Balance of Hormones regulate growth and development

Environmental factors regulate hormone levels

light- e.g. phototropism
gravity- e.g. gravitropism
temperature

Mode of action of each hormone

1. Signal **perception** by a receptor on or in target cells.
2. Signal **transduction** and amplification via
   a. R --> -->2 messengers ---> modify/activate protein
   b. R -----> ------> gene expression
3. **Response**: changes in protein activities and/or gene expression

---→ -→ growth/development response

Balance of hormones regulate growth and development

Balance of IAA: cytokinin
determines root or shoot development.

e.g. in pith tissue

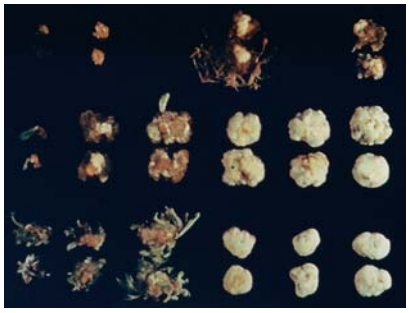
Hormones are used to regenerate transgenic plants.

23-. Shoot and root development depend on ratio of IAA/CK

[Auxin] ⟶ rooting

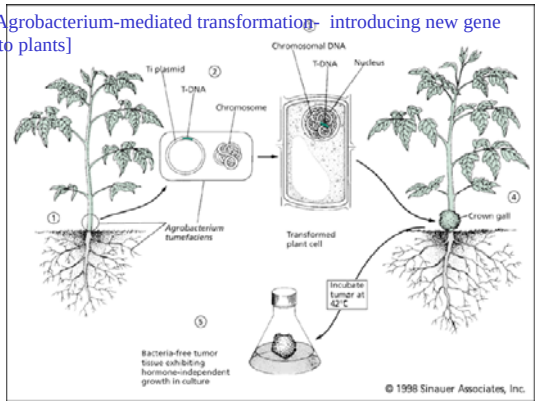
[Cytokinin] ↓

shoot

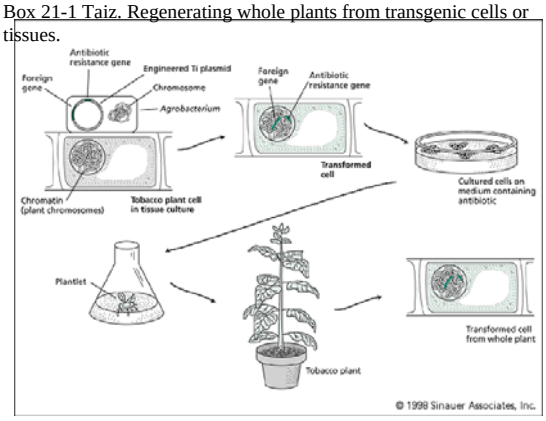


21-4 Taiz. Tumour induction by cytokinin.

[Agrobacterium-mediated transformation- introducing new gene into plants]



Box 21-1 Taiz. Regenerating whole plants from transgenic cells or tissues.



Balance of hormones regulate growth and development

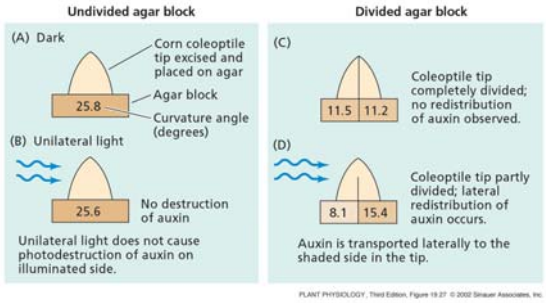
1. Shoot and root development depend on ratio of Auxin IAA: cytokinin
2. Leaf abscission depends on auxin and ABA
3. Seed development and germination depends on relative amount of ABA and GA

Effect depends on

- **Type of hormones**
- **conc**
- **balance of hormones**

19- 27. Light causes redistribution of auxin to shaded side in phototropism.

Environmental signals regulate hormone levels. How?



How can one monitor hormone levels in plants?

