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REVIEW OF GIBBERELLIN SIGNALING

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Abstract - This review covers recent advances in gibberellins (GA) signaling. Hormones gibberellins (GAs) are a class of diterpenoid acids that control many aspects of plants' life, including both developmental processes and stress responses. Nowadays, we have a good understanding of how GA levels are regulated and how this information is translated into physiological responses, mainly through genetic and biochemical approaches carried out during the last two decades in rice and Arabidopsis. Here, we review the current knowledge of the GA signaling, pathway from GA metabolism to the downstream responses and pay special attention to the regulatory molecular mechanisms. GA biosynthesis starts in plastids, whereas it's last steps, and also the GA inactivation, takes place in the cytosol. Importantly, the expression of gene coding enzymes that catalyze limiting steps, for example, the soluble GA 20-oxidases, is usually regulated by environmental cues, making the GA level very sensitive to changes in the environment. The binding of the hormone to the GID1 receptor provokes the degradation of the master negative regulators in the pathway, the transcriptional regulators DELLA proteins, and GA-promoted responses proceed. The biochemical basis of the GID1-GA-DELLA regulatory module is well established, but how DELLA proteins regulate downstream events is a matter of current intensive research. In this regard, the regulation of transcription factors' activity through direct physical interaction seems to be an extended yet not unique mechanism of DELLA action. Finally, how all this wealth of information is being used with biotechnological purposes is revised.

Key words *Development, Gibberellins, Metabolism, Signaling, and Stress*

I. INTRODUCTION

1.1. Gibberellins Signaling

Gibberellins (GAs) are a large family of tetracyclic diterpenoid plant growth regulators. Since its original discovery >130 GAs have been identified in plants, fungi and bacteria, although only a few GAs have biological activity (Yamaguchi, 2008); many non-bioactive GAs exist in plants, and these act as precursors for the bioactive forms or are de-activated metabolites. Gibberellins (GAs) are plant hormones that are essential for many developmental processes in plants, including seed germination, stem elongation, leaf expansion, trichome development, pollen maturation and the induction of flowering (Achard and Genschik , 2009). The major bioactive GAs, which includes GA1, GA3, GA4 and GA7, are derived from a basic diterpenoid carboxylic acid skeleton, and commonly has a C3 hydroxyl group (Yamaguchi, 2008). During the past decade, most of the components of the GA signaling pathway have been identified from genetic screens in rice and Arabidopsis.

In recent years there have been impressive advances in our understanding of how the GA-signal is transduced, subsequently leading to changes in GA-responsive growth and development. Studies in this field have in particular emphasized the central role played by the DELLA proteins, which function as repressors of GA-mediated responses (Thomas & Sun, 2004). However, until recently, the components responsible for perceiving bioactive GAs had remained elusive. An exciting recent study by Ueguchi-Tanaka *et al.* ; 2005) has now resulted in the identification of a soluble GA receptor from rice. This review highlights the newly discovered molecular mechanism of GA-induced proteolysis of GA signaling repressors, and the recent microarray and biochemical studies that have identified new GA-

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responsive genes and factors that regulate transcription of these genes. The role of the plant specific family of GRAS proteins in these processes has become apparent. In addition this review, the importance of DELLA proteins has been highlighted and a model of gibberellins signaling pathways in plants has been shown.

II. LITRATURE REVIEW

2.1. GA perception and signaling

The power of using mutants of Arabidopsis to determine hormone action has been applied usefully to GA signaling, with two broad group's identified: the GA-insensitive dwarfs and the constitutive GA-response mutants. The GA insensitive dwarfs resemble GA-deficient mutants but are not rescued by added GA. In contrast, the GA constitutive response mutants all appear as if they have been exposed to GA (for example, they all have elongated stems) in the absence of any treatment with the hormone. These latter mutants show resistance to inhibitors of GA biosynthesis, so demonstrating that there is additionally a GA-independent activation of GA responses. We do not wish to describe all of these mutants here, so the reader is referred to the review of Sun (2000). However, certain key studies are described as they contribute to the emerging view of the molecular mode of signaling of GA. We begin with the study by Peng *et al.*; 1997) who examined the **gai** mutant, described previously by Pengand Harberd (1993), and showed that it had reduced responsiveness to GA.

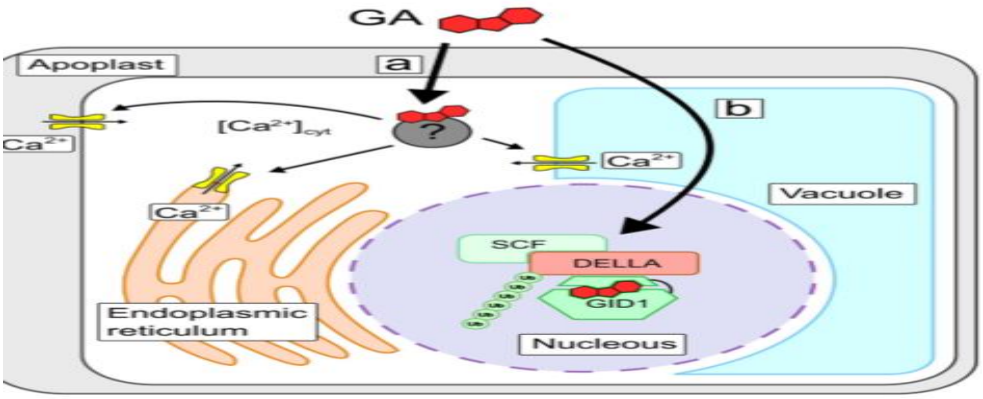
Thus, the GA perception mechanism differs from that of auxin, which serves as the "molecular glue" that brings together a substrate protein and an F-box protein without changing the structure of either protein or requiring the involvement of a third protein (Hedden, 2008; Tan *et al.*, 2007). In contrast, the GA receptor can be activated by the allosteric effector GA to function as the "ubiquitination chaperone" that stimulates substrate recognition by the SCF complex (Lumba *et al*., 2010; Murase *et al*., 2008).

