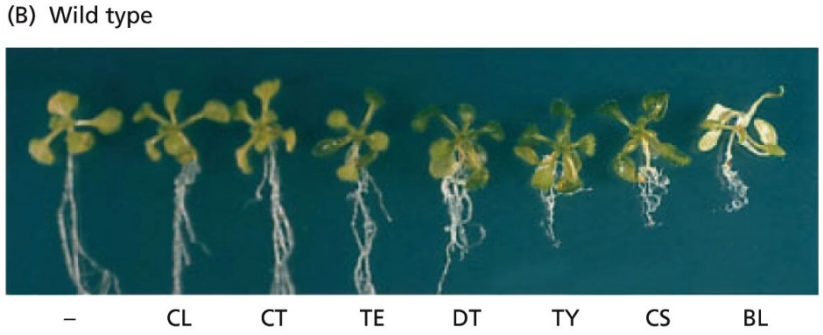
Contents

Brassinosteroids : Regulators of Cell Expansion and Development

- Biosynthesis, Metabolism and Transport of BRs
- BR Signaling Pathway
- Effects on Growth and Development
  - ① Shoot growth
  - ② Root growth
  - ③ Xylem differentiation
  - ④ Germination

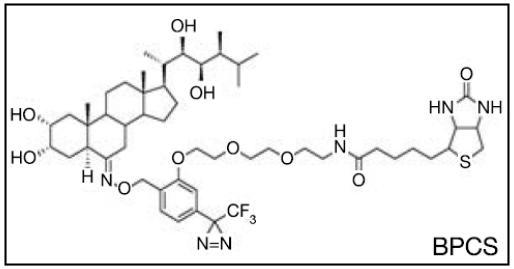
BR receptor, BRI1

- ***bri1* (*br*assinosteroid-*i*nsensitive *1*)**
  - ✓ From a screen of approximately 70,000 EMS-mutagenized seedlings of *Arabidopsis*
  - ✓ In the presence of 10^{-6} or 10^{-7} M BRs, elongation of wildtype roots inhibited by up to 75%.
  - ✓ *bri1* mutant exhibits normal root elongation in the presence of BL.
  - ✓ *bri1* mutant is an extreme dwarf that reaches less than 10% of the height of wild-type

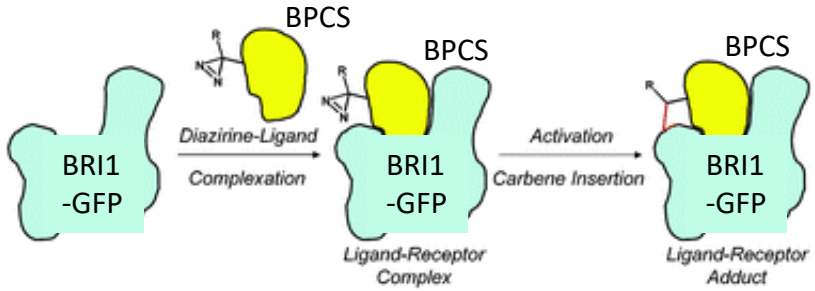


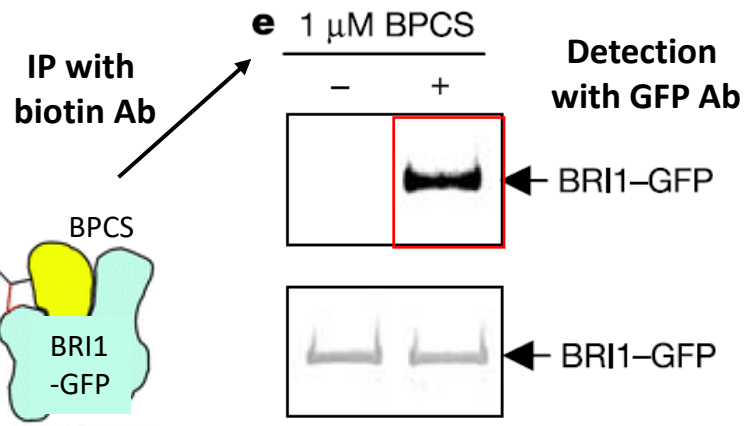
- **biotin-tagged photoaffinity castasterone (BPCS)**
- ✓ an analogue of castasterone

① Carbene-generating phenyldiazirine moiety
: allow the formation of a covalent bond between BPCS and specific proteins upon irradiation with UV



② Biotin tag





Clouse et al., 1996

Kinoshita et al., 2005

BR receptor, BRI1

- **BRI1**

✓ Located in plasma membrane & endosome

✓ Leucine-rich repeat (LRR)-receptor kinase

✓ Dual specificity

① Serine (S)/Threonine (T)

② Tyrosin (R)

