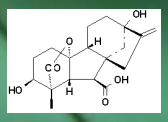
Gibberellin Insensitive Dwarf1 encodes a soluble receptor for gibberellin

Miyako Ueguchi-Tanaka, Motouki Ashikari et al. *Nature* **437**, 693-698 (29 September 2005)

Presented by: Robert Berkey

Gibberellins

- GAs are a large family of tetracyclic, diterpenoid plant hormones that induce many responses
  - Seed germination, stem elongation, leaf expansion, pollen maturation, flowering
- Over 100 GAs identified from plants, fungi, and bacteria
  - Only a few have been shown to have intrinsic activity (GA_1 and GA_4)
- First discovered in Japan in 1935- common condition in rice called "foolish seedling" disease
  - Plants grew much taller than normal
- Found in young leaves, roots, developing seeds, and fruits
- Biosynthesis well understood
  - synthesized from geranylgeranyl diphosphate produced mainly through the methylerythritol phosphate pathway
- perception of GA and signaling of GA is not



Gibberellic Acid (precursor)

**regulated in part by changing the cellular concentration of bioactive GAs

Key Terms

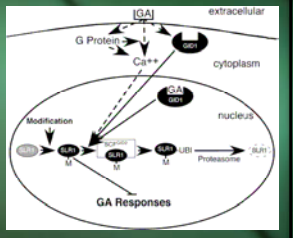
- GA- Gibberellic Acid
- SLR1- putative transcriptional regulator, DELLA protein, degraded during GA signaling cascade
- GID2- F-Box subunit protein that interacts with SCF in SLR1 degradation via proteasome
- GID1- speculated as a GA soluble receptor molecule upstream of SLR1 in GA signal pathway
- DELLA proteins- proteins that are believed to function as suppressors of GA signaling. Their degradation leads to GA responses

Known Information/Previous Work

- *slr1*- rice mutant shows constitutive GA response phenotype
  - Putative transcriptional regulator
  - Orthologous to Arabidopsis GAI and RGA, wheat Rht, maize D8, and barley SLN
- DELLA subfamily of GRAS regulatory protein family, share common sequence
- DELLA proteins suspected as suppressors of GA signaling

GID2 Gene

- GA-insensitive dwarf mutant *gid2* identified and characterized
  - Encodes a putative F-box subunit of an SCF E3 ubiquitin ligase
  - Interacts with a rice Skp1 homolog
  - Found high levels of SLR1 in *gid2* mutants
- Proposed that GA treatment induces degradation of SLR1 through SCF^{GID2} proteasome pathway



Unknown Information & Goal

- The molecular mechanisms of GA perception are unknown currently
- No GA-binding proteins isolated or genetic evidence of proteins acting as GA receptors
  - GA is a hydrophobic carboxylic acid
    - Soluble in inter and intracellular compartments
    - May cross plasma membrane by passive diffusion
  - Plant probably have both membrane bound and soluble GA receptors
- **Goal**: Screen rice *gid* mutants in order to investigate GA signaling mechanisms

gid1 *mutant*

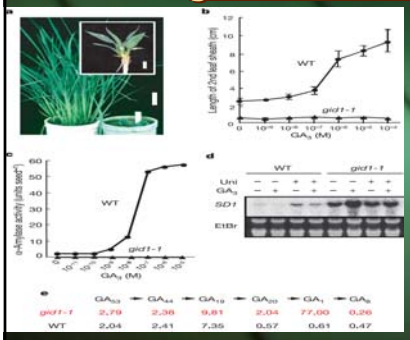


Figure 1.

****Gid1* is an insensitive mutant*

SLR1 Epistatic to GID1, Degradation Possibly Mediated by GID1

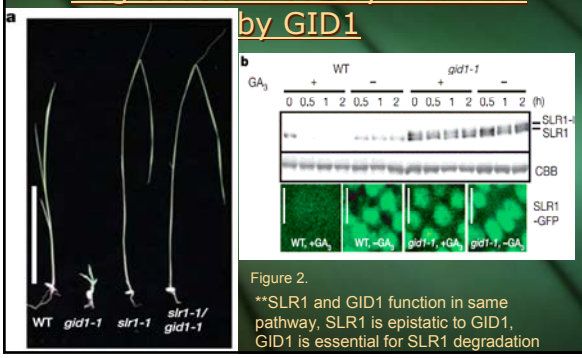


Figure 2.

