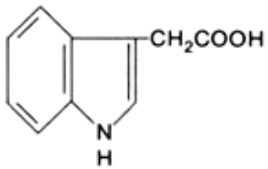
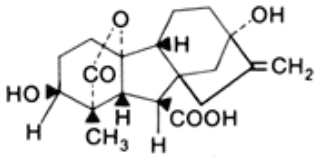
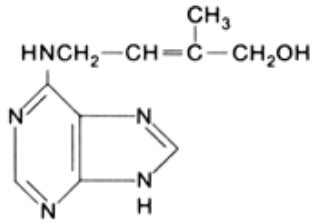
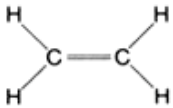
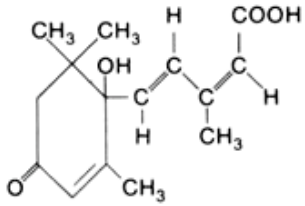
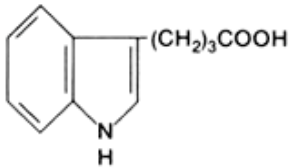
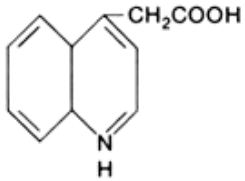
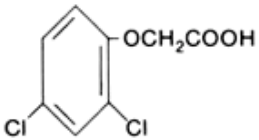
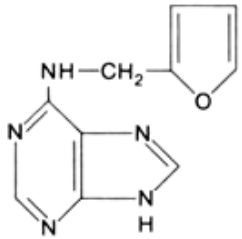
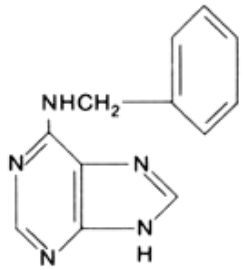
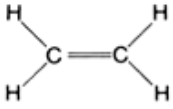
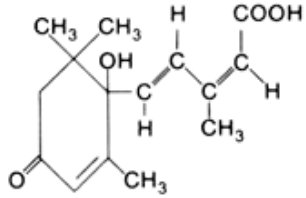
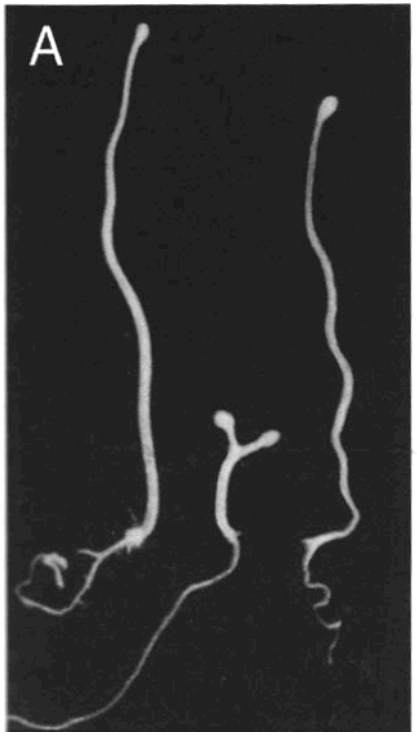
Lecture 16: Plant hormone signal mechanisms

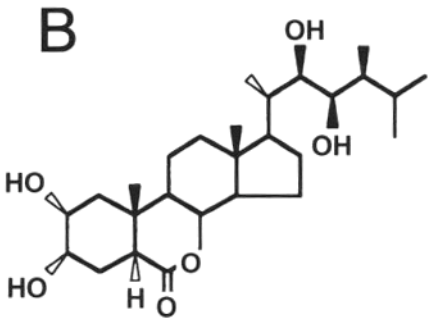
1. Ethylene
2. Brassinosteroid

Five classical plant hormones

	AUXIN	GIBBERELLIN*	CYTOKININ	ETHYLENE	ABSCISIC ACID
NATURALLY-OCCURRING	 Indoleacetic Acid (IAA)	 GA_3 (Gibberellic Acid)	 Zeatin	 Ethylene	 Abscisic Acid
SYNTHETIC	 Indolebutyric Acid (IBA)  a-Naphthaleneacetic Acid (NAA)  2,4-Dichlorophenoxyacetic Acid (2,4-D)	*There are 52 or more forms of gibberellin, all of which slightly differ only in structure from the GA_3 shown here.	 6-Furfurylamino Purine (kinetin)  6-Benzylamino Purine (BA)	 Ethylene	 Abscisic Acid

Brassinolide is a recently rediscovered hormone





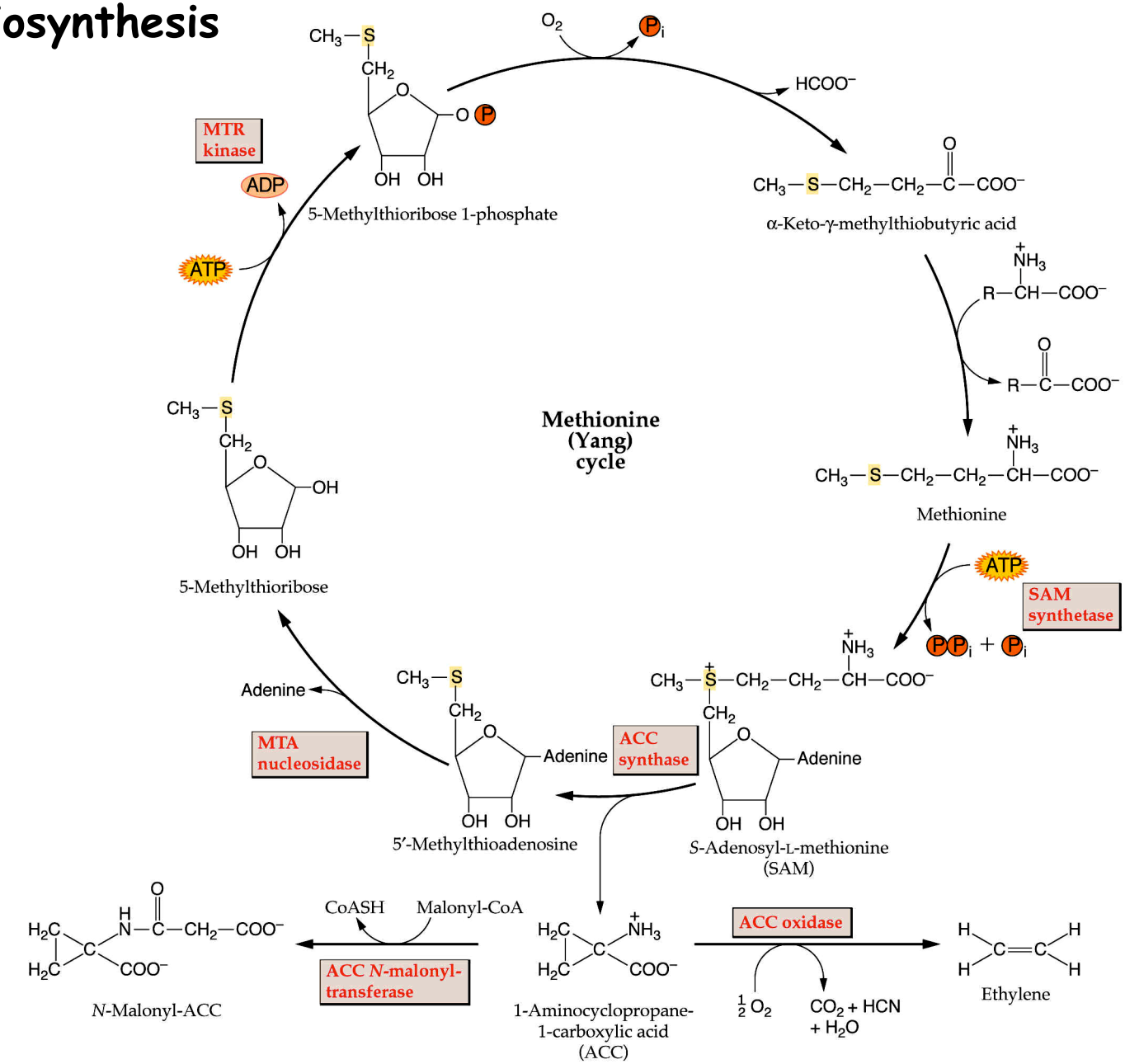
Brassinolide

C_2H_4

↓

Responses to Ethylene	Fruit ripening Senescence of leaves, flowers Abscission of leaves, flowers, fruits Altered geotropism in roots and stems Promotion of seed germination Inhibition/promotion of cell division and cell elongation Induction of phytoalexins and other disease resistance factors Initiation of roots Inhibition/promotion of flowering Epinasty of leaves Bud dormancy release Sex shifts in flowers

