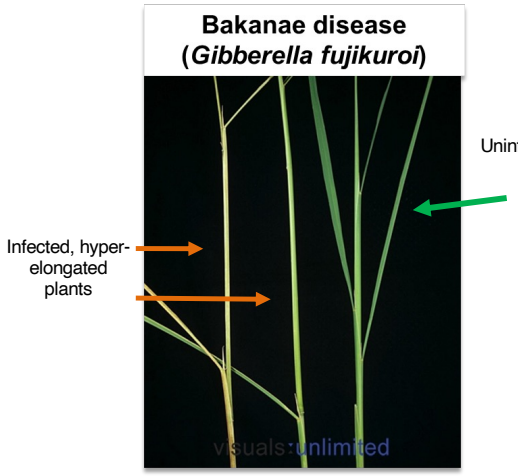
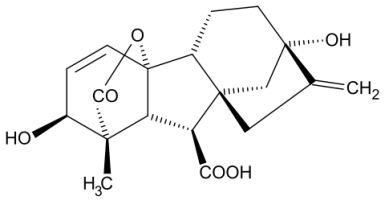
S2011
Hormones in Plant Development

8. Gibberellin

Gibberellins were identified as fungal compounds that promote stem elongation

Bakanae disease means "foolish seedling", because infected plants elongate too rapidly, and are unable to support themselves; they are also male sterile.





A Gibberellin (GA_3)

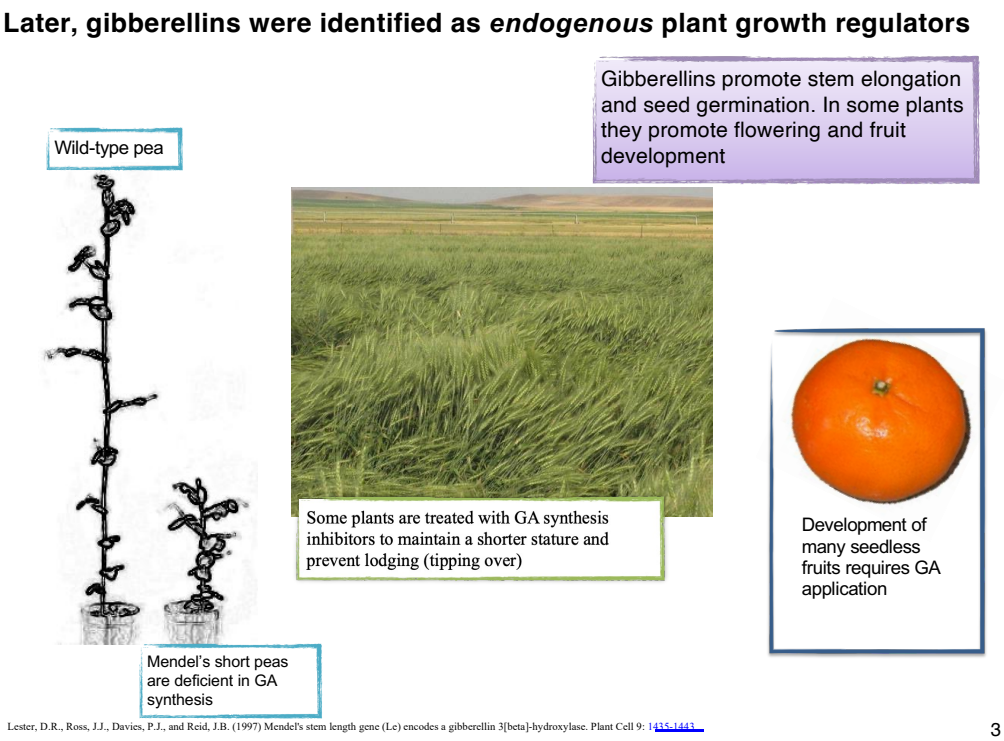


Figure 1. Mendel's Stem Length Trait.

Lines 205f (Le/Le; tall) and 205 (le/le; dwarf) of pea show the difference in stem length controlled by the *Le* locus.

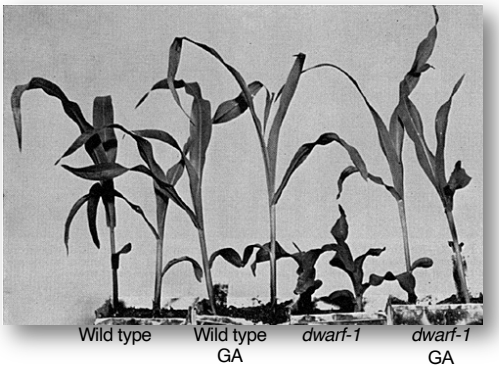
The manipulation of GA levels is tremendously important for agriculture

Distinguished plant breeder and Nobel Laureate
Norman Borlaug **1914-2009**

One of the most significant accomplishments of 20th century science was the development of semi-dwarf grain varieties which are deficient in GA synthesis or response

In 1970 Norman E. Borlaug was awarded the Nobel Peace Prize for a lifetime of work to feed a hungry world. He created the World Food Prize, because there is no Nobel prize for Agriculture

GA synthesis and homeostasis



In 1956, B.O. Phinney found that some dwarf mutants of *Zea mays* could be rescued by GA application

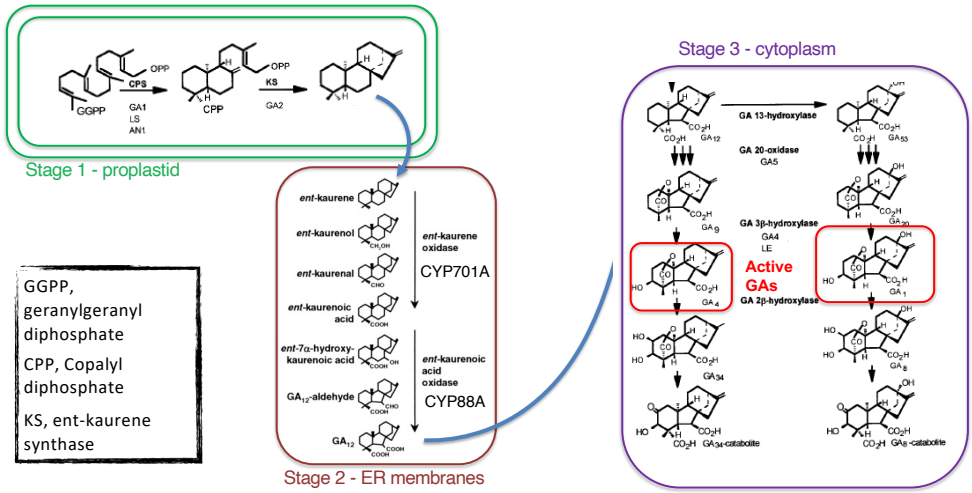
The pathway of GA biosynthesis in plants was determined in part by analysis of GA-deficient dwarfs; an early use of the "chemical genetics" approach

Phinney, B.O. (1956). Growth response of single-gene dwarf mutants in maize to gibberellic acid. Proc. Natl. Acad. Sci. USA 42: 185-189.

The use of genetics to elucidate biosynthetic pathways had been described earlier by Beadle and Tatum. For an animated tutorial of Beadle and Tatum's work see https://www.dnalc.org/resources/nobel/beadle_tatum.html

FIG.1-Response of normal and *dwarf-I* seedlings to a single application of 10g of gibberellic acid (pure sample) per plant. Treatments given at the time of emergence of the first leaf. Plants shown are 2 weeks old.

The GA biosynthetic pathway is complex



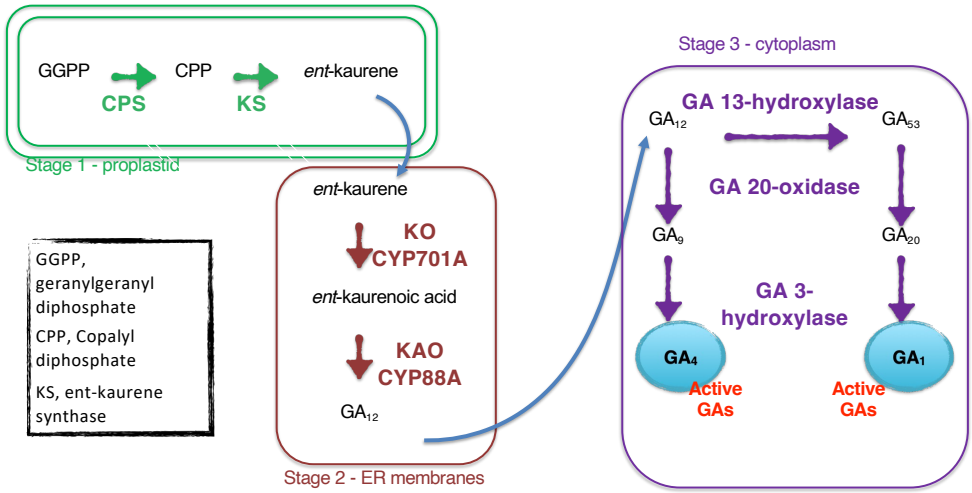
Hedden, P., Proebsting, W.M. (1999) Genetic analysis of gibberellin biosynthesis. Plant Physiol. 119: 365-370.
Helliwell, C.A., Chandler, P.M., Poole, A., Dennis, E.S., and Peacock, W.J. (2001). The CYP88A cytochrome P450, ent-kaurenoic acid oxidase, catalyzes three steps of the gibberellin biosynthesis pathway. Proc. Natl. Acad. Sci. USA 98: 2065-2070.

GAs are products of the diterpenoid pathway

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

Cytochrome P450-mediated steps of the GA biosynthesis pathway showing the steps catalyzed by KO and KAO.

The GA biosynthetic pathway is complex



Hedden, P., Proebsting, W.M. (1999) Genetic analysis of gibberellin biosynthesis. Plant Physiol. 119: 365-370.
Helliwell, C.A., Chandler, P.M., Poole, A., Dennis, E.S., and Peacock, W.J. (2001). The CYP88A cytochrome P450, ent-kaurenoic acid oxidase, catalyzes three steps of the gibberellin biosynthesis pathway. Proc. Natl. Acad. Sci. USA 98: 2065-2070.

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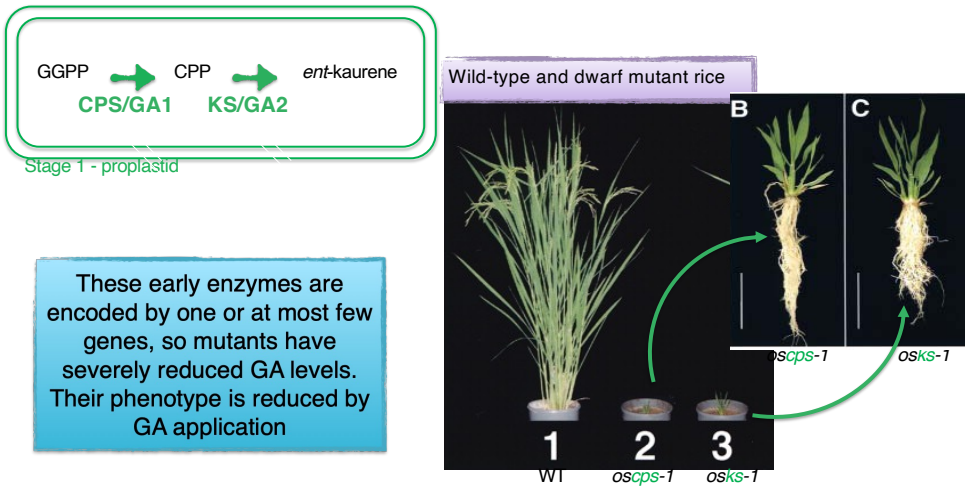
Cytochrome P450-mediated steps of the GA biosynthesis pathway showing the steps catalyzed by KO and KAO.

Loss-of-function mutants of CPS or KS are severely dwarfed



Sakamoto, T., et al. (2004). An overview of gibberellin metabolism enzyme genes and their related mutants in rice. Plant Physiol. 134: 1642-1653.

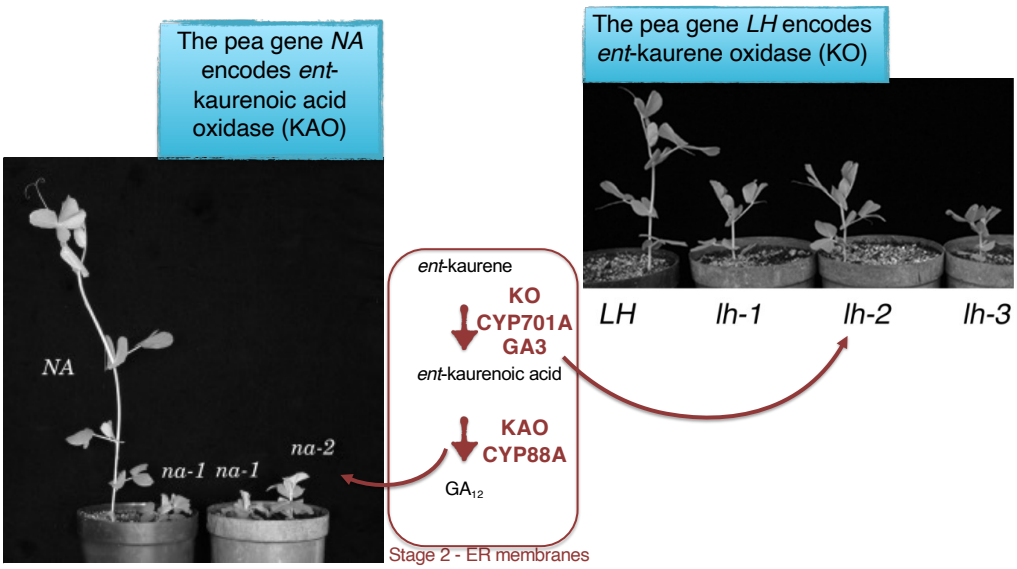
In the central picture the dwarf oscps-1 and osks-1 mutants are so small they are difficult to see; larger images of these plants are shown to the right. Rice (Oryza sativa) genes often start with “Os” to indicate they are from rice.

Figure 5. Phenotypes of rice GA-deficient dwarf mutants.

A, Comparison of gross morphology between mutant plants and their original cultivars. 1, Nipponbare (original strain for oscps-1, osks1-

1, osko2-1, and oskao-1); 2, oscps-1 (null allele); 3, osks1-1 (null allele). B, C, Close-up view of oscps-1, osks1-1, respectively. Arrows indicate panicles containing fertile seeds. Bar represents 5 cm.

Similarly, loss-of-function mutants of KO or KAO are severely dwarfed



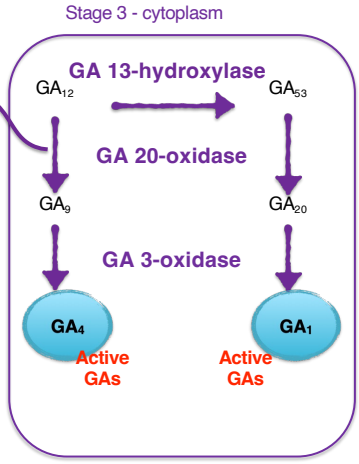
Davidson, S.E., Elliott, R.C., Helliwell, C.A., Poole, A.T., and Reid, J.B. (2003). The pea gene NA encodes ent-kaurenoic acid oxidase. Plant Physiol. 131: 335-344.
Davidson, S.E., Smith, J.J., Helliwell, C.A., Poole, A.T., and Reid, J.B. (2004). The pea gene LH encodes ent-kaurene oxidase. Plant Physiol. 134: 1123-1134.

Figure 2. The phenotype of 21-d-old seedlings of wild-type NA (WL1769) and two independent mutants, *na-1* (WL1766) and *na-2* (L81).

Figure 2. Photographs of 16-d-old pea seedlings with leaf at fifth node fully expanded.

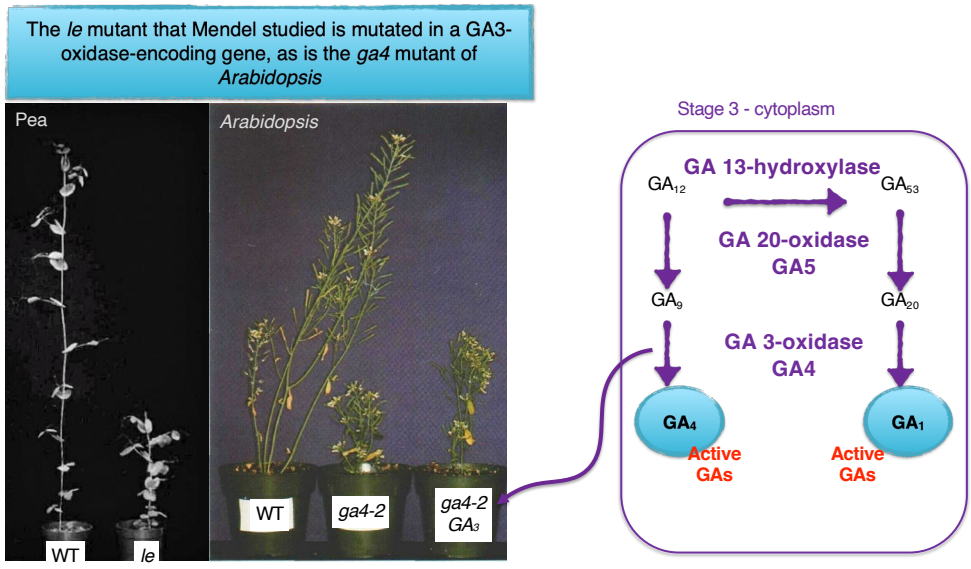
The later enzymes are encoded by multiple genes with differing expression patterns

The "green revolution" *semidwarf1* rice variety is mutated in a GA20ox that is expressed in shoots but not reproductive tissues, leading to increased grain yields



Sasaki, A., Ashikari, M., Ueguchi-Tanaka, M., Itoh, H., Nishimura, A., Swapan, D., Ishiyama, K., Saito, T., Kobayashi, M., Khush, G. S., Kitano, H. and Matsuoka, M. (2002) A mutant gibberellin-synthesis gene in rice. Nature 416, (6882) 701-702.

Similarly, mutants in *GA3ox* genes can have reduced height but not seed production



Lester, D.R., Ross, J.J., Davies, P.J., and Reid, J.B. (1997) Mendel's stem length gene (Le) encodes a gibberellin 3[beta]-hydroxylase. Plant Cell 9: 1435-1443.
Chiang, H.H., Hwang, I., and Goodman, H.M. (1995). Isolation of the Arabidopsis GA4 Locus. Plant Cell 7: 195-201.

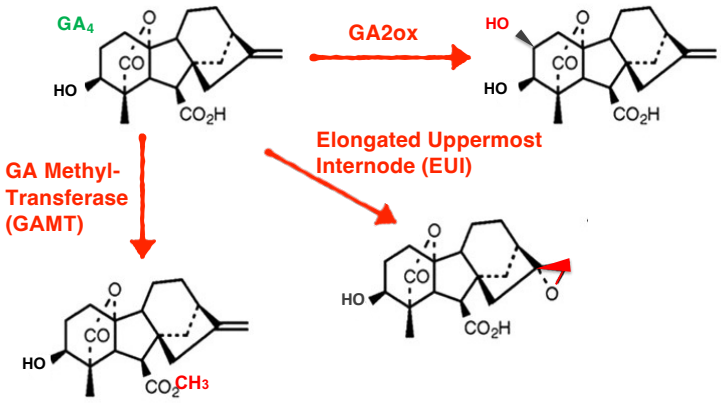
Figure 1. Mendel's Stem Length Trait.

Lines 205f (Le/Le; tall) and 205 (le/le; dwarf) of pea show the difference in stem length controlled by the *Le* locus.

Figure 1. T-DNA-Tagged Mutant Is an Allele of the GA4 Locus.

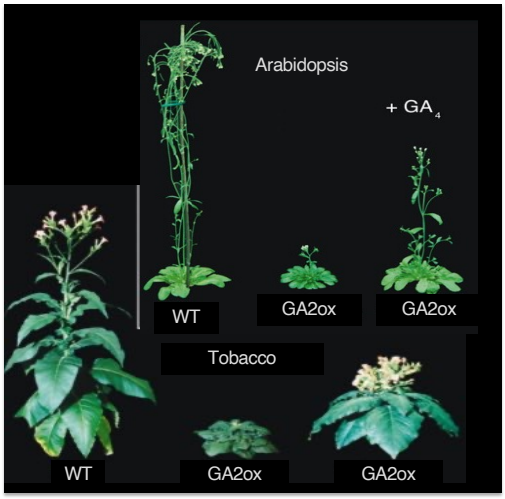
The T-DNA-tagged allele *ga4-2* responded to GA3 treatment with shoot elongation (+ GA3). WT, canonical wild type, Landsberg er

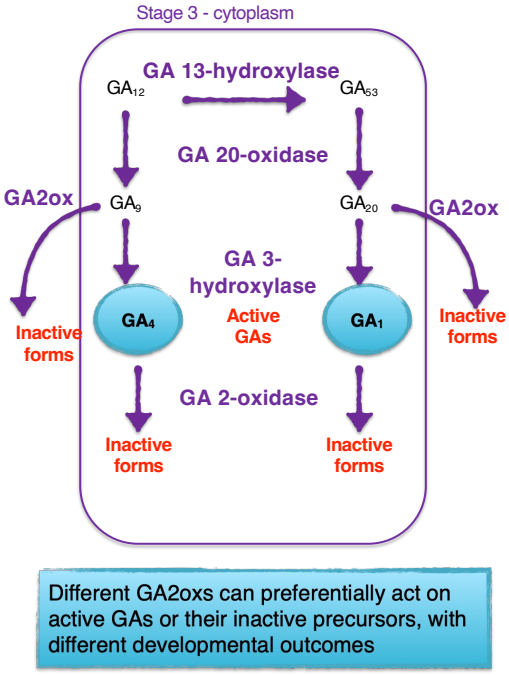
GAs can be deactivated by several different enzymes



The chemical modification carried out by these enzymes to deactivate GA4 are shown in red.

