Ubiquitin becomes ubiquitous in GA signaling

Tohru Ariizumi[a] and Camille M. Steber[ab]

[a]Dept. of Crop and Soil Science, Washington State University, Pullman WA 99164-6420
[b]USDA-ARS, Washington State University, Pullman WA 99164-6420

Research in a wide range of plant species has converged on a story where the hormone gibberellin (GA) stimulates critical stages in plant growth and development by triggering the destruction of negative regulatory DELLA proteins. GA stimulates seed germination, stem elongation, transition to flowering, and flower development in most plant species. Genes of the DELLA family act as repressors of these GA responses. It appears that GA stimulates GA responses by causing DELLA protein destruction via the ubiquitin-proteasome pathway in diverse species including rice, barley, and *Arabidopsis* (Gubler et al., 2002; McGinnis et al., 2003; Sasaki et al., 2003). This essay will use *Arabidopsis* as an example to discuss the current model for GA signaling via DELLA protein destruction, and will discuss the general notion of controlling plant growth and development by protein destruction.

DELLA proteins are a subfamily of the GRAS (GAI, RGA, and SCARECROW) family of putative transcription factors named for the conserved DELLA amino acid sequence found within the N-terminal domain (Peng et al., 1999; Pysh et al., 1999). The N-terminus is composed of the conserved DELLA domain required for negative regulation of protein accumulation by GA. The C-terminus is composed of the GRAS functional domain. There are five DELLA proteins in *Arabidopsis*, RGA (Repressor of ga1-3), GAI (GA-insensitive), RGL1 (RGA-like 1), RGL2 and RGL3 (reviewed by Thomas et al., 2005). These five proteins share high sequence homology, and the DELLA domain sequence, nuclear localization signal and GRAS functional domains are highly conserved in all five proteins.

Genetic analyses have demonstrated that the DELLA proteins act as negative regulators of GA responses. Loss-of-function mutations in DELLA genes result in a decreased requirement for GA, while mutations in the DELLA regulatory domain result in constitutive GA signaling (reviewed by Thomas et al., 2005). For example, loss of RGA function results in a reduced requirement for GA in stem elongation, whereas a deletion of the 17 amino acid DELLA regulatory domain causes a gain-of-function GA-insensitive dwarf phenotype (Dill et al., 2001). Although the functions of the five DELLA genes are partially overlapping, some of the DELLA proteins do have predominant roles in particular GA responses (Figure 1). Insights into these specialized roles have been obtained using double mutant studies with the severe GA-biosynthesis mutant of *Arabidopsis ga1-3*. The reduced GA levels in the recessive *ga1-3* mutant cause failure to germinate, dwarfism, late flowering, and reduced fertility. These phenotypes are rescued to varying degrees by loss-of-function mutations in DELLA genes. For example, mutations in *RGL2* can partially rescue *ga1-3* germination without GA application (Lee et al., 2002; Tyler et al., 2004; Cao et al., 2005). This rescue is enhanced by mutations in *RGA*, *RGL1*, and *GAI*. Thus, *RGL2* is the major negative regulator of seed germination, but other DELLA proteins also contribute. Similarly, *RGA* and *RGL2* are the major negative regulators of flower development and fertility, but *RGL1* also plays a role (Cheng et al., 2004; Tyler et al., 2004; Yu et al., 2004). *RGA* and *GAI* are the major negative regulators of stem elongation, but *RGL1* can also play a role (Dill and Sun, 2001; King et al., 2001; Wen and Chang, 2002; Cheng et al., 2004). No phenotypes have been reported for the RGL3 loss-of-function mutation.