Ethylene Biosynthesis



Triple responses to ethylene

-inhibition of stem elongation

-radial swelling of the stem

-apical hook formation

ethylene insensitive

WT

Two types of mutants in ethylene mutants

Type 1: constitutive response

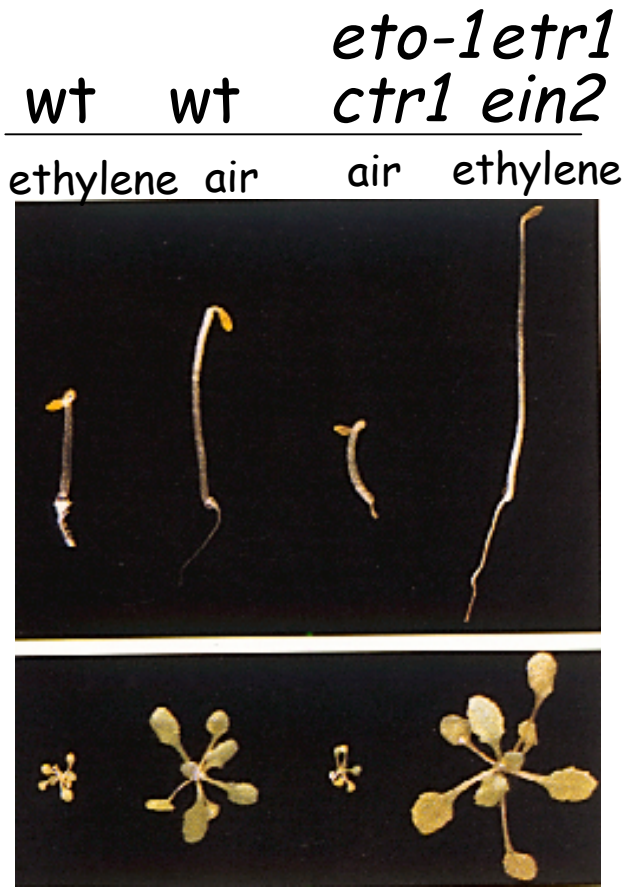
eto1 (ethylene over-producer)

ctr1 (constitutive ethylene response)

Type 2: ethylene insensitive

etr1 (ethylene resistance)

ein2, 3, 4,5 (ethylene insensitive)



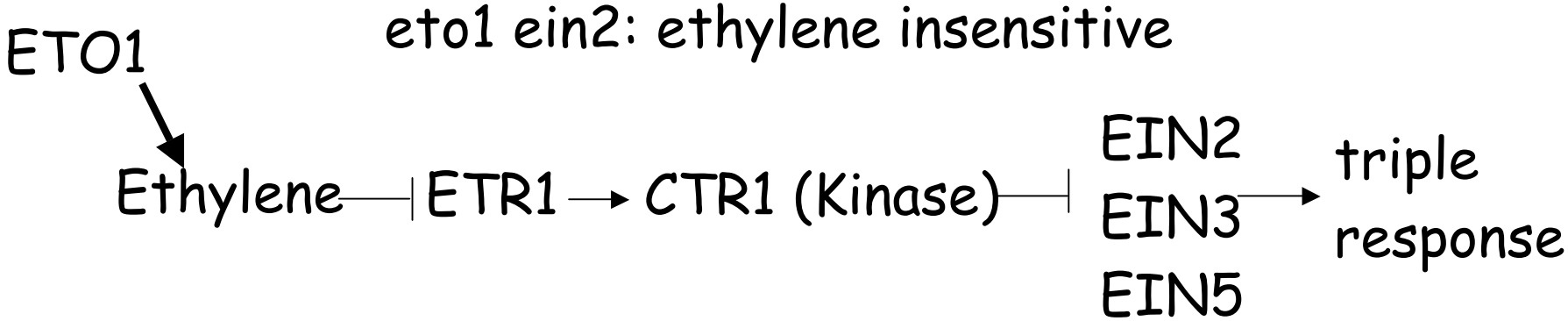
eto1: **phenotype can be blocked by ethylene synthesis inhibitors**

ctr1: **phenotype is unaffected by ethylene synthesis inhibitors**

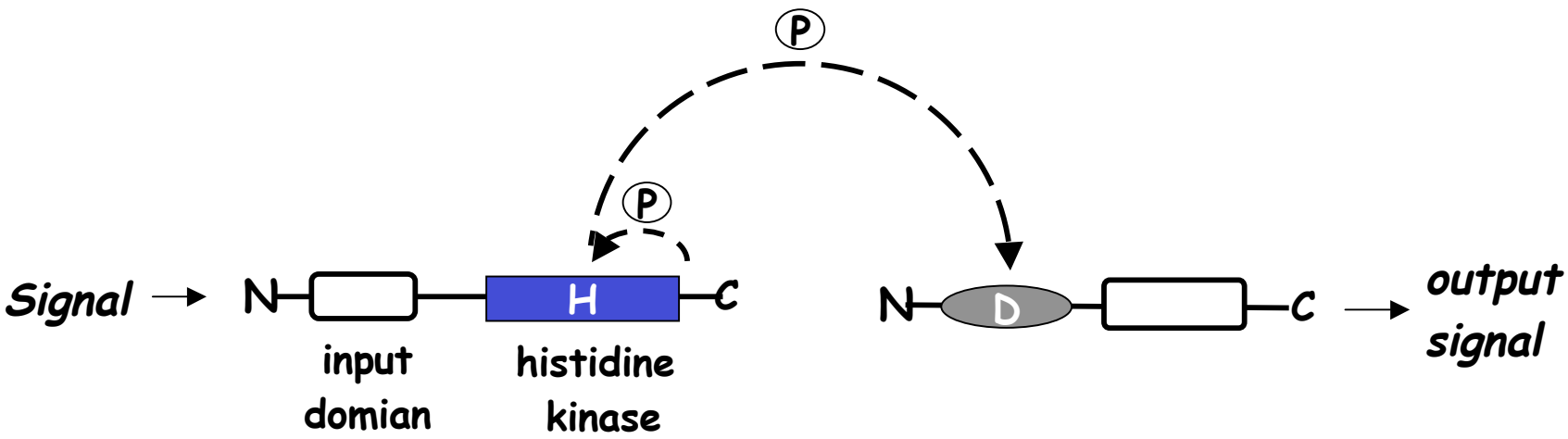
Epistasis pathway established by double mutant analyses

ctr1: constitutive responses
eto1: constitutive responses:
etr1: ethylene insensitive
ein2: ethylene insensitive

etr1 ctr1: constitutive response
ein2 ctr1: ethylene insensitive
eto1 etr1: ethylene insensitive
eto1 ein2: ethylene insensitive