Dwarfism is conferred by overexpression of the GA deactivating enzyme GA 2-oxidase





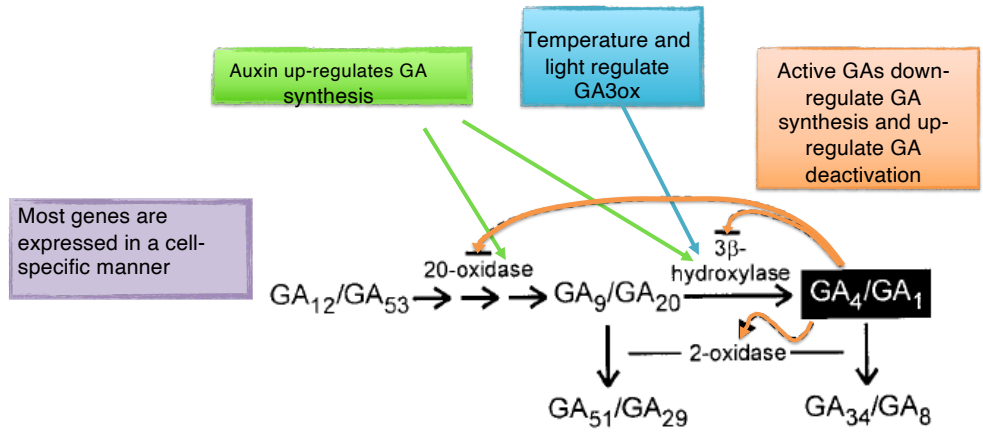
Different GA2oxs can preferentially act on active GAs or their inactive precursors, with different developmental outcomes

Schomburg, F.M., Bizzell, C.M., Lee, D.J., Zeevaart, J.A.D., and Amasino, R.M. (2003). Overexpression of a novel class of gibberellin 2-oxidases decreases gibberellin levels and creates dwarf plants. Plant Cell 15: 151–163.

Responses of AtGA2ox8ACT mutants to GA4 at 12 and 14 days, respectively, after GA application. Plants were transferred from short days to long days at the start of GA treatment.

Tobacco containing the 35S::AtGA2ox7 or 35S::AtGA2ox8 cDNA construct. WT, Wisconsin 38.

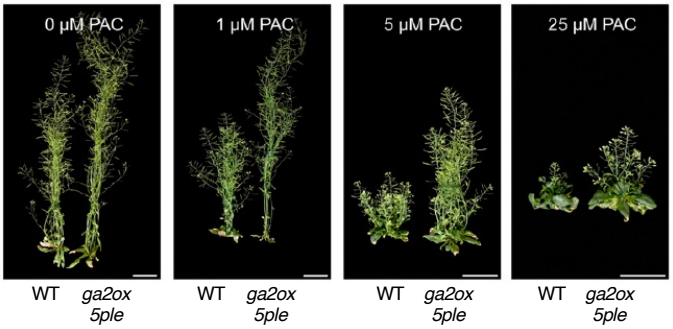
GA biosynthesis and deactivation are tightly controlled

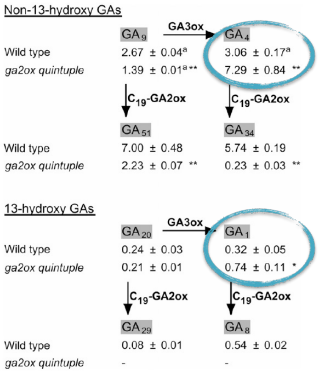


Thomas, S.G., Phillips, A.L., and Hedden, P. (1999). Molecular cloning and functional expression of gibberellin 2- oxidases, multifunctional enzymes involved in gibberellin deactivation. Proc. Natl. Acad. Sci. USA 96: 4698-4703

FIG. 5.
Model showing feedback and feedforward regulation of GA biosynthesis.

Paclobutrazol inhibits GA synthesis





Paclobutrazol is often sprayed on plants to interfere with GA synthesis and prevent branch growth (e.g. in fruit trees)

Rieu, I., et al. (2008). Genetic analysis reveals that C19-GA 2-Oxidation is a major gibberellin inactivation pathway in Arabidopsis. Plant Cell 20: 2420-2436.

Figure 7. Phenotype of the GA2ox Mutants under Low GA Conditions.

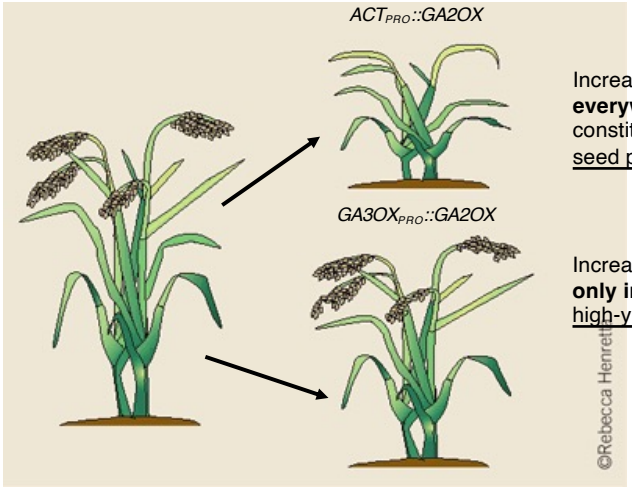
Growth characteristics of wild-type and *ga2ox* quintuple plants grown on different concentrations of PAC. Whole-plant phenotypes, with each pane showing a wild-type (left) and *ga2ox* quintuple plant (right)

Figure 3. Profile of Endogenous GAs in Wild-Type and *ga2ox* quintuple Plants.

The GA levels (in rosette and bolt of flowering plants) are means of four biological replicates

in ng/g dry weight ± SE, except where indicated. a, Three biological replicates only; *, significantly different from the wild type (t test: $P < 0.05$); **, significantly different from the wild type (t test: $P < 0.01$); , not detected.

Tissue-specific hormone modulation can optimize growth and crop yields

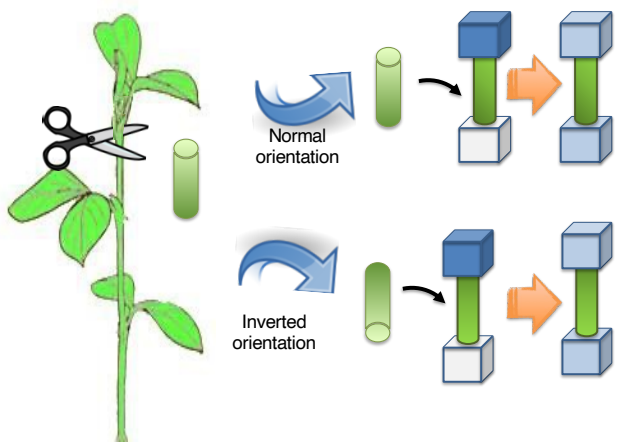


Increasing GA catabolism **everywhere** using a constitutive promoter affects seed production

Increasing GA catabolism **only in internodes** produces high-yielding dwarf rice plants

Hedden, P. (2003) Constructing dwarf rice. Nature Biotech. 21: 873 - 874 .

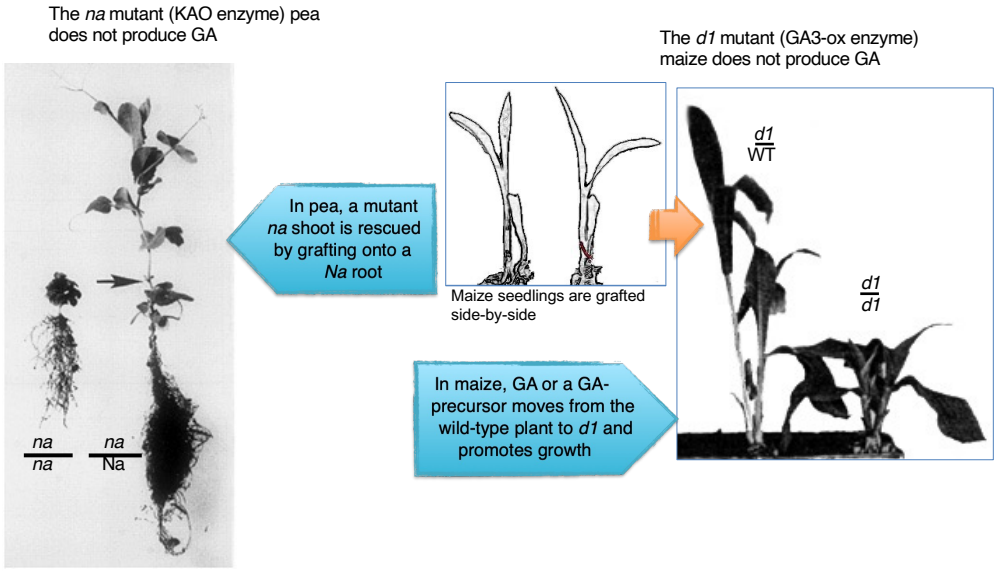
Unlike auxin, GA is not transported in a polar way



The **same amount of GA** moves from the upper donor block to the lower block no matter the polarity of the stem segment. By contrast, auxin moves much more efficiently from stem apex to base

Kato, J. (1958) Non polar transport of gibberellin through pea stem and a method for its determination. Science 128: 1008-1009.

GAs are graft-transmissible; they can move long distances



Proebsting, W.M., et al. (1992). Gibberellin concentration and transport in genetic lines of pea : Effects of grafting. Plant Physiol. 100: 1354-1360.
Katsumi, M., et al. (1983). Evidence for the translocation of gibberellin A3 and gibberellin-like substances in grafts between normal, dwarf1 and dwarf5 seedlings of Zea mays L. Plant Cell Physiol. 24: 379-388
Chen Y, Hou M, Liu L, Wu S, Shen Y, Ishiyama K, Kobayashi M, McCarty DR, Tan BC. The maize DWARF1 encodes a gibberellin 3-oxidase and is dual localized to the nucleus and cytosol. Plant Physiol. 2014 Dec;166(4):2028-39. doi: 10.1104/pp.114.247486. Epub 2014 Oct 23. PMID: 25341533; PMCID: PMC4256885.

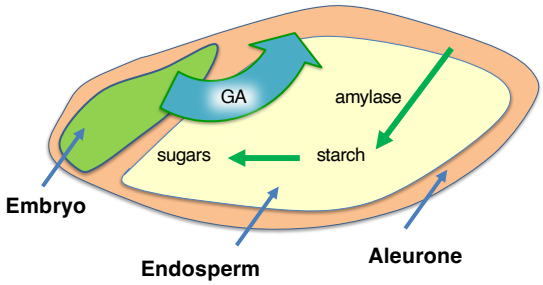
The *na* mutant (KAO enzyme) pea shoot does not produce GA, but when grafted onto Na roots it elongates, indicating that GA has been translocated from root to shoot. Similarly, the maize *d1* mutant is dwarfed when grafted to another *d1* mutant, but elongates when grafted to a wild-type plant, showing translocation of GA from the WT graft partner to the *d1* mutant.

See also Ragni, L., Nieminen, K., Pacheco-Villalobos, D., Sibout, R., Schwechheimer, C. and Hardtke, C.S. (2011). Mobile Gibberellin

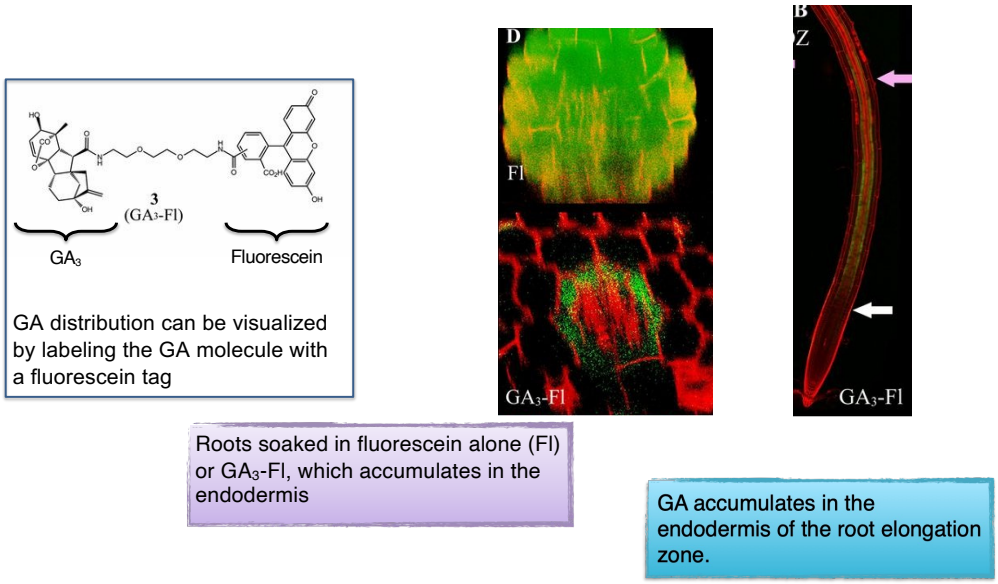
Directly Stimulates Arabidopsis Hypocotyl Xylem Expansion. Plant Cell. 23: 1322-1336.

GA moves from embryo to aleurone in germinating monocot seeds

GA is synthesized in the embryo and moves into the cells of the aleurone to induce production of α-amylase, an enzyme that breaks down starch



GAs actively accumulate in the root endodermis, indicating transport



Shani, E., Weinstein, R., Zhang, Y., Castillejo, C., Kaiserli, E., Chory, J., Tsien, R.Y. and Estelle, M. (2013) Gibberellins accumulate in the elongating endodermal cells of Arabidopsis root. Proc. Natl. Acad. Sci. USA 110: 4834–4839.

(A) Molecular structure of GA3-Fl.

Fig. 2. Distribution of GA3-Fl and GA4-Fl in the root.

(B) Confocal image of fluorescence distribution in elongating endodermal cells of roots treated with GA3-Fl (green) (5 µM, 2 h). White arrows mark transition from MZ to EZ; pink arrow marks transition from EZ to DZ. Cell walls were stained with propidium iodide (red).

(D) Fl and GA3-Fl distribution in radial confocal sections of the elongation zone. GA3-Fl

accumulates in the endodermal cells.

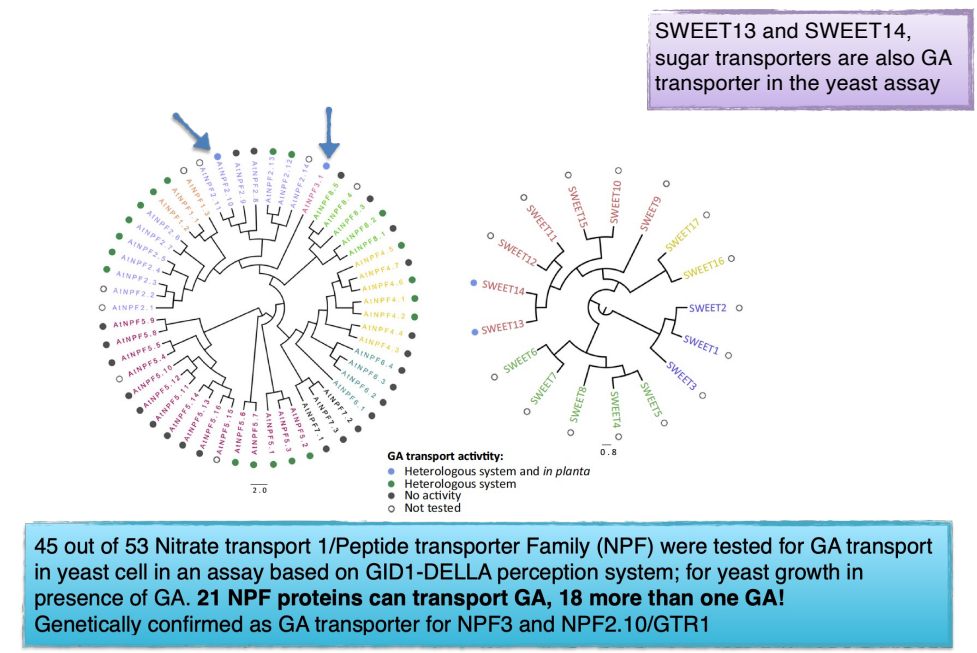


Figure 2. Identified Gibberellin Transporters. Phylogenetic trees of known gibberellin (GA) transport protein; the NPF and SWEET families in Arabidopsis. Color-coded circles indicate GA transport activity. Heterologous systems include yeast and Xenopus oocytes. The NPF and SWEET proteins also transport additional diverse substrates.

GA synthesis, homeostasis and transport - summary

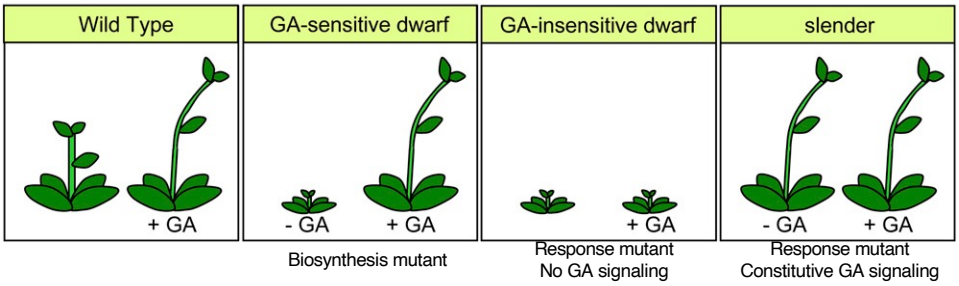
✓GA synthesis mutants are dwarfed, and may be or not be affected in reproductive development

✓Active GA levels are controlled by regulated synthesis and deactivation

✓GA moves within tissues and few potential transport proteins were identified

✓Control of active GA accumulation through chemical and genetic means greatly contributes to agricultural productivity

GA responses

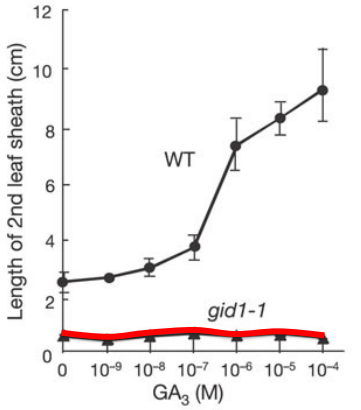


Harberd, N.P., Belfield, E., and Yasumura, Y. (2009). The angiosperm gibberellin-GID1-DELLA growth regulatory mechanism: How an "inhibitor of an inhibitor" enables flexible response to fluctuating environments. Plant Cell 21: 1328-1339.

(B) Schematic representation of plants in GA-related mutant categories. Normal (wild type) plants respond to exogenous GA (+GA) by increased growth. GA-sensitive dwarf mutants are GA-deficient (–GA) and grow in response to exogenous GA. GA-insensitive dwarf mutants do not grow in response to exogenous GA. Finally, slender mutant growth mimics that of GA-treated normal plants, even when additional mutations or chemical growth retardants cause GA deficiency.

GIBBERELLIN INSENSITIVE DWARF1 (*GID1*) encodes a GA receptor

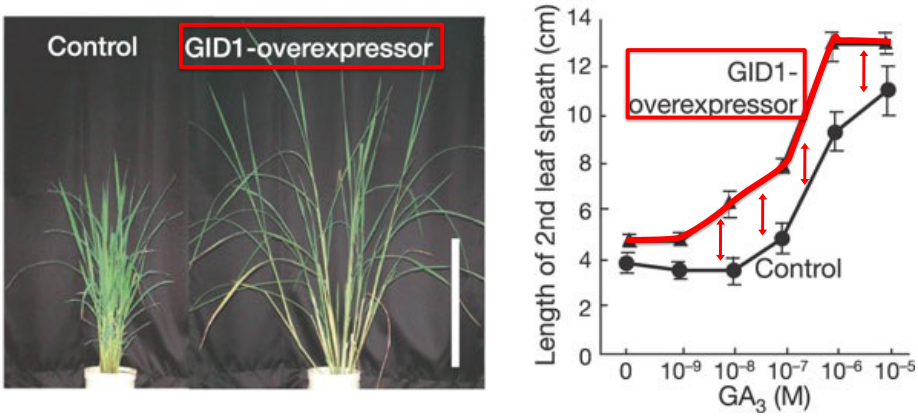
Unlike biosynthesis mutants, the rice *gid1* mutant is not rescued by GA. It is **gibberellin-insensitive**



Ueguchi-Tanaka, M., et al. (2005) GIBBERELLIN INSENSITIVE DWARF1 encodes a soluble receptor for gibberellin. Nature 437: 693-698.

Figure 1 | GA-insensitive phenotype of *gid1-1*. Wild type (left) and *gid1-1* (right) plants. Scale bar, 10 cm. Inset: higher magnification of *gid1-1*. Scale bar, 1 cm. b, GA_3-induced elongation of the second leaf sheath (mean ± s.d.; n = 10).

Overexpression of GID1 makes plants hypersensitive to GA



These observations are consistent with GID1 being a GA receptor

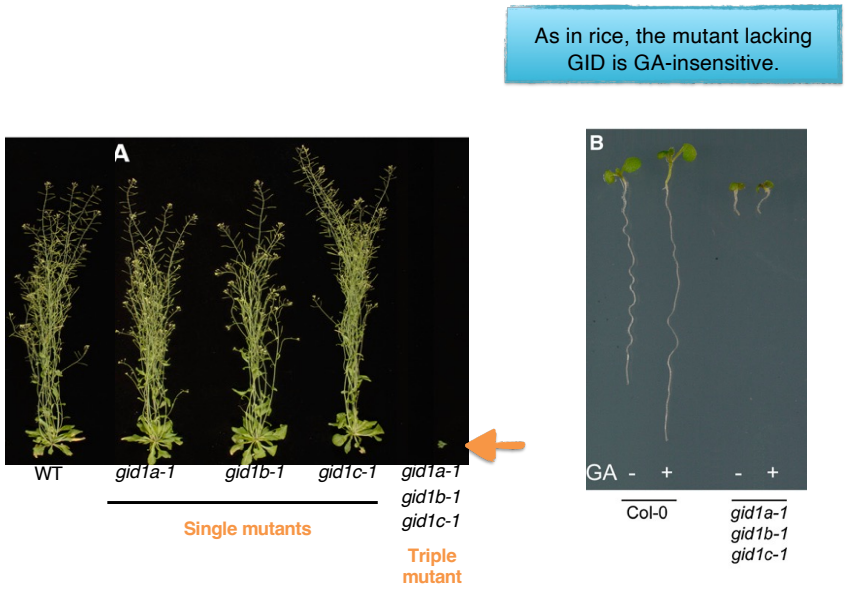
Ueguchi-Tanaka, M., et al. (2005) GIBBERELLIN INSENSITIVE DWARF1 encodes a soluble receptor for gibberellin. Nature 437: 693-698.