2.2. Gibberellin Signaling Pathway

As in the case of the elucidation of the GA metabolic pathway, genetic analyses carried out in Arabidopsis and rice have been fundamental to identify the core components of the GA signaling pathway, basically through the isolation and characterization of dwarf, GA-insensitive mutants. The components that form the basic skeleton of the pathway are the GA receptor GID1 (Ueguchi-Tanaka *et al.*, 2005), the transcriptional regulators DELLA proteins (Peng *et al*. 1997), and the F-box proteins GID2/SLEEPY1 (SLY1) (McGinnis *et al*., 2003; Sasaki *et al.* , 2003).

In essence, binding of GAs to the GID1 receptor allows its interaction with DELLA proteins, which are the negative regulators in the pathway. Once this tertiary complex is formed, DELLAs are ubiquitinated and degraded by the proteasome, a process mediated by the interaction of DELLAs with GID2/SLY1, thus releasing the brake on GA responses imposed by their activity (Daviere and Achard, 2013).

In response to diverse internal and external stimuli, cells generate transient increases in cytosolic Ca2+ ([Ca2+] cyt), varying in amplitude, frequency, duration, intracellular location and timing. One physiological response of plant cells to GAs is an increase in [Ca2+]cyt; however, previous studies using fluorescent Ca2+ indicators suggest that the increase occurs an hour to several hours after GA application, too slow for Ca2+ to act as a secondary messenger of GA signaling. We reexamined the effects of GAs on an increase in [Ca2+]cyt using the Ca2+ sensor protein aequorin in Arabidopsis (Arabidopsis thaliana**) (Okada K *et al* ., *2017)* .**



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Source: (Okada K *et al.*, 2017)

Fig. 1. DELLA-dependent and -independent GA signaling pathway. The GA-induced increase in [Ca2+]cyt occurs through the activation of Ca2+ channels within a few minutes, independently of DELLA (pathway a). The main pathway of GA signaling depends on DELLA degradation, which occurs >30 min after GA treatment (pathway b).

2.3. GA Biosynthesis and Catabolism

GA biosynthesis and catabolism pathways have been studied extensively by gas chromatography-mass spectrometry analysis of GA content, purification of GA metabolism enzymes, isolation of GA-deficient mutants, and cloning of the corresponding genes (Hedden and Phillips, 2000; Yamaguchi, 2008). Biosynthesis of GA in higher plants can be divided into three stages: (i) biosynthesis of ent -kaurene from geranyl geranyl diphosphate (GGDP) in proplastids, (ii) conversion of ent -kaurene to GA12 via cytochrome P450 mono oxygenases, and (iii) formation of C20- and C19-GAs in the cytoplasm.

GA signaling pathways are very conservative in the plant world, probably due to the structural and functional conservatively of DELLA proteins; this has been shown for wheat, maize, and barley (Peng, J.R *et al*., 1999, Chandler P.M *et al*., 2002, Gubler *et al*., 2002). This information is also confirmed by data based on the amino acid homology of DELLA proteins, which were found in soybeans, tomatoes, grapes, and plants of the Argyroxiphium genus (Boss, P.K *et al ., 2002,* Bassel, G.W *et al* ., 2004)

The GA biosynthetic pathway has been elucidated by a combination of biochemical and genetic approaches. The first few steps of the pathway, from Tran's geranyl geranyl diphosphate to GA12-aldehyde, are common to all species. The final steps to produce active GAs are species specific but in most cases require activity of the GA 20-oxidase (GA20ox) and GA3ox enzymes. In contrast, the enzyme GA2ox antagonizes GA activity by deactivating GAs. The level of endogenous active GA is governed by feedback regulation, where active GAs suppress the expression of the GA20ox and GA3ox genes and promote the expression of the GA2ox gene (David W. and Naomi O. , 2007) .

Currently, we have a good understanding of the GA metabolic pathway. A combination of the biochemical and molecular approaches that led to the purification of some enzymes and their genes in species, such as pumpkin, using classic forward genetics performed mainly in Arabidopsis and rice, has allowed the discovery of the main players involved in the GA biosynthetic and catabolic pathways (Fig. 2).