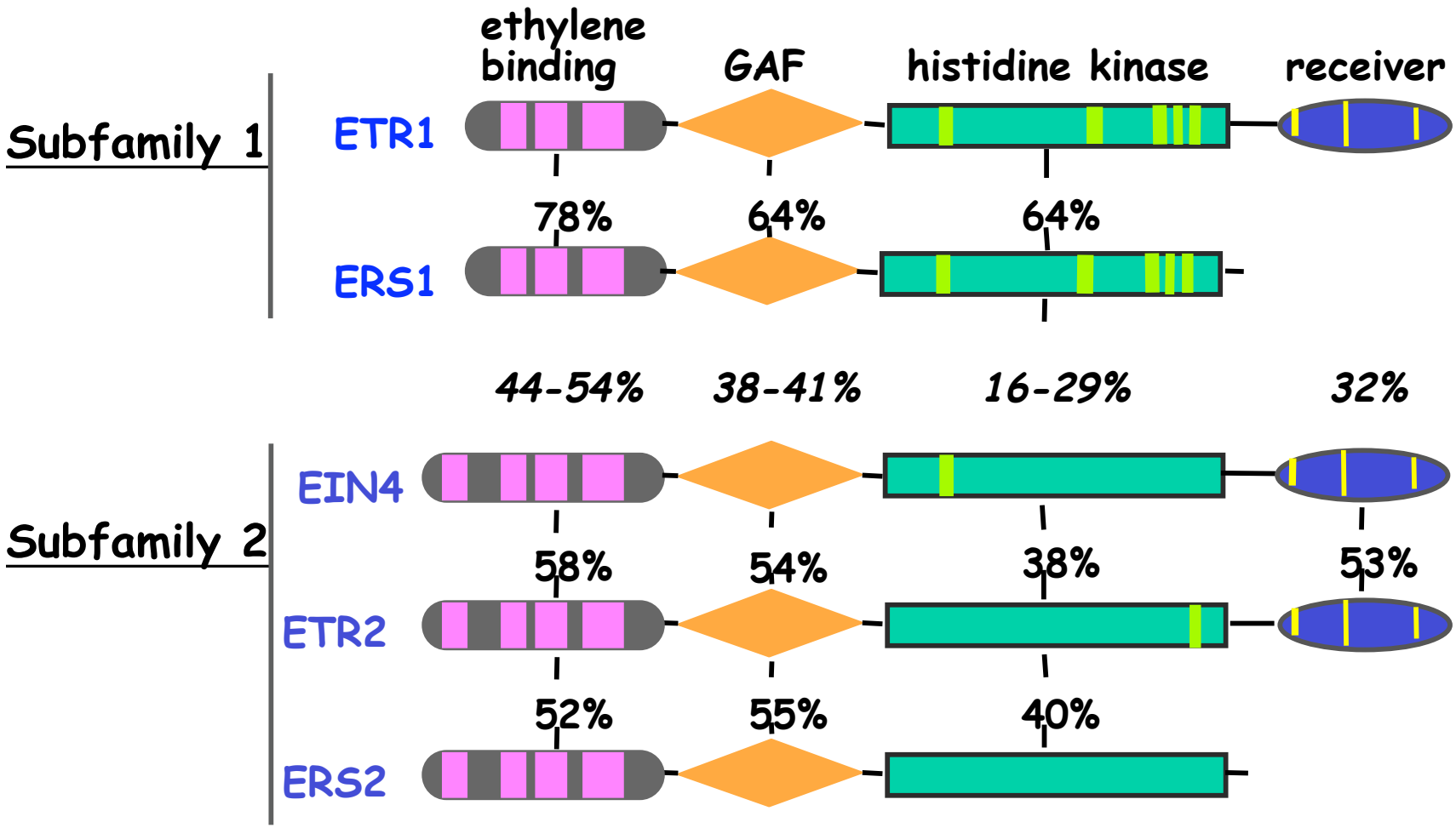
Two component system



Sensor

Response regulator

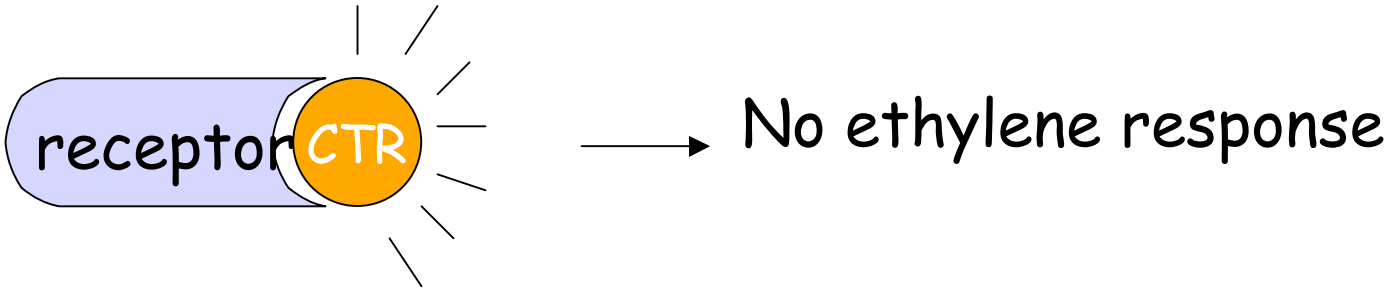
Arabidopsis ethylene receptor family



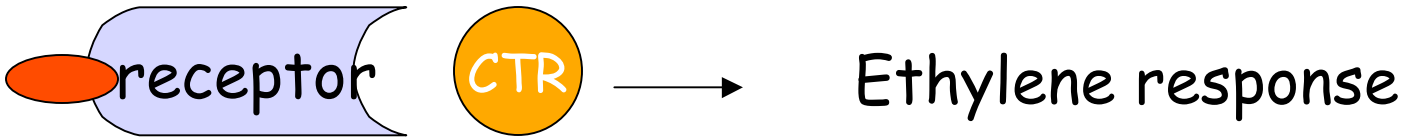
-*etr1* is a dominant mutation

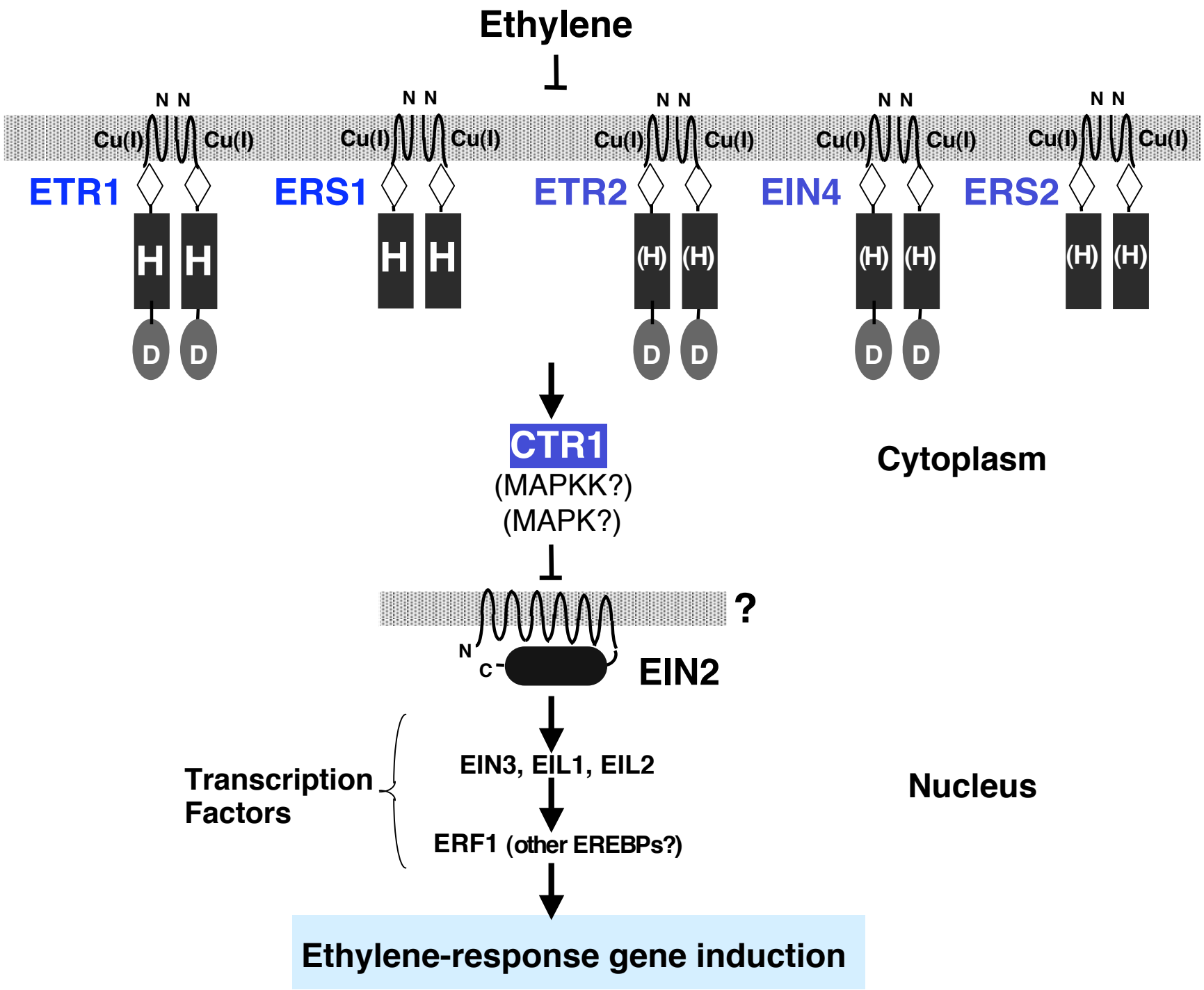
-loss of *etr* and its redundant receptors gives rise to constitutive phenotypes