Having more receptors means that at any given hormone concentration more hormone will be bound to receptors to elicit signaling, so the response will be greater.

Figure 5 | Phenotype of a GID1 overexpressor.

b, Gross morphology of GID1-overexpressor and control plants. Scale bar, 50 cm. c, Elongation of the sheath of the second leaf with exogenous GA_3 (mean ± s.d.; n = 5).

Arabidopsis has three redundantly-acting *GID1* genes



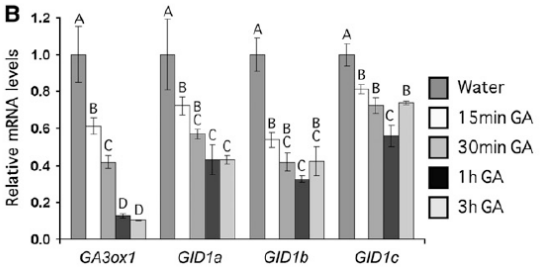
Griffiths, J. et al. (2006) Genetic characterization and functional analysis of the GID1 gibberellin receptors in Arabidopsis. Plant Cell18: 3399–3414.

Any of the single mutants in Arabidopsis show normal growth and GA response, indicating that there is functional redundancy. The triple mutant is tiny, and indicated by the arrow in the left figure.

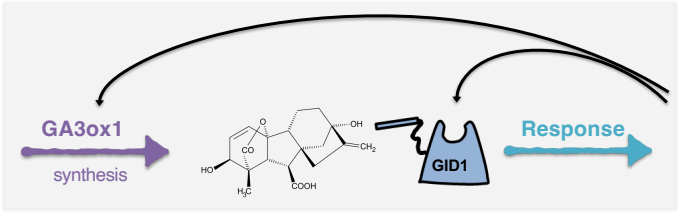
(A) Aerial portions of 37-d-old wild-type and homozygous mutant plants. Genotypes are indicated in (A).

(B) Representative 5-d-old seedling primary roots of selected genotypes, grown on vertical MS plates and treated with (+) or without (-) 0.2 uM GA_4 as indicated. Bar = 1 cm.

***GID1* expression is negatively regulated by GA**



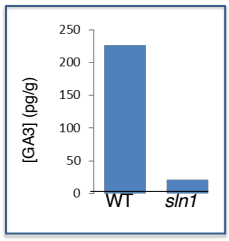
GA **down-regulates** expression of genes involved in its own synthesis (e.g *GA3ox*) and response (e.g. *GID1a,b,c*); these are important negative feedback controls

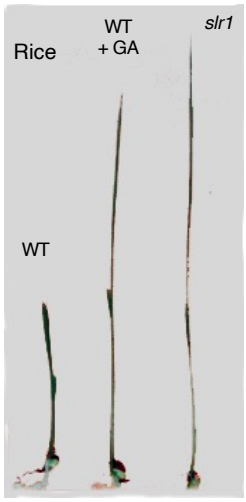


Griffiths, J. et al. (2006) Genetic characterization and functional analysis of the GID1 gibberellin receptors in Arabidopsis. Plant Cell18: 3399–3414

(B) GA treatment down-regulates GA3ox1 and GID1a-1c mRNA levels in *ga1-3* (in the Ler background). The means of three replicates of quantitative RT-PCR ± SE are shown. Relative mRNA levels of individual genes after GA treatment were calculated in comparison to the water-treated control at each time point. Similar results were obtained when quantitative RT-PCR was performed using a second set of samples.

The rice and barley *slender1* mutants have a constitutive GA response





GA accumulation in *sln* plants is much lower than in WT, indicating that it is a signaling mutant and not a GA-overproduction mutant

Fu, X., Richards, D.E., Ait-ali, T., Hynes, L.W., Ougham, H., Peng, J., and Harberd, N.P. (2002). Gibberellin-mediated proteasome-dependent degradation of the barley DELLA protein SLN1 repressor. Plant Cell 14: 3191-3200.
Ikeda, A., et al. (2001). slender Rice, a constitutive gibberellin response mutant, is caused by a null mutation of the SLR1 gene, an ortholog of the height-regulating gene GAI/RGA/RHT/D8. Plant Cell 13: 999-1010.

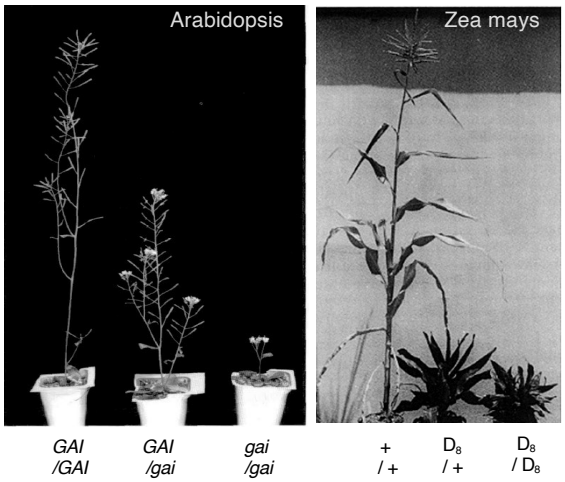
Figure 1. The *sln1-1* Mutant Allele.

(A) Five-day-old seedlings homozygous for SLN1 or *sln1-1.*

Figure 1. Phenotype of slender Rice and Its Original Wild Type, Nipponbare.

(B) Shoot elongation of wild type treated with (middle) or without (left) 10 □uM GA3 and *slender* without GA3 treatment (right). The photograph was taken 1 week after germination.

Semi-dominant GA-insensitive dwarfs have been identified



These mutants do **NOT** respond to exogenous GA and accumulate very high levels of GA, indicating that they are not biosynthesis mutants

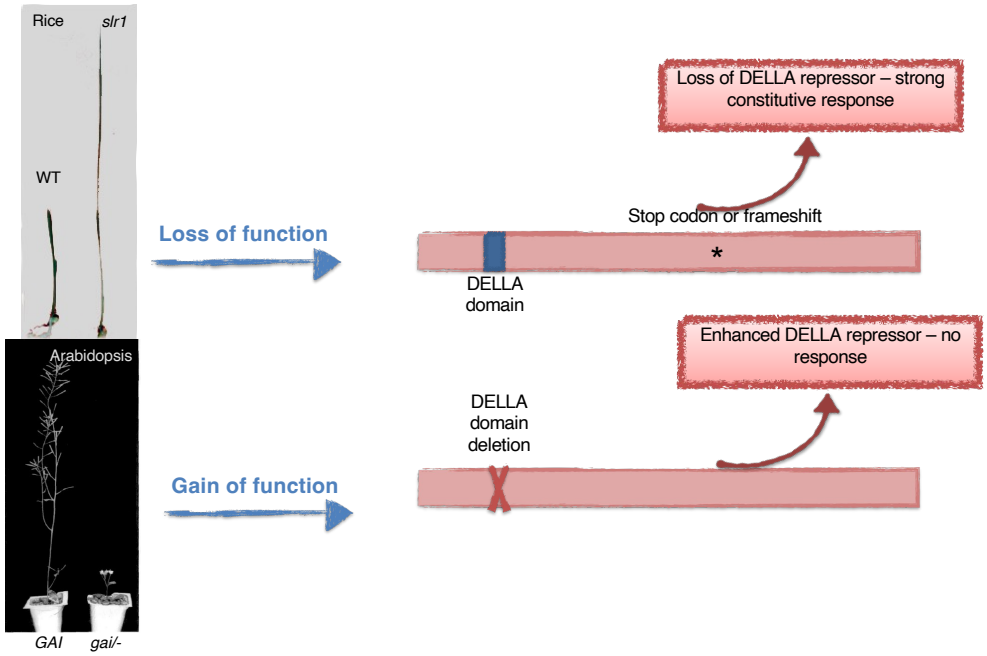
Peng, J., and Harberd, N.P. (1993). Derivative alleles of the Arabidopsis Gibberellin-Insensitive (gai) mutation confer a wild-type phenotype. Plant Cell 5: 351-360.
Fujioka, S., Yamane, H., Spray, C.R., Katsumi, M., Phinney, B.O., Gaskin, P, MacMillan, J., and Takahashi, N. (1988) The dominant non-gibberellin-responding dwarf mutant (D8) of maize accumulates native gibberellins. Proc. Natl. Acad. Sci. 85: 9031-9035.

The heterozygous plants show an intermediate phenotype, indicating that the dwarfing allele is semi-dominant.

Figure 1. Plants Heterozygous and Homozygous for *gai*.
Shown from left to right are *GAI/GAI*, *gai/GAI*, and *gai/gai* plants segregating in the progeny of a self-pollinated *gai/GAI* plant.

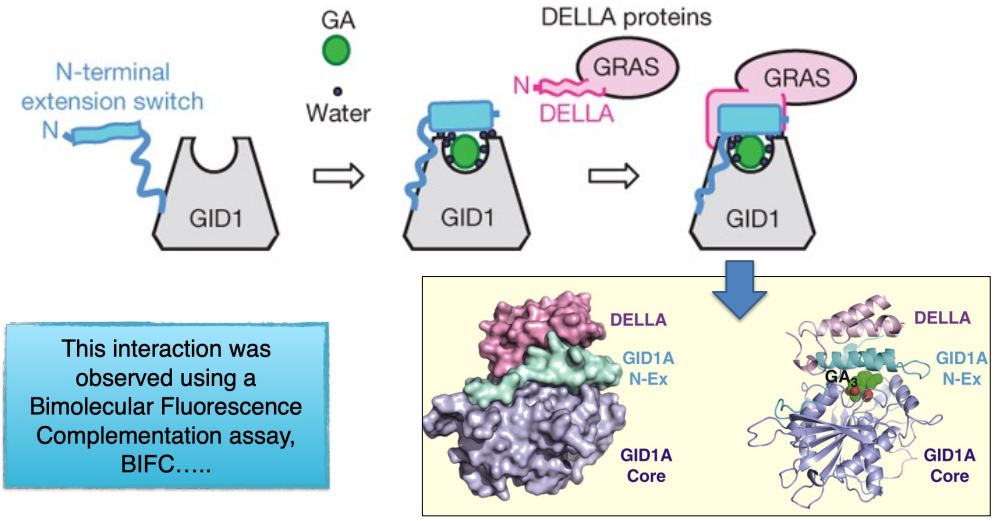
FIG.2. Green house-grown normal (+/+) and dwarf (both D8/+ and D8/D8 genotypes) plants at maturity.

GA-INSENSITIVE1 and *SLENDER1* both encode "DELLA" repressors



Peng, J., and Harberd, N.P. (1993). Derivative alleles of the Arabidopsis Gibberellin-Insensitive (gai) mutation confer a wild-type phenotype. Plant Cell 5: 351-360.
Ikeda, A., et al. (2001). slender Rice, a constitutive gibberellin response mutant, is caused by a null mutation of the SLR1 gene, an ortholog of the height-regulating gene GAI/RGA/RHT/D8. Plant Cell 13: 999-1010.

The receptor GID1 binds to DELLAs in presence of GA



Murase, K., Hirano, Y., Sun, T.-p., and Hakoshima, T. (2008). Gibberellin-induced DELLA recognition by the gibberellin receptor GID1. Nature 456: 459-463.
Sun, T.-p. (2011). The molecular mechanism and evolution of the GA GID1 DELLA signaling module in plants. Curr. Biol. 21: R338-R345.

GRAS is a conserved domain of DELLA proteins

Figure 6: A model of GA-regulated GID1–DELLA protein interactions.

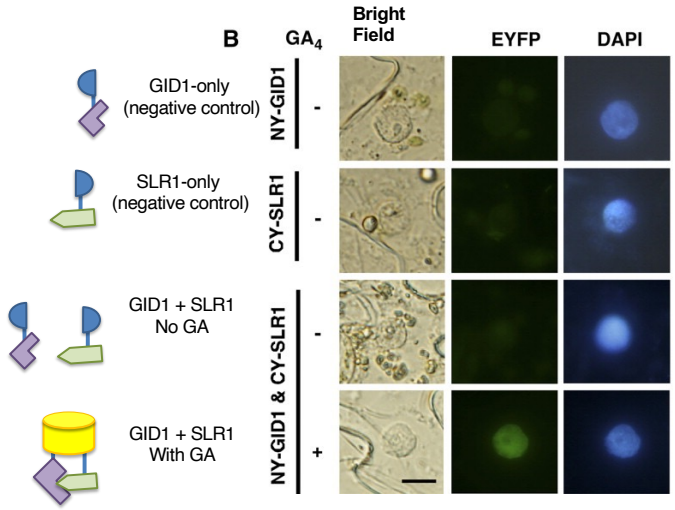
A model for the GA-dependent GID1–DELLA interaction. GA binding first induces a conformational change in the N-Ex of GID1 for DELLA binding, which promotes binding of the GRAS domain of the DELLA protein to GID1.

Figure 2. The GA–GID1–DELLA complex.

Crystal structure of the complex that contains GA_3, AtGID1A and the DELLA domain of GAI (an AtDELLA). (left) The molecular surface of

the complex. (right) Ribbon representation. The carboxy-terminal GID1 core domain is labeled in blue, the GID1 amino-terminal extension (N-Ex) is in cyan, and the DELLA domain is in pink. The bound GA_3 is represented as a space-filling model with carbon in green and oxygen in red.

The interaction between GID1 (receptor) and SLR1 (DELLA) is GA-dependent



Ueguchi-Tanaka, M., et al. (2007) Molecular interactions of a soluble gibberellin receptor, GID1, with a rice DELLA protein, SLR1, and gibberellin. Plant Cell 19: 2140-2155.

EYFP refers to “enhanced YFP” – a brighter form of YFP. Note that in some cases the proteins can interact without GA – see Yamamoto et al., (2010) Plant Cell 22: 3589 – 3602.

Figure 1. GA-Dependent Interaction between GID1 and SLR1 in Vivo.

(B) BiFC analysis of in vivo interaction between GID1 and SLR1 in N. benthamiana leaf epidermis (Abe et al., 2005). Bright-field image; EYFP, EYFP fluorescence; DAPI, 49,6-diamidino-2-phenylindole (DNA marker)

NY-GID1, expression of N□EYFP-GID1 alone; CY-SLR1, expression of C□EYFP-SLR1 alone; NY-GID1 and CY- SLR1, coexpression of N□EYFP-GID1 and C□EYFP-SLR1; NY-GID1 and CY-DDELLA, coexpression of N□EYFP-GID1 and C□EYFP-DDELLA□SLR1

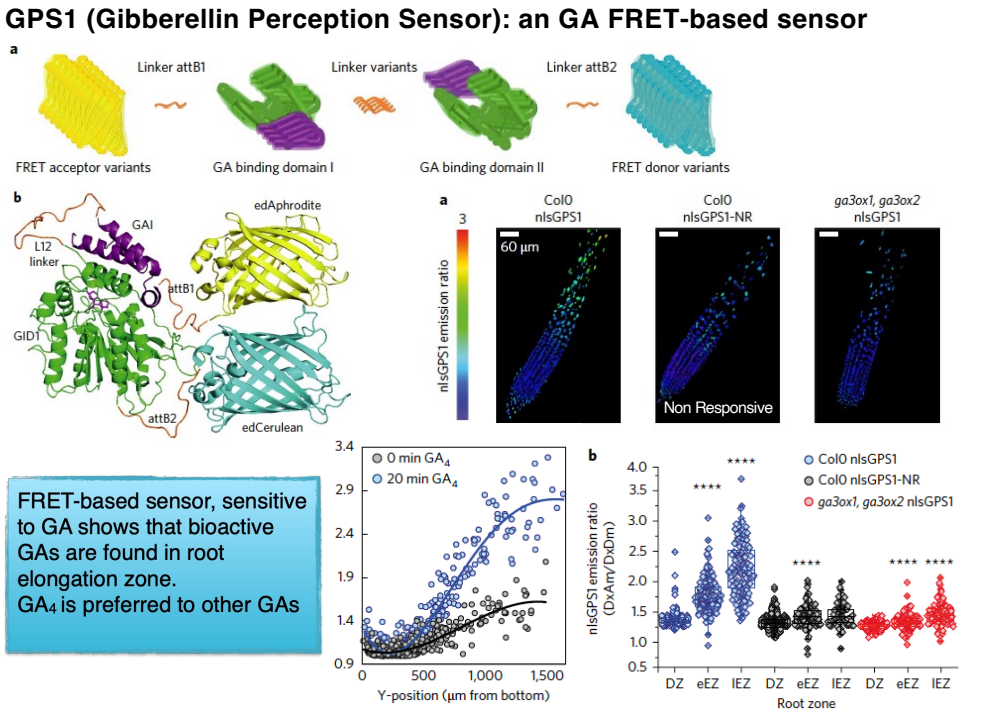


Fig. 1 | Engineering and GA response of GPS1 and GPS1-NR.

a, Representation of variant domains included in potential FRET biosensors for GA: GA binding domain variants (GA binding domain I and II connected through linker variants) were fused via attB1 and attB2 linkers to fluorescent protein FRET pairs (variant donor and acceptor fluorescent proteins).

b, Structural model of GPS1 bound to GA4. The GID1C protein (green), GAI truncation (purple) and GA4 (magenta) representations

are from a published structure of an AtGID1A, AtGAI truncation, GA4 ternary complex (PDB 2ZSI31). The edAphrodite (yellow) representation is from a published structure of Venus (PDB 1MYW55) and the edCerulean representation is from a published structure of Cerulean (PDB 2WSO56). The representations of three linkers (translated attB1, L12 and translated attB2) were built in PyMol software.

Right

Fig. 3 | Emission ratios of nlsGPS1 in root tips.

a, Three-dimensional images of nlsGPS1 or nlsGPS1-NR emission ratios of roots 3 days post sowing in wild-type Col0 or ga3ox1, ga3ox2 mutant backgrounds.

b, Beeswarm and box plot of nlsGPS1 or nlsGPS1-NR emission ratios for nuclei of late division zone (DZ, 100–200 μ m

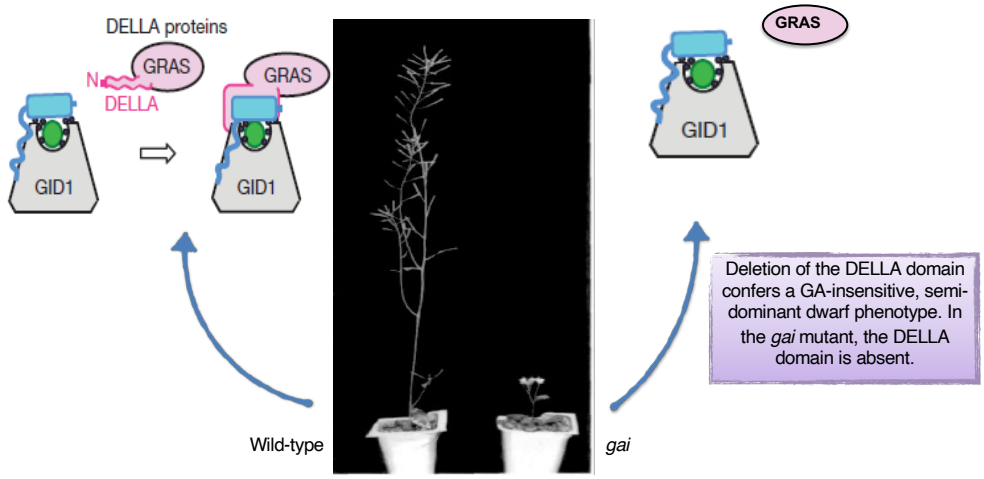
from quiescent centre), early elongation zone (eEZ, 200–400 μ m from quiescent centre) and late elongation zone (lEZ, 400–800 μ m from quiescent centre). n > 68 nuclei from three independent seedlings for each genotype and root zone. For

Col0 nlsGPS1, eEZ versus DZ Kruskal-Wallis test ****P value < 0.0001, Cohen's d = 1.83; and lEZ versus eEZ Kruskal-Wallis test ****P value < 0.0001, Cohen's d = 1.16. For Col0 nlsGPS1-NR, eEZ versus DZ Kruskal-Wallis test ****P value < 0.0001, Cohen's d = 0.60; and lEZ versus eEZ Mann-Whitney U test P value = 0.41, Cohen's d = 0.13. For nlsGPS1 ga3ox1, ga3ox2, eEZ versus DZ Kruskal Wallis test ****P value < 0.0001, Cohen's d = 0.73; and lEZ versus eEZ Kruskal-Wallis test ****P value < 0.0001, Cohen's d = 0.84.

Fig. 4 | Emission ratios of nlsGPS1 in roots before and after treatment with GAs.