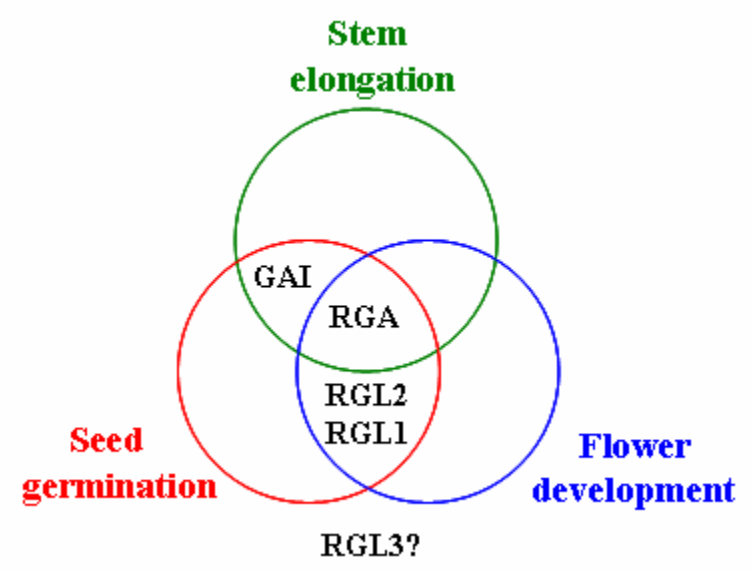

Fig. 1 The overlapping functions of Arabidopsis DELLA proteins in control of GA-stimulated stages in plant development. RGA negatively regulates stem elongation, seed germination and flower development. GAI is involved in stem elongation and seed development. RGL1 and RGL2 are involved in seed germination and flower development. The role of RGL3 is unknown.

How are DELLA proteins regulated in the GA signaling pathway? Although DELLA proteins repress GA responses in various stages of plant development, the DELLA proteins RGA, RGL2, and GAI all rapidly disappear following GA application (Dill et al., 2004; Fu et al., 2004; Tyler et al., 2004). This GA-triggered degradation relieves "growth repression" by DELLA proteins. GA appears to target DELLA proteins for destruction by ubiquitination.

Ubiquitin is a 76 amino acid protein that is covalently linked to proteins in order to flag them for proteolytic processing or destruction (reviewed by Smalle and Vierstra, 2004). Ubiquitin is covalently attached to a target protein via an ATP-dependent three-step cascade. In the final step, an E3 ubiquitin ligase catalyzes transfer of ubiquitin from the E2 ubiquitin conjugating enzyme to the target protein. Ubiquitin is attached to the target via an isopeptide bond between the C-terminal glycine of ubiquitin and a lysine residue of the target protein. Additional ubiquitin moieties can be added to lysine residues within ubiquitin. Formation of a polyubiquitin chain of 4 or more targets a protein to the 26S proteasome for destruction. The 26S proteasome essentially serves as a recycling machine, degrading proteins into amino acids.

The link between the ubiquitin-proteasome and specific signaling pathways is the E3 ubiquitin ligase. It is the E3 that recognizes and ubiquitinates a specific protein target. There are many types of E3 ubiquitin ligases. Examples of E3s include HECT domain, RING or U-box, Anaphase Promoting Complex, and SCF (Skp1, cullin, F-box) ubiquitin ligases. SCF complexes have been shown to be important regulators of DELLA proteins

in Arabidopsis and in rice (McGinnis et al., 2003; Sasaki et al., 2003). SCF complexes are composed of four subunits, a Skp1, cullin, RING finger protein and F-box protein (Figure 2, Zheng et al., 2002). It is the F-box protein that binds to a specific protein target via a C-terminal protein-protein interaction domain. The F-box protein binds to Skp1 (referred to as ASK or *Arabidopsis* Skp1 in *Arabidopsis*) via a conserved N-terminal F-box domain. Skp1 tethers the F-box to the N-termination of cullin. Cullin binds the RING finger protein which in turn binds to an E2 conjugating enzyme. The function of the E3 is to catalyze the transfer of Ubiquitin to the target protein, such as a DELLA protein. There are 698 predicted F-box proteins in the *Arabidopsis* genome (Gagne et al., 2002).