****SLR1 and GID1 function in same pathway, SLR1 is epistatic to GID1, GID1 is essential for SLR1 degradation*

GID1 Functions in GA Perception, Not SLR1 Degradation

- In gid1 mutants, no SLR1 degradation… GID1 directly involved in degradation or acts earlier in pathway
- Dwarfism less severe in *gid2-2* than *cps* and *gid1-3* and higher SLR1 accumulated
  - SLR1 dependent suppression of GA action weaker in *gid2-2* than *cps* and *gid1-3*
- Cps mutant- no active GAs made, signaling can't be activated
- GA signal will reach SLR1 in *gid2-2*, with no SLR1 degradation
- *gid1-3* phenotype similar to *cps* in respect to dwarfism and SLR1 accumulation
- **Speculation**: SLR1-dependent suppression of GA may be regulated by GA itself AND that SLR1 is less effective in *gid2-2* than *cps*
- GID1 most likely functions in GA recognition rather than SLR1 degradation

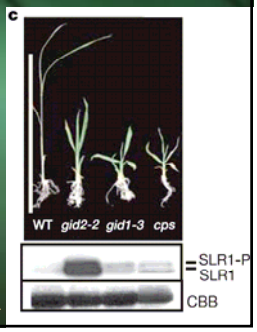
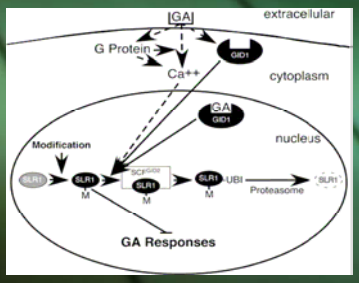


Figure 2.

GA Signaling Pathway



GID1 Protein Structure

- One intron & two exons
- 354 amino acid polypeptide
- Four *gid1* alleles
  - 1-1: nucleotide sub.
  - 1-2: nucleotide sub.
  - 1-3: 150 bp deletion
  - 1-4: 302 bp deletion
- 3 homologs found in Arabidopsis, none in rice
  - Unknown proteins
- Homology with hormone sensitive lipase (HSL) family
  - HGG motif
  - GXSXG motif

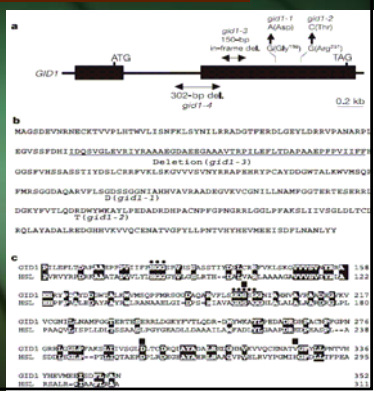


Figure 3.

Transgenic GID1-GFP Protein Localization

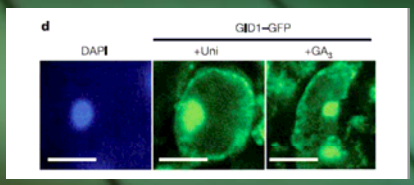


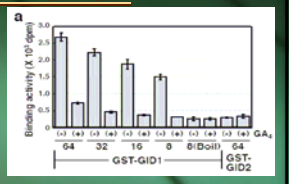
Figure 3.

- transgenic GID1-GFP protein expression under pAct1 promoter
- Primarily localized in the nuclei, faint cytosolic signal
  - No change with GA3 or Uniconazol treatment

GID1 Kinetics-GID1 in Perception of GA

- Size exclusion chromatography, glutathione *S*-transferase
- Radioactive GA_3
- Competition was GA specific when using non labeled GA_3
- heat denatured GST-GID1 or GST-GID2 had specific binding

Figure S2.



- Looking at binding saturability
- b) scatchard plot analysis

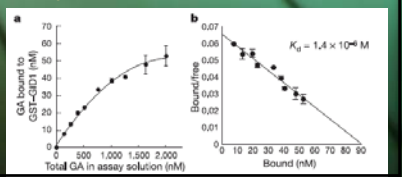


Figure 4.