- Monitor auxin levels using a reporter gene GUS or GFP.
- A promoter that is activated by auxin is fused to GUS or GFP.
  a. DR5:: GUS (enzyme gives blue color)
  b. DR5:: GFP (green fluorescence protein)
- Plant is transformed with a. or b. construct. Gene is incorporated into genome and inherited. Progeny plants carry a or b.

Auxin level changes in response to light and to gravity are due to auxin transport.

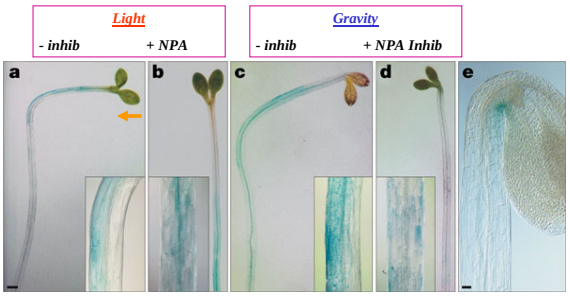
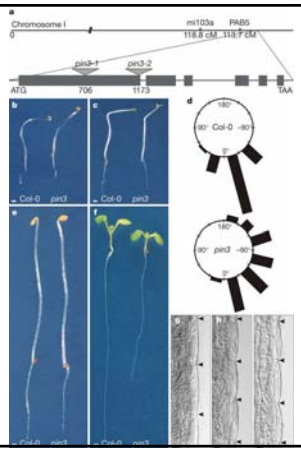


Figure 1 Auxin response during differential hypocotyl growth. a–d, Expression of the DR5::GUS reporter in hypocotyl of untreated (a, c) or auxin efflux inhibitor (NPA)-treated (b, d) wild-type seedlings upon stimulation by light (a, b) or gravity (c, d). Insets show details of DR5::GUS expression. Scale bars, 400 µm. e, Asymmetric expression of DR5::GUS reporter in the apical hook of an etiolated seedling. Scale bar, 50 µm.

Friml J. et al 2002. Nature



Evidence auxin transport is critical for phototropism

Genetic evidence:
Mutants of auxin transport respond poorly to light and to gravity

Figure 2. pin3 mutants.

b, c, **pin3 hypocotyls are defective in gravitropic (b) as well as phototropic** (c) responses.

d, pin3 mutants are defective in root gravitropism

Friml J. et al 2002. Nature

Auxin levels can be changed by auxin efflux carriers

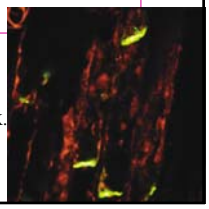
PIN1 Basipetal auxin transport in the stem

PIN2 Basipetal auxin transport

PIN3 Lateral auxin efflux in gravitropic responses

PIN1-GFP is localized to base of cell

PIN3-GFP is localized to lateral side of cell

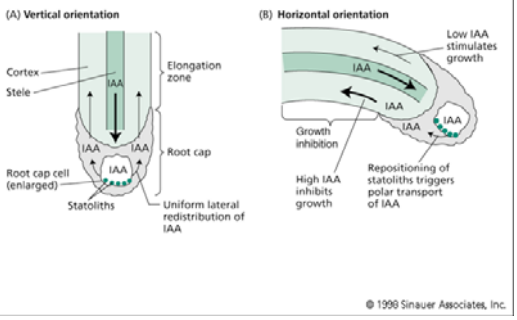


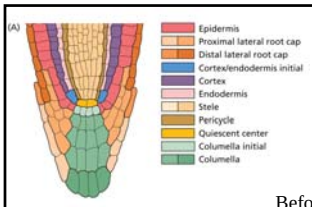
Friml, J. , Wisniewska, J. , Benkova, E. , Mendgen, K. (2002) Nature 415, 806-809.

Geldner N. 2001 Nature

19-33 Taiz. Why do roots placed horizontally bend downwards?