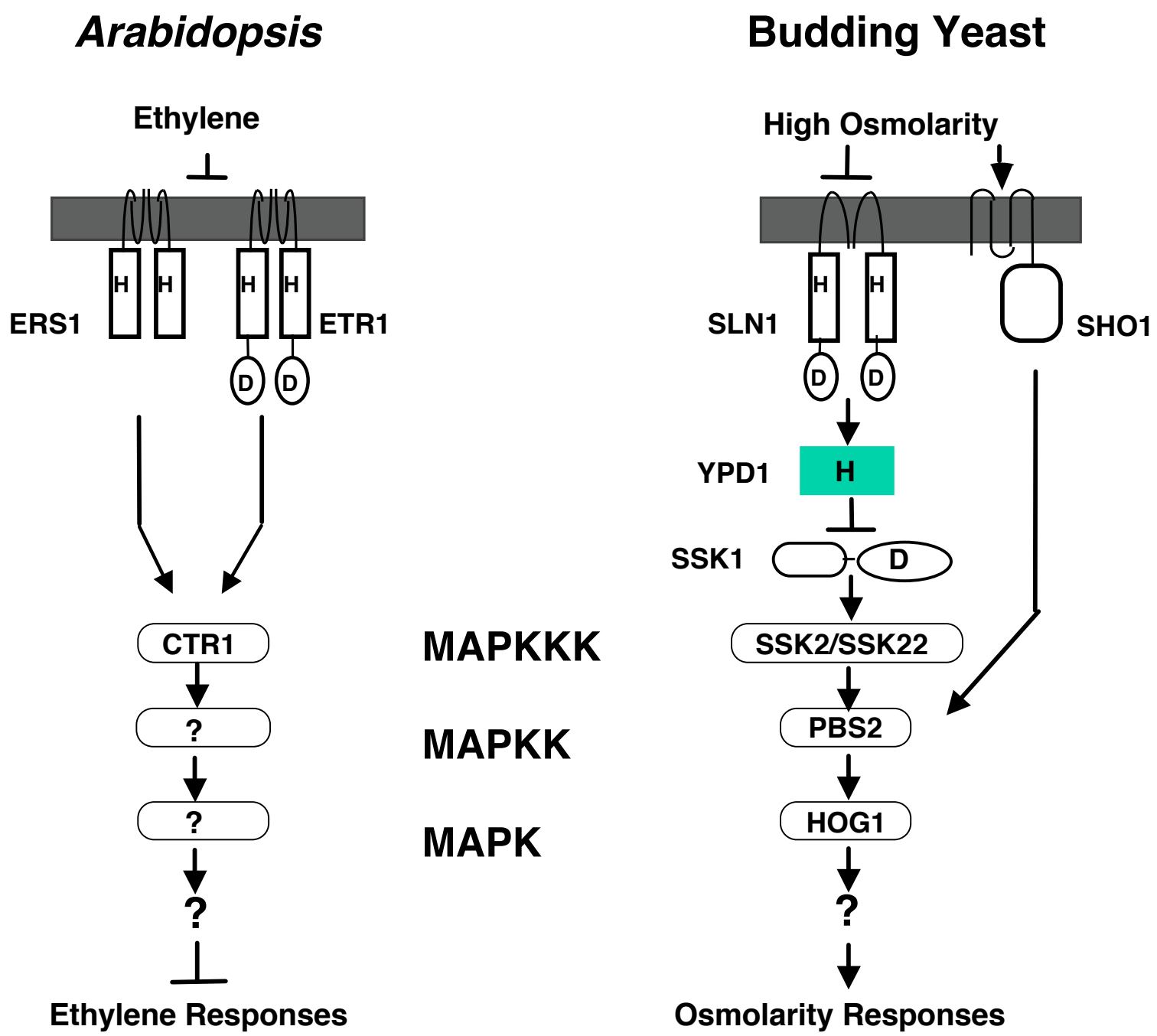
A. No ethylene



B. Yes ethylene




Ethylene
N N
Cu(I)
Cu(I)
ETR1
ERS1
ETR2
EIN4
ERS2
H
(H)
D
CTR1
(MAPKK?)
(MAPK?)
Cytoplasm
?
N
C
EIN2
Transcription Factors
EIN3, EIL1, EIL2
ERF1 (other EREBPs?)
Nucleus
Ethylene-response gene induction


Arabidopsis
Budding Yeast
Ethylene
High Osmolarity
H
H
H
H
ERS1
ETR1
D
D
SLN1
SHO1
YPD1
SSK1
CTR1
?
?
?
MAPKKK
MAPKK
MAPK
SSK2/SSK22
PBS2
HOG1
Ethylene Responses
Osmolarity Responses

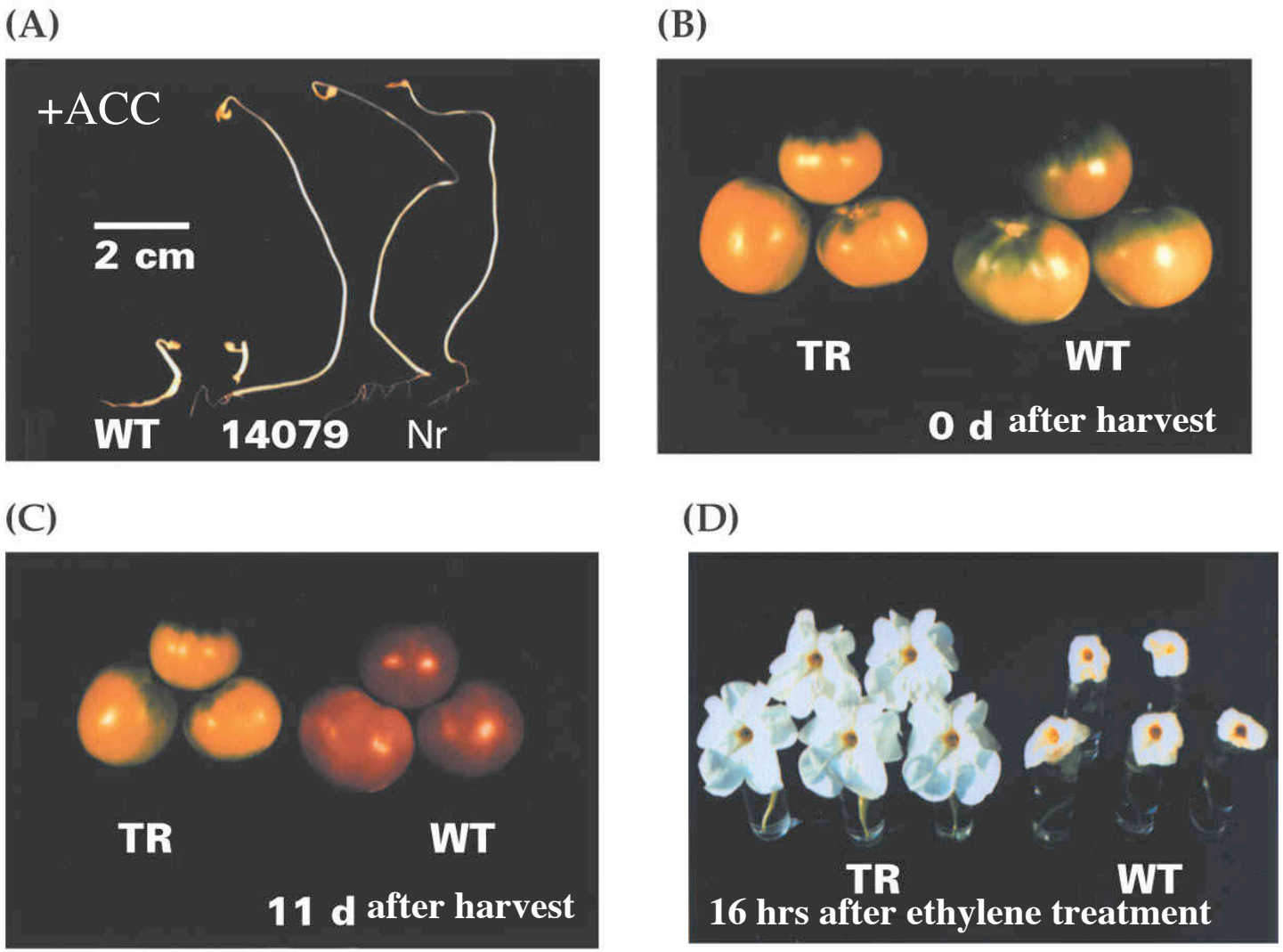
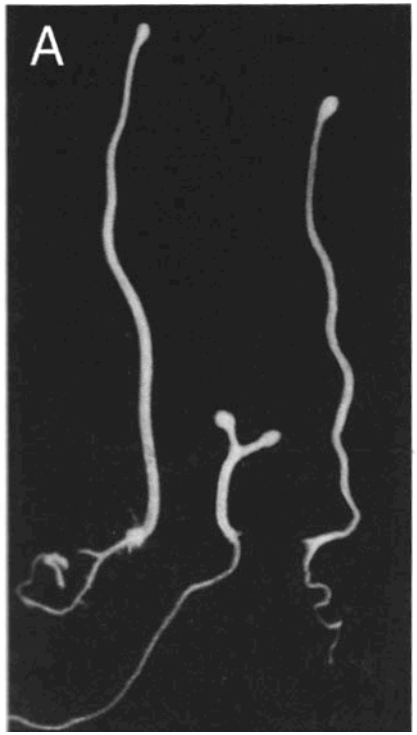