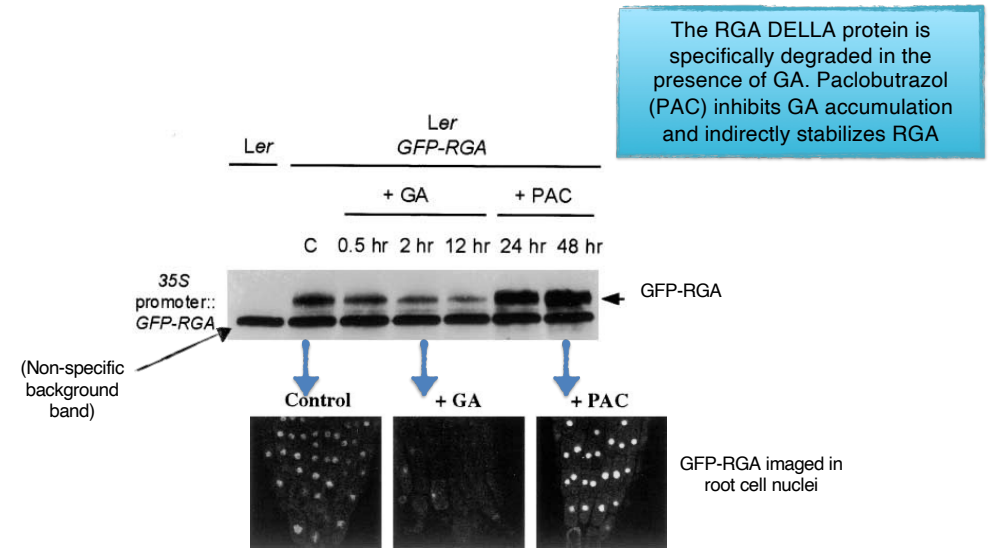
nlsGPS1 emission ratios for individual nuclei before (grey) and after (blue) GA4 treatment in relation to distance from the root tip (micrometres from bottom). Complete experiments were repeated at least three times with similar results.

The DELLA domain is necessary for GID1-binding



Peng, J., and Harberd, N.P. (1993). Derivative alleles of the Arabidopsis Gibberellin-Insensitive (gai) mutation confer a wild-type phenotype. Plant Cell 5: 351-360.

GA induces DELLA protein degradation



Silverstone, A.L., Jung, H.-S., Dill, A., Kawaide, H., Kamiya, Y., and Sun, T.-p. (2001). Repressing a repressor: Gibberellin-induced rapid reduction of the RGA protein in Arabidopsis. Plant Cell 13: 1555-1566.

Figure 6. Immunoblot Analysis of GFP-RGA Levels.

The blots contained 50 u□g of total protein extracted from L*er* and transgenic seedlings carrying either the 35S::GFP-RGA fusion gene. Lane C, water- treated control. The times after GA or PAC treatment were as labeled. A rat anti-GFP antiserum and a peroxidase-conjugated goat anti-rat IgG were used as primary and secondary antibodies, respectively. The arrows indicate the GFP-RGA fusion protein (91 kD). The additional lower

band in all lanes represents nonspecific background protein because it is present in L*er* as well.

Figure 4. Effects of GA and PAC Treatment on the RGA Promoter– Expressed GFP-RGA Protein.

Roots of transgenic plants (L*er* background) expressing the RGA promoter::GFP-RGA fusion were observed using confocal laser microscopy. Shown are the fluorescent images of root tips that were untreated (Control), treated with 100 uM GA_3 for 2 hr (+ GA), or incubated with 100 uM PAC and 0.01% Tween 20 for 48 hr (+PAC).

SLEEPY and _GID2_ encode a F-box protein, components of the SCF ubiquitin ligase complex

The *sleepy* and *gid2* mutants resemble mutants with stabilized DELLA-proteins. GA-induced elongation is inhibited

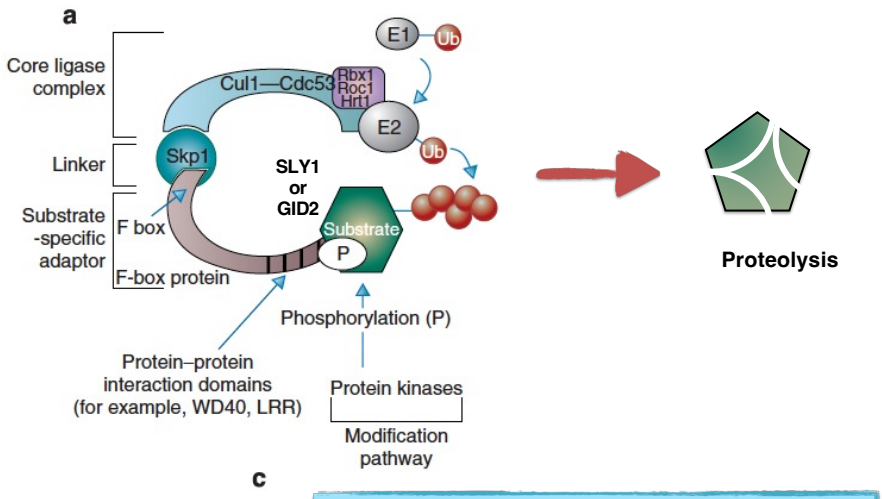
GID2 is the rice orthologue of SLEEPY

McGinnis, K.M., Thomas, S.G., Soule, J.D., Strader, L.C., Zale, J.M., Sun, T.-p., and Steber, C.M. (2003). The Arabidopsis SLEEPY1 gene encodes a putative F-box subunit of an SCF E3 ubiquitin ligase. Plant Cell 15: 1120-1130.
Sasaki, A., et al. (2003) Accumulation of phosphorylated repressor for gibberellin signaling in an F-box mutant. Science 299: 1896-1898.

sly1-10 (left) and wild-type Ler (right) plants are shown. Ler and homozygous mutant plants were grown on soil for 60 days under a long-day photoperiod. Bar = 15 mm.

Gross morphologies of wild type, gid2-1, plants in 2-week-old seedlings.

SLEEPY/GID2 is a component of the SCF ubiquitin ligase complex $SCF^{SLY1/GID2}$

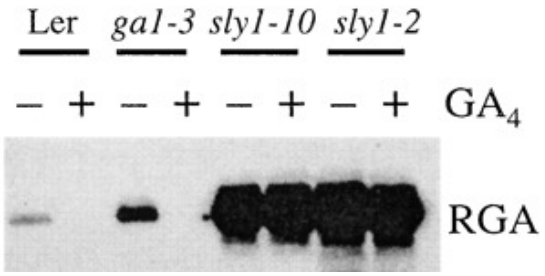


SCF ubiquitin ligase complex
(Named for **S**KP1, **C**UL1 and **F**-box proteins)

The $SCF^{SLY1/GID2}$ complex targets DELLA proteins for ubiquitination and proteolysis

SLEEPY is required for GA-mediated proteolysis of DELLA proteins

sleepy mutants do not degrade the RGA DELLA protein in response to GA



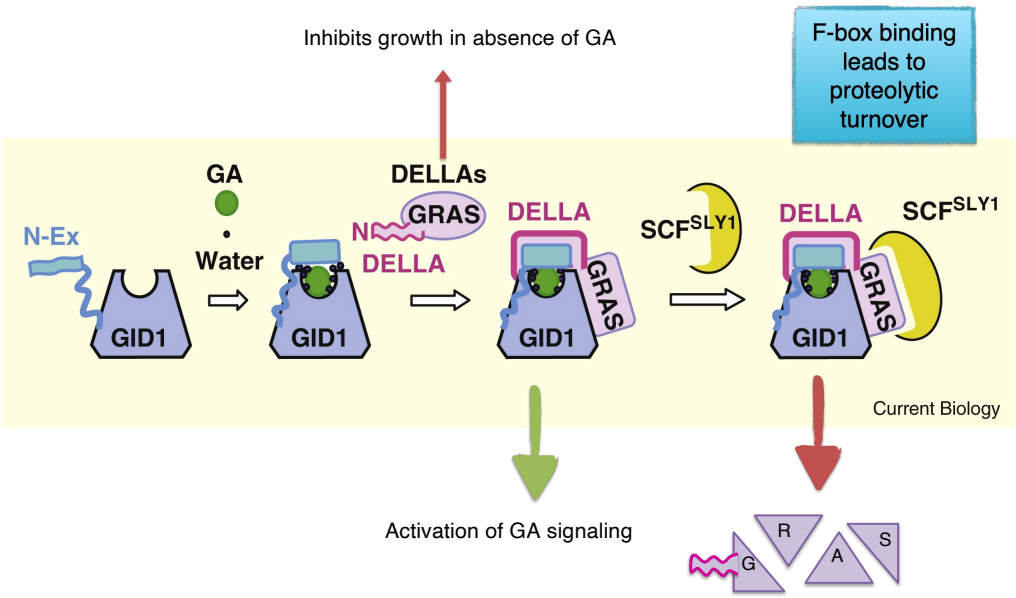
McGinnis, K.M., Thomas, S.G., Soule, J.D., Strader, L.C., Zale, J.M., Sun, T.-p., and Steber, C.M. (2003). The Arabidopsis SLEEPY1 gene encodes a putative F-box subunit of an SCF E3 ubiquitin ligase. Plant Cell 15: 1120-1130.

Figure 6. RGA Protein Levels, but Not the RGA mRNA, Are Highly Increased in *sly1* Mutants.

(A) Eight-day-old seedlings were treated with (+) or without (-) 1 uM GA4 for 2 h, and protein extracts were fractionated by 8% SDS-PAGE. The protein blot was probed with an anti-RGA antibody. Ponceau staining was used to confirm equal loading of the blot (data not shown).

The *ga1-3* biosynthesis mutant has higher accumulation of RGA1 because it accumulates less GA than wild-type plants.

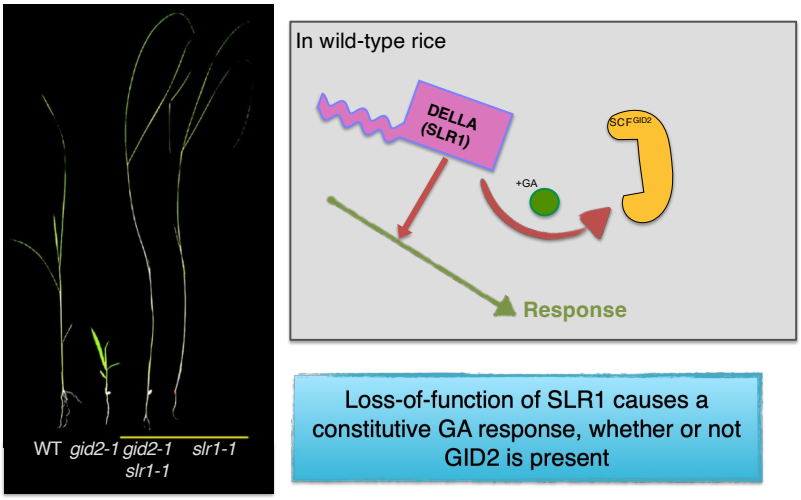
DELLAs complexed with GID1 are recognized by the F-box protein



Sun, T.-p. (2011). The molecular mechanism and evolution of the GA GID1 DELLA signaling module in plants. Curr. Biol. 21: R338-R345

A model for the GA-dependent GID1–DELLA interaction and subsequent SCF^{SLY1} binding. GA binding first induces a conformational change in the N-Ex of GID1 for DELLA binding, which promotes binding of the GRAS domain of the DELLA protein to GID1. This stable complex enables efficient SCF^{SLY1} recognition and subsequent degradation of DELLA by the proteasome.

In support of this model, the *slr1* / *gid2* double mutant looks like *slr1*

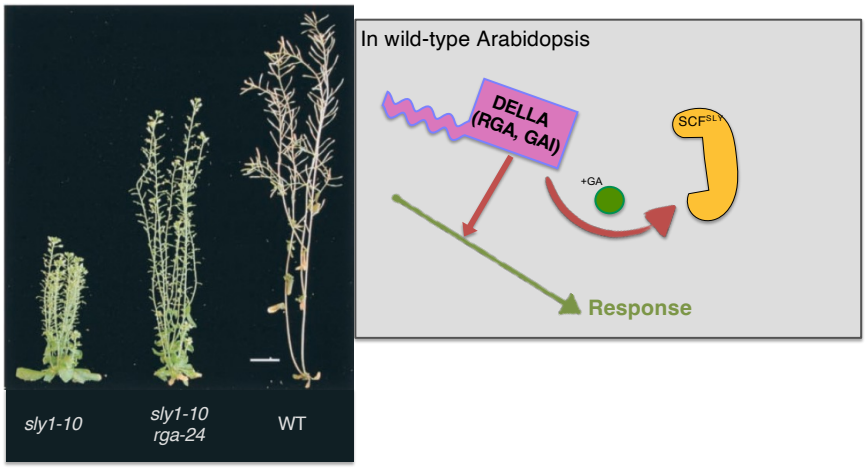


Sasaki, A., et al. (2003) Accumulation of phosphorylated repressor for gibberellin signaling in an F-box mutant. Science 299: 1896-1898

The *gid2* mutant is dwarfed because it is defective in degradation of DELLAs like SLR1. When the SLR1 protein is absent, as in the *slr1-1* mutant, it doesn't matter if there is GID2 or not because its function is to remove SLR1.

Gross morphologies of wild type, *gid2-1*, *gid2-1/slr1-1*, and *slr1-1* plants (left to right) in 2-week-old seedlings. Bar = 10 cm.

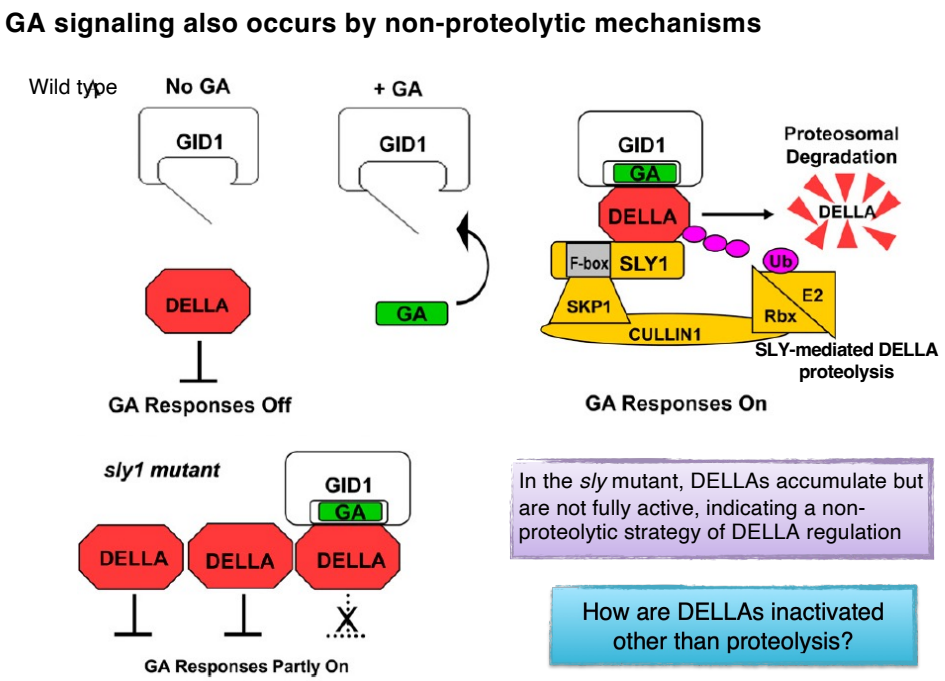
Similarly, in *Arabidopsis*, the *sleepy* mutant is partially reverted by DELLA loss-of-function mutants



McGinnis, K.M., Thomas, S.G., Soule, J.D., Strader, L.C., Zale, J.M., Sun, T.-p., and Steber, C.M. (2003). The Arabidopsis SLEEPY1 gene encodes a putative F-box subunit of an SCF E3 ubiquitin ligase. Plant Cell 15: 1120-1130.

Suppression of *sly1-10* by *rga-24.*

sly1-10 (left), the *sly1-10 rga-24* double mutant (center), and wild-type Ler (right) plants are shown. *rga-24* partly rescues the dwarf phenotype of *sly1-10,* but not poor fertility. Ler and homozygous mutant plants were grown on soil for 60 days under a long-day photoperiod. Bar = 15 mm.



Hauvermale, A.L., Ariizumi, .T. and Steber, C.M. (2012) Gibberellin signaling: a theme and variations on DELLA repression. Plant Physiol. 160: 83-92

Figure 1. Proteolysis-dependent and -independent GA signaling models.

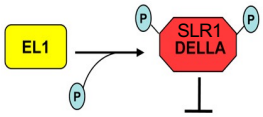
A, The canonical GA signaling model illustrating GA-dependent GID1-DELLA complex formation resulting in DELLA recognition and ubiquitylation by the SCF^{SLY1} E3. Polyubiquitylation leads to DELLA proteolysis by the 26S proteasome, thereby lifting DELLA repression of GA responses.

B, Proteolysis-independent GA signaling in *sly1* mutants occurs when GID1-GA-DELLA complex formation blocks DELLA repression of GA

responses without DELLA destruction.

DELLA activity and stability is affected by phosphorylation

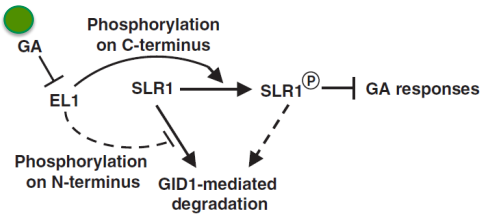
EARLY FLOWERING1 encodes a protein kinase (EL1) that phosphorylates SLR1



The rice *early flowering1* mutant is GA-hypersensitive

WT *el1*

Phosphorylation stabilizes and activates SLR1



Dai, C. and Xue, H.W. (2010) Rice early flowering1, a CKI, phosphorylates DELLA protein SLR1 to negatively regulate gibberellin signalling. EMBO J. 29(11):1916-1927
Hauvermale, A.L., Arizumi, .T. and Steber, C.M. (2012) Gibberellin signaling: a theme and variations on DELLA repression. Plant Physiol. 160: 83-92.

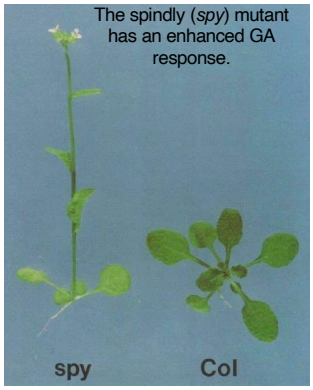
EL1-mediated phosphorylation of DELLA activates DELLA as a repressor of GA responses.

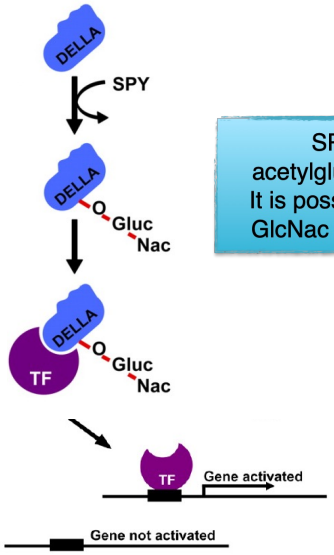
Observations of the inflorescence development of WT and *el1* plants at 50 or 55 days confirmed the earlier flowering of el1 (Bar = 5 mm). After 55 days of cultivation, the young panicles of *el1* plants were almost fully developed—much earlier than WT.

Hypothetical model of EL1 function in GA signalling. EL1 is repressed by GA and phosphorylation of EL1 on the SLR1 C-

terminus sustains its active form. Phosphorylation on the SLR1 N-terminus suppresses the GID1– GA-mediated degradation of SLR1, resulting in the suppressed GA responses.

SPINDLY may also regulate DELLA proteins post-translationally





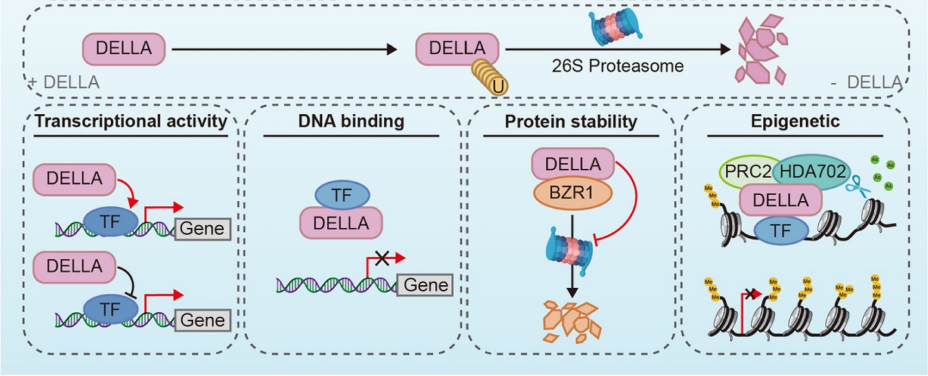
SPY encodes an *N*-acetylglucosamine transferase. It is possible that the addition of GlcNac activates DELLA action

Jacobsen, S. E. and Olszewski, N. E. (1993) Mutations at the SPINDLY locus of Arabidopsis alter gibberellin signal transduction. Plant Cell 5: 887-896.
Harberd, N.P., Belfield, E., and Yasumura, Y. (2009). The angiosperm gibberellin-GID1-DELLA growth regulatory mechanism: How an "inhibitor of an inhibitor" enables flexible response to fluctuating environments. Plant Cell 21: 1328-1339.

DELLAs are growth inhibitors that act (at least in part) by interfering with the activity of growth-promoting transcription factors. In addition, the growth inhibitory properties of DELLAs may be enhanced by O-GlcNAc modification due to SPY.

spy-1 and wild-type Columbia (Col) plants photographed 18 days after germination.

DELLA proteins have a central role in GA signaling



Wang, J.-D., Liu, Q.-Q. & Li, Q.-F. DELLA family proteins function beyond the GA pathway. *Trends Plant Sci.* (2025) doi:10.1016/j.tplants.2025.01.007.