The first stage in the GA biosynthesis pathway takes place in plastids and starts with the synthesis of ent -kaurene from geranylgeranyl diphosphate (GGDP), a common precursor for diterpenoids, chlorophylls, or carotenoids (Lichtenthaler , 1999). Most of the GGDP devoted for the GA biosynthesis is provided by the methylerythritol phosphate pathway in the plastid, although there is also a minor contribution from the cytoplasmic mevalonate pathway (Kasahara *et al*.; 2002). Two terpene synthases participate in the conversion of GGDP to ent -kaurene: ent -copalyl diphosphate synthase (CPS) and ent- kaurene synthase (KS) (Sun and Kamiya 1994; Saito *et al*., 1995; Yamaguchi *et al*., 1998b). These two steps were defined genetically with the GA-sensitive, severe dwarf Arabidopsis mutant's ga1 and ga2 (Koornneef and Van der Veen, 1980). CPS and KS are both encoded by a single gene in Arabidopsis as in many plant species, thus explaining the strong phenotype conferred by the null alleles. The expression pattern of CPS is cell-type specific in Arabidopsis with very low levels of transcript throughout development and high expression associated to active growing tissues (Silverstone *et al*., 1997a). A similar expression pattern has been described for KS gene but with the overall amount of transcript being higher than that of CPS (Silverstone *et al*., 1997a; Yamaguchi *et al.* , 1998b) , suggesting that the expression and location of CPS control the synthesis of ent- kaurene, what is supported by the dramatic increase in ent -kaurene accumulation in Arabidopsis lines over expressing CPS , whereas no changes are detected in lines over expressing KS (Fleet *et al*., 2003). Interestingly, over expression of either CPS or KS genes in transgenic Arabidopsis lines does not result in increased levels of GAs, indicating that these two steps are not limiting (Fleet *et al*., 2003).

In the next stage, ent -kaurene is converted to GA 12 by the consecutive action of two cytochrome P450 monooxygenases: the ent -kaurene oxidase (KO) catalyzes the conversion of ent- kaurene to ent-kauronic acid (Helliwell *et al.* , 1998) , which is subsequently converted to GA 12 by an ent -kauronic acid oxidase (KAO) (Helliwell *et al.* , 2001a). The step catalyzed by KO was defi ned genetically with the GA-sensitive dwarf mutant ga3 (Koornneef

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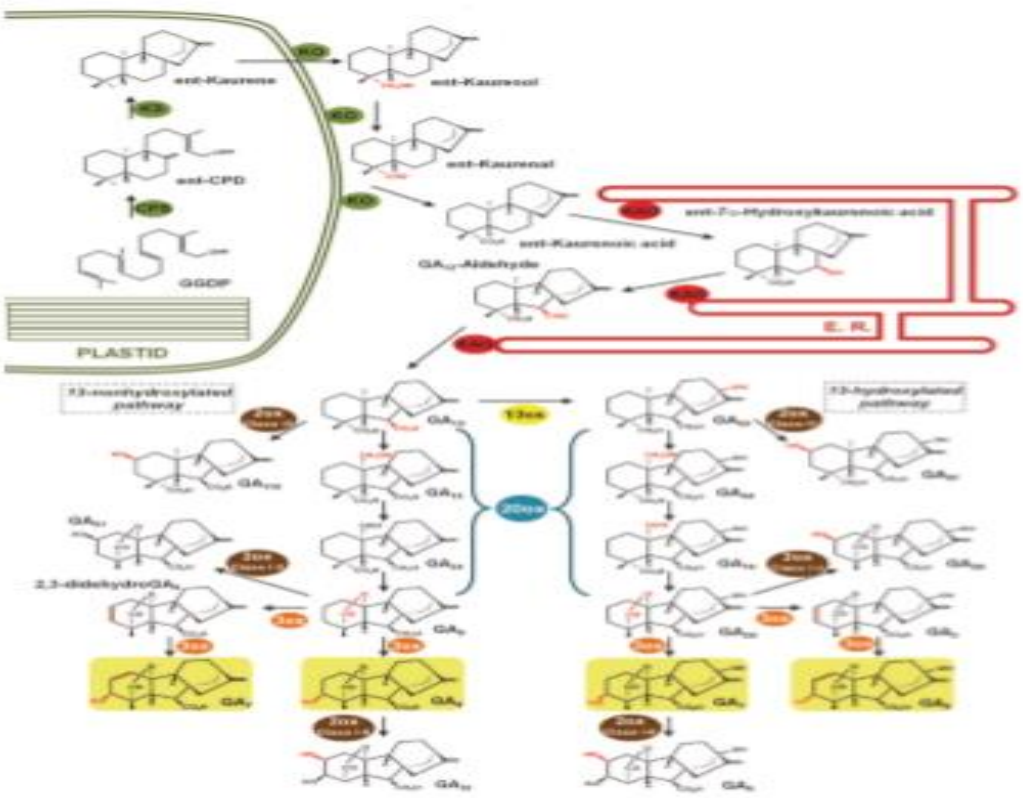
and Van der Veen, 1980). Transient expression experiments of green fl uorescent protein fusions indicate that KO is mainly present in the cytosolic side of the outer membrane of the plastid, whereas KAO is located in the endoplasmic reticulum (ER) (Helliwell *et al.*, 2001b) . KO is encoded by a single gene in most species whereas KAO is encoded by two gene copies in some species, such as Arabidopsis (Yamaguchi, 2008). In this species, both AtKAO1 and AtKAO2 are expressed in all tissues examined (Helliwell *et al.*, 2001a) whereas some specificity has been found for the expression of these genes in pea, for instance, PsKAO2 is detected only in seeds, thus explaining the normal seed development in the dwarf mutant na , which is defective in PsKAO1 (Davidson *et al.*, 2003).



