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Role of central nodes in growth regulatory networks: A review

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Abstract
Gibberellins (GAs) are a family of diterpenoid compounds produced by plants. The major bioactive GAs are GA_1, GA_4 and most agriculturally used is GA_3 which affect wide range of processes during seed germination, plant development, leaf expansion, stem elongation to flower initiation and development of flowers, fruits and seeds. DELLA proteins belong to a subfamily of the plant-specific GRAS protein (GAI, RGA and SCARECROW) and are negative regulators of GA signaling that act immediately downstream of the GA receptor. DELLA repressors have an N-terminal DELLA regulatory domain containing the conserved amino acid sequence Asp-Glu-Leu-Leu-Ala and C- terminal GRAS functional domain. The first DELLA protein isolated was the *ga-1*(gibberellins deficient mutant) of *Arabidopsis* as inframe deletion motif and its orthologues in other species encode nucleus-localized proteins that act as transcription factor and appearS to be negative regulators which includes several RGA (repressor-of-ga1-3), RGL1 (RGA-like1), RGL2 and RGL3 in the GA-signal transduction pathway. The role of DELLA mediated GA signaling in relation to the gibberellin- stimulated synthesis and secretion of alpha- amylase in aleurone layers are explained by biochemical and molecular mechanisms.

Keywords: gibberellins, DELLA proteins, germnation

Introduction
GA-DELLA pathway is involved in regulation of nitrogen deficiency-induced anthocyanin accumulation. They reported that DELLA proteins positively regulat nitrogen deficiency-induced anthocyanin accumulation through directly interaction with PAP1 to enhance its transcriptional activity on anthocyanin biosynthetic gene expressions Zhang *et al*., (2017) [38]. Identification of a new semi-dwarf mutant *ds-3* and its inheritance. A single nucleotide substitution in the N-terminal VHYNP domain of an *RGA* homolog confers the dwarfism of *ds-3*. Other *RGA* homologues in *B. napus* and characterized their expression patterns and activity in GA signaling pathway. Piskurewicz and Lopez-Molina (2009) [25] reported that GA-signaling repressor RGL3 represses testa rupture in response to changes in GA and ABA levels in *Arabidopsis.* Chen *et al.* (2012) [4] role of DELLAs in seed fatty acid (FA) metabolism by enhancement of gibberellin (GA) signaling through DELLA mutation or exogenous gibberellic acid (GA_3) which results in the up-regulated expression of transcription factors for embryogenesis and seed development genes involved in the FA biosynthesis pathway and five GDSL-type *Seed Fatty Acid Reducer (SFAR)* genes in *Arabidopsis thailana*.
The NaCl treatment in soybean cultivars (ND 12 and HD 19) represses seed germination by decreasing the GA / ABA ratio, levels of GA biosynthesis genes, increases GA inactive gene and *GmRGL* transcription expression gene (DELLA) which represses seed coat rupture during seed germination increased (Shu *et al.*, 2017) [27, 28]. The identification of the *DS-3* gene and characterization of thes *RGA* genes will facilitate the improvement of lodging resistance in oilseed rape (Zhao *et al*., 2017) [39, 40]. In the 1950s the second group of hormones, the gibberellins (GAs), was characterized. The gibberellins are a large group of related compounds (more than 125 are known) that, unlike the auxins, are defined by their chemical structure rather than by their biological activity. Gibberellins are most often associated with the promotion of stem growth, and the application of gibberellin to intact plants can induce large increases in plant height.
The biosynthesis of gibberellins is under strict genetic, developmental, and environmental control, and numerous gibberellin-deficient mutants have been isolated. Mendel's tall/dwarf alleles in peas are a famous example. Such mutants have been useful in elucidating the complex pathways of gibberellin biosynthesis. They have a role in regulating various physiological processes, including seed germination, mobilization of endosperm storage

reserves, shoot growth, flowering, floral development, and fruit set.

Rice farmers in Asia had long known of a disease that makes the rice plants grow tall but eliminates seed production. In Japan this disease was called the "foolish seedling," or *bakanae*, disease. This chemical was isolated from filtrates of the cultured fungus and called *gibberellin* after *Gibberella fujikuroi*, the name of the fungus. the structural feature that all gibberellins have in common, and that defines them as a family of molecules, is that they are derived from the *ent*kaurene

Ring structure

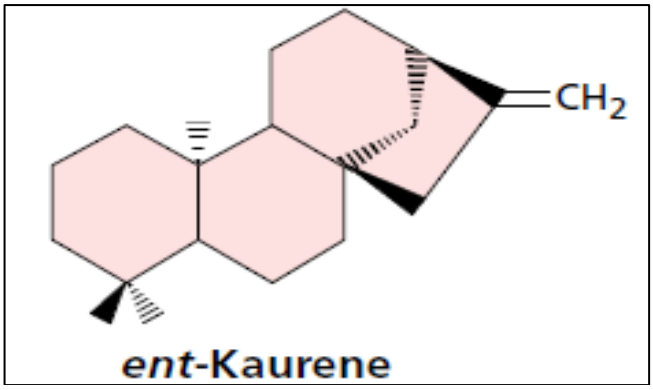


Fig 1: Ent-Kaurene

There are other variations in the basic structure, especially the oxidation state of carbon 20 (in C_{20}-GAs) and the number and position of hydroxyl groups on the molecule. Despite the plethora of gibberellins present in plants, genetic analyses have demonstrated that only a few are biologically active as hormones. All the others serve as precursors or represent inactivated forms.

Gibberellins Promote Seed Germination

Seed germination may require gibberellins for one of several possible steps: the activation of vegetative growth of the embryo, the weakening of a growth-constraining endosperm layer surrounding the embryo, and the mobilization of stored food reserves of the endosperm.

Some seeds, particularly those of wild plants, require light or cold to induce germination. In such seeds this dormanc can often be overcome by application of gibberellin. Since changes in gibberellin levels are often, but not always, seen in response to chilling of seeds, gibberellins may represent a natural regulator of one or more of the processes involved in germination.

Biosynthesis and Metabolism of Gibberellin

Gibberellins Are Synthesized via the Terpenoid Pathway in Three Stages. Gibberellins are tetracyclic diterpenoids made up of four isoprenoid units. Terpenoids are compounds made up of five-carbon (isoprene) building blocks. Researchers have determined the entire gibberellin biosynthetic pathway in seed and vegetative tissues of several species by feeding various radioactive precursors and intermediates and examining the production of the other compounds of the pathway (Kobayashi *et al*. 1996).