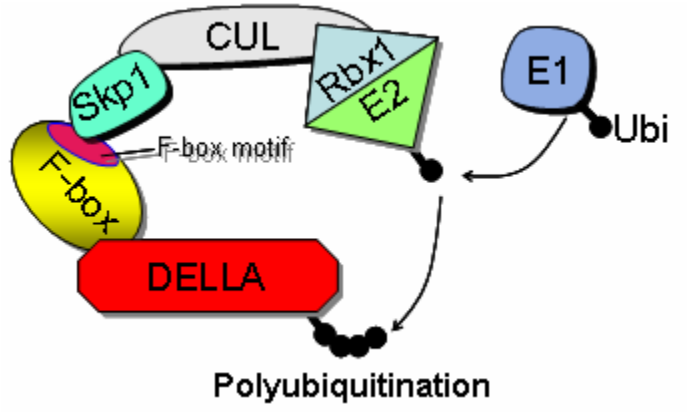

Fig. 2 The structure of the SCF E3 ubiquitin ligase complex based on the SCF^{Skp2} crystal structure (Zheng et al, 2002). The DELLA protein is the target for destruction. The F-box protein SLY1 binds DELLA via its C-terminal domain and interacts with ASK1 through the N-terminal F-box motif. The Cullin (CUL1) backbone binds the ASK1 and ring-finger protein RBX1. RBX1 binds the E2 ubiquitin conjugating enzyme, the source of ubiquitin. Ubiquitin (Ub) binds E1 (or Ubiquitin activating enzyme) via thiolester bond to the E1. Next, the ubiquitin moiety is transferred to the E2, which binds to the SCF E3 which catalyzes transfer of Ub from E2 to the DELLA substrate.

The DELLA proteins of GA signaling are negatively regulated by the F-box gene *SLEEPY1* (*SLY1*) gene in *Arabidopsis* (McGinnis et al., 2003; Dill et al., 2004; Fu et al., 2004). The predicted SLY1 protein contains an F-box motif located in N-terminal region, and appears to interact with the GRAS domain of DELLA proteins via the C-terminus LSL region (Dill et al., 2004; Fu et al., 2004). Because SLY1 is a negative regulator of the DELLA negative regulators, it is a positive regulator of GA signaling. Consistent with this, loss-of-function *sly1* mutant phenotypes resemble the *ga1-3* GA biosynthesis mutant in that they are dwarf plants with poor seed germination, reduced fertility, and delayed flowering. However, while the *ga1-3* mutant phenotypes are rescued by GA application, the *sly1* mutant is not. Thus, the *sly1* mutant is GA-insensitive. Other evidence indicating that SCF^{SLY1} acts as an E3 ubiquitin ligase in GA signaling include 1)

DELLA proteins accumulate at high levels in *sly1* mutant plants, 2) GA application does not cause DELLA proteins to disappear in the *sly1* mutant, and 3) the SLY1 protein directly interacts with the DELLA proteins (McGinnis et al., 2003; Dill et al., 2004; Fu et al., 2004; Tyler et al., 2004).

Although the SCF^{SLY1} E3 ubiquitin ligase is a major regulator of DELLA proteins, it is possible that DELLA proteins are also targeted for destruction by other E3 ubiquitin ligases including SCF^{SNE}, and the U-box PHOR1. SNE (SNEEZY) is a homolog of SLY1 in *Arabidopsis* that can partially rescue the *sly1* mutant phenotype when overexpressed (Fu et al., 2004; Strader et al., 2004). PHOR1 (PHOTOPERIOD RESPONSE PROTEIN 1) is a putative U-box E3 ubiquitin ligase that was originally identified as a GA signaling gene involved in short photoperiod induction of tuber formation in potato (Amador et al., 2001). It is possible that PHOR1 may regulate DELLA proteins by ubiquitination (Monte et al., 2003).