Source : Eugenio G et *al.*, 2014

Fig. 2: The GA metabolic pathway. CPS ent -copalyl diphosphate synthase, KO ent -kaurene oxidase, KAO ent-kaurenoic acid oxidase, 13ox GA 13-oxidase, 20ox GA 20-oxidase, 3ox GA 3- oxidase, 2ox GA 2-oxidase. Active GAs is highlighted in yellow. Modifi cations in GA molecules due to the preceding enzymatic activity appear in red. E.R. Endoplasmic reticulum

2.4. Model of plant gibberellins signaling pathways

GA signaling pathways are very conservative in the plant world, probably due to the structural and functional conservatively of DELLA proteins; this has been shown for wheat, maize, and barley (Chandler, P.M., 2002). The DELLA proteins (RGA, GAI, RGL1, RGL2, and perhaps RGL3) are putative transcriptional regulators that directly or indirectly inhibit GA-activated genes (Tai-ping Sun and Frank Gubler , 2004).

The mechanisms of GA perception are conserved, showing agreement in Arabidopsis and rice. The GID1 gene was identified through map based cloning of a GA-insensitive mutant in rice, where there is a single copy of the gene (UeguchiTanaka *et al*., 2005). GA-insensitive GID1 mutants have defined a single barley homolog, GSE1 (Chandler *et al.*, 2008), and three Arabidopsis homologs, GID1a, GID1b, and GID1c (Willige *et al*., 2007).

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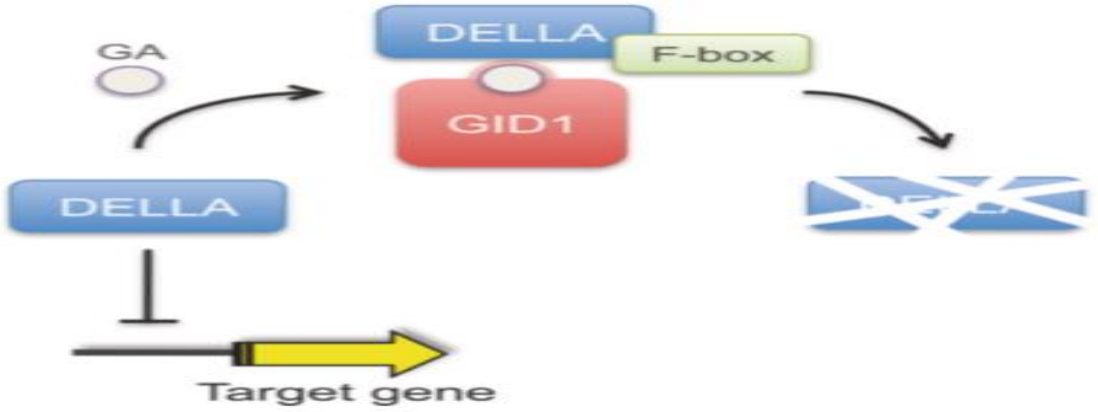

Source: (E.G. Minguet *et al*., 2014)

Fig.3 Scheme of the GA signaling pathway. When GA levels are low, DELLAs accumulate and regulate transcription of target genes. On the contrary, when hormone levels increase, the GA-loaded GID1 receptor is able to interact with the DELLA protein, thus facilitating its ubiquitination and degradation mediated by the F-box protein SLY1

2.5. DELLA-dependent and -independent gibberellin signaling

The founder member of the DELLA family of transcriptional regulators was the Arabidopsis GAI (Peng *et al.*, 1997). GAI was originally isolated in Arabidopsis as a semi dominant, GA-insensitive, and dwarf mutant, gai - 1 (Koornneef *et al.*, 1985). Mutant plants showed the morphological features typically caused by GA deficiency: reduced stature, dark-green color, and compactness, among others. However, two features in gai - 1 indicated that this mutant was not impaired in the GA metabolism: (1) the insensitivity to the hormone and (2) the accumulation of high levels of active GAs (Talón *et al.*, 1990), the latter indicating that it affected the feedback mechanism that normally operates to control the GA homeostasis (Hedden and Phillips , 2000). All these evidences together pointed out that this mutation hit in a protein with a central, negative role in either GA perception or signaling (Peng *et al.*, 1997). However, it was not until the isolation of a null allele of GAI, gai - t6, when it was unambiguously shown that the GAI protein performs a negative role in GA signaling, since the mutation conferred certain GA-independent growth: gai – t 6 plants were partially resistant to the growth-restraint effect of the GA biosynthesis inhibitor PAC (Peng *et al*., 1997). This ability of gai - t6 was shared with the newly identified recessive alleles of another locus, RGA (Silverstone *et al*., 1997b), that were identified based on their ability to suppress, to a certain extent, the dwarf phenotype of the GA-deficient mutant ga1 - 3.

DELLAs, a subset of the plant-specific GRAS family of putative transcription regulators, are key intracellular repressors of GA responses (Peng *et al*., 1997; Silverstone *et al*., 1998; Ogawa et al., 2000; Ikeda et al., 2001; Chandler *et al*., 2002). DELLAs repress seed germination, growth and almost all known GA-dependent processes, whereas GA relieves their repressive activity (Achard and Genschik , 2009).

All DELLA repressors have an N-terminal DELLA regulatory domain containing the conserved amino acid sequence Asp-Glu-Leu-Leu-Ala (DELLA) and a C-terminal GRAS (for GAI, RGA, and SCARECROW) functional domain (Pysh *et al*., 1999; Itoh *et al*., 2002). The N-terminal DELLA regulatory domain is an intrinsically disordered domain that folds and becomes structured upon GID1 protein binding (Sun *et al.*, 2010).