Figure 1. DELLA protein acts as an integration node of multiple signaling pathways. Gibberellic acid

(GA)-induced degradation of DELLA protein is the key event in the GA signaling pathway. In addition, several

other signals, including temperature, light, strigolactone (SL), and stress signals, can also affect the stability of

DELLA proteins independently of GA. The DELLA proteins influence downstream

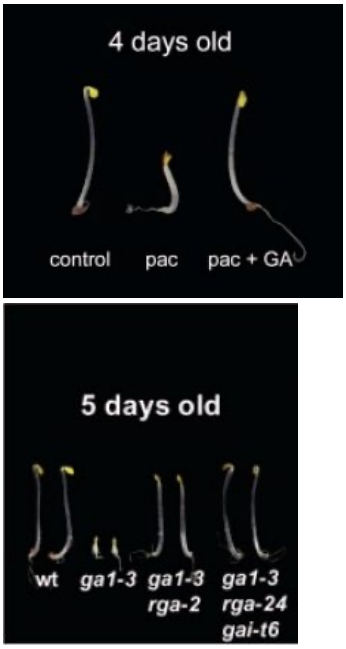
gene expression through

multiple strategies, such as affecting the transcriptional activity or DNA-binding ability of transcription factors

(TFs), regulating the protein stability and epigenetic modification of transcription factors, thereby coordinating

plant growth and stress resistance.

GAs repress photomorphogenesis and DELLAs promote it



GA is required for etiolation.
Etiolation is a dark-growth response that includes elongated shoot growth and unopened leaves.
The GA-deficient *ga1-3* mutant or treatment with the GA biosynthesis inhibitor PAC do not show this normal dark growth pattern, but can be rescued with DELLA mutants or exogenous GA.

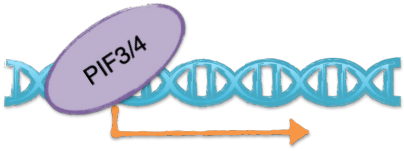
Alabadi, D., Gil, J., Blazquez, M.A., and Garcia-Martinez, J.L. (2004). Gibberellins repress photomorphogenesis in darkness. Plant Physiol. 134: 1050-1057.

Effect of paclobutrazol (PAC) on photomorphogenesis of dark-grown Arabidopsis wild-type (WT) seedlings. A, Phenotypes of representative 4-d-old dark-grown seedlings for each treatment.

Effect of Arabidopsis GA biosynthesis and signaling mutants on photomorphogenesis in darkness. A, Phenotypes of representative 5-d-old dark-grown Arabidopsis GA biosynthesis and signaling mutants.

The PIF3 and PIF4 transcription factors are necessary for etiolation

PIF3 and PIF4 activate the transcription of growth-promoting genes, leading to elongation of the hypocotyl in the dark

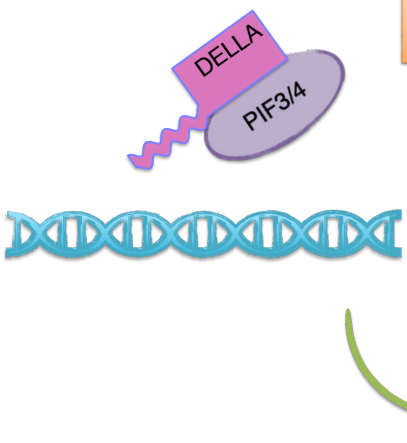


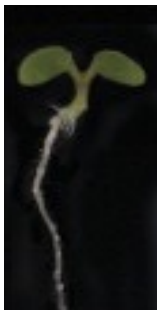
Davière, J.-M., de Lucas, M., and Prat, S. (2008) Transcription factor interaction: a central step in DELLA function. Curr. Opin. Genet. Devel. 18: 295–303.

PIF stands for phytochrome-interacting factor

DELLA proteins bind PIF3 and PIF4 and interfere with their action

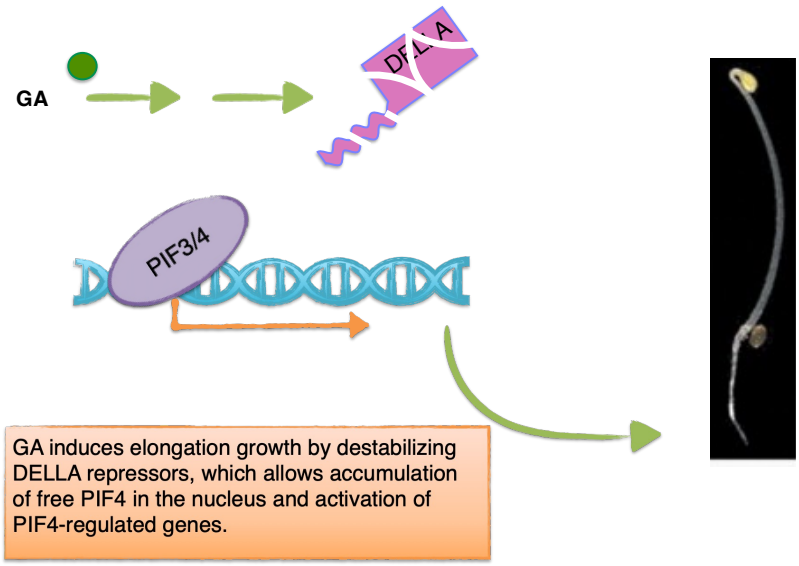
DELLAs repress PIFs transcriptional activity by binding their DNA-recognition domain, hence sequestering them into inactive complexes unable to bind DNA.





Davière, J.-M., de Lucas, M., and Prat, S. (2008) Transcription factor interaction: a central step in DELLA function. Curr. Opin. Genet. Devel. 18: 295–303

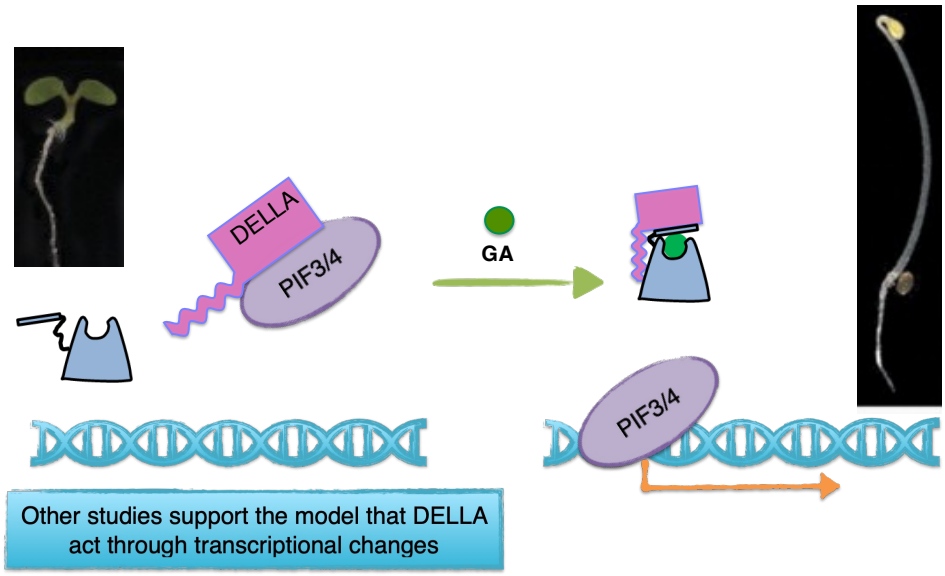
GA promotes DELLA degradation, allowing PIF3 and PIF4 to act



GA induces elongation growth by destabilizing DELLA repressors, which allows accumulation of free PIF4 in the nucleus and activation of PIF4-regulated genes.

Davière, J.-M., de Lucas, M., and Prat, S. (2008) Transcription factor interaction: a central step in DELLA function. Curr. Opin. Genet. Devel. 18: 295–303.

These studies reveal that DELLAs can affect growth through their actions on other proteins



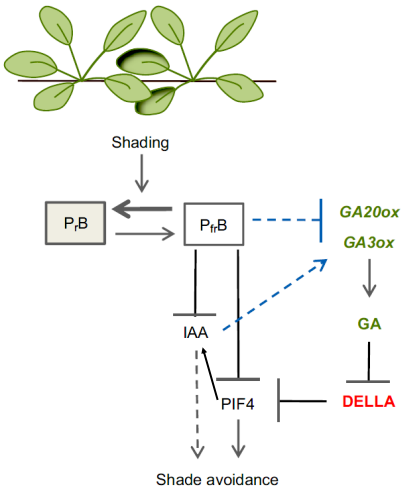
Davière, J.-M., de Lucas, M., and Prat, S. (2008) Transcription factor interaction: a central step in DELLA function. Curr. Opin. Genet. Devel. 18: 295–303.

See for example Cao et al, (2006) Plant Physiol 142: 509 – 535, and Gallego-Bartolomé et al., (2011) PloS ONE 6: e23918.

The shade-avoidance response is mediated by DELLAs

In unshaded conditions, phytochrome accumulates in the active P_{fr} form, GA doesn't accumulate, and DELLAs inhibit PIF4 action.

Vegetative shading causes phytochrome to convert to the P_r form, GA accumulates and PIF4 is activated, stimulating stem and petiole elongation.



Colebrook, E.H., Thomas, S.G., Phillips, A.L., and Hedden, P. (2014) The role of gibberellin signalling in plant responses to abiotic stress. J. Exp. Biol. 217: 67-75

Fig. 3. Phytochrome B-mediated signalling and the involvement of GA in the shade avoidance response. A low red/far-red light ratio converts the active Pfr form to inactive Pr (indicated by the thick grey arrow), allowing the gene activation function of PIF4. This light regime also promotes GA biosynthesis through transcriptional activation of GA20ox and GA3ox genes, resulting in degradation of DELLA proteins, which otherwise inhibit PIF4 function by sequestration. The mechanism for the transcriptional activation is uncertain but it may be mediated by auxin (IAA), whose

synthesis and distribution is promoted by far-red light. Normal arrows and T-bars indicate positive and negative interactions, respectively. Blue arrows denote transcriptional activation. Dashed arrows indicate unclear or indirect relationships.

Perception and signaling - Summary

- The GA receptor GID1 forms a complex with inhibitory DELLAs via the DELLA domain
- Plants expressing stabilized mutant proteins without a DELLA domain are GA-insensitive and dwarf
- Plants expressing loss-of-function DELLAs show a GA-hypersensitive response (e.g. *slender1*)
- DELLAs can directly bind to transcription factors or regulators of transcription factors and interfere with their function

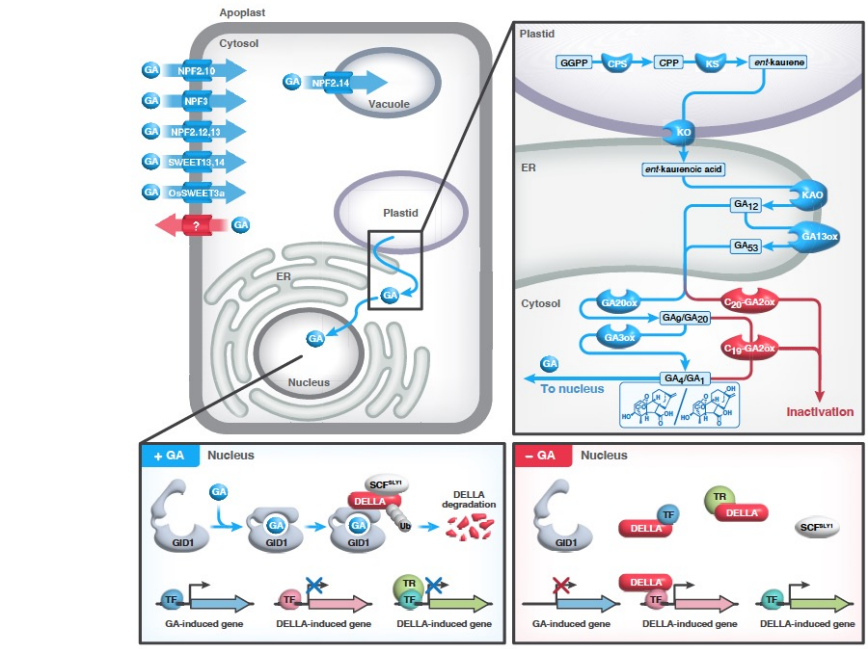


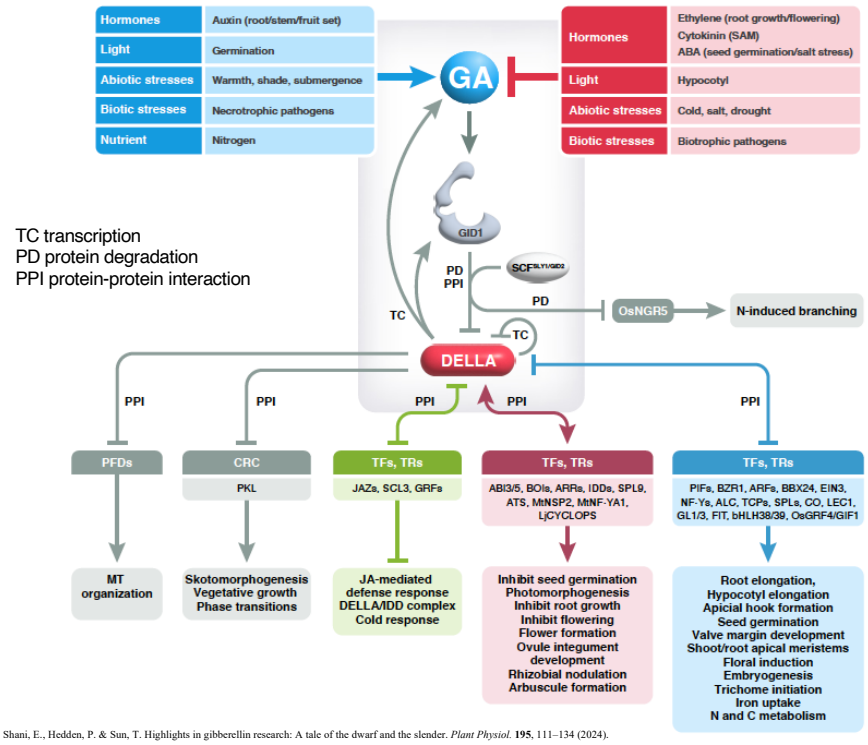
Figure 2. GA metabolism, transport, perception, and signaling in plant cells. GA biosynthesis takes place in three cellular compartments: *ent*-Kaurene is synthesized from GGPP by CPS and KS in the plastid; *ent*-Kaurene is converted to *ent*-kaurenoic acid by KO on the outer plastid membrane, which is connected to the ER; *ent*-Kaurenoic acid is converted to GA12 by KAO and GA12 to GA53 by GA13ox in the ER; GA12 and GA53 are converted to bioactive GA4 and GA1, respectively, by GA20ox and GA3ox in the cytoplasm. GA4

and GA1 as well as their immediate precursors GA9 and GA20, respectively, are oxidized on C-2 by C19-GA2ox, resulting in inactivation, while C20-GA2ox acts on earlier C20-GA precursors. In addition to de novo biosynthesis, GA can be imported into the cell by GA transporters nitrate and peptide transporter families (NPFs) and SWEETs or transported into the vacuole as labeled. GA perception and signaling occur in the nucleus where GA binding to its receptor GID1 (+GA) promotes DELLA degradation via Skp, Cullin, F-box (SCF)SLY1/GID2-mediated polyubiquitination and subsequent proteolysis by the 26S proteasome. When GA levels are low (– GA), DELLAs accumulate to high levels. Three distinct modes of DELLA action are shown: (i) DELLA represses transcription by blocking DNA binding and sequestering transcription factors (TF in blue) from target promoters; (ii) DELLA induces transcription by recruiting TFs (in pink); and (iii) DELLA induces transcription by sequestering transcription repressors (TR in green) from target promoters. GGPP, *trans*-geranylgeranyl diphosphate; CPP, *ent*-copalyl

diphosphate; CPS, *ent*-copalyl diphosphate synthase; KS, *ent*-kaurene synthase; KO, *ent*-kaurene oxidase; KAO, *ent*-kaurenoic acid oxidase; GA13ox, GA 13-oxidase; GA20ox, GA 20-oxidase; GA3ox, GA 3-oxidase; GA2ox, GA 2-oxidase. GA biosynthesis enzymes are labeled in blue, and the deactivation enzymes are labeled in red. GA, gibberellin; TF, transcription factor; TR, transcription repressors; ER, endoplasmic reticulum.

GAs' roles in whole-plant physiology

- GAs control ***growth and elongation*** by cell expansion and cell division
- GAs mediate ***stress responses*** through DELLA proteins
- GAs promote ***seed germination*** and reserve mobilization
- GAs promote ***flowering***
- GAs interact with ***brassinosteroids*** at transcriptional level



Shani, E., Hedden, P. & Sun, T. Highlights in gibberellin research: A tale of the dwarf and the slender. *Plant Physiol.* **195**, 111–134 (2024).

Figure 3. Interaction network between the GA-GID1-DELLA signaling module and various internal and external cues. Signals that increase bioactive GA levels are labeled in blue, while signals that decrease GA levels are shown in red. GA-GID1 triggers DELLA degradation via SCFSLY1/GID2-mediated polyubiquitination. GA-GID1 also induces OsNGR5 degradation in a SCFGID2-dependent manner to inhibit nitrogen-induced shoot branching in rice. DELLAs interact antagonistically or additively with a

myriad of transcription factors (TFs), transcription regulators (TRs), CRC, and PREFOLDINs (PFDs) to modulate specific developmental processes. Most of the DELLA interactors are Arabidopsis proteins, except those that are labeled (Os, *Oryza sativa*; Mt, *Medicago truncatula*; Lj, *Lotus japonicus*). See Boxes 1 and 2 for details. PD, protein degradation; PPI, protein–protein interaction; TC, transcription; SAM, shoot apical meristem; MT, microtubule; GA, gibberellin; ABA, abscisic acid.

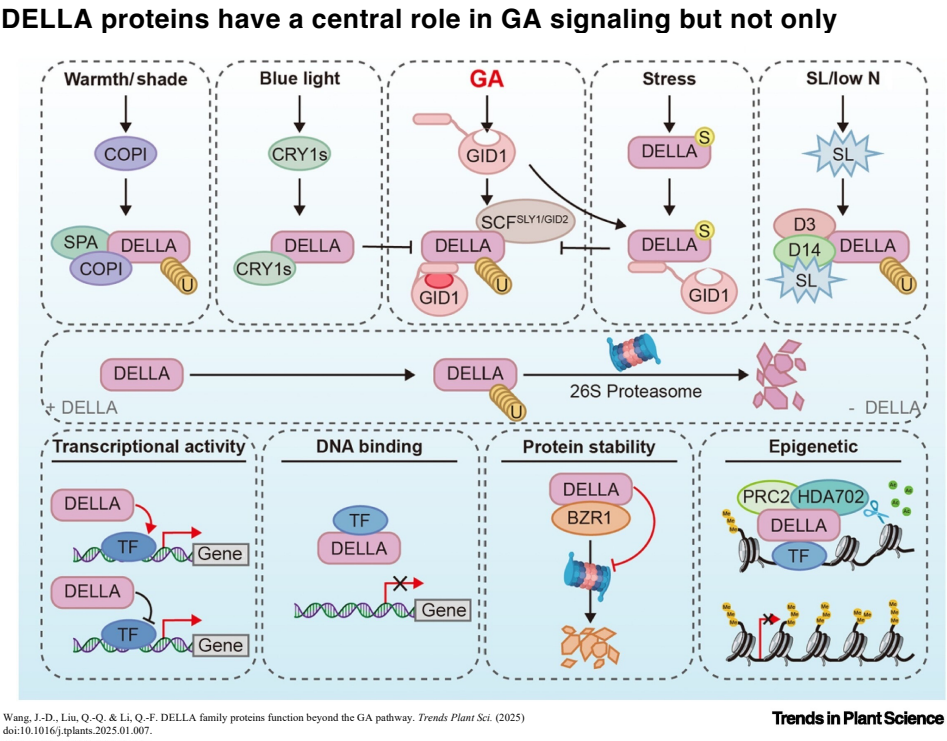


Figure 1. DELLA protein acts as an integration node of multiple signaling pathways. Gibberellic acid

(GA)-induced degradation of DELLA protein is the key event in the GA signaling pathway. In addition, several

other signals, including temperature, light, strigolactone (SL), and stress signals, can also affect the stability of

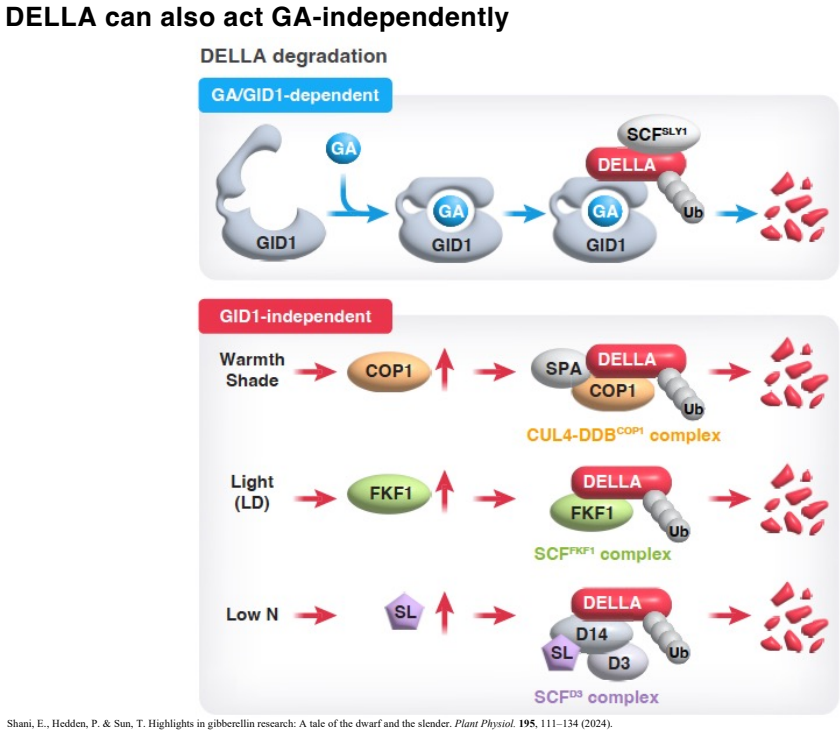
DELLA proteins independently of GA. The DELLA proteins influence downstream

gene expression through

multiple strategies, such as affecting the transcriptional activity or DNA-binding ability of transcription factors

(TFs), regulating the protein stability and epigenetic modification of transcription factors, thereby coordinating

plant growth and stress resistance.