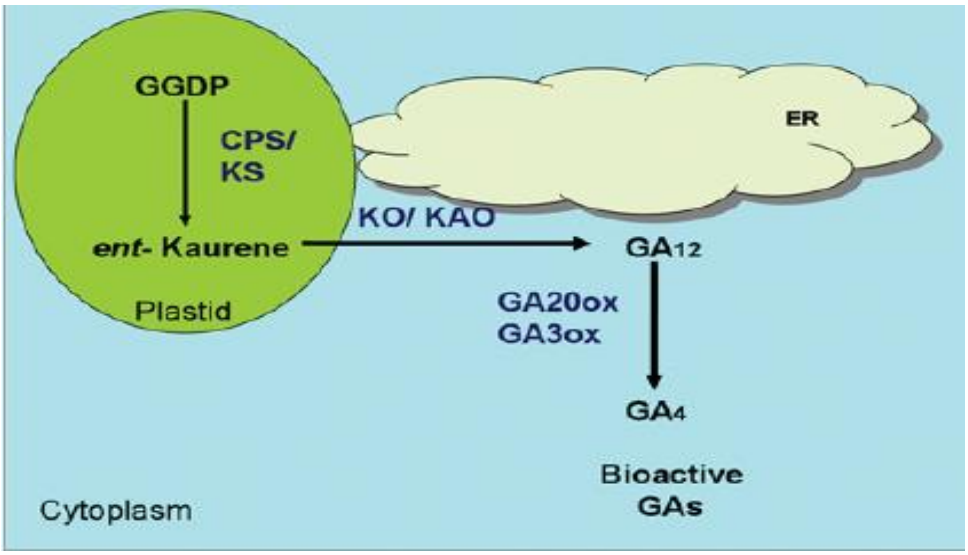


Fig 2: The three stages of gibberellin biosynthesis

The three stages of gibberellin biosynthesis. In stage 1, geranylgeranyl diphosphate (GGPP) is converted to *ent*-kaurene via copalyl diphosphate (CPP) in plastids. In stage 2, which takes place on the endoplasmic reticulum, *ent*-kaurene is converted to GA12 or GA53, depending on whether the GA is hydroxylated at carbon 13.

In most plants the 13-hydroxylation pathway predominates, though in *Arabidopsis* and some others the non-13-OH pathway is the main pathway. In stage 3 in the cytosol, GA12 or GA53 are converted other GAs. This conversion proceeds with a series of oxidations at carbon 20. In the 13 hydroxylation pathway this leads to the production of GA20. GA20 is then oxidized to the active gibberellin, GA1, by a 3β-hydroxylation reaction (the non-13-OH equivalent is GA4). Finally, hydroxylation at carbon 2 converts GA20 and GA1 to the inactive forms GA29 and GA8, respectively.

The Enzymes and Genes of the Gibberellin Biosynthetic Pathway Have Been Characterized

The enzymes of the gibberellin biosynthetic pathway are now known, and the genes for many of these enzymes have been isolated and characterized. Most notable from a regulatory standpoint are two biosynthetic enzymes-GA 20-oxidase (GA20ox) 3 and GA 3-oxidase (GA3ox)-and an enzyme

involved in gibberellins metabolism, GA 2-oxidase (GA2ox):

- GA 20-oxidase, catalyzes all the reactions involving the successive oxidation steps of carbon 20 between GA53 and GA20, including the removal of C-20 as CO2.
- GA 3-oxidase functions as a 3β-hydroxylase, adding a hydroxyl group to C-3 to form the active gibberellin, GA1. The evidence demonstrating that GA1 is the active gibberellins.
- GA 2-oxidase *inactivates* GA1 by catalyzing the addition of a hydroxyl group to C-2.

Della Protiens

DELLA proteins are negative regulators of diverse GA responses; GA stimulates germination by causing the disappearance of DELLA proteins. The DELLA family is defined by an N terminal DELLA domain equired for GA regulation and a C-terminal GRAS [GAI (GA-INSENSITIVE), RGA (REPRESSOR OF GA 1–3), and SCARECROW] domain required for function of these putative transcription factors.

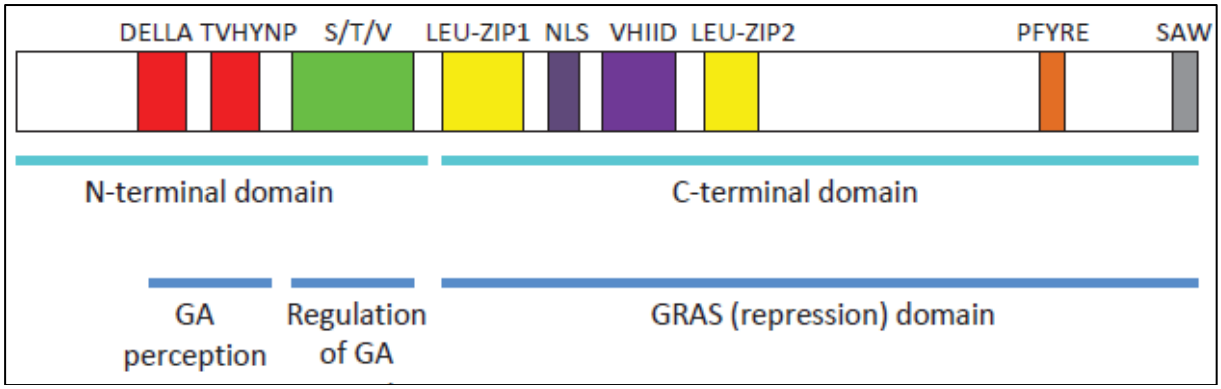


Fig 3: C-terminal GRAS [GAI (GA-INSENSITIVE), RGA (REPRESSOR OF GA 1–3), and SCARECROW]

Mutations in the DELLA domain result in gain-of-function GA signaling phenotypes resembling GA deficiency, whereas loss-of-function mutations in the GRAS domain typically result in an enhanced or constitutive GA response.

Although a single DELLA gene exists in rice and barley, the *Arabidopsis* genome contains five DELLA genes. *RGL* (*RGA-LIKE*) *2* appears to be the major DELLA protein regulating seed germination because loss of *RGL2* function partly restores germination in GA-deficient seeds, but single mutations in the other family members are not sufficient. However, combinations of *RGA*, *GAI*, and *RGL1* can enhance this seed germination rescue.

RGL2 mRNA and protein levels increase during cold imbibition, then decrease rapidly as germination approaches after transfer to 23^0C. RGL2 protein disappears within 5 h of GA application to imbibing GA-deficient (*ga1-3)* seeds, long before germination occurs. This pattern of expression suggests that RGL2 is a potent repressor of seed germination whose repression is lifted by GA-stimulated DELLA degradation.

Conversely, ABA stabilizes at least one DELLA, an RGA::GFP protein fusion. Recent evidence suggests that DELLA proteins promote seed dormancy through inhibition of cotyledon expansion prior to germination, possibly by repressing expression of hydrolytic and wall-modifying enzymes. GA relieves DELLA repression of seed germination through proteolysis by the 26S proteasome, triggered via polyubiquitination by the SCFSLY1/GID2 (Skp1-Cdc53/CUL-1/F-box protein, in this case SLY1/GID2) E3 ubiquitin ligase.

Arabidopsis SLY1 and rice GAINSENSITIVE DWARF 2 (GID2) are homologous F-box subunits of an SCF E3 ubiquitin ligase that ubiquitinates DELLA proteins, thereby targeting them for destruction in the presence of GA. The GAsignal is received by the *GID1*GA recepeptors. The GID1 protein undergoes a GA-dependent interaction with DELLA proteins, which promotes the interaction of DELLA with the F-box protein.

DELLA function may be regulated by mechanisms other than destruction. Although rescue of *sly1* dormancy by *RGL2* mutations suggests that *sly1* dormancy is due to RGL2 over accumulation, weak alleles and long after-ripened *sly1* seeds are able to germinate, although they retain high levels of RGL2 and RGA protein. This suggests that the RGL2 protein that accumulates in *sly1* mutant seeds is not fully active as a repressor of GA signaling. RGL2 may be subject to additional posttranslational regulation or germination may be regulated by parallel pathways.