DELLA proteins act as growth repressors by inhibiting GA signaling in response to developmental and environmental cues (Takeshi Ito *et al*., 2018). Rice (Oryza sativa) contains one DELLA, SLENDER1, while Arabidopsis thaliana contains five DELLAs, GIBBERELLIN-INSENSITIVE (GAI), REPRESSOR OF ga1–3 (RGA), RGA-LIKE1 (RGL1), RGL2, and RGL3, which display partially

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overlapping but distinct functions in repressing GA responses (Dill A and Sun T, 2001) . The high homologous DELLA protein repressors GAI, RGA, and, probably, RGA LIKE1 (RGL1) serve as elongation repressors (Dill, A. and Sun T, 2001), Sasaki Aetal 2003). RGA, RGL1 and RGL2 jointly repress petal and stamen development (Cheng H *etal* ., 2004) , Tyler L *etal* ., 2004) and RGL2 is a key negative regulator of grain germination (Lee S *et al* ., 2002) The functional role of RGL3 has not been determined yet Hussain A *et al* ., 2007). Many of the mutations that modify GA sensitivity affect genes encoding members of the RGA/GAI family. The RGA/ GAI family is a subset of the larger GRAS family (Pysh *et al*., 1999). SLEEPY1 (SLY1) and GIBBERELLIN INSENSITIVE DWARF2 (GID2) are F-box proteins in Arabidopsis and rice, respectively (Sasaki A *et al*., 2003). Recently, recessive GA-insensitive dwarf mutants of rice, gibberellin-insensitive dwarf1 (gid1), exhibiting phenotypes observed in GA-deficient plants, were identified (Sasaki *et al*., 2001). Upon GA-binding to a soluble GA receptor, GIBBERELLIN INSENSITIVE DWARF1 (GID1), DELLAs are recruited to the SCFSLY1/GID2 ubiquitin E3 ligase complex for poly ubiquitination and is subsequently degraded by the 26S proteasome (Griffiths J *et al*., 2006).

The uniqueness of the DELLA domain hints that this region may specify the role of the DELLA subfamily of GRAS proteins in GA response. Recent studies of the dwarf mutants containing the mutations in the DELLA domain illustrate that this domain is important for the inactivation of the DELLA proteins by the GA signal. The initial evidence came from the discovery that the gain-of-functiongai-1 allele contains an in-frame deletion in the GAI gene, which results in the loss of 17 amino acids spanning the DELLA motif (Peng *et al.*, 1997). (Peng *et al.*, 1997) hypothesized that deletions in the gai-1 protein make it a constitutive repressor of GA response. Similar internal deletions or N-terminal truncations in other DELLA proteins in different species also results in a GA-unresponsive dwarf phenotype (reviewed in Olszewski N, 2002).

2.6. Positive regulation of GA signaling

Genetic studies suggest that SLY1 in Arabidopsis and its ortholog GID2 in rice are positive regulators of GA signaling. Both SLY1 and GID2 encode homologous F-box proteins and function as subunits of the SCF E3 ligase complex, which is required for GA-mediated degradation of DELLA proteins (Dill *et al*., 2004). The sly1 null mutant fails to degrade DELLA proteins and exhibits GA-insensitive dwarf phenotypes (McGinnis *et al*., 2003). However, the sly1-10 dwarf phenotype is suppressed in the gai-t6 rga-24 double mutant (Dill *et al*., 2004; Fu *et al*., 2004). The direct interaction between SLY1 or GID2 and DELLA proteins has been demonstrated using the yeast two-hybrid assay, and further co immune precipitation analysis confirmed their roles in recruiting DELLA proteins and targeting them for degradation by SCFSLY1/ GID2 E3 ubiquitin-ligase proteins complex (Dill *et al*., 2004; Fu *et al*., 2004; Sasaki *et al*., 2003).

Positive regulation of GA signaling GAMYB is a GA-regulated MYB transcription factor that was first identified as an activator of α -amylase expression in barley aleurone cells (Cerco ´s M *et al* ., 1999) . GA-unresponsive dwarf mutants have identified several positive regulators of GA signaling. The dwarf1 (d1) (Mitsunaga S *et al* ., 1994) and GA-insensitive dwar f2 (gid2) (Sasaki A *et al., 2003)* mutants in rice and the sleepy1(sly1) (Steber CM. , 1998) mutant in Arabidopsis have a semi dwarf phenotype, but they cannot be rescued by GA treatment. Pharmacological studies in cereal aleurones suggest that the hetero trimeric G protein plays a role in GA signaling (Jones HD *et al*., 1998). This hypothesis is supported by the finding that D1 encodes a putative α-subunit of the hetero trimeric G protein (Fujisawa Y *et al*., 1999; Ashikari M et *al*., 1999). However, an alternative GA signaling path way must exist because the d1 null mutant is not as dwarf as a severe GA biosynthetic mutant, even though D1 seems to be a single gene in the rice genome (Ueguchi-Tanaka M *et al* ., 2000) .