The GA signaling pathway is highly conserved within the plant kingdom. The SLY1 homolog in rice, the F-box protein GID2 (GIBBERELLIN INSENSITIVE DWARF2), was independently identified as a GA signaling gene that functions in the same way as SLY1 (Sasaki et al., 2003). Like SCF^{SLY1}, SCF^{GID2} ubiquitin ligase targets the DELLA protein, SLR1 (SLENDER RICE1) for degradation via 26S proteosome in rice plants. SLR1 also shares functional domains with *Arabidopsis* DELLA proteins. In addition to rice, DELLA proteins are structurally and functionally conserved in wheat, maize and barley (Peng et al., 1999; Chandler et al., 2002; Fu et al., 2002; Gubler et al., 2002). DELLA proteins have also been identified in soybean, tomato, grape, and Hawaiian Silver Sword based on amino acid homology (Boss and Thomas, 2002; Remington and Purugganan, 2002; Bassel et al., 2004).

The first step in GA signaling is perception of GA by a receptor. A GA receptor, GID1 (GIBBERELLIN INSENSITIVE DWARF1), has been identified in rice (Ueguchi-Tanaka et al., 2005). Although *GID1* encodes a soluble protein with homology to the mammalian hormone sensitive lipase (HPL), it does not appear to function as a lipase. While GID1 protein localizes mainly to the nucleus, GID1 can also be detected in the cytoplasm. There are three GID1 homologues in *Arabidopsis*, AtGID1A, AtGID1B, and AtGID1C, that can function as GA receptors (Nakajima et al., 2006). GID1 has a high affinity for bioactive GAs such as GA_3, GA_4 and GA_1, whereas it has a low affinity for inactive GAs. Once GID1 binds a bioactive GA, its interaction with the DELLA becomes stronger and more stable. Somehow this interaction appears to trigger DELLA ubiquitination and degradation through $SCF^{GID2/SLY1}$ and the proteasome pathway. The precise mechanism by which GA stimulates DELLA protein ubiquitination by $SCF^{GID2/SLY1}$ is not yet known. Although previous models suggested that phosphorylation of the DELLA protein was required for its recognition by $SCF^{GID2/SLY1}$ (Fu et al., 2004; Gomi et al., 2004), more recent data suggests that this is not the case (Hussain et al., 2005; Itoh et al., 2005).

A current model of the GA signaling pathway based on recent data from rice and Arabidopsis is shown in Figure. 3. In the absence of GA, DELLA protein negatively

regulates GA responses. In the presence of GA, GA binds the GID1 receptor in the cytoplasm and/or in nucleus. GID1-GA can then bind DELLA protein(s) probably in the nucleus, and this interaction triggers ubiquitination of DELLA by the $SCF^{GID2/SLY1}$ and its degradation by the 26S proteasome. The role of other posttranslational modifications, such as phosphorylation or O-GluNAc modification by the SPY (SPINDLY) protein in control of DELLA protein activity remain unclear (Swain et al., 2002; Hussain et al., 2005; Itoh et al., 2005). Although the discovery of a GA receptor has given us a better understanding of its signaling pathway, several questions remain. First, it is important to learn whether plants have other GA receptors, because several classic experiments employing cereal aleurone cells strongly suggest the existence of a membrane-bound GA receptor (Gilroy and Jones, 1994). It is not yet known how GA enters the cell to bind the cytoplasmic or nuclear localized GID1 GA receptor. It is not yet known whether GID1-GA binding to DELLA directly or indirectly stimulates ubiquitination by $SCF^{GID1/SLY1}$. Although sequence homology and nuclear localization suggest that DELLA proteins are transcription factors, this has not yet been proven. Also, the direct target(s) of DELLA protein regulation have not been identified. Finally, while it is known that DELLA proteins repress GA responses, it is not known if GA signaling genes ever act to stimulate GA responses.