RGL2 is subject to phosphorylation, and phosphomimic mutations stabilize the RGL2 protein. Mutations in the *Arabidopsis* O-linked β-N-acetylglucosamine (O-GlcNAc) transferase SPINDLY (SPY) lead to GA-independent germination and increased stability of DELLA proteins, suggesting that O-Glc-NAc modification may both activate DELLAs and stimulate their turnover. An alternative pathway mediating GA effects on dormancy release is suggested by microarray analyses demonstrating DELLA-independent GA-induced gene expression during stratification. Because *sly1* mutants are ABA hypersensitive, ABA catabolism may constitute one parallel pathway.

The della repressors of GA signaling are regulated by the ubiquitin-proteasome pathway

The N-terminal DELLA regulatory domain is an intrinsically disordered domain that folds and becomes structured upon GID1 protein binding (Sun *et al*., 2010). Mutations in the DELLA and TVHYNP regions of the regulatory domain interfere with the ability to bind the GID1 receptor, leading to a semidominant GA-insensitive dwarf phenotype due to the inability to down-regulate the DELLA repressor (Peng *et al*., 1997; Dill and Sun, 2001; Itoh *et al*., 2002; Silverstone *et al*., 2007; Asano *et al*., 2009) [24, 7, 18, 29].

Similar phenotypes have been detected in the PFYRE and SAW regions of the GRAS domain, which form secondary interactions with GID1 (Hirano *et al*., 2010) [15]. The DELLA regulatory domain also contains a Ser/Thr/Val-rich domain

believed to be involved in the regulation of DELLA function by phosphorylation, since deletion of both the TVHYNP and Ser/Thr/Val domains blocks DELLA phosphorylation (Itoh *et al*., 2005; Silverstone *et al*., 2007; Dai and Xue, 2010) [17, 29].

The DELLA gene of barley is called SLENDER1 (SLN1) and the DELLA gene of rice is called SLENDER RICE1 (SLR1), "tall" phenotypes resulting from loss-of-function mutations in the GRAS functional domain. The roles of the five Arabidopsis DELLA repressor genes have been determined based on the ability of loss-of-function alleles to rescue phenotypes of the ga1-3 GA biosynthesis mutant.

Mutations in REPRESSOR OF GA1-3 (RGA), GA INSENSITIVE (GAI), and RGA-LIKE (RGL1) rescue plant height; mutations RGA, RGL2, and RGL1 rescue flowering; while mutations in RGL2, RGA, GAI, and RGL3 rescue seed germination (Dill and Sun, 2001; King *et al*., 2001; Lee *et al*., 2002; Cheng *et al*., 2004, Tyler *et al*., 2004; Cao *et al*., 2005; Piskurewicz and Lopez-Molina, 2009) [7, 19, 21, 5, 25].

DELLA RGL2 has the strongest effect on seed germination, whereas DELLA RGA has the strongest effect on plant height. Promoter-swap experiments have shown that the partially specialized functions of these two Arabidopsis DELLA proteins appear to result mainly from tissue-specific gene expression (Gallego-Bartolomé *et al*., 2010) [11]. GA lifts DELLA repression of GA responses by targeting DELLA for destruction via the ubiquitinproteasome pathway. This model was originally based on the observation that rescue of dwarfism due to GA deficiency by GA treatment was associated with DELLA protein disappearance (Silverstone *et al*., 2001; Itoh *et al*., 2002) [18].

The ubiquitin-proteasome pathway was implicated when it was found that mutations in the F-box genes SLEEPY1 (SLY1) of Arabidopsis and the rice homolog GID2 resulted in a GA-insensitive phenotype associated with an inability to target DELLA for destruction (McGinnis *et al.*, 2003; Sasaki *et al*., 2003) [23, 26]. SLY1/GID2 is the F-box subunit of an SCF (for SKP1, CULLIN, and F-BOX) E3 ubiquitin ligase that catalyzes the polyubiquitylation of DELLA protein (McGinnis *et al*., 2003; Sasaki *et al*., 2003; Dill *et al*., 2004; Fu *et al*., 2004; Gomi *et al*., 2004; Hussain *et al*., 2009) [23, 26, 8, 10, 12, 16]

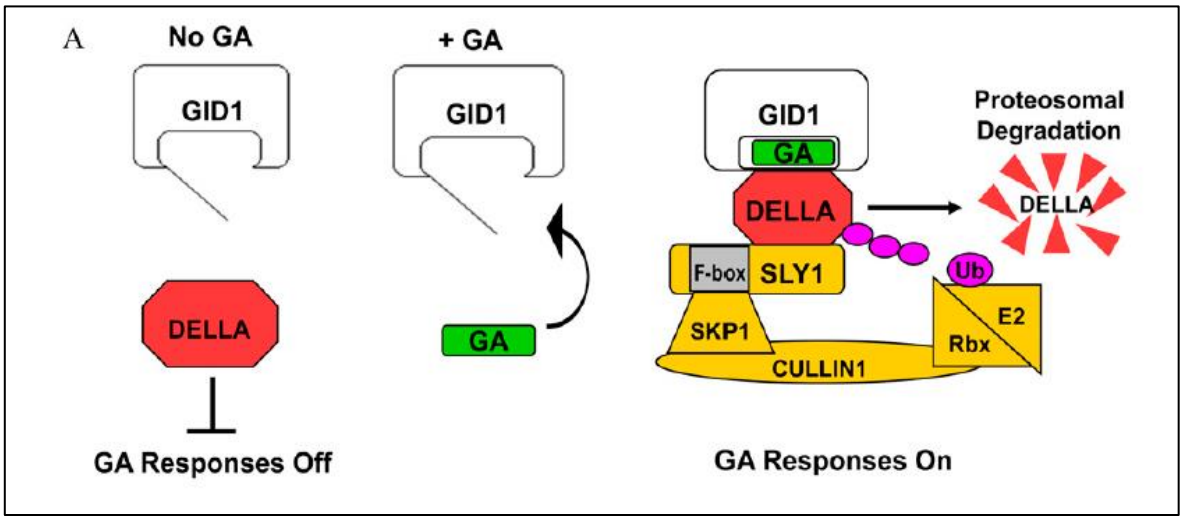


Fig 4 A: The canonical GA signaling model illustrating GA-dependent GID1-DELLA complex formation resulting in DELLA recognition and ubiquitylation by the SCFSLY1 E3. Polyubiquitylation leads to DELLA proteolysis by the 26S proteasome, thereby lifting DELLA repression of GA responses.

Nonproteolytic Mechanisms for Ga Signaling: Variations on the Theme

If all of GA signaling occurred through DELLA proteolysis, then the level of DELLA protein accumulation should always correlate with the severity of GAinsensitive phenotypes. This is not the case in sly1 and gid2 F-box mutants, which accumulate much more DELLA protein but show less severe GA-insensitive phenotypes than either GA biosynthesis or GA receptor null mutants (McGinnis *et al*., 2003; Willige *et al*., 2007; Ueguchi-Tanaka *et al*., 2008) [23].

It appears that DELLA can be inactivated by a nonproteolytic mechanism such that not all of the DELLA protein that accumulates in sly1/gid2 mutants is functional as a repressor of GA signaling (Ariizumi and Steber, 2007; Ariizumi *et al*., 2008; Ueguchi-Tanaka *et al*., 2008) [2]. A model in which DELLA repression is blocked by GID1- GA-DELLA complex formation alone was proposed based on the following evidence:

1. sly1/gid2 phenotypes are rescued by GID1 gene overexpression without a reduction in DELLA protein levels
2. GA signaling in sly1/gid2 requires all of the elements required for GID1-GA-DELLA complex formation, including GA hormone synthesis, the GID1 gene, and the DELLA domain required for DELLA-GID1 protein interaction.

