal biosynthesis route is highlighted in vellow. The plant pathways are indicated by dashed gray arrows. GGDP, geranylgeranyl diphosphate; CPS, ent-copalyl diphosphate synthase; KS, ent-kaurene synthase; KO, ent-kaurene oxidase; KAO, ent-kaurenoic acid oxidase; GA3ox, GA 3-oxidase: GA20ox, GA 20-oxidase; GA13ox, GA 13-oxidase.

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the plant enzymes in amino acid sequence. Remarkably, P450-1 has 3^β-hydroxylase activity in addition to KAO activity and produces GA₁₄ (**Figure 5**). Thus, 3β -hydroxylation is catalyzed by a P450 at an early step of the pathway in G. fujikuroi, in contrast to plant GA3oxs that are soluble 20DDs and act at the final step to produce bioactive GAs (**Figure 1**). GA_{14} is converted to GA_4 by another P450, P450-2 (112). Thus, GA 20 oxidation in G. fujikuroi is also catalyzed by a P450, unlike the case with plant GA20oxs. GA₄ is then converted to GA₇ by GA₄desaturase and finally to GA₃ by P450-3 through 13-hydroxylation (111) (Figure 5). Notably, these GA biosynthesis genes are clustered on a single chromosome in fungi, whereas they are randomly located on multiple chromosomes in plants (30, 47). Taken together, these substantial differences in genes and enzymes suggest that plants and fungi have evolved their complex GA biosynthesis pathways independently.

Gibberellin Biosynthesis in Lower Plants

The moss *Physcomitrella patens* is a model organism of bryophytes (12). Some plant hormones, including auxin, cytokinin, and abscisic acid, are biosynthesized and function as growth regulators in P. patens. However, whether GAs act as growth regulators in mosses was unknown. Recently, a cDNA clone encoding ent-kaurene synthase was identified from *P. patens*, which indicates the occurrence of at least an early GA intermediate in the moss (27). Curiously, P. patens *ent*-kaurene synthase is a bifunctional enzyme with both CPS and KS activity, as is the case of fungal CPS/KSs discussed above (Figure 5). Both GA₁ and GA₄ were identified in sporophytes of some tree fern species (132, 133). In addition, the lycophytes Selaginella moellendorffii and Selaginella kraussiana (members of the oldest lineages of vascular plants) possess functional GID1 and DELLA homologs that are capable of forming a complex with GA (137). However, such functional GID1/DELLA proteins are not found in P. patens, suggesting that GAstimulated GID1-DELLA interactions presumably arose in the land plant lineage after the bryophyte divergence (115, 137). Given these observations, it is important to assess the biological relevance of *ent*-kaurene biosynthesis in *P. patens* through reverse genetics.

SUMMARY POINTS

- 1. The majority of the genes encoding enzymes in the gibberellin (GA) metabolism pathway have now been identified in model plant species.
- 2. New GA deactivation enzymes have recently been discovered, including C₂₀-GA-specific GA 2-oxidase (class III), 16α ,17-epoxidase (EUI/CYP714D1), and GA methyltransferases (GAMTs). These findings indicate the diversity of deactivation mechanisms and emphasize the presence of a number of branches in the GA metabolism pathway.
- Some transcription factors that directly regulate GA metabolism genes have been identified. In addition, transcriptional regulators that coordinate the balance of two hormones (GA and another hormone) have been discovered, including KNOTTED1like homeobox (KNOX) proteins, PHYTOCHROME-INTERACTING FACTOR 3-LIKE 5 (PIL5), and FUSCA3 (FUS3).
- 4. There is now compelling evidence that bioactive GAs act as key mediators in growth responses to environmental cues, such as light and temperature.

5. Profound differences in the GA pathways and enzymes between *Arabidopsis* and the fungus *Gibberella fujikuroi* clearly indicate that higher plants and fungi have evolved the GA pathway independently.

FUTURE ISSUES

- 1. The identification of additional enzymes, such as GA 13-oxidase and GA-conjugating enzymes, will be necessary to understand the whole picture of the GA metabolism pathway.
- 2. In many cases, GA biosynthesis and deactivation genes are reciprocally regulated by a common signal (**Figure 4**). Elucidation of the molecular mechanisms underlying this transcriptional regulation will be important to learn how hormone concentrations are effectively altered by the signal.
- 3. To better understand the regulation of the GA metabolism pathway, clarification of the localization of various GAs at finer spatial resolution in plants is necessary. Because GAs exist in plant tissues at extremely low levels, this will require improvements in GA analytical systems.
- 4. Although GAs are thought to act occasionally like paracrine signals do, it is not known how GAs move in plants. It will be an important challenge to elucidate the molecular mechanisms of GA movement/transport.

DISCLOSURE STATEMENT

The author is not aware of any biases that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

MEXT of Japan supported work in the author's lab.

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Volume 59, 2008

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Errata

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