GID1 Kinetics

Table 1 Competition for $^3H_4$-16,17-dihydro-GA_4 binding to GID1 by GAs

GAs	IC_{50}	Relative percentage
Biologically active GAs*		
GA_4	2×10^{-7} M	100
H_2-GA_4	1×10^{-6} M	20
GA_1	4×10^{-6} M	5
GA_3	4×10^{-6} M	5
Weakly biologically active GAs*		
GA_{35}	1×10^{-5} M	2
GA_{37}	2×10^{-5} M	1
Biologically inactive GAs*		
GA_4-Me	3×10^{-5} M	0.6
GA_9	2×10^{-4} M	0.1
GA_{51}	$>2 \times 10^{-4}$ M	<0.1
3-epi-GA_4	$>2 \times 10^{-4}$ M	<0.1

Estimated K_d values:

GA_3: 4*10^-6 M
GA_4: 2*10^-7 M

**GID1 has a higher affinity for biologically active GAs

- Half time for association and dissociation between GST-GID1 and radio labeled GA_4
- Rapid, sensitive system
- Mutant GST-GIDs had no binding activity, therefore there mutations result in GA insensitivity

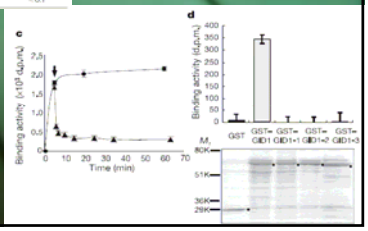
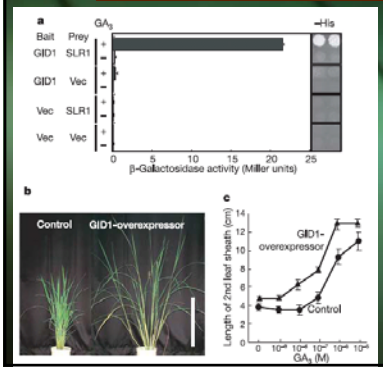


Figure 4.

GID1 is a GA Receptor



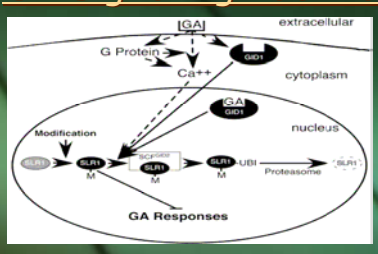
- Is signal transduced downstream to SLR1?
- Y-2-H
- GID1 interacts with SLR1 when GA_3 present
- GID1 interacts with SLR1 and transduces GA signal to SLR1 leading to SLR1 degradation and GA signal in plant
- Overexpression-GA hypersensitive phenotype
- 10X more sensitive to GA in overexpression lines

Figure 5.

Conclusions

- GID1 is a soluble GA receptor
  - Loss of function mutations > dwarf phenotypes and GA insensitivity
  - GST-GID1 interacts with active GAs but no inactive GAs
  - Mutated GST-GID1 proteins lack ability to bind GA
  - GID1 interacts with SLR1 in a GA dependent manner leading to GA response
  - GID1 overexpression shows GA hypersensitivity
- Alternative receptors (membrane bound) could still exist or interacting factors with GID1
- Conserved motifs between HSL family and GID1 probably mediate substrate-enzyme interactions

GA Signaling Pathway



- GID1 present in nucleus, when binds to active GA can now interact with SLR1 which becomes degradable
- Unclear whether GA-GID1 stabilizes SLR1 or whole interaction is targeted by SCF^{GID2}

Accomplished since 2005

- Probenazole-inducible protein (PBZ1)
  - Tanaka et. Al., Plant Cell Environment (2006)
  - Found up regulation of PBZ1 in *gid1* mutant and by GA_3 addition
    - Also induced by cold stress or rice blast fungus
  - PBZ1 expression regulated by GA signaling and stress stimuli and gid1 involved in tolerance to cold stress and resistance to blast fungus
- Characterization of Arabidopsis GA receptors
  - Three (GA) receptor genes AtGID1a, AtGID1b and AtGID1c with overlapping function
  - Griffiths et al., Plant Cell (2006)
  - Nakajima et al., Plant Journal (2006)

Future Directions

- Isolate and characterize other GA receptor proteins or factors interacting with GID1 or GID1-GA
- Many different GAs, active and inactive, are they all targeted to GID1 with different affinities or is GID1 specific for a few like GA_3 and GA_4
  - Structural analysis of GID1-GA interactions
- What is the mechanism of signal transduction from GID1 to SLR1
  - Does GID1-GA target SLR1 to SCF^{GID2} for degradation or is whole complex targeted
- similarities and differences in pathway between 3 Arabidopsis homologs of GID1 in rice