In light of this, it appears that GID1 causes GA signaling in sly1 mutants by binding DELLA protein rather than by triggering DELLA proteolysis (Fig. 1B). Unlike Arabidopsis GID1a and GID1c, the GID1b protein binds GA with a higher affinity and shows some ability to interact with DELLA in the absence of GA. This explains why GID1b overexpression better rescues GAinsensitive sly1 mutant phenotypes (Ariizumi *et al*., 2008; Yamamoto *et al*., 2010) [2].

This GA-independent GID1b-DELLA binding may provide a low level of GA signaling in the absence of GA. Arabidopsis GID1b tends to have low expression levels, suggesting that control of GID1b accumulation may be essential to regulating basal GA responses (Griffiths *et al*., 2006; Willige *et al*., 2007) [13]. Because DELLA cannot be degraded in the sly1/gid2 F-box mutants, this background provides a tool for examining GA signaling mechanisms that precede DELLA destruction or may occur in situations where DELLA

destruction is delayed by ethylene signaling or lack of auxin signaling (Achard *et al*., 2003; Fu and Harberd, 2003) [1, 9]. Determining the contribution of proteolysis-independent DELLA downregulationto GA signaling without using a sly1/gid2 background requires the ability to directly assay DELLA protein function as well as its accumulation. Phenotypic assays are not useful here, since phenotype is the sum of events over time and can be influenced by multiple signaling pathways. Future work will need to develop a direct assay for DELLA function or use DELLA regulated promoters as reporters.

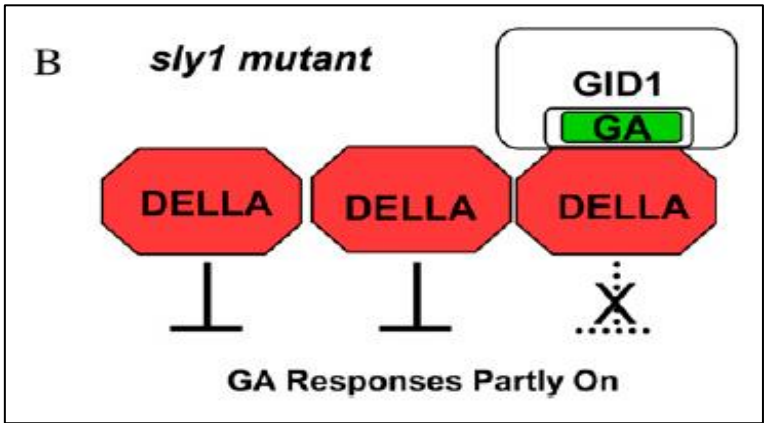


Fig 4B: Proteolysis-independent GA signaling in sly1 mutants occurs when GID1-GA-DELLA complex formation blocks DELLA repression of GA responses without DELLA destruction.

C. Phosphorylation and O-Glcnac Modification of Della Protein Activity

Evidence also suggests that DELLA activity can be influenced by phosphorylation and O-GlcNAc (for Olinked N-acetylglucosamine) modification. Studies indicate that DELLA is phosphorylated, but the precise role of this modification in controlling DELLA activity or protein degradation is unclear.

When DELLA phosphorylation was first observed in rice and in Arabidopsis, it was initially hypothesized that DELLA phosphorylation might increase DELLA affinity for the F-box protein SLY1/GID2, since target protein phosphorylation often stimulates F-box protein target binding in other systems (Sasaki *et al*., 2003; Fu *et al*., 2004; Smalle and Vierstra, 2004) [26, 10].

Initially, it appeared that rice DELLA SLR1 and Arabidopsis DELLA GAI showed stronger F-box protein binding when phosphorylated (Fu *et al*., 2004; Gomi *et al*., 2004) [10, 12]. However, kinetic studies of the SLR1-GID2 protein protein interaction demonstrated no difference in F-box GID2 affinity for phosphorylated or nonphosphorylated DELLA SLR1 (Itoh *et al*., 2005a) [17]. Protein phosphatase inhibitors appear to block the degradation of barley DELLA SLN1 and Arabidopsis DELLAs RGL2 and RGA, suggesting that phosphorylated DELLA is more resistant to degradation (Fu *et al*., 2002; Hussain *et al*., 2005; Wang *et al*., 2009) [16, 33].

The interaction network between the GA–GID1–DELLA signaling module and other internal and external cues.

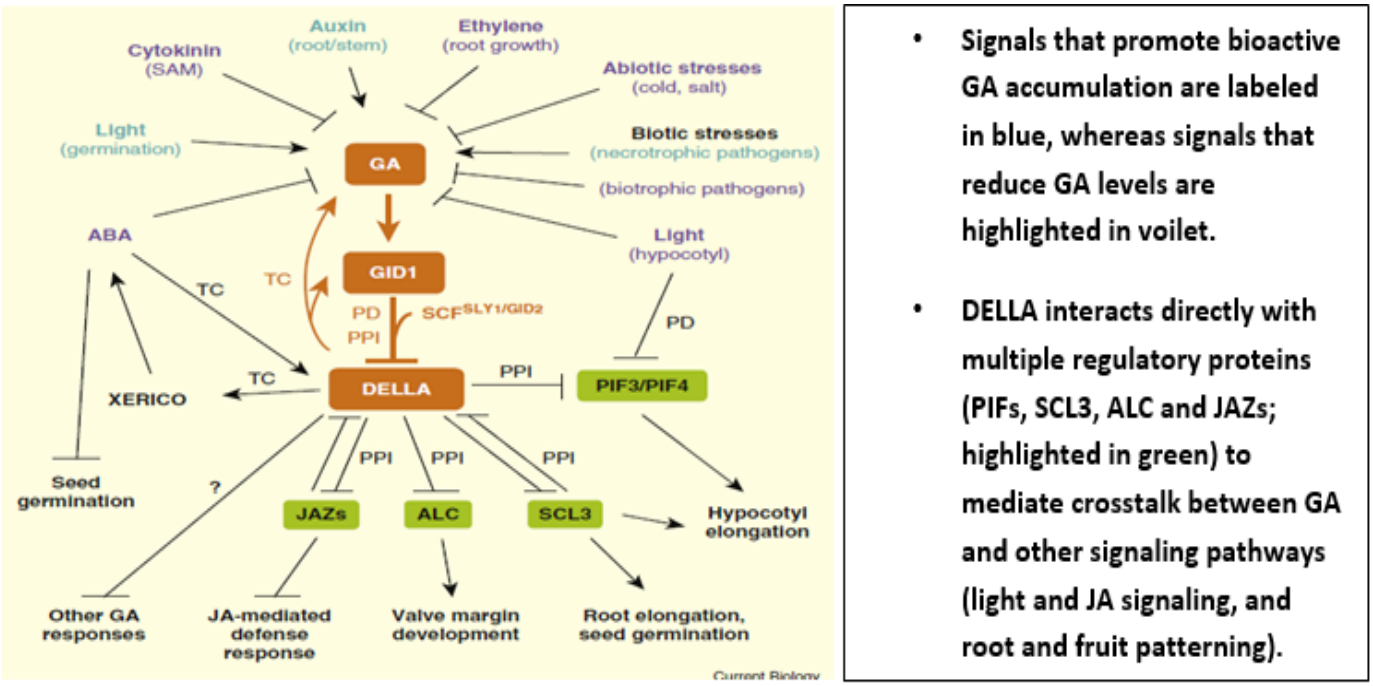


Fig 4C: The GA–GID1–DELLA regulatory module is highlighted in orange. Signals that promote bioactive GA accumulation are labeled in blue, whereas signals that reduce

GA levels are highlighted in purple. DELLA interacts directly with multiple regulatory proteins (PIFs, SCL3, ALC and JAZs; highlighted in green) to mediate crosstalk between GA and other signaling pathways (light and JA signaling, and root and fruit patterning). Activation or inhibition could be via different modes of action: PD, protein degradation; PPI, protein–protein interaction; TC, transcription. SAM, shoot apical meristem; ABA, abscisic acid; JA, jasmonic acid.