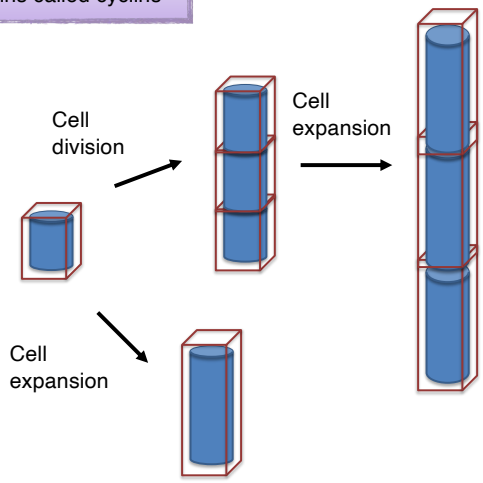
Shani, E., Hedden, P. & Sun, T. Highlights in gibberellin research: A tale of the dwarf and the slender. *Plant Physiol.* **195**, 111–134 (2024).

Figure 4. GA/GID1-dependent versus GA/GID1-independent DELLA degradation. GA/GID1-dependent proteolysis of DELLA is mediated by SCFSLY1/GID2. DELLA can be destabilized by signals including warmth, shade, LD light, and low nitrogen conditions. COP1 and its associated CUL4-DDBCOP1 ubiquitin E3 ligase complex mediate warmth- and shade-induced DELLA degradation in Arabidopsis. The SCFFKF1 ubiquitin E3 ligase complex mediates LD-induced

DELLA proteolysis in Arabidopsis. Low nitrogen conditions induce the biosynthesis of SL in rice, which binds to its receptor D14 and promotes the D14-DELLA interaction and DELLA degradation mediated by SCFD3. GA, gibberellin; Ub, ubiquitin.

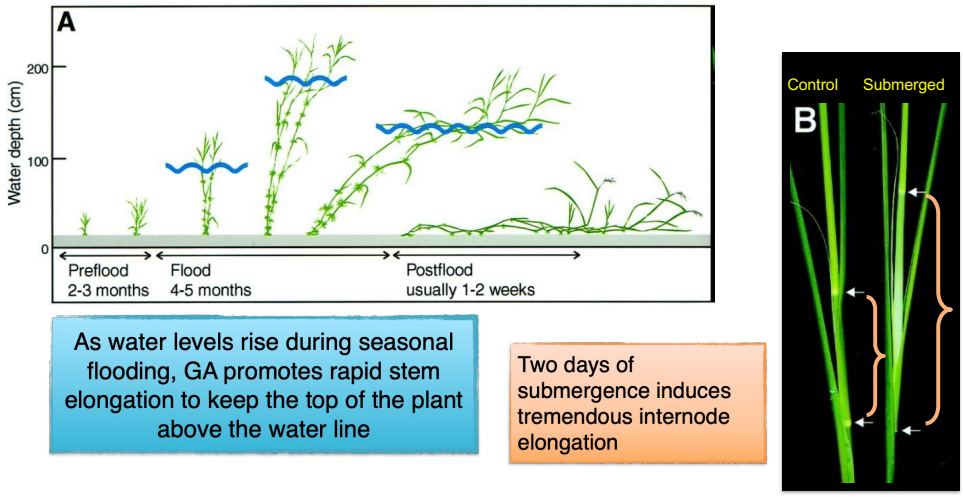
GAs promote growth through cell expansion and division

GAs induce expression of cell-cycle regulatory proteins called cyclins



GAs promote elongation by cell wall loosening and stabilizing the orientation of cortical microtubules, which help direct growth

GAs promote elongation and submergence avoidance in rice



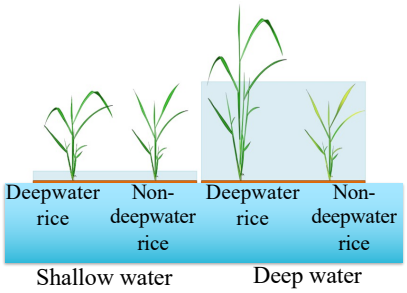
Kende, H., van der Knaap, E., and Cho, H.-T. (1998). Deepwater rice: A model plant to study stem elongation. Plant Physiol. 118: 1105-1110.

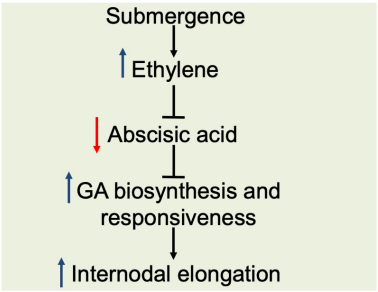
Figure 1. A, The growth cycle of a deepwater rice plant. Young plants are allowed to establish themselves before the annual flood arrives. As the floodwaters rise, rapid internodal growth permits the plants to maintain part of their foliage above the water. Adventitious roots develop at the submerged nodes. After the flood recedes, the upper internodes show gravitropic sensitivity and grow upward (modified with permission from Catling, 1992). B, Youngest internode of an air-grown (left) and a submerged plant (right). The upper and lower arrows indicate the

positions of the highest and second highest node, respectively. The whitish tissue of the internode on the right corresponds to about 10 cm of new growth during 2 d of submergence.

GAs mediates elongation deepwater rice

Deepwater rice can elongate their stems up to 4 meters



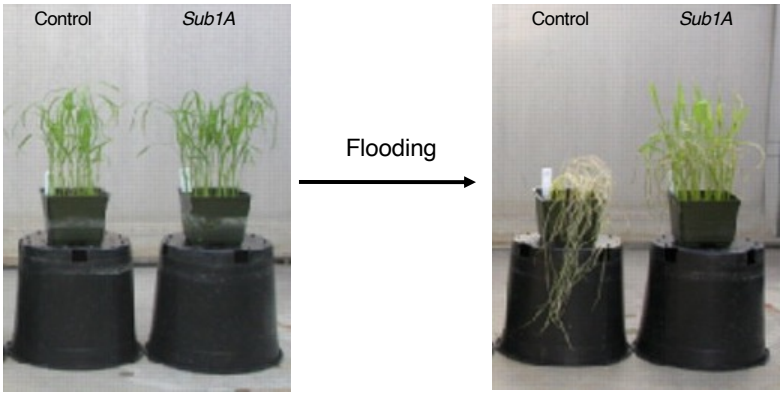


This response is in part mediated by **ethylene**, with **increases GA biosynthesis** and sensitivity, and thus **internode elongation**

Minami, A. et al. (2018) Time-course transcriptomics analysis reveals key responses of submerged deepwater rice to flooding. Plant Phys 176: 3081-3102 See also Mickelbart et al Nature Reviews Genetics 16, 237–251 (2015)

Rice that carry the *Sub1A* gene can survive flooding by *not* growing

Sub1A codes for an ERF transcription factor

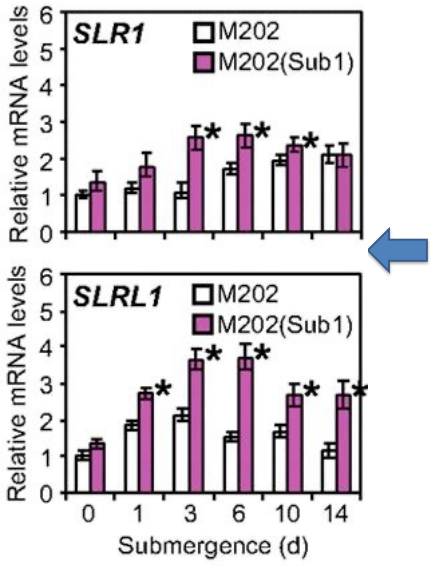


Fukao, T. and Bailey-Serres , J. (2008). Submergence tolerance conferred by Sub1A is mediated by SLR1 and SLRL1 restriction of gibberellin responses in rice. PNAS USA 105: 16814-16819

Constitutive-induced expression of Sub1A confers growth restriction and survival of prolonged submergence.

Plants before submergence (left), and after 7 days of recovery from 16 days of submergence (right). Fourteen-day-old [LG, control] and 21-day-old (Ubi:Sub1A) plants at a similar developmental age were subjected to submergence tests.

Sub1A-carrying plants have enhanced DELLA expression



Submerged rice expressing high levels of *Sub1A* show increased mRNA and protein accumulation of the GA-response repressors SLR1 and SLRL1

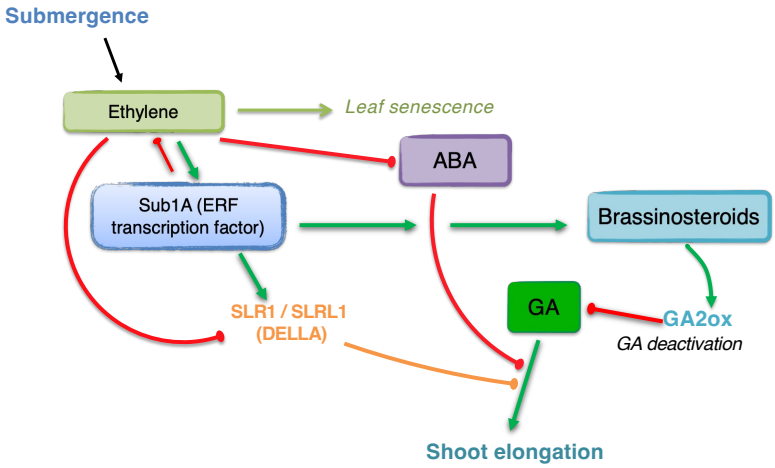
Fukao, T. and Bailey-Serres , J. (2008). Submergence tolerance conferred by Sub1A is mediated by SLR1 and SLRL1 restriction of gibberellin responses in rice. PNAS USA 105: 16814-16819

Sub1A dampens GA response during submergence.

Relative transcript levels of *SLR1* and *SLRL1* in aerial tissue during submergence. Fourteen-day-old [M202, M202(Sub1)] were submerged for up to 14 days. Aerial tissue was collected at the specific time points and subjected to quantitative RT-PCR analysis. Relative levels of individual transcripts were calculated by comparison to the nonsubmerged control (M202 at day 0). Data represent mean ± SD from 3 independent biological replicates, and

an asterisk indicates a significant difference between M202 vs. M202(Sub1) (P < 0.01).

Sub1A promotes flooding tolerance through several mechanisms



Fukao, T., Yeung, E. and Bailey-Serres, J. (2011). The submergence tolerance regulator SUB1A mediates crosstalk between submergence and drought tolerance in rice. Plant Cell. 23: 412-427.
Fukao, T., Yeung, E. and Bailey-Serres, J. (2012). The submergence tolerance gene SUB1A delays leaf senescence under prolonged darkness through hormonal regulation in rice. Plant Physiol. 160: 1795-1807.
Schmitz, A.J., Folsom, J.J., Jikamaru, Y., Ronald, P. and Walia, H. (2013). SUB1A-mediated submergence tolerance response in rice involves differential regulation of the brassinosteroid pathway. New Phytol. 198: 1060-1070.

Under salt stress, survival is enhanced by reduced GA response

GA treatment decreases survival in wild-type plants grown on NaCl

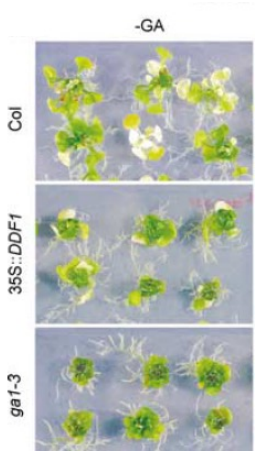
Magome, H., S. Yamaguchi, et al. (2004). dwarf and delayed-flowering 1, a novel Arabidopsis mutant deficient in gibberellin biosynthesis because of overexpression of a putative AP2 transcription factor. Plant J. 37: 720-729.

The white tissues are dead or dying as a result of NaCl treatment.

b) Physiological changes of plants transferred to plates containing 170 mM sodium chloride. Photographs were taken on day 13 post-transfer. +GA indicates the treatment with 10^{-6} M GA_3.

Under salt stress, survival is enhanced by reduced GA synthesis

GA-deficient plants survive better



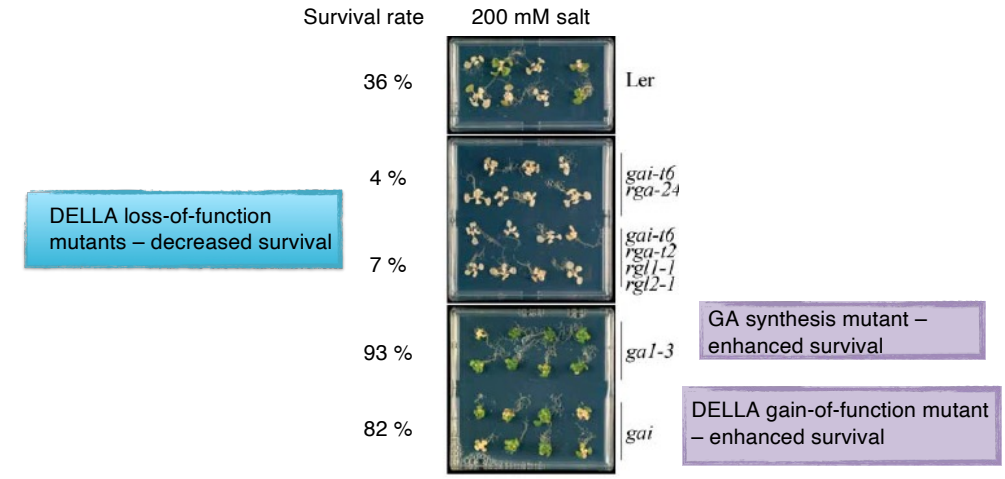
These plants over-express a transcription factor, DDF1, that induces expression of *GA2ox*, reducing GA levels

GA biosynthesis mutant.

Magome, H., S. Yamaguchi, et al. (2004). dwarf and delayed-flowering 1, a novel Arabidopsis mutant deficient in gibberellin biosynthesis because of overexpression of a putative AP2 transcription factor. Plant J. 37: 720-729

b) Physiological changes of plants transferred to plates containing 170 mM sodium chloride. Photographs were taken on day 13 post-transfer.

At high salt levels, DELLA activity is necessary for survival



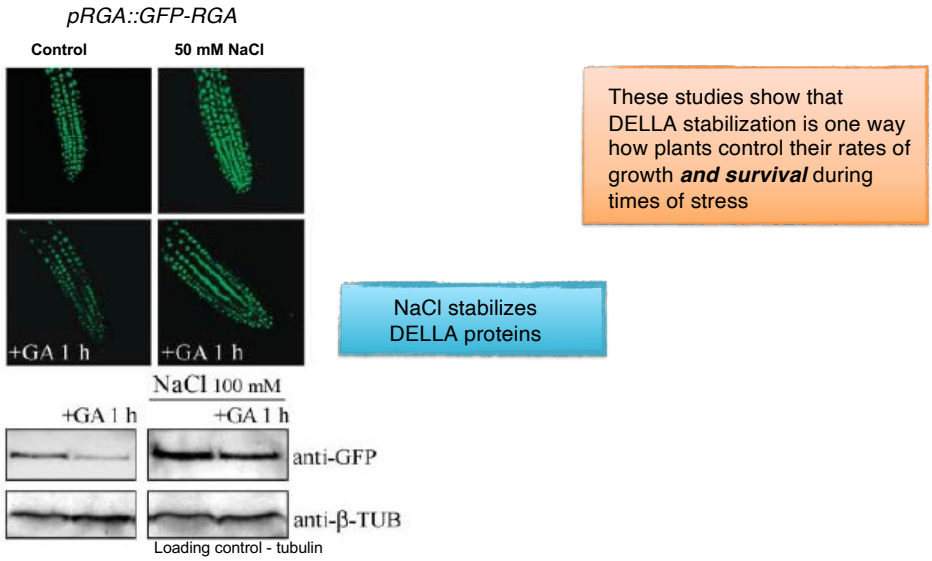
Achard, P., et al. (2006). Integration of plant responses to environmentally activated phytohormonal signals. Science 311: 91-94

Fig. 4. DELLA-dependent survival of toxic salt concentrations.

Survival of representative plants of various genotypes (as indicated) on high-salt medium (200 mM). Photograph taken 10 days after transfer of plants to high-salt medium. Live plants are green; dead plants are white.

Numbers of plants of various genotypes [expressed as rate of survival (%)] that survive growth on high-salt medium.

DELLA proteins are stabilized by NaCl stress



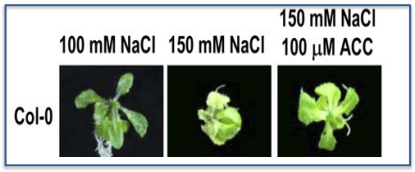
Achard, P., et al. (2006). Integration of plant responses to environmentally activated phytohormonal signals. Science 311: 91-94

Fig. 1. Salt slows vegetative growth by enhancing DELLA function.

(top) GFP fluorescence (viewed by fluorescence confocal microscopy) in tips of *pRGA:GFP-RGA* primary seedling roots after a 1-hour treatment with 10 uM GA_3 (+GA) (or control) in the presence or absence of NaCl, together with (bottom) immunodetected (by an antibody to GFP) GFP-RGA in salt-treated (or control) GA-treated (or control) *pRGA:GFP-RGA* roots. b-tubulin (b-TUB) serves as a sample-loading control.

Note that the GA-induced decrease in fluorescence in control plants does not occur in NaCl-treated plants. The same result is shown below by western blotting; in the presence of NaCl GA does not induce DELLA degradation.

Ethylene increases salt tolerance through DELLAs



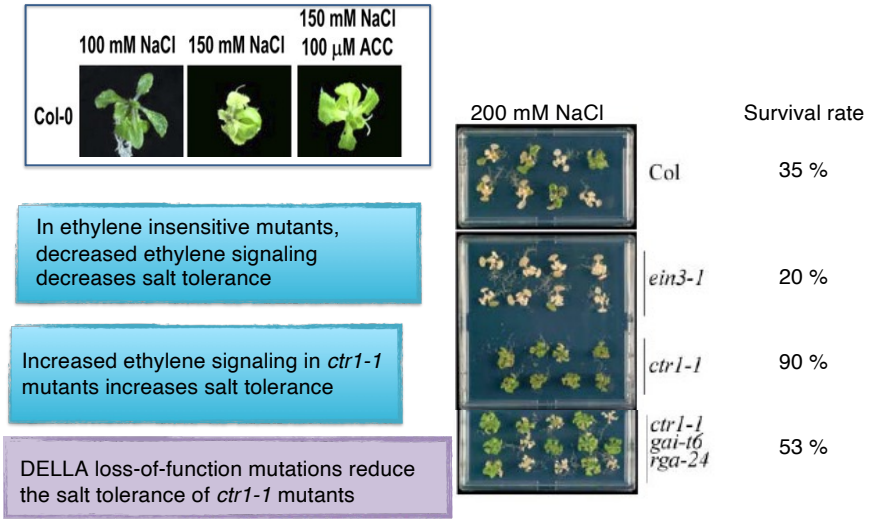
In wild-type plants, ethylene increases salt tolerance. (ACC is an ethylene precursor converted to ethylene by the plant)

Cao, W.-H. et al., (2007) Modulation of ethylene responses affects plant salt-stress responses. Plant Physiol. 143: 707-719.

The plants treated with ACC are better able to tolerate salt.

The 150 mM NaCl-treated wild type seedling was transferred onto NaCl plus ACC to observe phenotypic change.

Ethylene increases salt tolerance through DELLAs



Cao, W.-H. et al., (2007) Modulation of ethylene responses affects plant salt-stress responses. Plant Physiol. 143: 707-719.;
Achard, P., et al. (2006). Integration of plant responses to environmentally activated phytohormonal signals. Science 311: 91-94.

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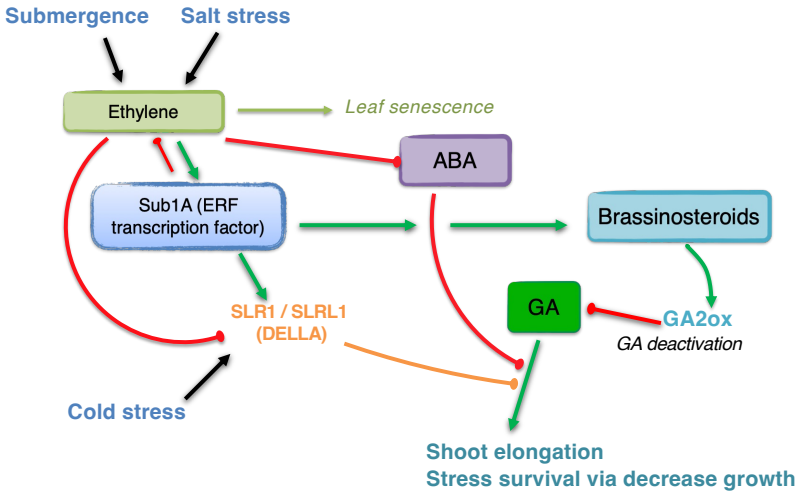
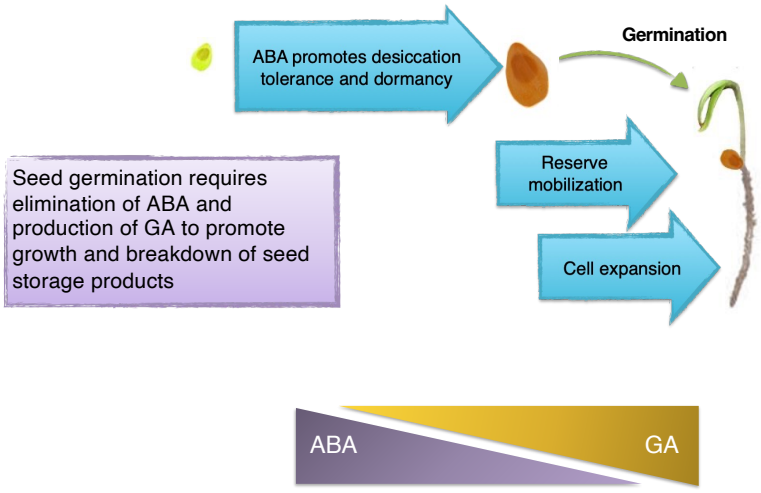
Numbers of plants of various genotypes [expressed as rate of survival (%)] that survive growth on high-salt medium.