Gravitropism depends on distribution of hormones

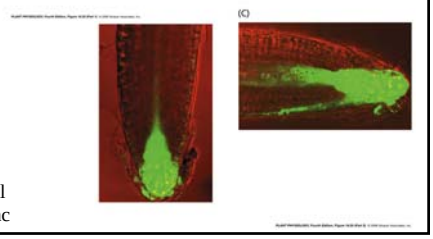




Gravity changes auxin distribution

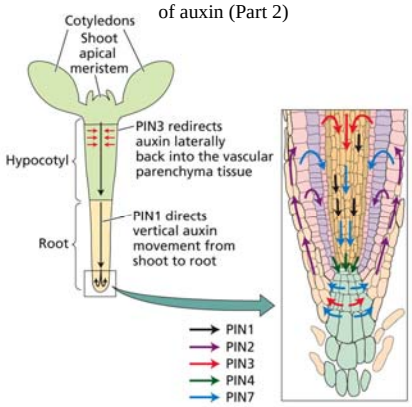
Before

gravistimulation
After 3 h

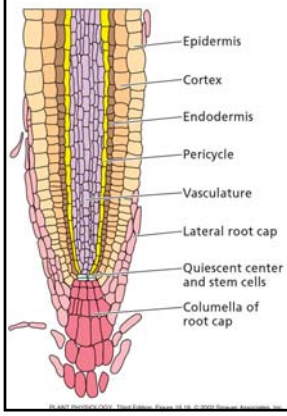


DR5::GFP signal reflect auxin conc

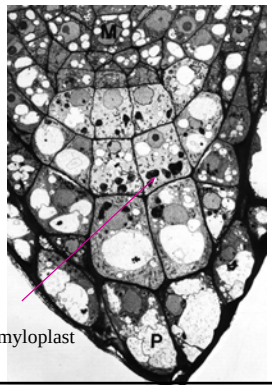
19.15 In Arabidopsis, PIN transport proteins direct the flow of auxin (Part 2)



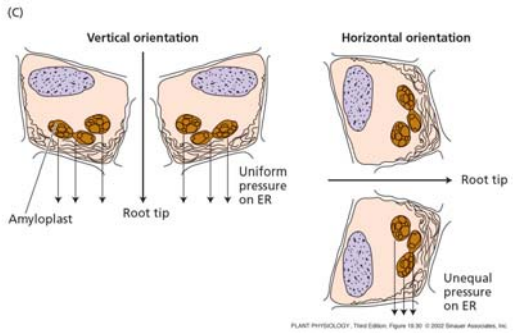
Q: What is the sensor for gravity?



19-30. Taiz. Root cap cells are important for sensing gravity



19-30. Taiz. Gravity may be sensed by a root cap cell according to the distribution of starch-storing plastids



Mode of Action of Hormones

1. *Signal perception by a receptor in/on target cell*
2. *Signal transduction and amplification*
3. *Responses: a. molecular*
   - *early gene expression*
   - *late gene expression*
   - *proteins activated*
   - *repressor removed*

   b. Cellular & Physiological response

Auxin

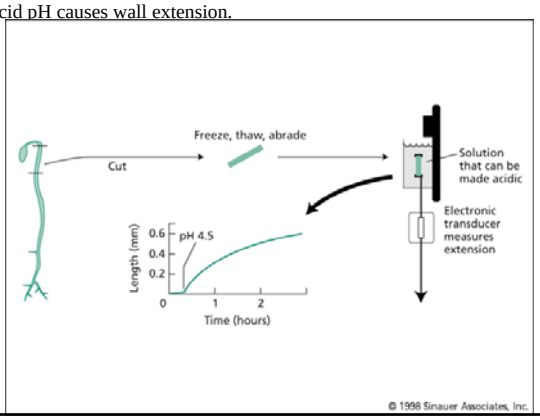
Stimulate cell elongation

How? Two modes of action

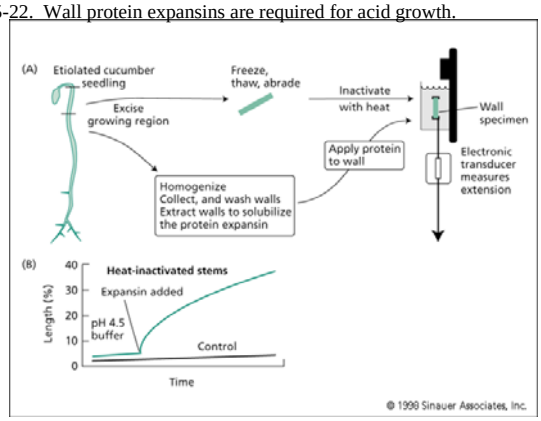
a. Stimulates H^+ efflux (Membrane protein trafficking)