The first supporting evidence for this idea came from two studies reporting an interaction between DELLA and PHYTOCHROME INTERACTING FACTORs (PIFs), belonging to subfamily 15 of bHLH transcription factors in Arabidopsis. The DELLA–PIF interaction inhibits PIFinduced hypocotyl elongation by blocking the transcription of PIF's target genes.

In an effort to elucidate how DELLA proteins regulate plant growth and development, 14 putative DELLA target genes were identified by microarray studies using an inducible system . Chromatin immunoprecipitation (ChIP)– quantitative PCR analysis confirmed that DELLA associates with several promoters of its target genes.

Surprisingly, expression of all 14 genes is upregulated by DELLA and downregulated by GA. Several DELLA target genes encode GA biosynthesis enzymes or GA receptors, suggesting that DELLA plays a role in maintaining GA homeostasis by feedback regulation of positive components in the upstream GA pathway. Other DELLA-induced target genes encode putative transcription factors/regulators, or RING-type ubiquitin E3 ligases. XERICO, one of the DELLA target genes encoding an E3 enzyme, promotes accumulation of abscisic acid that antagonizes GA effects. Therefore, DELLA may restrict GA-promoted processes by modulating both GA and abscisic acid pathways. SCARECROW-LIKE 3 (SCL3), another DELLA-induced target gene, surprisingly functions as a positive regulator of GA signaling and an attenuator of DELLA proteins.

SCL3 is also a GRAS family member, although it does not contain the GA-responsive DELLA domain. The scl3 mutant is sensitive to the GA biosynthesis inhibitor paclobutrazol in seed germination, and hypocotyl and root elongation. Co-immunoprecipitation and transient expression assays indicate that SCL3 antagonizes DELLA function in modulating target gene expression by direct protein–protein interaction.

Importantly, the SCL3–DELLA interaction not only plays a role in controlling downstream GA responses, but also is involved in maintaining GA homeostasis by regulating expression of upstream GA biosynthetic genes. The upregulation of SCL3 mRNA levels by DELLA may be another part of the feedback mechanism to maintain GA homeostasis.

In the primary root, SCL3 mRNA is mainly expressed in the endodermis, which appears to be the primary site of GA-induced DELLA degradation. Expression of a stabilized DELLA mutant protein in the endodermis (but not in other cell types) inhibits root elongation.

Studies with various mutants indicate that SCL3 plays a role in determining the timing of the root ground tissue divisions, acting downstream of SCR and SHORT-ROOT (SHR), both of which are GRAS proteins that are essential for endodermis specification and stem-cell maintenance . Therefore, during root development, the SCL3–DELLA interaction integrates GA signaling activities with the developmental program controlled by SCR and SHR.

Table 1: Della proteins function as growth-repressors of GA signaling, which are highly conserved in *Arabidopsis* and other crop species.

CROP	DELLA PROTEIN GENE	LOCATION	FUNCTION
Rice	SLR1 SLENDER RICE 1	Throughout the plant	Hypocotyl elongation
Barley	SLN1 SLENDER RICE 1	elongation zone in the basal region of the leaves	Germination and seedling elongation
Maize	D8 Dwarf mutant	Kernels	sturdy stature in cropping
Wheat	Rht1 Reduced height	stem	Dwarf nature
Grape	VvGAI	fruit	Parthenocarpic fruits
Cotton	GhSLR1a and -b. SLENDER	Epidermal cells on surface of ovule differentiate	Fibre development

Gibberellin Signal Transduction: Cereal Aleurone Layers

The biochemical and molecular mechanisms, which are probably common to all gibberellin responses, have been studied most extensively in relation to the gibberellin-stimulated synthesis and secretion of α-amylase in cereal aleurone layers (Jacobsen *et al*. 1995) [14].

Gibberellin from the Embryo Induces α-Amylase Production by Aleurone Layers

Cereal grains (*caryopses*; singular *caryopsis*) can be divided into three parts: the diploid embryo, the triploid endosperm, and the fused testa–pericarp (seed coat–fruit wall). The embryo part consists of the plant embryo proper, along with its specialized absorptive organ, the *scutellum* (plural *scutella*), which functions in absorbing the solubilized food reserves from the endosperm and transmitting them to the growing embryo.

The endosperm is composed of two tissues: the centrally located starchy endosperm and the aleurone layer. The starchy endosperm, typically nonliving at maturity, consists of thin-walled cells filled with starch grains. The aleurone layer surrounds the starchy endosperm and is cytologically and biochemically distinct from it.

Aleurone cells are enclosed in thick primary cell walls and contain large numbers of protein-storing vacuoles called *protein bodies* enclosed by a single membrane. The protein bodies also contain phytin, a mixed cation salt (mainly Mg2+ and K+) of *my*inositolhexaphosphoric acid (phytic acid).