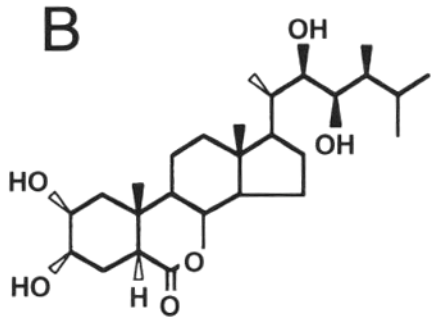
Figure 18.22, WT: wild-type.
14079 (TR): transgenic plant expressing *ETR1-1*

Brassinosteroid (BR)

(A) BR biosynthetic mutants

DET2 encodes steroid 5a reductase
***det2* is defective in biosynthesis of BR**
det2 phenotype can be rescued by BR





Brassinolide

BR-defective mutants: dark green (light grown), small cells, reduced apical dominance. reduced male fertility. dark grown plants have similar phenotype to cop/det/fus, a role in photomorphogenesis

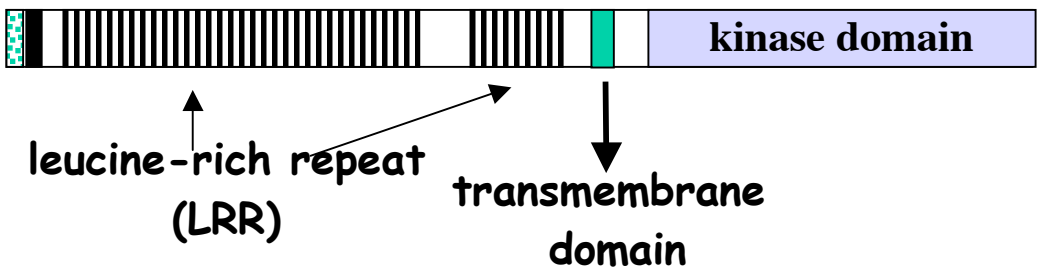
(B) BR signaling components

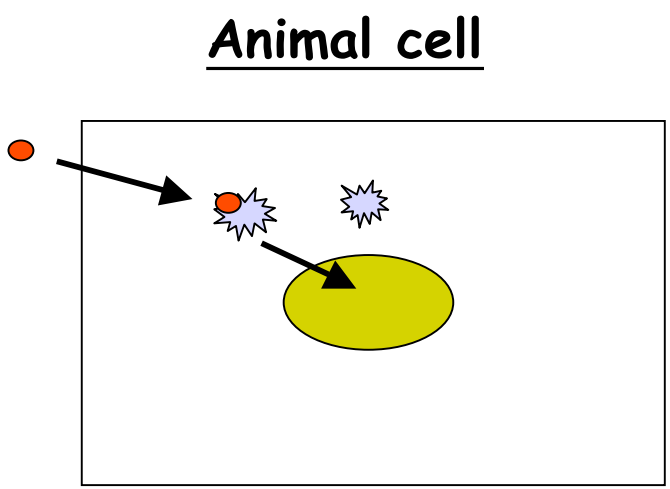
(I) BRI1 (BR receptor):

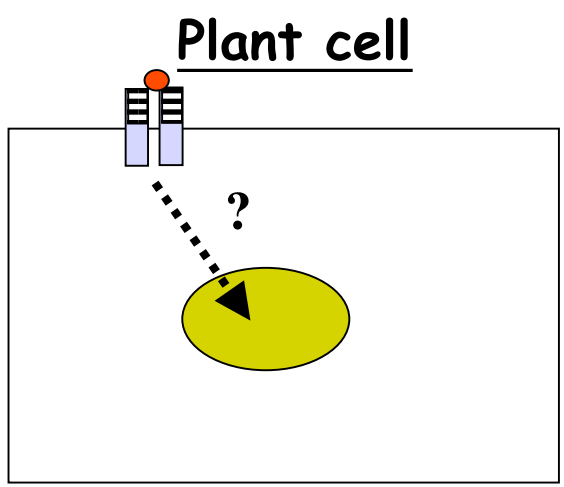
Receptor like (serine/threonine) kinase (RLK)

leucine-rich repeat (LRR) extracellular domains

localizes to plasma membrane (thus differs from animals)





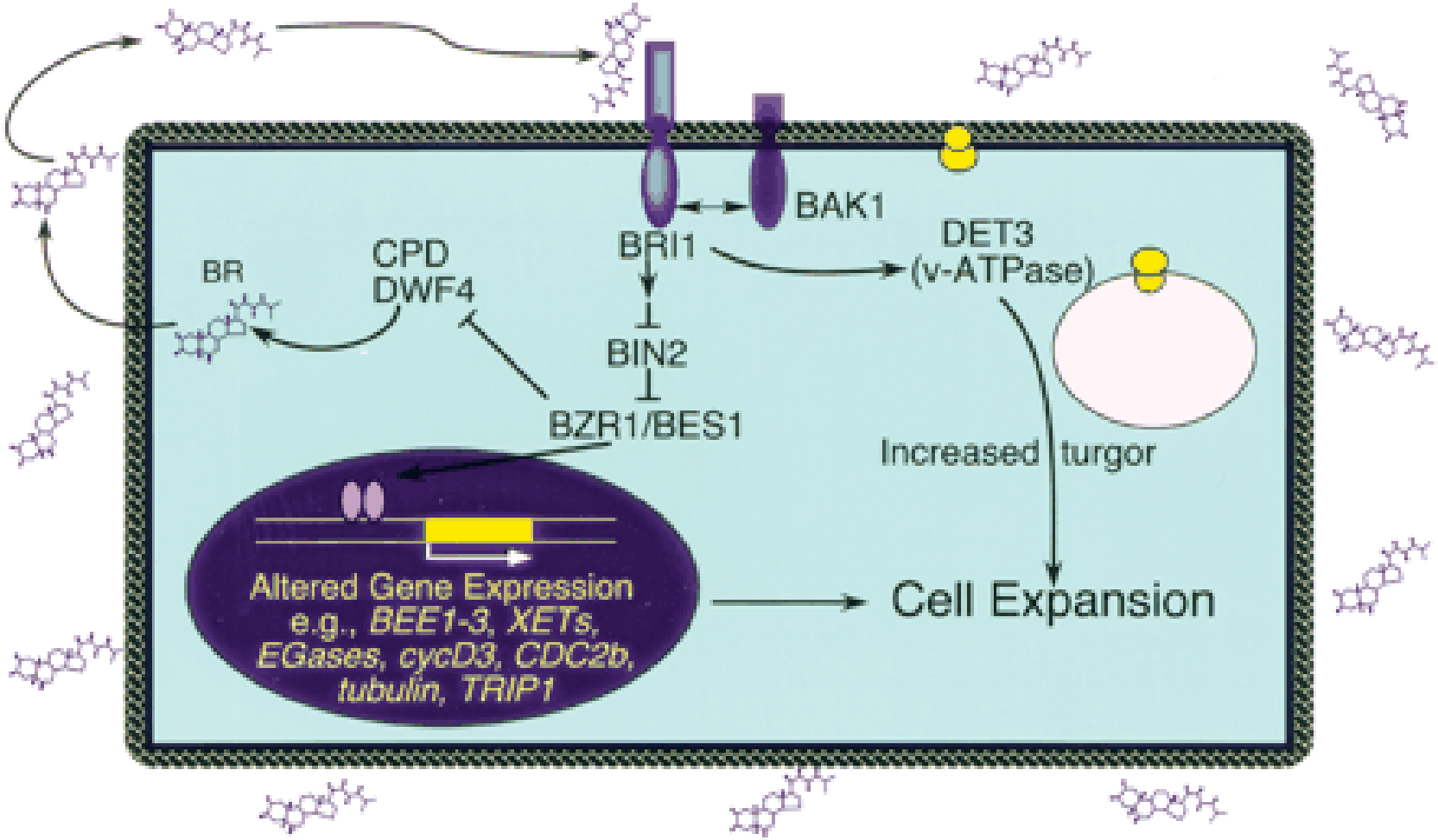


(II) BIN2: member of a 10-member family
glycogen synthase kinase

bin2: semidominant
phenotype similar to bri (BR receptor mutation)
negative regulator of BR signaling

(III)BEZ1 and BZR1: highly similar in protein seq (89% identity
6 member gene family
nuclear protein (upon BR treatment)
no DNA-binding domain
novel protein
Bez1 and bzr1: dominant mutant
constitutive response phenotype

A model of BR signaling pathway



How do you distinguish mutants defective in
BR synthesis (det2) from mutants defective in
BR perception (bri) and signal transdection (bin2-D)?