b. Cause changes in gene expression
Removal of repressors

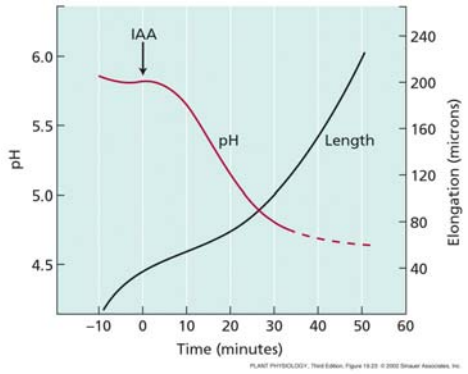
15-21. Taiz. What causes cell elongation?
Acid pH causes wall extension.



15-22. Wall protein expansins are required for acid growth.



19-23. Auxin induces H⁺ extrusion and increases cell extension



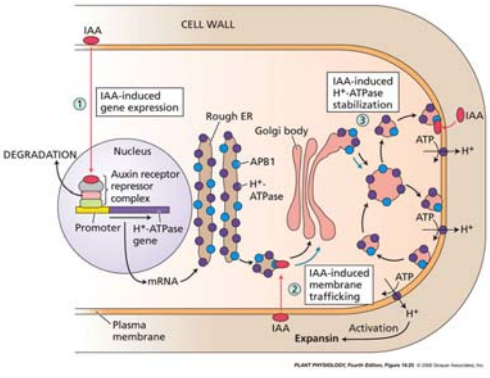
How does auxin stimulate cell elongation?
Acid growth theory

- **Auxin induce H⁺ extrusion**
- **Ion uptake --> osmotic water uptake**
- **Turgor pressure builds up**
- **Wall loosens, allowing cell wall to expand**

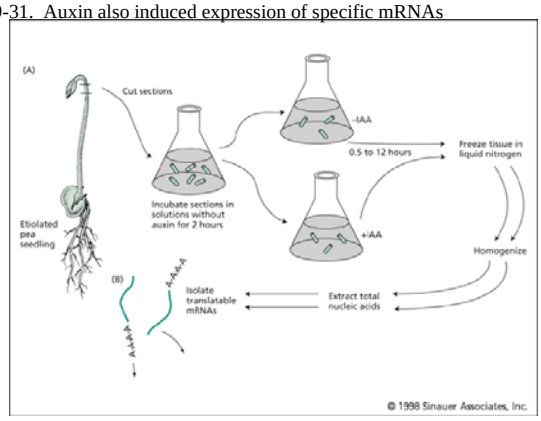
How does auxin stimulate H+ extrusion?
Hypotheses:

- **Activate the PM H⁺-pumping ATPase**
- **Increase synthesis of new PM H⁺ pumps**

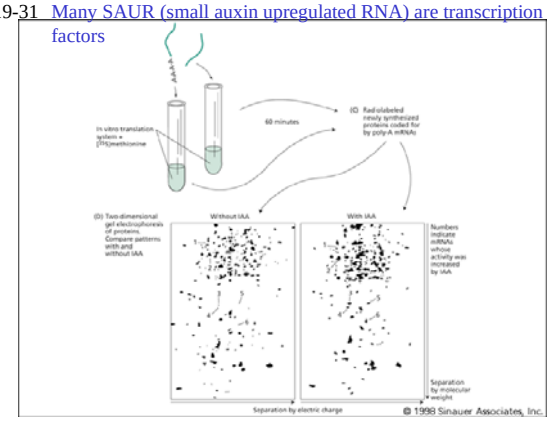
19-18. Model of auxin-induced H+ extrusion and cell expansion