PHOTOPERIOD-RESPONSIVE1 (PHOR1) encodes the armadillo-repeat (arm-repeat) protein, which is upregulated in potato leaves under conditions that induce tuberisation. PHOR1-antisense plants have a semi-dwarf phenotype similar to that of GA-deficient mutants and exhibit reduced GA responsiveness. A PHOR1::green fluorescent protein (GFP) construct was transported from the cytosol into the nucleus in response to GA treatment (Amador V., 2011) suggesting that PHOR1 acts as appositive regulator in GA signaling. The GA-insensitive dwarf1 (gid1) rice mutant has a GA insensitive dwarf phenotype (Sasaki A *et al*., 2001)**.** The GID1 gene encodes a member of the serine hydrolase family, which includes esterases, lipases, and proteases (Sasaki A *et al*., 2001, Ueguchi-Tanaka M *et al***., 2001)**

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2.7. Negative regulation of GA signalling

GA-insensitive mutants that are defective in the DELLA genes have been identified in screens of various plant species, such as Arabidopsis (repressor of ga1–3[rga] and gibberellic-acid insensitive [gai]), barley (slender1 [sln1]), maize (Dwarf 8 [D8]), wheat (Reduced height [Rht]), and rice (slr1) (Kenji Gomi and Makoto Matsuoka , 2003)

RGA and GAI are negative regulators of the gibberellins (GA) signal transduction pathway in Arabidopsis thaliana. These genes may have partially redundant functions because they are highly homologous, and plants containing single null mutations at these loci are phenotypically similar to wild type. Previously, rga loss-of-function mutations were shown to partially suppress defects of the GA-deficient ga1-3 mutant (Alyssa D. and Tai-ping S, 2001).

The DELLA proteins are members of the GRAS family, which also includes SCARECROW and SHORT ROOT (Pysh LD., 1999). In addition to the GRAS family consensus motifs, GA-signal-related DELLA proteins also contain unique motifs in their amino-terminal region called DELLA domains. These domains are absent from other GRAS proteins. The sequence of the Arabidopsis gai allele demonstrated that in-frame deletion mutations in the DELLA domain induced the GA-insensitive dwarf phenotype of gai mutants (Peng J *et al.*, 1997).

Similarly, wheat Rht-B1/Rht-D1 and maize D8 alleles also have an in-frame deletion in the DELLA or TVHYNP domain, respectively (Peng J *et al.*, 1999). Several negative regulators of GA signaling have been isolated by characterization of the recessive (loss-of-function) slender mutants and the dominant (gain-of function) GA-unresponsive dwarf mutants. One of the slender mutants, spindly (spy) in Arabidopsis, was first identified as a mutant seed that germinated in the presence of PAC (Jacobsen SE *et al.*, 2003). Additional spy alleles have been isolated as suppressors of the GA-deficient mutant ga1-3 and a GA-unresponsive dwarf gai-1 (Silverstone AL *et al.*, 1997; Wilson RN and Somerville CR. , 1995)

Another negative regulator of GA signaling, SHORT INTERNODES (SHI) in Arabidopsis has been identified by the dwarf phenotype of the dominant *shi* mutant that over expressed the *SHI* gene (Fridborg I *et al.*, 1999). SHI contains a zinc- finger motif, suggesting its potential role in transcriptional regulation or ubiquity-mediated proteolysis. Transient expression of SHI in barley aleurone cells inhibits GA induction of α-amylase expression, further supporting its role in GA signaling (Fridborg I., 2001). However, the loss-of-function *shi* alleles show no obvious phenotype, probably owing to functional redundancy of several homologous genes in Arabidopsis (Fridborg I *et al* . ., 2001).

Another negative regulator of GA signaling, RGA, by screening for Arabidopsis mutants that were able to suppress the GA-deficient phenotype of ga1-3 (Silverstone *et al*., 1997). The homozygous rga/ga1-3 double mutants, while still none germinating and male sterile, have larger leaves and a semi dwarf stature. Cloning of RGA revealed that RGA and GAI are 82% identical at the amino acid level and have hallmarks of transcriptional regulators, such as a nuclear localization signal, homopolymeric serine and threonine sequences, leucine heptad repeats, and an SH2-like domain (Peng *et al.* , 1997, 1999)

2.8. Genes of GA biosynthesis and their regulation

The availability of complete genome sequences for Arabidopsis and rice has enabled the identification of most of the genes involved in GA biosynthesis and deactivation in these species (Hedden *et al.*, 2001; Sakamoto *et al*., 2004). However, the list of genes (Table 1) is not complete and genes encoding enzymes with novel functions in GA biosynthesis or previously unknown classes of the known enzymes are still likely to be discovered. A common feature is that enzymes catalysing early steps in the pathway are encoded by single or limited numbers of genes, while the 2ODDs are encoded by gene families, the members of which differ in their spatial and temporal patterns of expression. This is consistent with these later genes being the primary sites of regulation. In Arabidopsis, CPS, KS and KO are present as single copies, while there are two fully redundant KAO genes. Although there are four CPS-like genes in rice (Sakamoto *et al*., 2004), only one, OsCPS1, appears to be involved in GA biosynthesis (Otomo *et al*., 2004). Similarly, mutant studies indicate that only one member from each of the nine-member KS-like and four-member KO-like gene families has a major role in GA production in rice (Sakamoto *et al*., 2004). In contrast to Arabidopsis, rice has a single KAO gene. Null mutations in these early genes cause severe pleiotropic phenotypic abnormalities, such as extreme stunting, that are characteristic of GA deficiency whereas the effects from loss of a functional 2ODD gene are much less severe, indicating that the paralogues are partially redundant, as a result of overlapping expression patterns or movement of intermediates between tissues. GA