Lecture 17: Plant hormone signaling mechanism II
GA, Auxin, cytokinin, and ABA

<u>Gibberellic Acid</u>

floral induction
germination
hypocotyl
elongation
leaf expansion
apical dominance
floral development
fruit maturation
internode elongation

GA biosynthetic mutants

GA1: encodes for ent-CDP synthase

ga1-3: loss-of-function mutant of GA1

deficient for GA,
do not germinate,
short and dark green leaves
dwarf
reduced apical dominance
can be rescued by GA application

wt *ga1-3*

GA signaling mutants

(I) RGA, RGL1, GAI all encode *GRAS (GAI RGA Scarecrow)* transcription factor and serve as negative regulators of GA responses

gai: *dominant allele*

mimics the effect of GA-deficiency
harbors high levels of GA
can't be rescued by GA application, defective in GA signaling

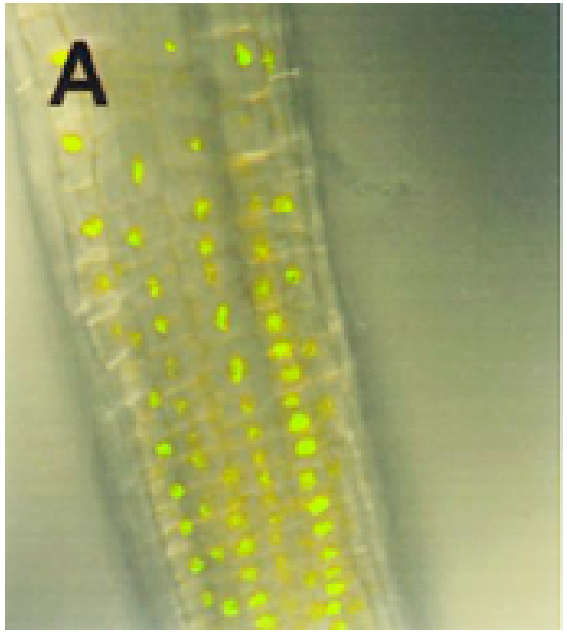
lose-of function allele

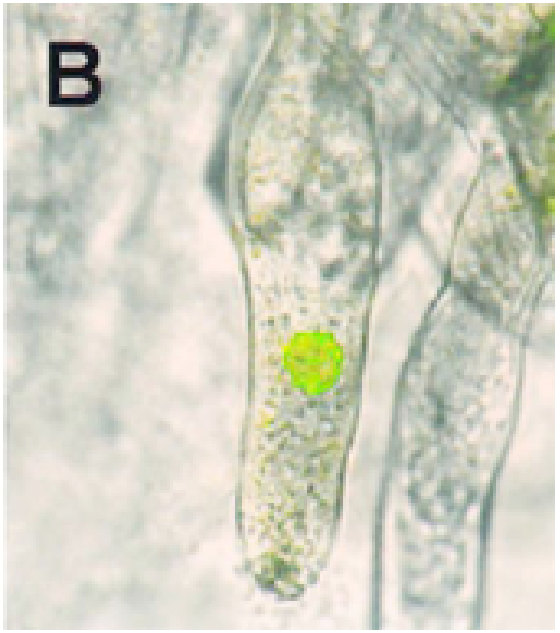
tall plants
insensitive to GA biosynthesis inhibitor paclobutrazol