19-31. Auxin also induced expression of specific mRNAs



19-31 Many SAUR (small auxin upregulated RNA) are transcription factors



Auxin induces expression of early and late genes

Early genes*- [primary response genes]*

expressed in 5-60 min after adding auxin

include transcription factors
have roles in intercellular communication
stress adaptation

Late Genes [secondary response genes]
target genes

Auxin receptor identified in 2005. TIR1 receptor

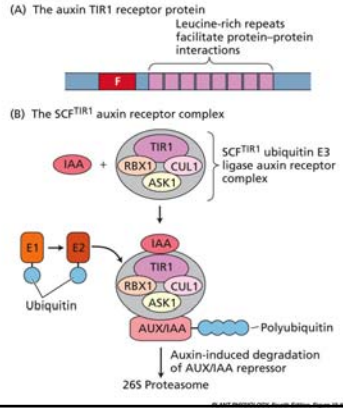
Auxin binds to TIR1 complex.

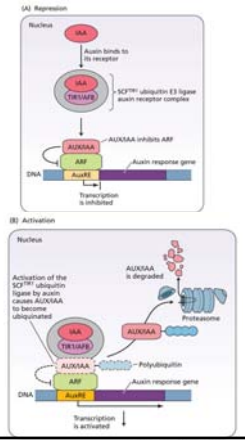
Auxin activates TIR1 (F-box protein)

TIR1 complex targets the degradation of IAA repressor proteins.

Removal of repressor activates transcription of early genes.

19.40 The auxin receptor





Model for auxin binding to TIR auxin receptor

Removal of repressor protein by degradation

Binding of TIR1 to IAA7 depends on auxin conc

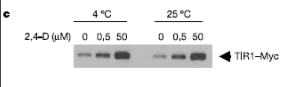
- Plant (expressing TIR1-myc) extract
  + GST-IAA7 (repressor)
  + 0 - 50 auxin
- GST-IAA7 will pull down X if it binds to IAA7.
- Bound protein identified by SDS-PAGE & Immunoblot with anti-myc is TIR1

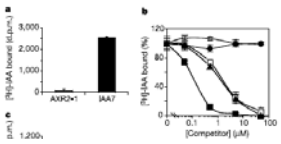
•3H-IAA interacts with TIR1 complex.

•Crude extracts (TIR1-myc) + GST-IAA7 + 3H-IAA

•pull-down & count cpm (a)

•Competed by IAA, 2,4-D, NAA (b)

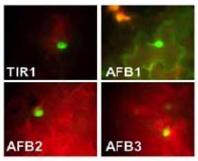




Dharmasiri et al 2005 Nature

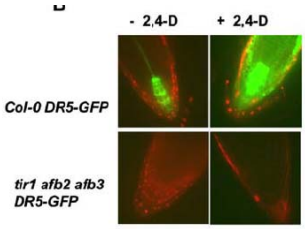
Where is TIR1 localized?

- GFP-TIR1 DNA is expressed in tobacco leaf cells. Located to nucleus
- 4 related F-box proteins in Arabidopsis are auxin receptors.
- So quadraple mutants were made to knock out all 4 proteins.