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biosynthesis and deactivation are regulated by numerous developmental and environmental factors, much of this regulation acting on the 2ODDs, the activity of which have a major influence on GA content. This is illustrated for the GA 20oxidase in Arabidopsis by work with transgenic plants. Over expression of a GA20ox gene caused increased GA4 content, accelerated bolting and longer stems (Huang *et al.*, 1998; Coles *et al.*, 1999), whereas increasing expression of CPS and KS resulted in higher amounts of ent-kaurene and GA12, but had no effect on the levels of bioactive GAs or the phenotype (Fleet *et al.*, 2003

Table 1. Comparison of GA-metabolic genes in Arabidopsis and rice

Enzyme	*Arabidopsis*				Rice			
	Gene name	*Arabidopsis* locus	AGI locus identifier	References	Gene name	Rice locus	Accession number	References
ent-Copalyl diphosphate synthase	*AtCPS*	*GA1*	At4g02780	(1)	*OsCPS*		AP004872	(2)
ent-Kaurene synthase	*AtKS*	*GA2*	At1g79460	(3)	*OsKS*		OSJN00255	(2)
ent-Kaurene 19-oxidase	*AtKO*	*GA3*	At5g25900	(4)	*OsKO*	*D35*	AP005471	(5)
ent-Kaurenoic acid oxidase	*AtKAO1*		At1g05160	(6)	*OsKAO*		AP000616	(2)
	AtKAO2		At2g32440	(6)				
GA 20-oxidase	*AtGA20ox1*	*GA5*	At4g25420	(7)	*OsGA20ox1*		AC096690	(8)
	AtGA20ox2		At5g51810	(7)	*OsGA20ox2*	*SD1*	AP003561	(9)
	AtGA20ox3		At5g07200	(7)	*OsGA20ox3*		AP005840	(2)
	AtGA20ox4		At1g60980	(10)	*OsGA20ox4*		AC124836	(2)
	AtGA20ox5		At1g44090	(10)				
GA 3-oxidase	*AtGA3ox1*	*GA4*	At1g15550	(12)	*OsGA3ox1*		AC144738	(13)
	AtGA3ox2		At1g80340	(14)	*OsGA3ox2*	*D18*	AP002523	(13)
	AtGA3ox3		At4g21690	(10)				
	AtGA3ox4		At1g80330	(10)				
GA 2-oxidase* (I)	*AtGA2ox1*		At1g78440	(15)	*OsGA2ox3*		AP003375	(16)
	AtGA2ox2		At1g30040	(15)	*OsGA2ox4*		AC132485	(2)
	AtGA2ox3		At2g34555	(15)				
(II)	*AtGA2ox4*		At1g47990	(10)	*OsGA2ox1*		AC119288	(17)
	AtGA2ox6		At1g02400	(10,16)	*OsGA2ox2*		AP003143	(16)
(III)	*AtGA2ox7*		At1g50960	(19)	*OsGA2ox5*		AP005187	(20)
	AtGA2ox8		At4g21200	(19)	*OsGA2ox6*		OSJN00156	(20)

Source : (P. Hedden and S.G. Thomas. , 2006)

2.9. Gibberellin transport

Plants produce and accumulate appropriate levels of bioactive GAs to ensure normal growth. The fine-tuning of gene expression in GA biosynthesis and metabolism pathways coordinately control the levels of GAs (Hedden and Thomas, 2012). In addition, studies suggest the existence of local and long-distance GA transport in plants (Dayan *et al.*, 2012; Regnault *et al.*, 2015; Shani *et al.*, 2013; Tal *et al.*, 2016). Biochemical and micrografting experiments have demonstrated the translocation of GAs from synthetic sites to the tissues and organs that require GAs for growth and development (Renault *et al.*, 2016).

Studies in pea provided the first evidence for the transport of GAs from root to shoot, by employing grafting experiments in a GA biosynthesis mutant **na** mutant that blocks ent-7α-hydroxykaurenoic acid conversion to GA12-aldehyde, resulting in reduced levels of active GAs within the shoot (Ingram and Reid, 1987). After application of [2H, 3H]-labeled GA1, GA19, and GA20 to the Na rootstocks (the donor) in na scions grafted to Na rootstocks (na/Na grafts), the labeled GAs could be detected in the na scions (the receiver) (Proebsting *et al.*, 1992). Moreover in na/Na grafts, GA1 concentration of the na scions was normal, GA20 content increased, but GA19 was hardly translocated to shoot apices of the na scions, suggesting that GA20 was the major transported GA in peas (Proebsting *et al.*, 1992).