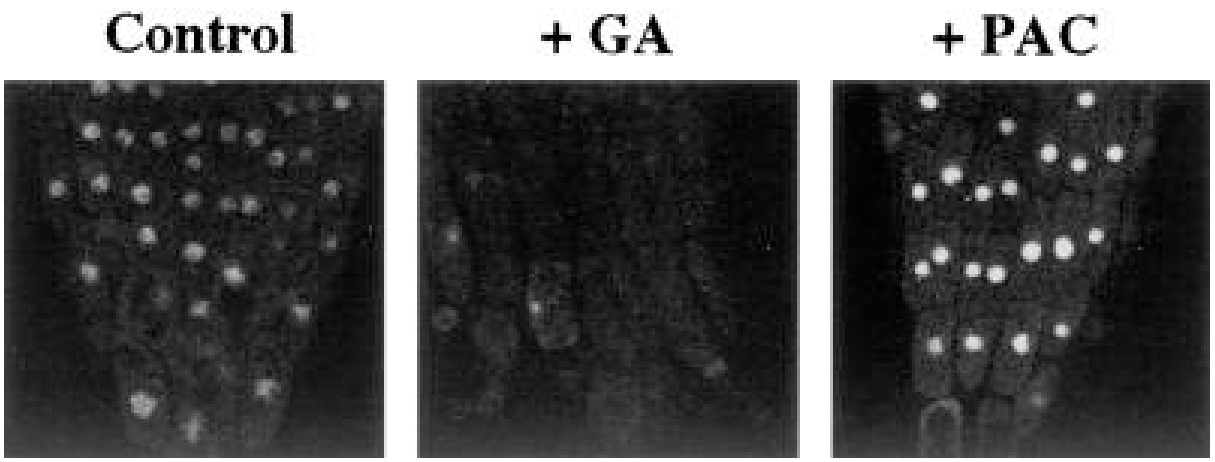
Epistasis with GA biosynthetic mutants

GA1 ⊣ RGA, GAI ⊣ GA response

RGA-GFP localization





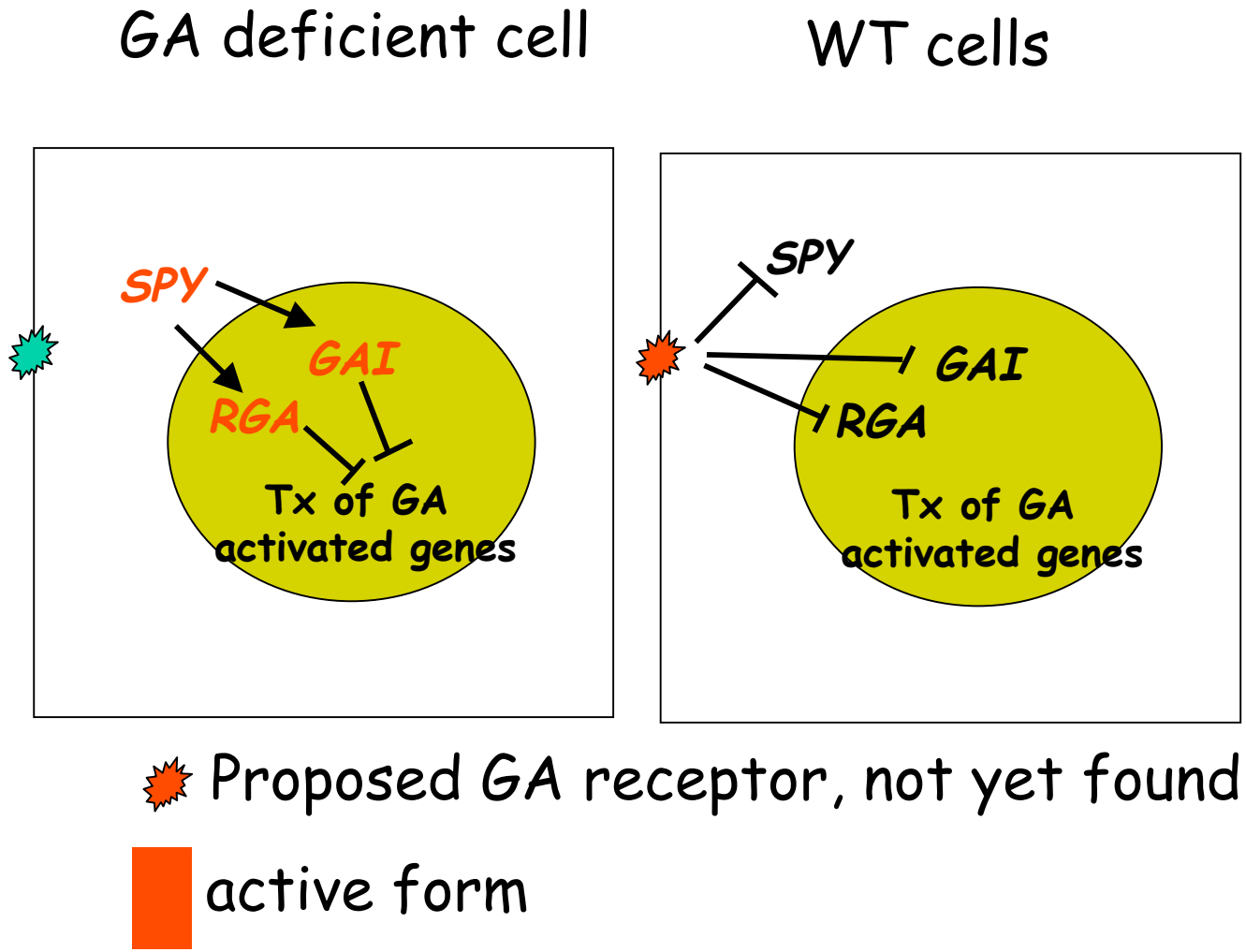

Control
+ GA
+ PAC

(II) SPINDLY (SPY)

Loss-of-*SPY* exhibits constitutive *GA* response

Encode OGT, which modifies other proteins in a manner similar to phosphorylation. Ie. *SPY* may add an O-GlcNAc moiety to other proteins to regulate their activities.

GA functions as an inhibitor of inhibitors



Auxin: the growth hormone, required for viability

phototropism
gravitropism
germination
delay leaf abscission
promote cell elongation
promote cell division
promote formation of lateral & adventitious roots
required for apical dominance
vascular differentiation

Polar transport of auxin: synthesized in shoot tip

Auxin signaling mechanism

ABP1: auxin binding protein 1 = Auxin receptor?

knockout mutants are lethal

Genes identified in auxin signaling pathway

ARF: auxin-response factors

23 member family in Arabidopsis

has DNA binding domains

can be either activators or repressors

AUX/IAA: 29 member in Arabidopsis

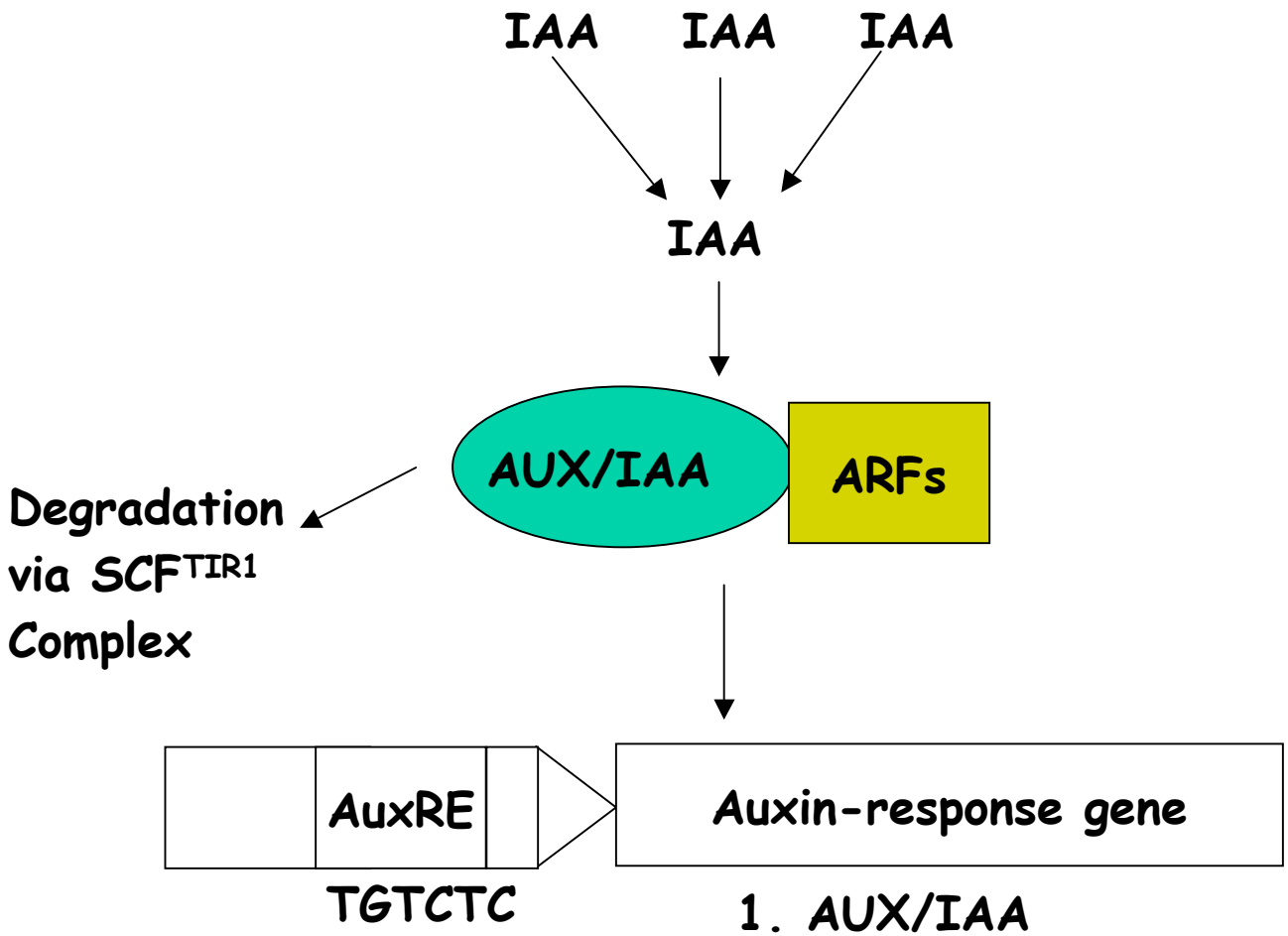
Has protein binding domain

heterodimerize with ARFs

rapid auxin-dependent turn-over of AUX/IAA

is dependent on ubiquitin-proteasome-pathway

Auxin signaling pathway



1. AUX/IAA
2. SAUR
3. GH3
4. GST (glutathion-S-transferase)
5. ACC synthase

ABA: Seed maturation and anti-stress signal

Initiation and maintenance of seed and bud dormancy

acquire desiccation tolerance by inducing LEA

promote seed storage proteins

prevent preharvest sprouting (vivipary)

Response to stress (water stress)

close stomata in response to water stress

No ABA receptor has been identified

<u>In Stomatal guard cells:</u>

ABA binding to receptor

↓

ROS (Reactive Oxygen Species)