✓ Domains

① Extracellular tandem 25 LRR motifs

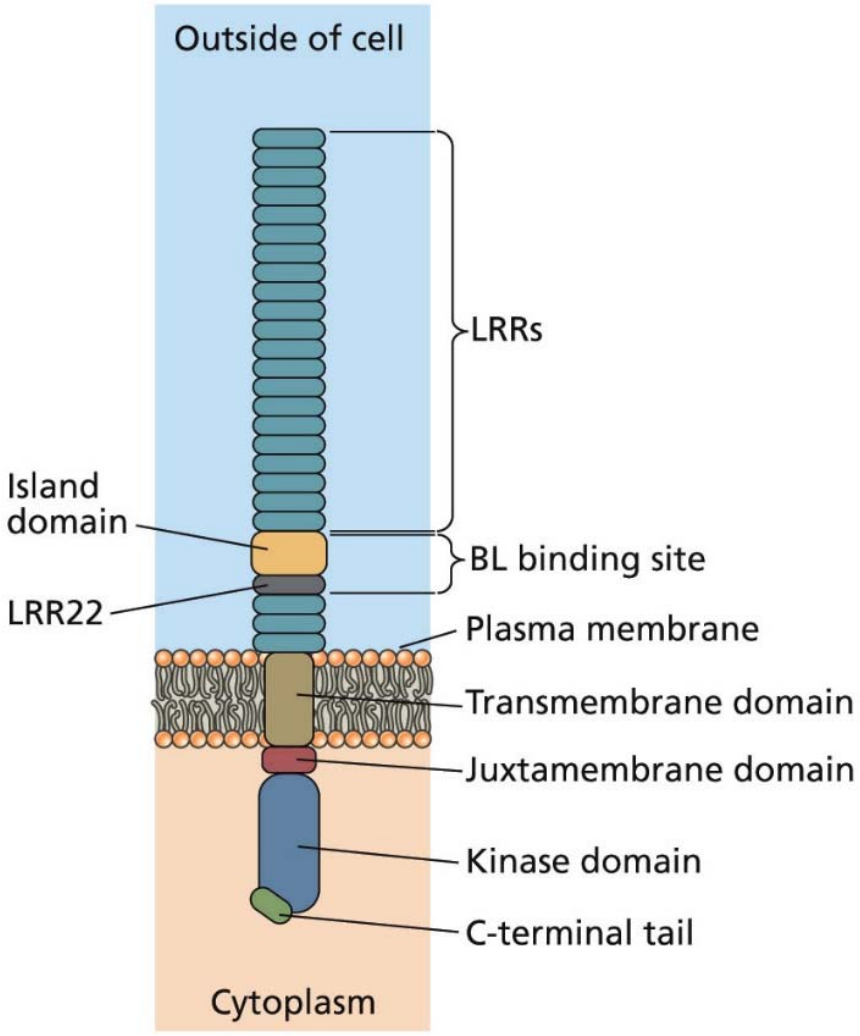
- Island domain (70 a.a.)
- LRR22 (24a.a.)

→ Minimum binding site for BRs (94 a.a.)

② Transmembrane domain

③ Cytoplasmic kinase

- Juxtamembrane region (JM)
- Kinase
- C-terminal tail (CT)