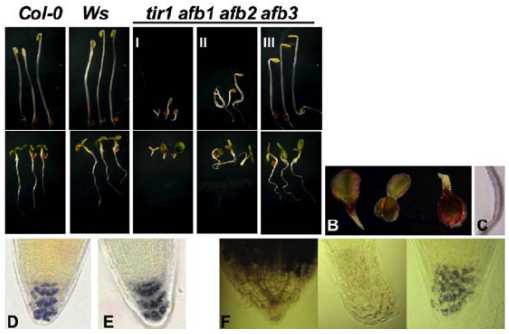


Dharmasiri et al 2005 Dev Cell

Auxin-responsive gene expression is severely reduced in tir1 mutant

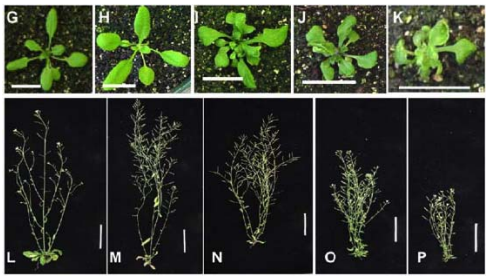


Seedling growth affected in tir1 (4 genes) mutants



Root tip in Wt (col, Ws), and in mutants

TIR1 and AFB genes act collectively



Wt-col Ws 2x mut 3x 4x

Specificity in IAAs and ARFs

- 23 ARFs, and 29 Aux/IAA proteins

- E.g.
- Root & Hypocotyl growth, ARF2, 7, 8, 19
- Tropism, ARF 2, 7, 19
- Embryogenesis, ARF5
- flower

Summary: Auxin action

- 1. Increase H+ pumping

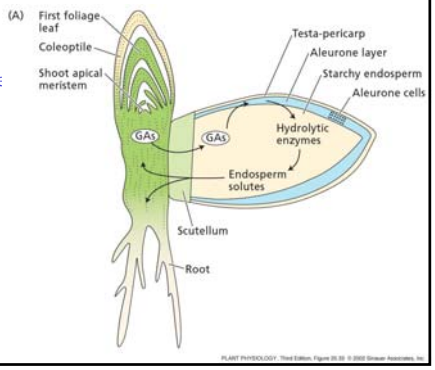
- 2. Change gene expression
  Auxin binding to TIR receptor stimulated repressor breakdown. ARF can then bind regulatory region to enhance expression of early response genes

GA
Gibberellin

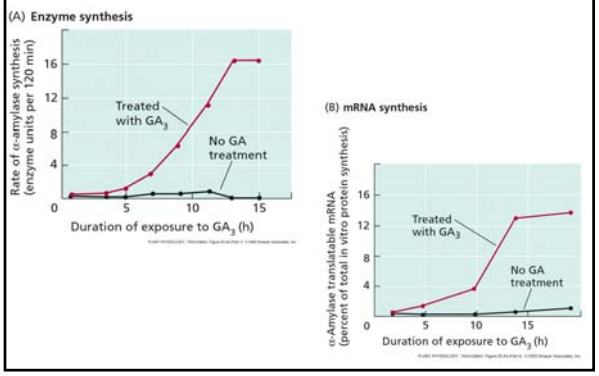
Chap. 20

20-33. GA and barley seed germination

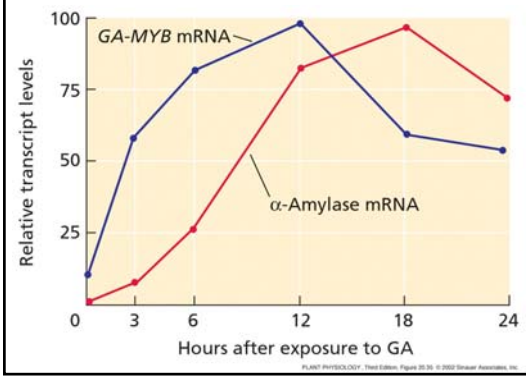
- Promote germination
- Activate vegetative growth
- Mobilize stored food
- Q? How does GA stimulate amylase activity?
- What is the signal transduction pathway?



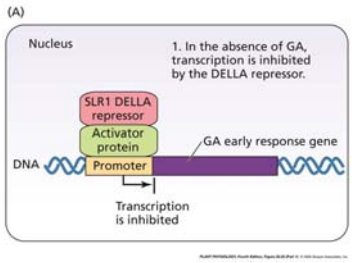
20-34



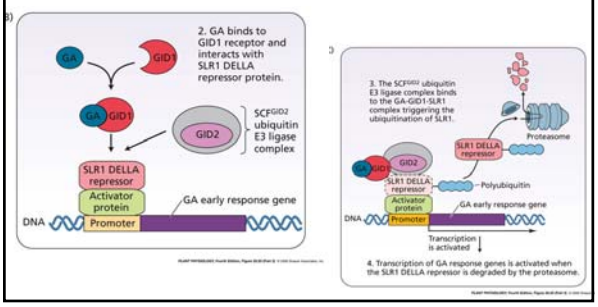
20-22. GA-Myb is an early response gene that regulates expression of a-amylase