Plants seem to have fully embraced the strategy of controlling protein function by destruction. Based on amino acid homology, an estimated 5% of the *Arabidopsis* genome consists of elements of the ubiquitin-proteasome pathway (Smalle and Vierstra, 2004). The use of the ubiquitin-proteasome to control GA signaling is conserved in species ranging from the monocots rice and barley to the dicot *Arabidopsis*. Why should plants control GA signaling and many other signals via the ubiquitin-proteasome pathway? At first glance, the strategy of controlling gene expression by protein destruction may seem wasteful. On the other hand, use of protein destruction may allow faster responses to the environment than changes in transcription. Since plants are photosynthetic organisms, they may be able to afford protein destruction as a ubiquitous strategy for rapid signal transduction.

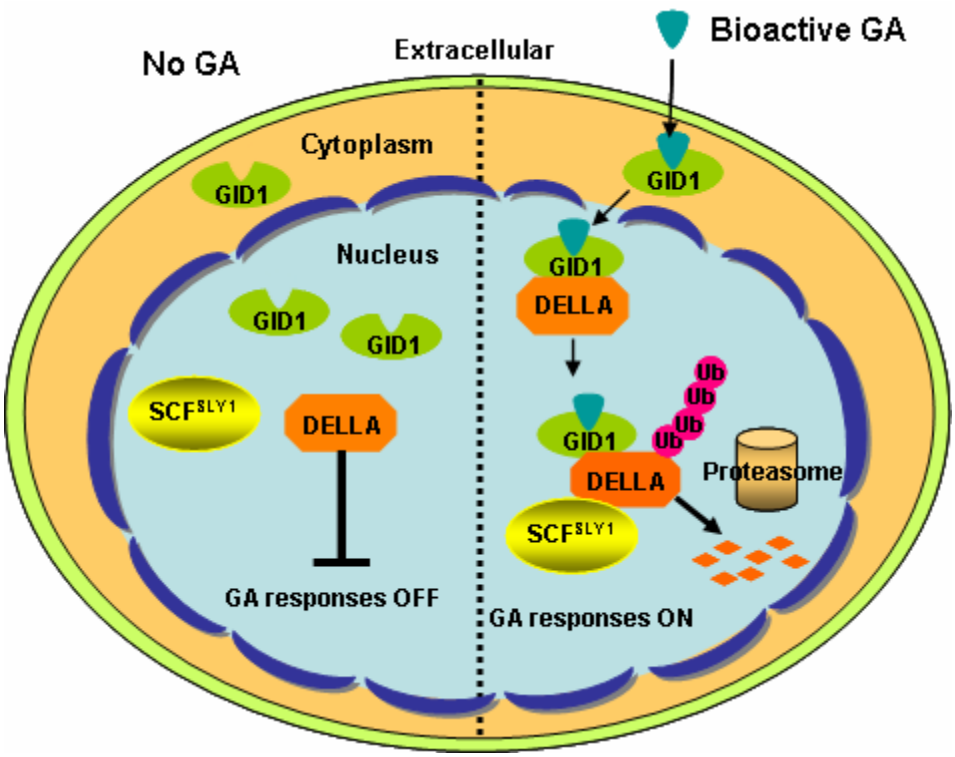

Fig. 3 A model for the GA signaling pathway in Arabidopsis. Left side: In the absence of bioactive GA, the SCF^{SLY1} E3 ubiquitin ligase cannot interact with DELLA proteins. Thus, the DELLA proteins persist in cells and repress GA responses such as seed germination, stem elongation and flowering. Right side: In the presence of bioactive GA, GID1 binds GA in the nucleus and/or cytoplasm. If the binding occurs in the cytoplasm, the GID1-GA complex probably transfers into nucleus to bind the nuclear localized DELLA proteins. Once the DELLA protein binds to GID1-GA, the SCF complex recognizes and ubiquitinates the DELLA protein. Ubiquitinated DELLA proteins are degraded by 26S proteasome allowing GA responses to occur.

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