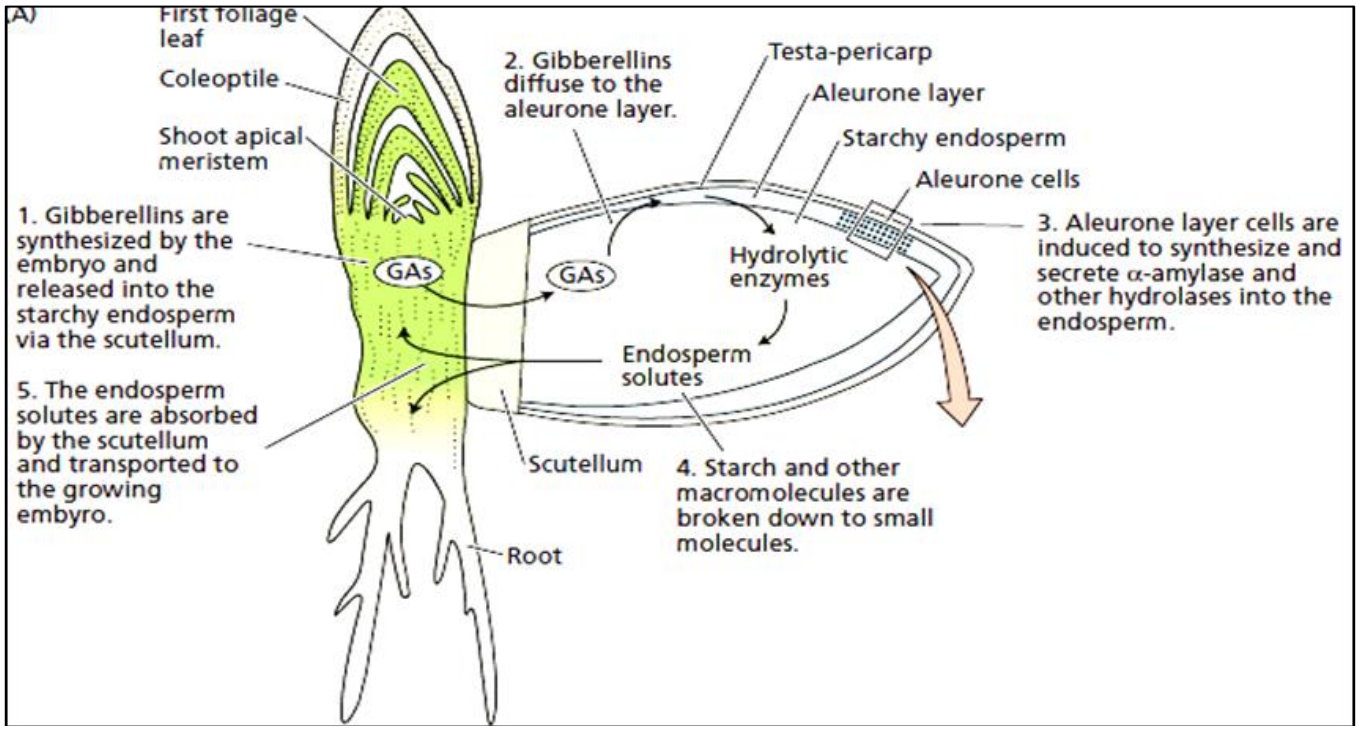


Fig 5: α-Amylase Production by Aleurone Layers

During germination and early seedling growth, the stored food reserves of the endosperm—chiefly starch and protein—are broken down by a variety of hydrolytic enzymes, and the solubilized sugars, amino acids, and other products are transported to the growing embryo. The two enzymes responsible for starch degradation are a -amylase and beta Amylase .

Alpha amylase hydrolyzes starch chains internally to produce oligosaccharides consisting of α-1,4-linked glucoseresidues. b-Amylase degrades these oligosaccharides from the ends to produce maltose, a disaccharide. Maltase then converts maltose to glucose. a-Amylase is secreted into the starchy endosperm of cereal seeds by both the scutellum and the aleurone layer. The sole function of the aleurone layer of the seeds of graminaceous monocots (e.g., barley, wheat, rice, rye, and oats) appears to be the synthesis and release of hydrolytic enzymes. After completing this function, aleurone cells undergo programmed cell death.

The significance of the gibberellin effect became clear when it was shown that the embryo synthesizes and releases gibberellins (chiefly GA1) into the endosperm during germination. Thus the cereal embryo efficiently regulates the mobilization of its own food reserves through the secretion of gibberellins, which stimulate the digestive function of the aleurone layer. Gibberellin has been found to promote the productionand/or secretion of a variety of hydrolytic enzymes that are involved in the solubilization of endosperm reserves; principal among these is a-amylase.

A GA-MYB Transcription Factor Regulates a -Amylase Gene Expression

The stimulation of a-amylase gene expression by gibberellins is mediated by a specific transcription factor that binds to the promoter of the a-amylase gene (Lovegrove and Hooley 2000) [22]. To demonstrate such DNA-binding proteins in rice, a technique called a *mobility shift assay* was used. This assay detects the increase in size that occurs when the a-amylase promoter binds to a protein isolated from gibberellin-treated aleurone cells.

The mobility shift assay also allowed identification of the regulatory DNA sequences (gibberellin response elements) in the promoter that are involved in binding the protein. Identical gibberellin response elements were found to occur in all cereal a-amylase promoters, and their presence was shown to be essential for the induction of a-amylase gene transcription by gibberellin.

These studies demonstrated that gibberellin increases either the level or the activity of a transcription factor protein that switches on the production of a-amylase mRNA by binding to an upstream regulatory element in the a-amylase gene promoter. The sequence of the gibberellin response element in the a-amylase gene promoter turned out to be similar to that of the binding sites for MYB transcription factors that are known to regulate growth and development in phytochrome responses.

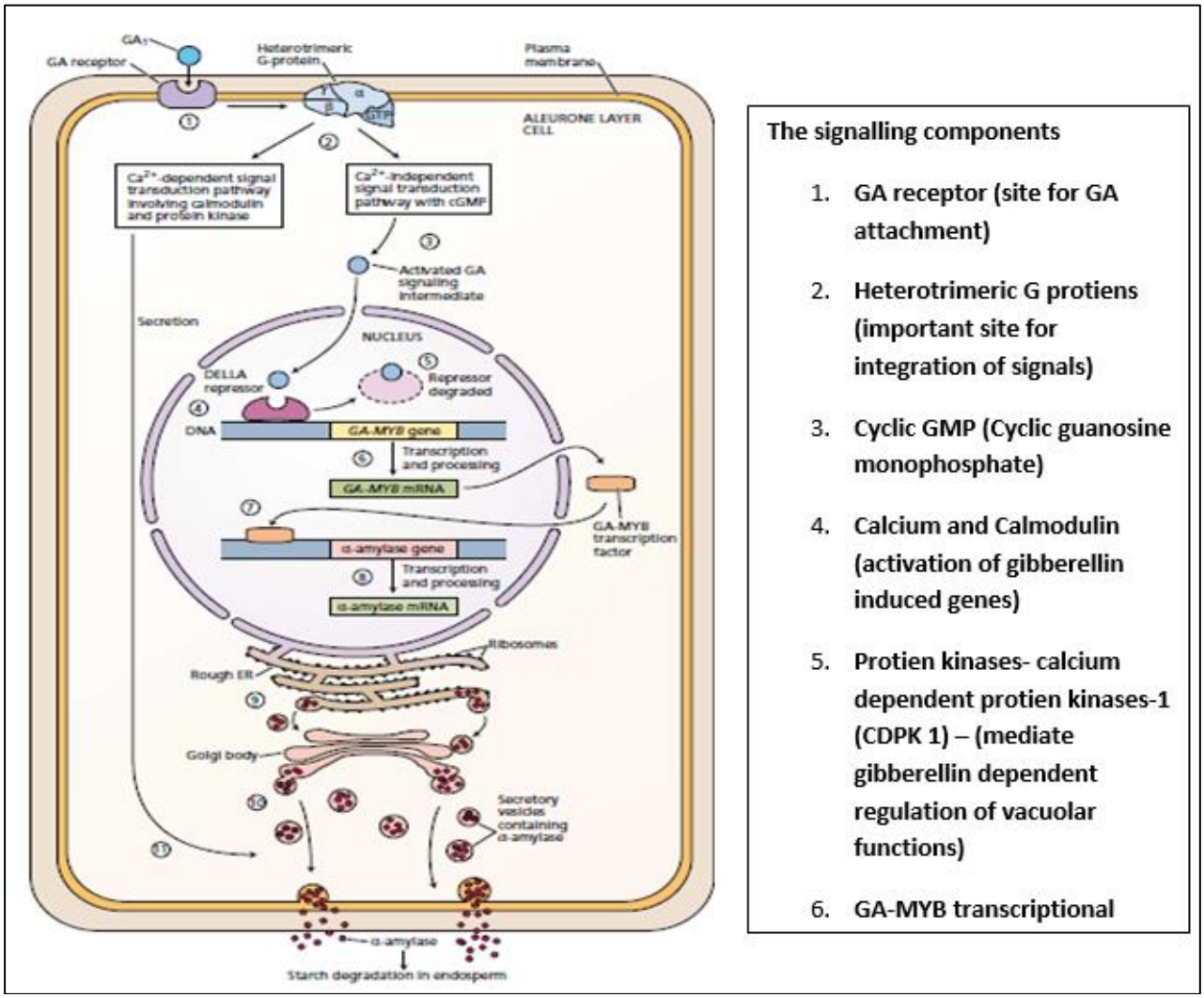
This knowledge enabled the isolation of mRNA for a MYB transcription factor, named GA-MYB, associated with the gibberellin induction of a-amylase gene expression. The synthesis of *GA-MYB* mRNA in aleurone cells increases within 3 hours of gibberellin application, several hours before the increase in a-amylase mRNA .