↓

Increased cytosolic Ca^{++}

Ca^{++} influx through Plasma-membrane

Ca^{++} release from vacuoles

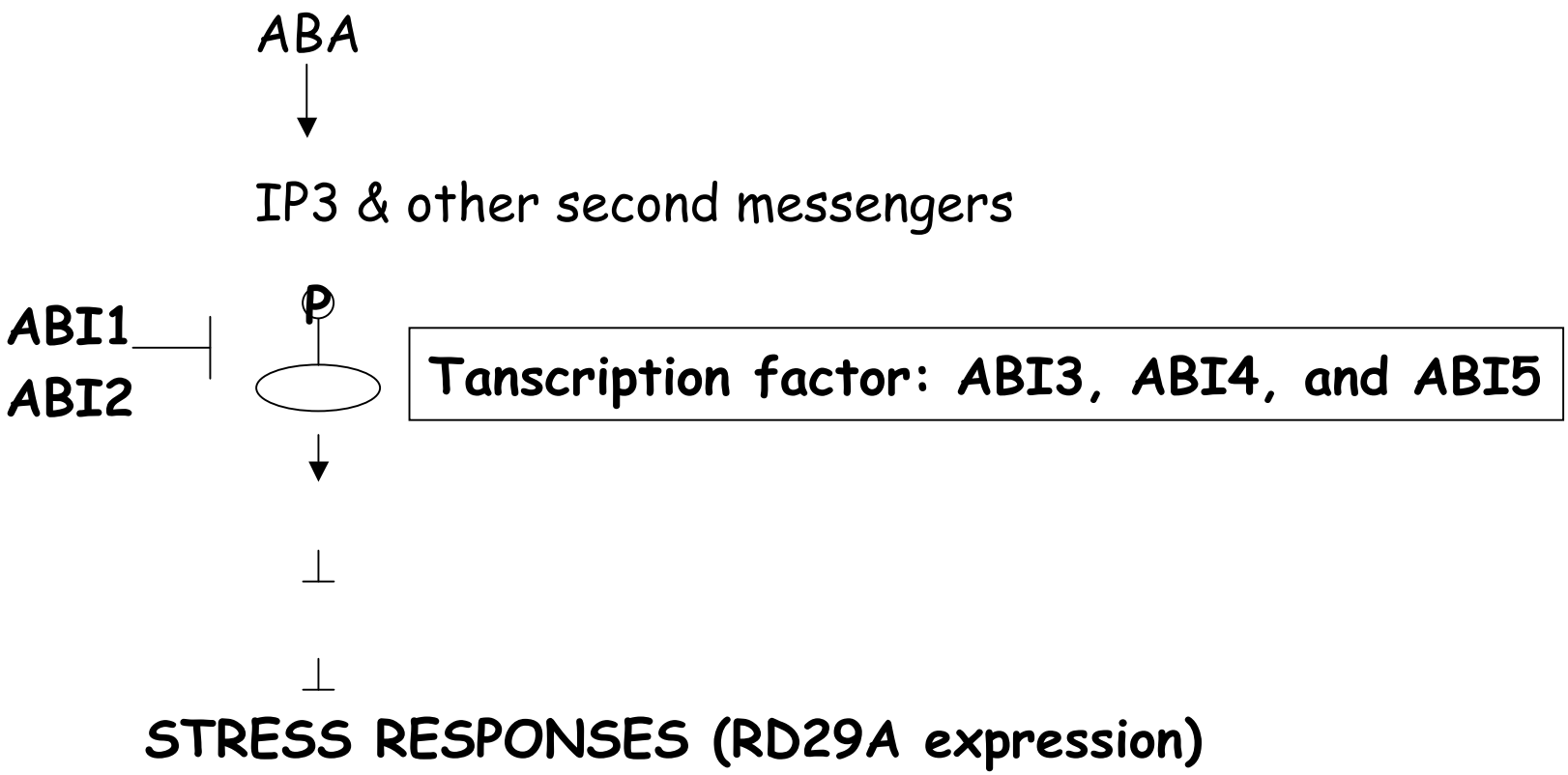
Increase cellular PH (7.67 to 7.94)

Membrane depolarization

↓

Stomata closing

SCHEMATIC MODEL OF ABA Pathway



ABI1 **and** ***ABI2*** **encode phosphatase**

Cytokinin: regulators of cell division

Cytokinin-over producer:

Shoot apical meristem produce more leaves
Leaves have higher chlorophyll levels & greener
Adventitious shoot may form from unwounded leaf
Leaf senescence is retarded
Aplical dominance is reduced
Root growth rate is reduced

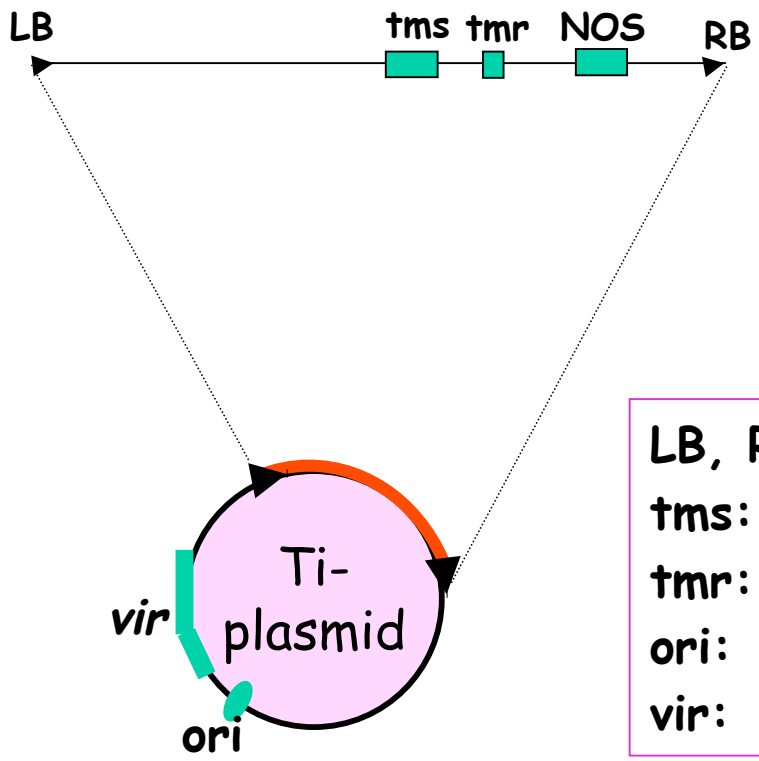
Morphogenesis in cultured tissues:

High auxin:cytokinin ratio: root
Low auxin/cytokinin ratio:shoot
Intermediate levels: callus

Agrobacteria causes crown-gall

tms: auxin synthesis (tms)

tmr: cytokinin syntesis



LB, RB: 25 bp repeat, left and right border

tms: tumor morphology shoot

tmr: tumor morphology root

ori: origin of replication

vir: virulence region

Receptor:

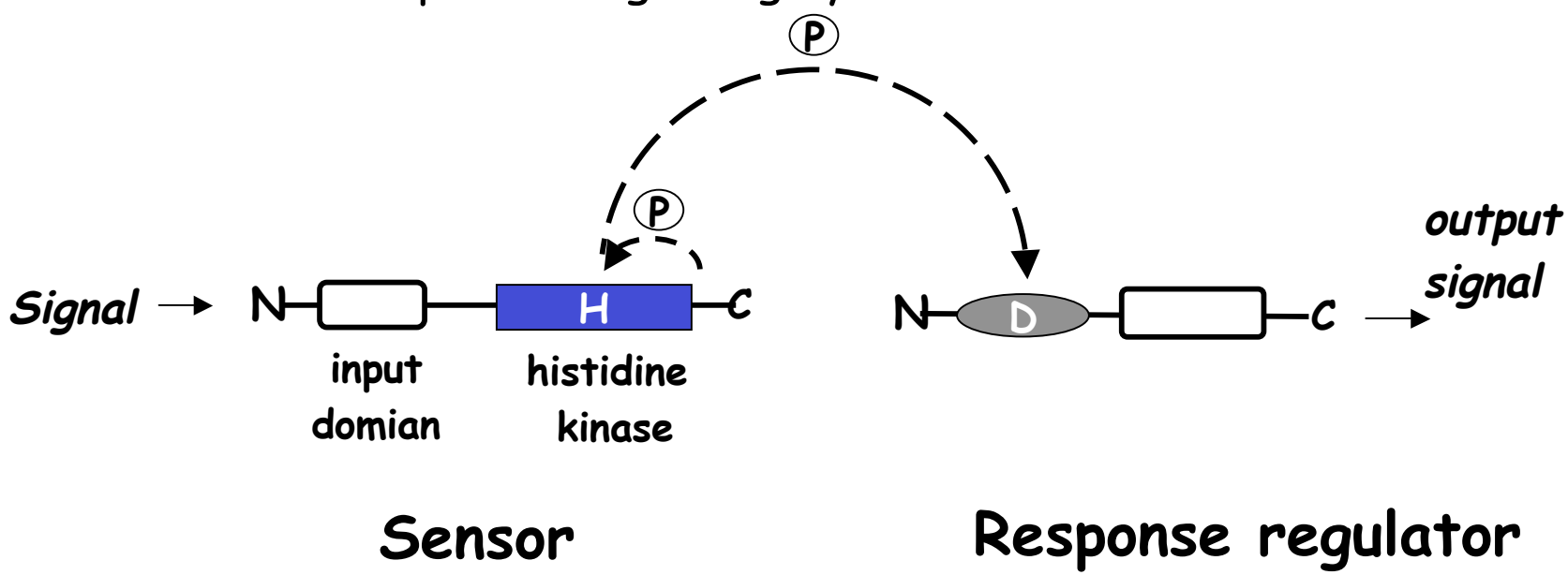
CKI1: (over-producers that confer cytokinin-independent growth)

CRE1: (mutants failed to produce shoots in response to cytokinin)

AHK2

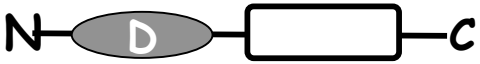
AHK3

All encode two component signaling system



Type A ARR

Type B ARR



Receiver Domain

output domain (Tx factor)

ARR: Arabidopsis Response Regulator

AHP: Arabidopsis Histidine Phosphotransfer