In the grass Lolium temulentum, GA5 was shown to be transported from the leaf to the shoot apex (King *et al.*, 2001). In Arabidopsis, GA4 application to a

single leaf significantly decreased the total number of leaves formed before flowering of the wild-type and induced flowering in the GA-deficient ga1-13 mutant, suggesting that GA4 likely acts as a mobile GA from the leaf to the shoot apex (Eriksson *et al*., 2006). In addition, de novo synthesis of bioactive GAs is not only necessary for stamen development, but also is transported to nearby tissues, such as petals, to support their growth (Hu *et al*., 2008). Further micro grafting experiments between the ga1-3 mutant and the wild-type Ler plants showed that the Ler scions could restore hypocotyls xylem expansion in the ga1-3 rootstocks, whereas impaired GA signaling did not affect xylem expansion systemically in Ler/ga1-3 grafts (Ler scions grafted to ga1-3 rootstocks). Thus, the mobility of the shoot-derived GAs contributes to regulate hypocotyl xylem expansion (Ragni *et al*., 2011). Similarly, leaf-derived GA1 and GA20 are mobile signals that induce GA-promoting internode elongation, cambial activity, and fiber differentiation in tobacco stems (Dayan *et al.*, 2012). The GA precursor GA12 is also a long-distance mobile GA signal through the vascular system. The shoot-to-root translocation of GA12 induces degradation of the DELLA proteins in roots (Regnault *et al*., 2015). Although endogenous GA12 easily moves throughout the plant and promotes the growth of recipient tissues and organs, the plant-produced GAs fail to compensate for the germination defects of progeny seeds in the GA-deficient ga1-3 mutant, suggesting that endogenous GAs are not transmitted to offspring in Arabidopsis (Regnault *et al*., 2016). The fluorescently labeled GA compounds (GA3-Fl and GA4-Fl) were shown to accumulate in the endodermis of the root elongation zone after application to Arabidopsis roots (Shani et al., 2013), which is consistent with previous studies that the endodermis is the major GA-responsive tissue in the roots (Shani *et al*., 2013; Zhang *et al.*, 2011b). Two transcription factors TEMPRANILLO1 (TEM1) and TEM2 negatively regulate trichome initiation from the mesophyll cells beneath the epidermis. Surprisingly, GA3-Fl accumulation in the mesophyll of rosette leaves in the Arabidopsis tem1-1 tem2-2 mutants was increased and distributed throughout a much larger leaf area in comparison with wild-type plants (Matías-Hernández *et al*., 2016). Indeed, TEM is known to inhibit GA biosynthesis, and also represses the expression of several GA transporters, including NITRATE TRANSPORTER1/PEPTIDE TRANSPORTER FAMILY (NPF) NPF2.3, NPF2.10, and NPF3.1 (Jiao, 2016). Therefore, TEM is essential for GA distribution.

Accumulating evidence suggests that the movement of GA across membranes does not occur by simple diffusion but requires transporter proteins that are strictly regulated during plant growth and development. The NPF family proteins were initially identified as nitrate or peptide transporters (Léran *et al.*, 2014; Tsay *et al*., 2007), and were later found to also transport auxin, ABA, GA, and/or JA (jasmonic acid) hormones (Chiba *et al*., 2015; Saito *et al.*, 2015). NPF3.1 has been proven to be a unique GA transporter in plants (Tal *et al.*, 2016). The AtNPF3.1 protein is targeted to the plasma membrane of root endodermis cells that accumulate bioactive GAs. Interestingly, expression of AtNPF3.1 is repressed by GA treatments, suggesting a feedback regulation. Another nitrate/peptide transporter GTR1 (glucosinolate transporter1, also known as NPF2.10) was identified as the high-affinity, proton-dependent glucosinolate-specific transporter (Nour-Eldin *et al.*, 2012).

Interestingly, GTR1 can also transport GA3. Consistent with this observation, the gtr1 mutant's exhibit severely impaired filament elongation and anther dehiscence (Saito *et al*., 2015). Therefore, levels of bioactive GAs in special tissues or cells are determined not only by local GA biosynthesis and catabolism but also by GA translocation through a GA transporter.

III. CONCLUSION

Gibberellins (GAs), a class of diterpenoid phytohormones, produced by plants and some fungi play an important role in modulating diverse processes throughout plant growth and development. So far, up to 136 different gibberellin molecules have been discovered, only a few of which are bioactive, such as GA1, GA3, GA4, and GA7. Recent studies on GA biosynthesis, metabolism, transport, and signaling, as well as cross talk between GA and other plant hormones and environmental cues have achieved great progress along with the advancement of molecular genetics and functional genomics. Accumulating evidences suggest that the "de-repression" model makes it possible to explain signal transduction mechanisms in GA action. Bioactive GAs promote plant growth and development by promoting the degradation of the DELLA proteins, a family of nuclear growth repressors. The GA signal is perceived by the soluble receptor protein GIBBERELLIN INSENSITIVE DWARF1 (GID1) that undergoes a conformational change and then promotes GA-GID1DELLA association with the Skp1-Cullin-F-box (SCF) E3 ubiquitin-ligase complex via the F-box protein (SLEEPY1 [SLY1] in

Arabidopsis and GIBBERELLIN INSENSITIVE DWARF2 [GID2] in rice), thereby targeting the DELLA proteins for degradation via the 26S proteasome pathway. Evidence also shows that GAs act as mobile molecules that can pass through the plasma membrane for cell-to-cell transport. In this chapter, we focus on findings on GA biosynthesis, perception, and signal transduction pathways, highlighting how the evolutionary conserved GA-GID1-DELLA regulatory module is connected to developmental and environmental responses.

DELLA proteins act as negative regulators in gibberellin (GA) signal transduction. GA-induced DELLA degradation is a central regulatory system in GA signaling pathway. Intensive studies have revealed the degradation mechanism of DELLA and the functions of DELLA as a transcriptional regulator. Meanwhile, recent studies suggest the existence of a DELLA-independent GA signaling pathway. In this review, we summarized the DELLA-independent GA signaling pathway together with the well-analyzed DELLA dependent pathway

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