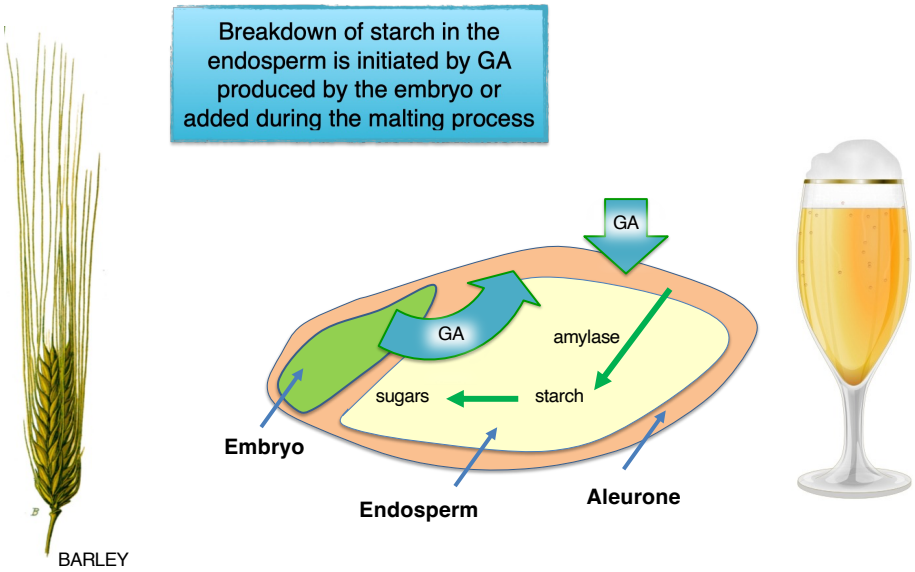
Growth and environmental stress: DELLAs integrate diverse signals



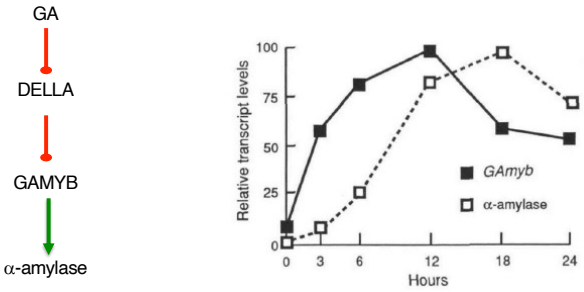
GA and ABA act antagonistically in the control of seed germination



During germination, GA induces expression of nutrient-mobilizing enzymes



In the aleurone GAMYB transcription factors activate seed storage genes



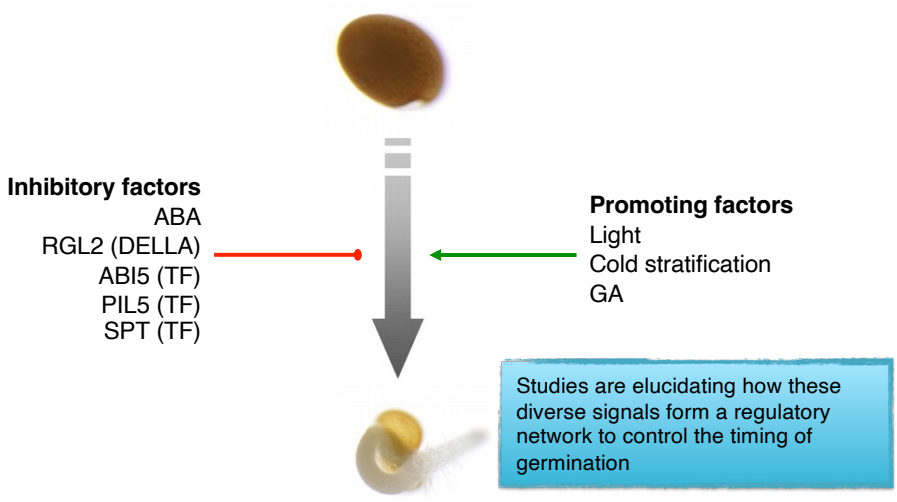
GA induces expression of the transcription factor GAMYB, which induces α-amylase expression

Gubler, F., Kalla, R., Roberts, J.K., and Jacobsen, J.V. (1995). Gibberellin-regulated expression of a myb gene in barley aleurone cells: Evidence for Myb transactivation of a high-pl α-amylase gene promoter. Plant Cell 7: 1879-1891.

Figure 5. Effect of GA_3 on GAmyb and α-Amylase Gene Expression in Barley Aleurone Layers.

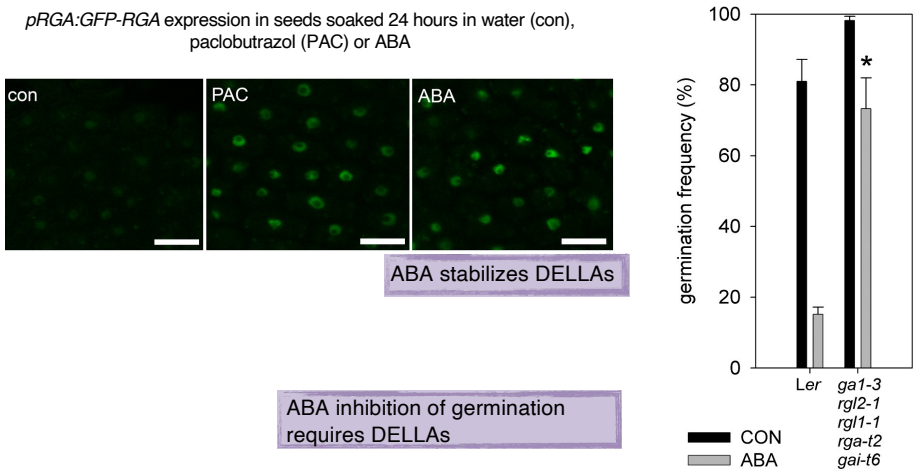
Quantitation of time course of GAmyb and α-amylase gene expression in GA_3-treated layers. mRNA levels were quantified by PhosphorImager and normalized to rRNA.

Germination involves integration of multiple signals



Oh, E., et al. (2007). PIL5, a phytochrome-interacting bHLH protein, regulates gibberellin responsiveness by binding directly to the GAI and RGA promoters in Arabidopsis seeds. Plant Cell 19: 1192-1208.

The germination-inhibitory effects of ABA are mediated by DELLAs



Penfield, S., Gilday, A.D., Halliday, K.J., and Graham, I.A. (2006). DELLA-mediated cotyledon expansion breaks coat-imposed seed dormancy. Curr. Biol. 16: 2366-2370.

Figure 1. The Seed Germination of Wild-type, *ga1-3*, and DELLA Mutant Combinations in the *ga1-3* Background

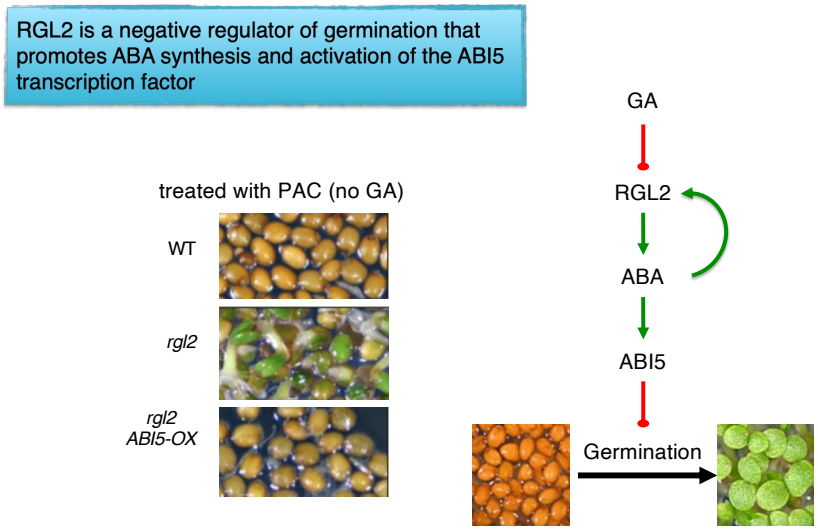
Mutant combinations containing *ga1-3* but lacking *rgl2-1* did not germinate under any conditions and are excluded from the presented data. Asterisks indicate lines with germination significantly greater than the wild-type after ABA treatment ($p < 0.001$).

The germination of cold stratified freshly harvested seed and stratified seed treated with 1 uM ABA on minimal water-agar

medium. Data represent mean and standard error of the germination of five or six independent seed batches.

pRGA:GFP-RGA fluorescence in 24-hr-imbibed seeds on water-agar media or media supplemented with 10 uM paclobutrazol or 10 uM ABA. Scale bars represent 10 um.

The DELLA protein RGL2 promotes ABA synthesis and signaling

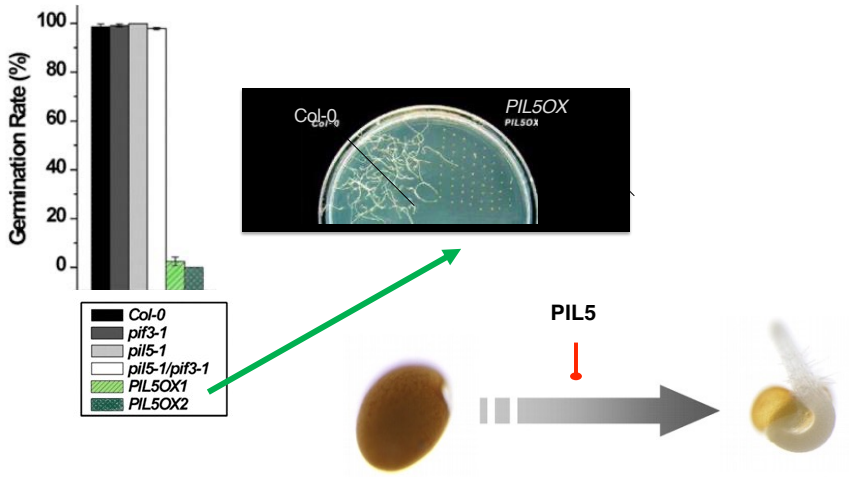


Piskurewicz, U., Jikumaru, Y., Kinoshita, N., Nambara, E., Kamiya, Y., and Lopez-Molina, L. (2008). The gibberellic acid signaling repressor RGL2 inhibits Arabidopsis seed germination by stimulating abscisic acid synthesis and ABI5 activity. Plant Cell 20: 2729-2745.

Figure 6. Transgenic ABI5 Expression in rgl2 Mutant Seeds Restores Wild-Type–Like Germination Responses under Low-GA Conditions.

(A) PAC-treated *rgl2-1/35S:HA-ABI5*, *rgl2-1*, and wild-type (Ler) plant material at 96 h after seed imbibition (PAC, u mM).

PIL5 is a transcription factor that inhibits germination



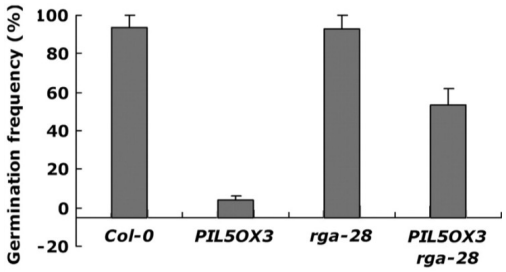
Oh, E. *et al.* Light activates the degradation of PIL5 protein to promote seed germination through gibberellin in Arabidopsis. *Plant J* **47**, 124–139 (2006).

Figure 3. PIL5 Is a Negative Component in PhyB-Mediated Promotion of Seed Germination.

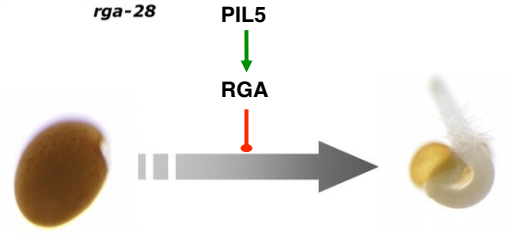
Germination patterns of the PIL5OX transgenic lines.

Quantification of the germination rates of the various mutants under different light conditions. Error bars ± SD.

Germination inhibition by PIL5 requires DELLAs



PIL5 overexpression (*PIL5OX3*) inhibits germination, but this inhibition is partly relieved in a *rga* mutant with reduced DELLA activity



Oh, E., et al. (2007). PIL5, a phytochrome-interacting bHLH protein, regulates gibberellin responsiveness by binding directly to the GAI and RGA promoters in Arabidopsis seeds. Plant Cell 19: 1192-1208.

Germination frequencies of Col-0, *PIL5OX3*, *rga-28*, and P*IL5OX3 rga-28* seeds after red light irradiation.

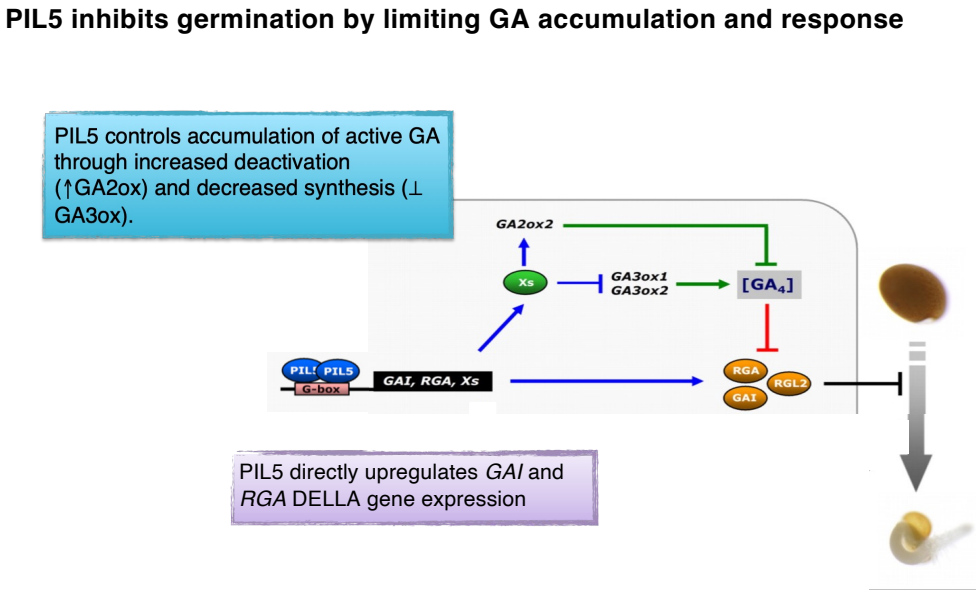
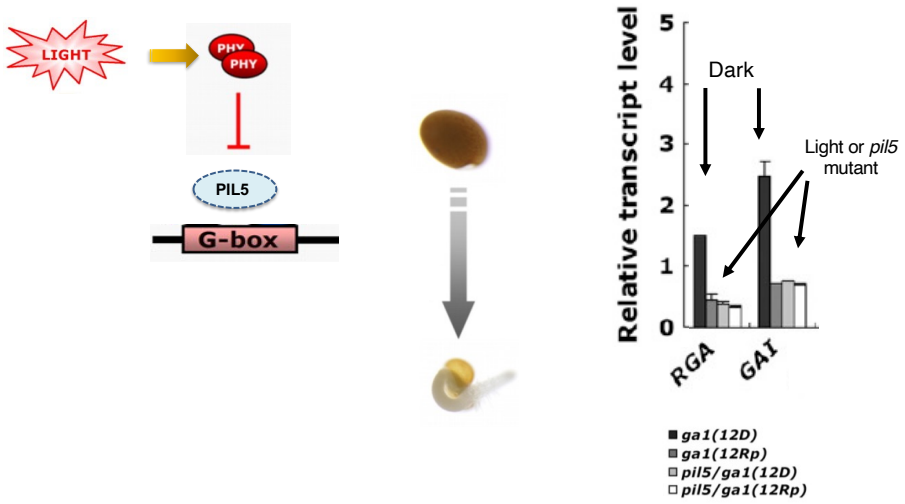


Figure 10. Proposed Molecular Events Leading to Seed Germination in Arabidopsis.

In the dark, PIL5 activates the expression of various genes, including GAI, RGA, and other unknown factors (Xs), by binding directly to their promoters through G-box elements, resulting in increased protein levels of GAI, RGA and X factors. The X factors repress GA biosynthetic and ABA catabolic genes and activate GA catabolic and ABA biosynthetic genes, resulting in decreased GA levels and increased ABA levels. The decrease in GA

stabilizes DELLA proteins, leading to increased DELLA protein levels and suppression of GA responses, including testa rupture and subsequent germination. Upon light irradiation, activated phytochromes induce PIL5 degradation, leading to decreased levels of GAI and RGA proteins and ABA. The level of RGL2, although not transcriptionally regulated by PIL5 directly, is also decreased due to the increased bioactive GA. As a result, various physiological processes are initiated, including the mobilization of storage molecules and the hydrolysis of cell walls, and the seeds eventually germinate. Red lines, events occurring at the protein level; blue lines, events occurring at the transcriptional level; green lines, events occurring via enzymatic activities. The potential regulatory circuit between GA and ABA is not shown here.

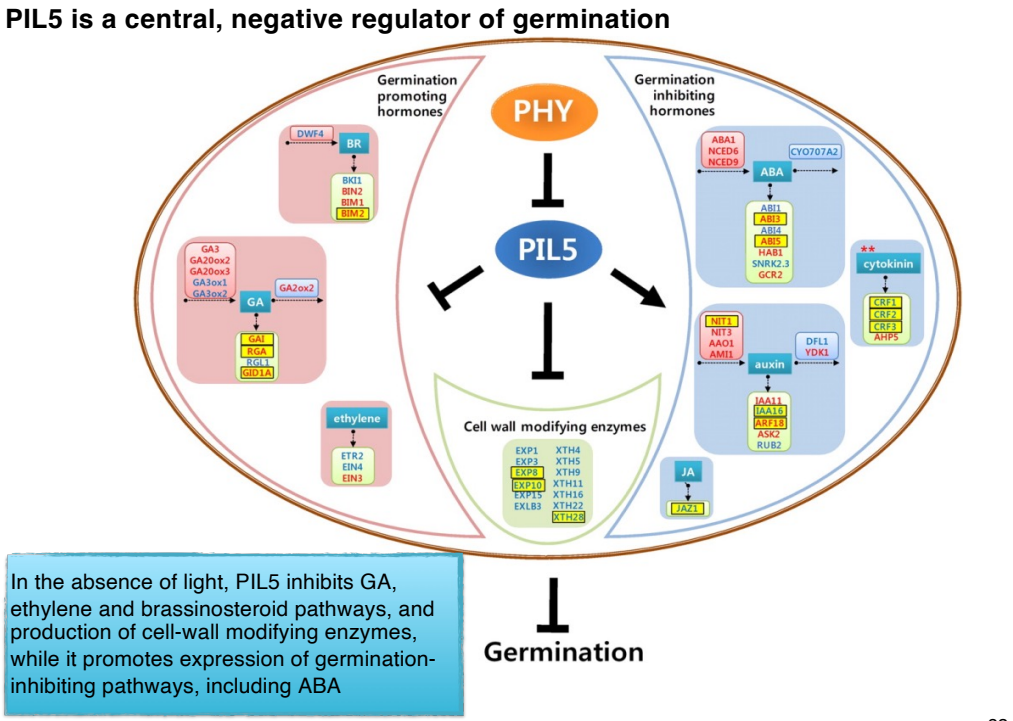
PIL5 is light labile; it represses germination exclusively in the dark



Oh, E., et al. (2007). PIL5, a phytochrome-interacting bHLH protein, regulates gibberellin responsiveness by binding directly to the GAI and RGA promoters in Arabidopsis seeds. Plant Cell 19: 1192-1208.

Relative expression levels of GA metabolic genes and GA signaling genes in *ga1* and *pil5 ga1* mutant seeds under PHYB-dependent germination conditions. The relative expression levels of the tested genes were normalized versus that of PP2A.

12D, seeds were irradiated with far-red light and then dark incubated for 12 h; 12Rp, seeds were irradiated with red light followed by far-red light and then dark incubated for 12 h.



Oh, E., H. Kang, et al. (2009). Genome-wide analysis of genes targeted by PHYTOCHROME INTERACTING FACTOR 3-LIKE5 during seed germination in Arabidopsis. Plant Cell 21: 403–419.

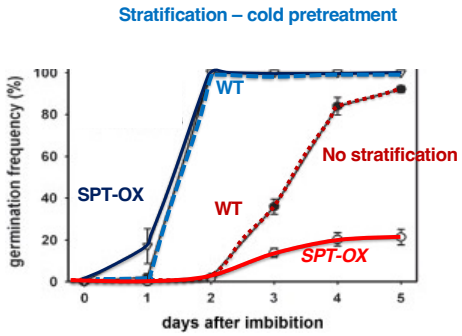
Figure 7. PIL5 Regulates Seed Germination by Coordinating Various Hormonal Signaling Pathways and Modulating Cell Wall Properties.