The inhibitor of translation, cycloheximide, has no effect on the production of *MYB* mRNA, indicating that *GA-MYB* is a *primary response gene*, or *early gene*. In contrast, the α-amylase gene is a *secondary response gene*, or *late gene*, as indicated by the fact that its transcription is blocked by cycloheximide.

In conclusion, gibberellin signal transduction in aleurone cells seems to involve G-proteins as well as cyclic GMP, leading to production of the transcription factor GAMYB, which induces a amylase gene transcription. a- Amylase secretion has similar initial components but also involves an increase in cytoplasmic calcium and protein phosphorylation.

1. GA1 from the embryo first binds to a cell surface receptor
2. The cell surface GA receptor complex interacts with a

heterotrimeric G protein, initiating two separate signal transduction chains
3. A calcium independent pathway, involving cGMP, results in the activation of a signaling intermediate
4. The activated signaling intermediate binds to DELLA repressor proteins in the nucleus
5. The DELLA repressors are degraded when bound to the GA signal
6. The inactivation of the DELLA repressors allows the expression of the *MYB gene*, *as well as* other genes, to proceed through transcription, processing, and translation.
7. The newly synthesized MYB protein then enters the nucleus and binds to the promoter genes for a-amylase and other hydrolytic enzymes.
8. a-Amylase and other hydrolases are synthesized on the rough ER.
9. Proteins are secreted via the Golgi.
10. The secretory pathway requires GA stimulation *via* a calcium–calmodulin dependent signal transduction pathway



GA induced destruction of GA signalling repressor-DELLA proteins in rice

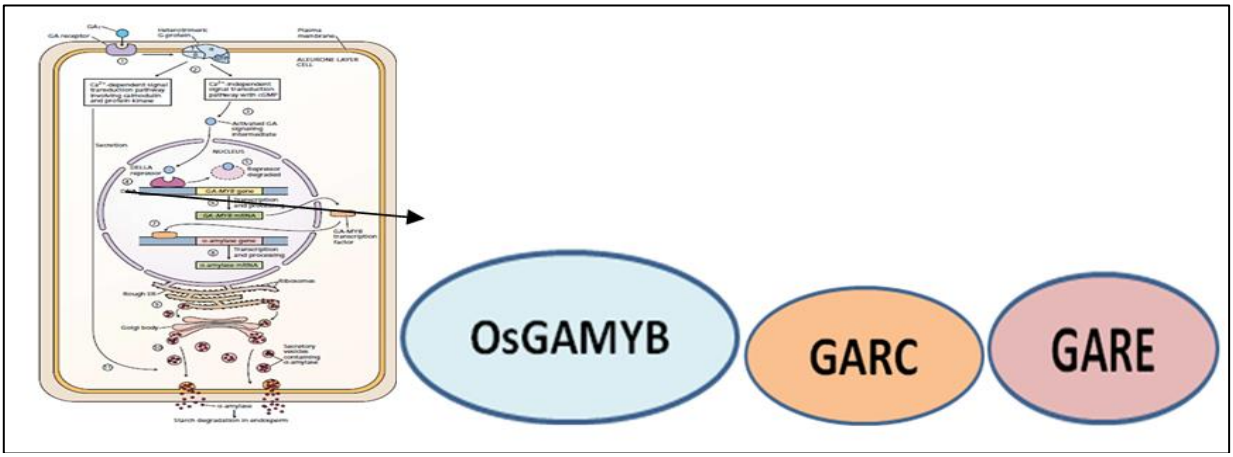


Fig 6: Regulation of a -Amylase Gene Expression

In the past decade, significant progress has been made, particularly in rice, in understanding GA perception, signal transmission, and modulation of GA-responsive targets. The present review specifically highlights the molecular mechanisms of GA-induced protein destruction of GA signaling repressor DELLA protein, and discusses the possible mechanisms underlying the regulation of GA-responsive gene expression in rice.

Most of the studies on the mechanisms for GA regulation of gene expression were performed in the cereal aleurone layer. The function of the cereal aleurone layers in germinated cereal grains is to synthesize and secrete hydrolytic enzymes to degrade the starch in the endosperm (Sun and Gubler 2004) [30]. It has been demonstrated that GAs can induce the expression of the genes encoding hydrolytic enzymes and the secretion of hydrolytic enzymes into the endosperm, where the starch and storage proteins are mobilized and degraded to facilitate embryo germination and seedling development.

GAMYB is the first identified positive transcription regulator of the GA-dependent expression of a-amylase and some other hydrolytic enzyme genes in cereal aleurone cells. In rice, one *GAMYB* gene, *OsGAMYB*, and two *GAMYB*-like genes, *OsGAMYBL1* and *OsGAMYBL2*, have been identified (Kaneko *et al.* 2004; Tsuji *et al*. 2006) [31]. High-level *OsGAMYB* expression was detected in aleurone cells, inflorescence shoot apical region, stamen primordia, and tapetum cells of the anther, whereas only lowlevel expression was found in organs at the vegetative stage or in the elongating stem in rice (Kaneko *et al*. 2004).

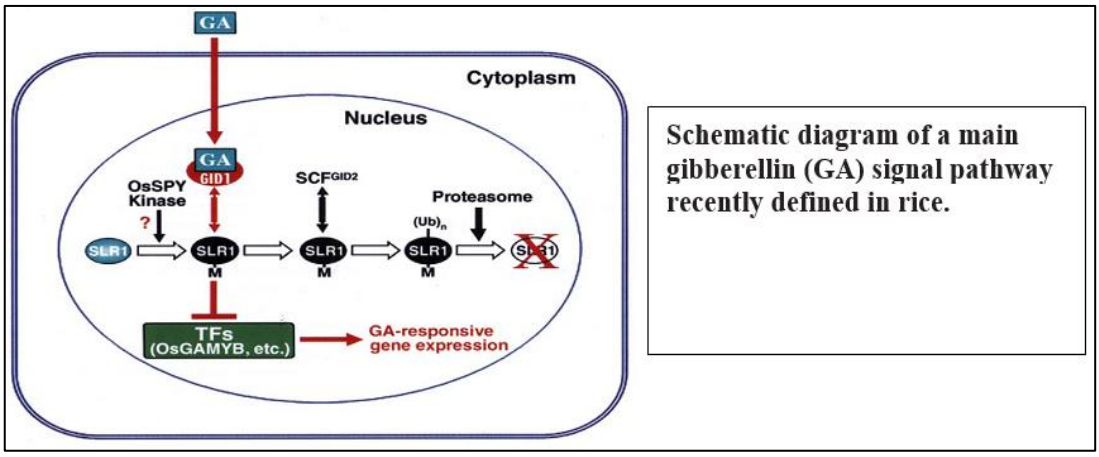
The lossof- function mutation by insertion of a retrotransposon, *Tos17*, abolishes the induction of a-amylase activity and *RAmy1A* (an a-amylase gene) expression in the endosperm in response to GA treatment, indicating an important role for OsGAMYB in the induction of a-amylase in the aleurone.