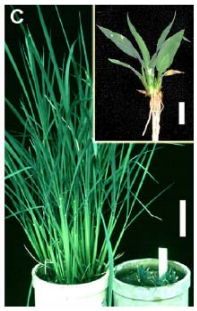
Model: GA signals degradation of repressors



GA binding to receptor targets repressor for degradation

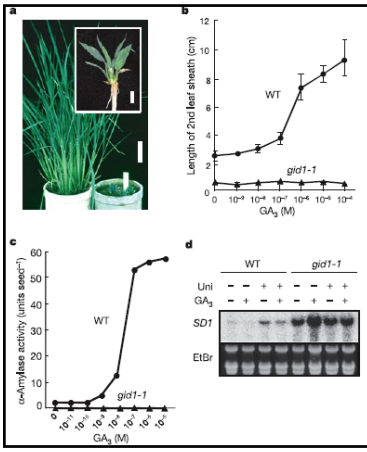


Rice GA receptor gid1-1 mutant is stunted



WT gid1 mutant

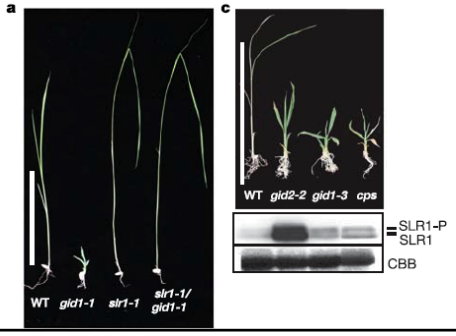
Ueguchi-Tanaka et al Matsuoka 2005 Nature



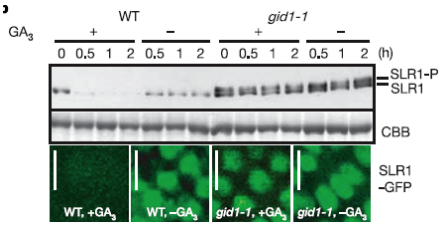
Interpret results

- (b) effect of GA conc on leaf length
- (c) effect of GA on embryo-less half seed

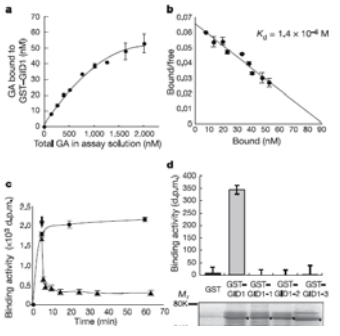
Other mutants: slr1-1 mutant is longer than Wt.



SLR protein is high in *gid1* mutant



^{3}H-GA binding to GST-Gid1



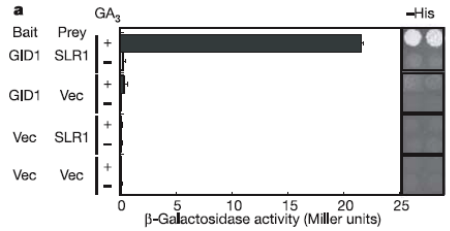
a) ^{3}H-GA binds to purified GID1 protein

b) Binding has high affinity

c) 3H-GA Binding is reduced with non-radio-labeled GA

d) Binding is high in purified wild-type GID1, but not detected in 3 mutated proteins: gid1-1, 1-2, 1-3

Gid1 binding to SLR1 is GA-dependent



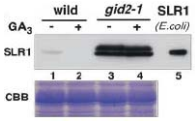
Binding of two proteins assayed by yeast 2 hybrid method.
Vec, vector only (negative control)

Gid2 mutant is insensitive to GA.
GID2 is an F-box protein
(a subunit of the ubiquination complex)

Immunoblot to test protein

Extract from Wt & gid2-1 mutant.
SDS-PAGE to separate protein.

Test for SLR1 with anti-SLR1.
SLR1 is high in gid2-1 mutant



Sakasi et al 2003 science

Gid2-1 mutant is small

Model of GA-induced amylase synthesis