PIL5 directly regulates various hormone signaling genes, including those involved in ABA (ABI3 and ABI5), auxin (IAA16 and ARF18), BR (BIM2), cytokinin (CRF1, CRF2, and CRF3), GA (GAI, RGA, and GID1A), and JA (JAZ1) signaling. Various hormone metabolism genes are also regulated indirectly by PIL5. In addition to altering the hormone-related genes, PIL5 inhibits the expression of genes

encoding cell wall–modifying enzymes either directly (EXP8, EXP10, and XTH28) or indirectly (four EXP genes and six XTH genes). Taken together, our results indicate that PIL5 inhibits seed germination by coordinating germination-promoting and -inhibiting hormone signals and by modulating cell wall properties. When phytochromes (PHY) are activated, they promote the degradation of PIL5, leading to seed germination. We did not include other seed germination-related PIL5 direct target and regulated genes in the diagram. The notations used in the figure are as follows. Different colored rounded boxes indicate the hormone anabolic genes (light red), hormone catabolic (light blue), and hormone signaling genes (light green). Genes that are upregulated by PIL5 are represented by red letters, whereas those that are downregulated are represented by blue letters. PIL5 direct target genes are enclosed in yellow rectangles. Asterisks indicate that the role of cytokinin signaling in seed germination is still controversial.

Like PIL5, SPATULA (SPT) represses GA synthesis in non-optimal germination conditions

The SPT transcription factor inhibits germination in the absence of cold stratification. SPT negatively regulates GA biosynthesis

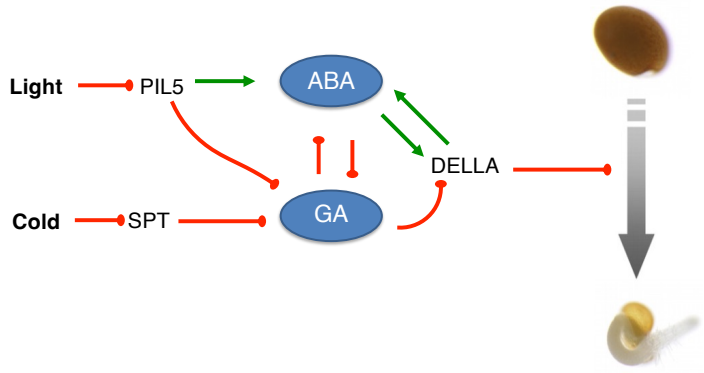


Penfield, S., Josse, E.-M., Kannangara, R., Gilday, A.D., Halliday, K.J., and Graham, I.A. (2005). Cold and light control seed germination through the bHLH transcription factor SPATULA. Current Biology 15: 1998-2006. 83

Arabidopsis plants usually flower during the long days of summer, and their seeds lie dormant during the winter months. In the lab, Arabidopsis seeds are induced to germination by stratification, which mimics the dormancy-breaking effects of winter.

The germination of 1-week-after-ripened Ler and 35S:SPT seed in white light. no stratification, without 3 nights of stratification; stratification, with 3 nights of stratification

GA, DELLAs and ABA contribute to a sophisticated signaling network controlling germination



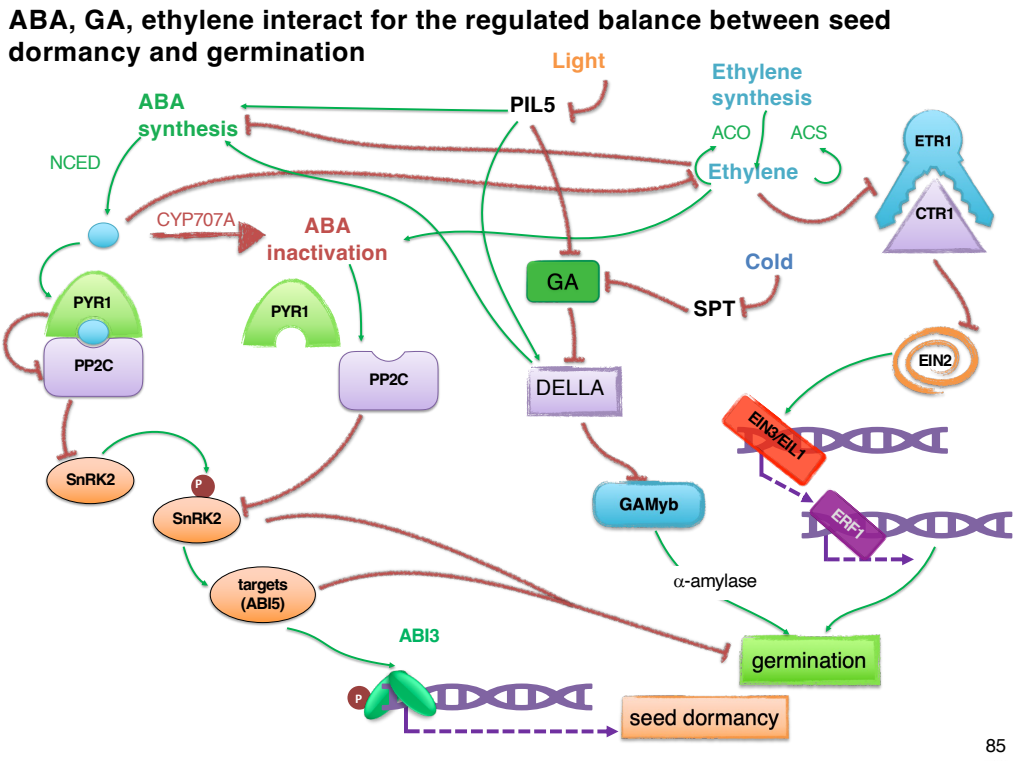
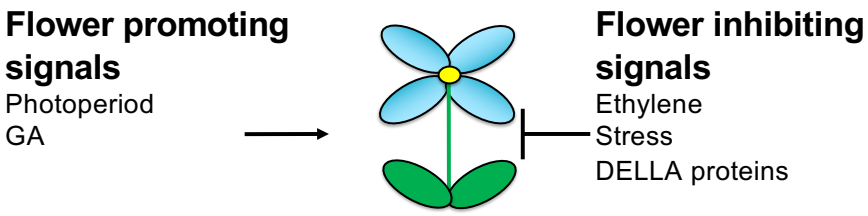


FIGURE 2 | Interactions between ethylene, abscisic acid, and nitric oxide signaling pathways in the regulation of seed germination and dormancy. This scheme is based on genetic analyses, microarray data, and physiological studies on seed responsiveness to ABA, ethylene, or NO. ABA binding to PYR/PYL/RCAR receptor induces the formation of a protein complex with PP2C and the inhibition of phosphatase activity. In the absence of ABA, PP2C dephosphorylate SnRK2. When ABA is present, PP2C binding to the receptor releases inhibition of SnRK2 activity,

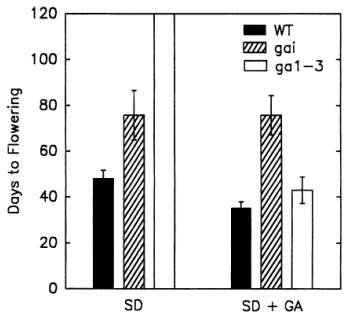
which can phosphorylate downstream targets, including ABI5-related transcription factors. Interactions between ABI3 and ABI5 mediate transcriptional regulation of ABA-responsive genes. Ethylene positively regulates its own biosynthesis, by acting on ACC synthesis catalyzed by ACS and subsequent conversion to ethylene by ACO. This last step is also subject to ABA inhibition. Ethylene is perceived by receptors (among which ETR1) located in the endoplasmic reticulum; its binding leads to the deactivation of the receptors that become enable to recruit CTR1. Release of CTR1 inhibition allows EIN2 to act as a positive regulator of ethylene signaling pathway. EIN2 acts upstream of nuclear transcription factors, such as EIN3, EILs, and ERBPs/ERFs. Ethylene down-regulates ABA accumulation by both inhibiting its synthesis and promoting its inactivation, and also negatively regulates ABA signaling. In germinating seeds, NO enhances ABA catabolism and may also negatively regulate ABA synthesis and perception. Moreover, NO promotes both ethylene synthesis and signaling pathway. ABA, abscisic acid; ABI3, ABA insensitive3; ABI5, ABA insensitive5; ACC, 1-aminocyclopropane 1-carboxylic acid; ACO, ACC

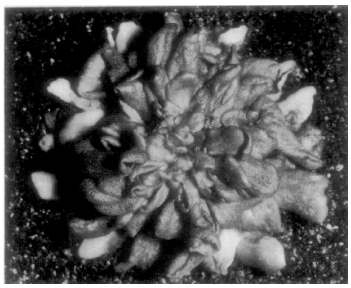
oxidase; ACS, ACC synthase; CTR1, constitutive triple response 1; CYP707A, ABA-8′-hydroxylase; EIL, EIN3-like; EIN, ethylene-insensitive; EREBP, ethylene-responsive element binding protein; ERF, ethylene response factor; Et, ethylene; ETR1, ethylene receptor1; NCED, 9-cis-epoxycarotenoid dioxygenase; NO, nitric oxide; PP2C, clade A type 2C protein phosphatases; PYR/PYL/RCAR, pyrabactin resistance1/PYR1-like /regulatory components of ABA receptor; SnRK2, group III sucrose non-fermenting-1-related protein kinase 2; a dashed line is used when regulatory targets are not precisely identified.

In some plants, GAs contribute to the control of flowering



In Arabidopsis, a long-day plant, GA is required for flowering in short days





GA synthesis mutant *ga1-3* after 113 d of growth in short days, and still no flowering!

Wilson, R.N., Heckman, J.W., and Somerville, C.R. (1992). Gibberellin is required for flowering in Arabidopsis thaliana under short days. Plant Physiol. 100: 403-408.

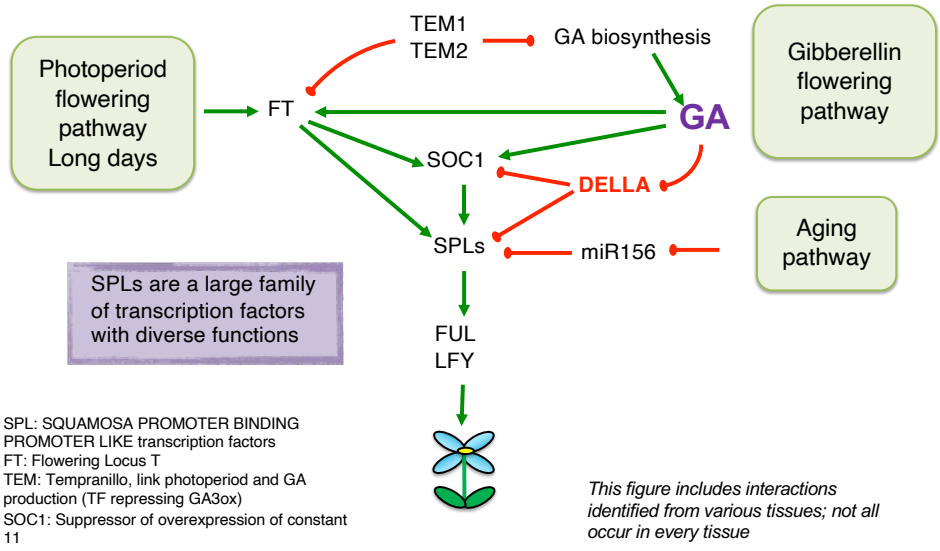
Under non-inducing short day (SD) photoperiods, the GA-deficient mutant *ga1-3* does not flower unless treated with exogenous GA.

Figure 1. The effect of exogenous GA3 on flowering time in SD. Each bar represents the mean of observations on 7 to 11 plants ± SE. GA-treated plants were sprayed weekly with the hormone as described. Untreated *ga1 -3* mutant plants failed to flower, but the plants began to senesce at the indicated time.

Figure 4. Mutant *ga1-3* after 113 d of growth

in SD at 21 to 23^{o}C.

Photoperiod, aging and GA act in separate but converging pathways



Osnato, M., Castillejo, C., Matías-Hernández, L., and Pelaz, S. (2012) TEMPRANILLO genes link photoperiod and gibberellin pathways to control flowering in Arabidopsis. Nat. Commun. 3: 808.
Porri, A., Torti, S., Romera-Branchat, M., and Coupland, G. (2012) Spatially distinct regulatory roles for gibberellins in the promotion of flowering of Arabidopsis under long photoperiods. Development 139: 2198-2209.
Jung, J.-H., Ju, Y., Seo, P.J., Lee, J.-H. and Park, C.-M. (2012). The SOC1-SPL module integrates photoperiod and gibberellic acid signals to control flowering time in Arabidopsis. Plant J. 69: 577-588.
Yu, S., Galvão, V.C., Zhang, Y.-C., Horrer, D., Zhang, T.-Q., Hao, Y.-H., Feng, Y.-Q., Wang, S., Schmid, M. and Wang, J.-W. (2012). Gibberellin Regulates the Arabidopsis Floral Transition through miR156-Targeted SQUAMOSA PROMOTER BINDING–LIKE Transcription Factors. Plant Cell. 24: 3320-3332.

SQUAMOSA PROMOTER BINDING PROMOTER LIKE (SPL) transcription factors

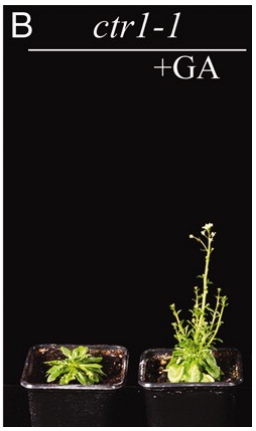
FT: Flowering Locus T

TEM: Tempranillo, link photoperiod and GA production (TF repressing GA3ox)

SOC1: Suppressor of overexpression of constant 11

Ethylene delays flowering but this effect is reversible by GA

Plants with constitutive ethylene signaling (*ctr1-1*) show delayed flowering which can be partly rescued by adding GA



The *ctr1-1* mutants accumulate reduced levels of bioactive GAs

		GA_1
WT	Mean	65.4
	s.d.	19.1
ctr1-1	Mean	16.0
	s.d.	2.3

Achard, P., Baghour, M., Chapple, A., Hedden, P., Van Der Straeten, D., Genschik, P., Moritz, T., and Harberd, N.P. (2007). The plant stress hormone ethylene controls floral transition via DELLA-dependent regulation of floral meristem-identity genes. Proc. Natl. Acad. Sci. 104: 6484-6489.

Fig. 1. Ethylene delays flowering by reducing bioactive GA levels.

Representative (5-week-old) *ctr1–1* mutant plants grown in SDs and treated with GA (+GA) or control.

Levels of GAs in WT Col and *ctr1–1* mutant plants (expressed as picograms per gram of fresh weight; ± SD; n = 5).

GA and DELLAs act downstream of ethylene to promote flowering

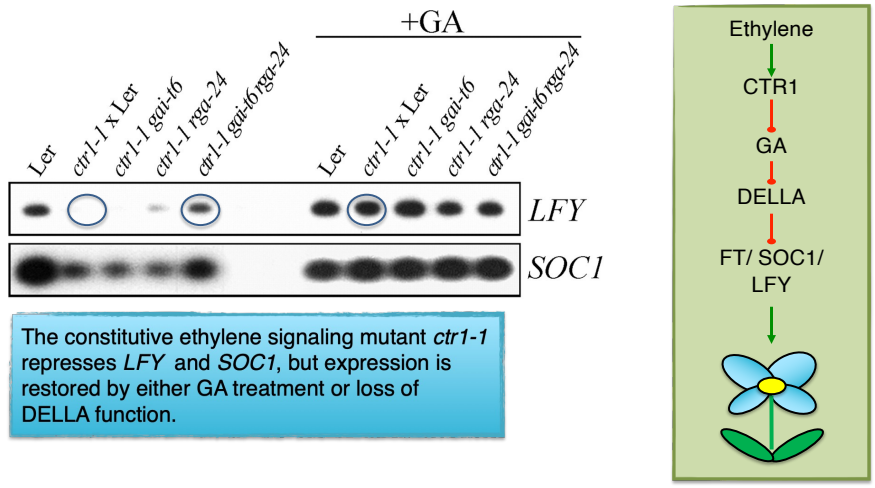
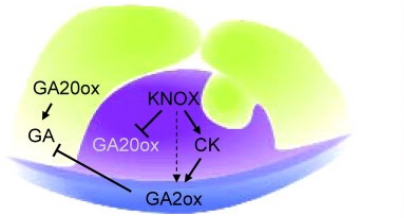


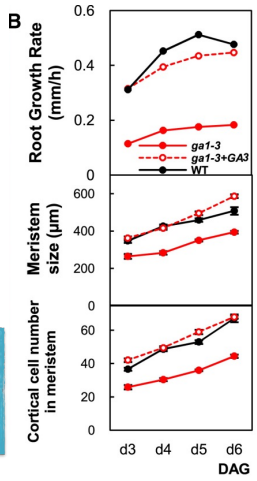
Fig. 3. Ethylene delays flowering via DELLA-dependent repression of LFY and SOC1 transcript levels.

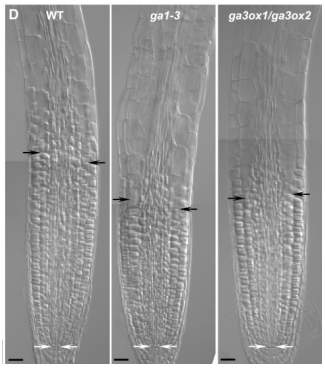
Levels of floral meristem identity LFY and SOC1 gene transcripts in SD, soil- grown, GA-treated WT Ler, *ctr1–1* x Ler, *ctr1–1 gai-t6*, *ctr1–1 rga-24*, and *ctr1–1 gai-t6 rga-24* mutant plants (and controls).

Gibberellins have roles at the shoot and root apical meristems



GA biosynthesis is repressed at the shoot apical meristem (purple) but promoted in development leaves (green)





GA at the root apex controls meristem size

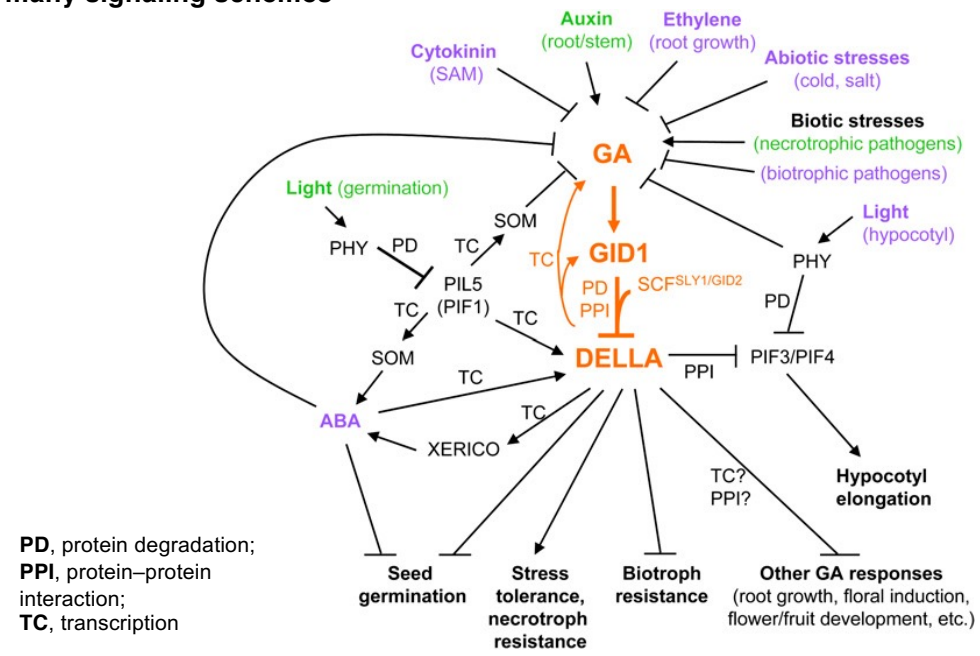
Jasinski, S., Piazza, P., Craft, J., Hay, A., Woolley, L., Rieu, I., Phillips, A., Hedden, P. and Tsiantis, M. (2005). KNOX action in Arabidopsis is mediated by coordinate regulation of cytokinin and gibberellin activities. Curr. Biol. 15: 1560-1565.
Ubeda-Tomás, S., Federici, F., Casimiro, I., Beemster, G.T.S., Bhalerao, R., Swarup, R., Doerner, P., Haseloff, J. and Bennett, M.J. (2009). Gibberellin signaling in the endodermis controls Arabidopsis root meristem size. Curr. Biol. 19: 1194-1199.

Figure 4. Model Depicting Interactions between KNOX Proteins, GA, and CK in the Shoot Apex KNOX proteins are expressed in the SAM (purple), where they activate CK biosynthesis and repress GA 20-oxidase gene expression and hence GA biosynthesis (GA20ox, gray), thus promoting meristem activity. CK also activates GA2ox (blue) expression, possibly stimulating GA deactivation. These interactions may confine active GA to the leaf (green). KNOX proteins may also activate GA2ox in a CK-independent manner (dashed arrow).

Figure 1. GA Regulates Root Meristem Size and Cell Production Rate

(B) The GA-deficient mutant *ga1-3* (Col-0 background) showed a reduction in its root growth rate (mm/h), meristem size (um), and cortical cell number in meristem from day 3 to day 6 after germination (DAG). GA treatment (GA3 1 uM) restored the mutant phenotype. Error bars represent SE (n > 15).

(D) Root meristem images (bright-field microscopy) of GA-deficient mutants *ga1-3* and g*a3ox1/ga3ox2* (both in Col-0 background) showing a reduction in meristem size with respect to control (wild-type, Col-0 background) of four DAG seedlings. White and black arrowheads indicate QC and TZ position, respectively. Scale bar represents 25 um.



Sun, T.-p. (2011). The molecular mechanism and evolution of the GA GID1 DELLA signaling module in plants. Curr. Biol. 21: R338-R345

Figure 3. The interaction network between the GA–GID1–DELLA signaling module and other internal and external cues.

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.

Physiological effects - summary

•GA contributes to growth and elongation through cell division and elongation

•DELLAs can delay growth, which enhances stress tolerance

•GA and ABA act antagonistically on seed germination, through effects on DELLAs and biosynthesis of both hormones

•GA can promote flowering, but this effect is also subject to input from other signaling pathways via DELLAs

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