In addition, mutation of OsGAMYB causes no significant defects in growth and development at the vegetative stage, but does result in shortened internodes and floral organ defects, especially a defect in pollen development after the phase transition to the reproductive stage. These observations demonstrate that, in addition to its role in the induction of α-amylase in the aleurone, OsGAMYB plays an important role in floral organ and pollen development.

The results of a number of studies have led to the proposal that various GA-responsive complexes (GARCs) mediate the regulation of GA-regulated gene expression by GAMYB. Several GA-responsive *cis*-acting elements (GARE) and GARE like elements (TAACAA/GA, or TAACGTA) have been identified in the promoters of hydrolase genes expressed in the aleurone.

In addition, GARE and CARE are also present in a cysteine proteinase gene *REP-1*, which is expressed in rice aleurone and is induced by GAs and repressed by ABA. These two elements have been identified as necessary and sufficient for conferring GA inducibility of the *REP-1* promoter. Mutations of CARE in the promoters of *RAmy1A* and *REP-1* result in loss of GA inducibility and GAMYB transactivation, suggesting that CARE is a regulatory element for the GA-inducible expression of hydrolase genes in germinating seeds. In anthers, many, but not all, GAMYB-regulated genes contain GARE-like elements and some do not contain pyrimidine boxes and TAT boxes (Tsuji *et al.* 2006) [31].

Other transcription factors may cooperate with OsGAMYB in regulating gene expression. OsDOF3 belongs to another class of transcription factors that are capable of binding pyrimidine boxes. Cooperative function of GAMYB and the pyrimidine boxbinding protein OsDOF3 has been demonstrated in rice aleurone cells (Washio 2003) [24]. Transient expression of OsDOF3 in the germinated aleurone enhances GAMYB induction of reporter expression in the absence of GA.



GA signal pathway in rice

Upon binding to a biologically active GA molecule, a soluble GA receptor (mainly nuclear) acquires the ability to interact with SLR1 in the nucleus. This interaction leads to the destruction of SLR1 through the SCFGID2 proteasome pathway. SLR1 up- or down-regulates downstream gene expression, possibly via interacting with nuclear transcription factors (such as OsGAMYB) and negatively affecting their activities.

Eventually, altered gene expression leads to a variety of GA responses, TF, transcription factor. The exact roles of post translational modifications (M) of SLR1 by phosphorylation catalyzed by an unknown protein kinase or addition of *O*GlcNAc catalyzed by OsSPY for the destruction of SLR1 by proteasome have yet to be defined. Gibberellin may also act via a [Ca2+]cyt/Ca2+-dependent protein kinase (CDPK)/calmodulin (CaM) pathway to regulate SLR1 stability, possibly mediated by heterotrimeric G proteins .

Systems biology for a second green revolution

Plant growth is regulated by complex networks in which GAs represent important hubs. Modeling suggests that changes in DELLA levels, rather than their absolute basal levels, impact

growth rates, and certain GA responses are dependent on a fragile signaling balance: modest increases in DELLA protein levels (20–40%) can have large effects on growth. It was therefore suggested that the growth-controlling network around and down-stream of DELLAs is highly dynamic. Considering the diversity of GA responses, these networks are most likely to be cell type-specific. Embedded in these networks is a robust feedback signaling mechanism activated by DELLAs to ensure GA homeostasis; disruption of feedback signaling components, such as SCARE-CROW-LIKE3 (SCL3), an attenuator of DELLAs, can significantly impact growth. Although components of these growth-regulating networks have been identified e.g., for leaf growth. The overall topology is still unclear and is further complicated by factors such as tissue specificity and environmental modulation. This complexity needs to be addressed using systems biology approaches as outlined below. Our transcriptome meta-analysis clearly shows the necessity of tissue- and condition-specific sampling. Furthermore, many known protein interactions involving DELLAs take place in specific tissues under specific conditions, such as the DELLA–PIF interaction in hypocotyls exposed to red light. Increasingly small amounts of material can be harvested and profiled, as evidenced by the high-resolution Arabidopsis root transcriptome map. Furthermore, laser capture micro dissection technology or immune purification of epitope-tagged ribosomal proteins should assist in increasing spatial resolution, in particular when addressing transcriptomes. To dissect the proteome or interactome at higher resolution, larger model systems hold great promise, as was recently demonstrated by high-resolution hormone measurements along the growing maize leaf. Affinity purification of protein complexes coupled with mass spectrometry (AP-MS) and transcriptomics have revealed changes in growth-promoting protein complexes and GA-regulated genes over the transition zone in maize leave. Although many studies have focused on transcripts, and DELLAs ultimately function as transcriptional regulators, it is evident that protein interactions and protein degradation play crucial roles in growth regulation by GAs. To further illustrate the importance of protein interactions, genetic screens in wheat and barley for new DELLA alleles with improved agricultural potential resulted in mutations in domains known to be involved in the interaction between DELLAs and PIFs. How-ever, few large-scale proteome studies have been per-formed in planta after GA perturbation, and all known DELLA interactors were found by performing yeast two-hybrid screens. Numerous attempts to use AP-MS to identify protein complexes involving DELLAs in cell cultures or complete seedlings have failed to return interactors, showing the need for tissue-specific proteome-wide approaches that could reveal more about the dynamics of growth-regulating complexes involving DELLAs. Finally, to fully understand the complex nature of hormonal growth regulation, mathematical modeling will be indispensable. Recently, two studies have used modeling to study feedback loops in GA signaling and the role of GA dilution in the expansion zone of the root, greatly advancing our knowledge about GAs and growth. These models can be used as starting points to study growth regulation by GAs at the cellular and organ level, and predict interesting leads for genetic engineering or molecular breeding. Ultimately, models at multiple scales will be needed, in which sub cellular processes are used as inputs for a cell based growth and differentiation model that in turn serves as inputs for organ or even plant growth models. DELLAs fueled the genetic gains developed to improve plant productivity during the first green revolution, based on general disruption of GA signaling. However, because of the complex interplay between GAs and carbon metabolism, it is uncertain how the current green revolution genes will function at higher CO_2 levels, given that in Arabidopsis, dwarfism resulting from low GA levels can be rescued by increasing CO_2. Moreover, a second green revolution is necessary to improve plant productivity and yield stability in a changing climate while lowering agronomic inputs such as water and energy-consuming fertilizers. The central role for GAs in adapting growth to the environment suggests that GAs will again be crucial. A better understanding of the growth-regulating networks in which DELLAs function could provide tremendous potential for this second green revolution.

Conclusion

DELLA lead a pivotal role in regulating multiple hormone signals and represent a central integrator of GA-dependent processes for seed germination, dormancy regulator, abiotic stress management and seed storage life as it integrates different signaling activities by direct protein–protein interaction with multiple key regulatory proteins by various pathways. These proteins are much useful in overcoming the problem of preharvest sprouting in various crops during seed production.

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