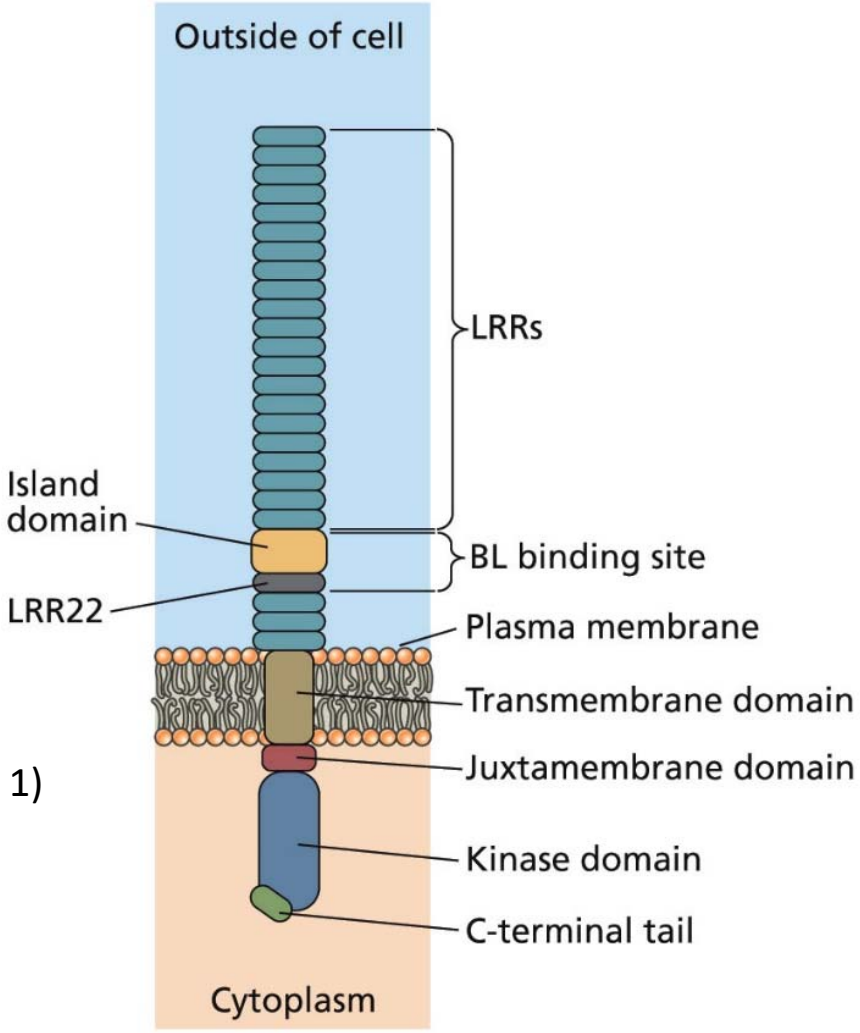


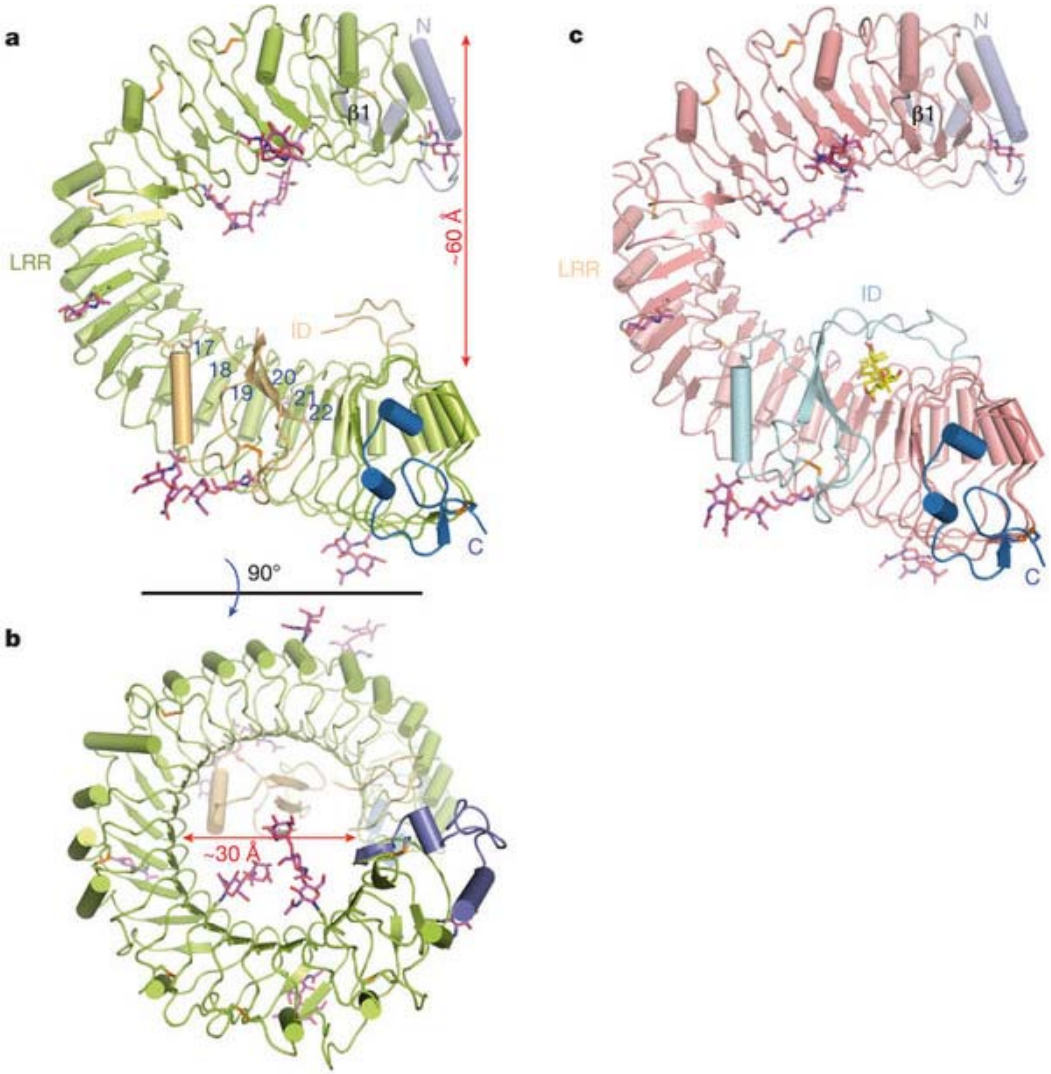
BR receptor, BRI1

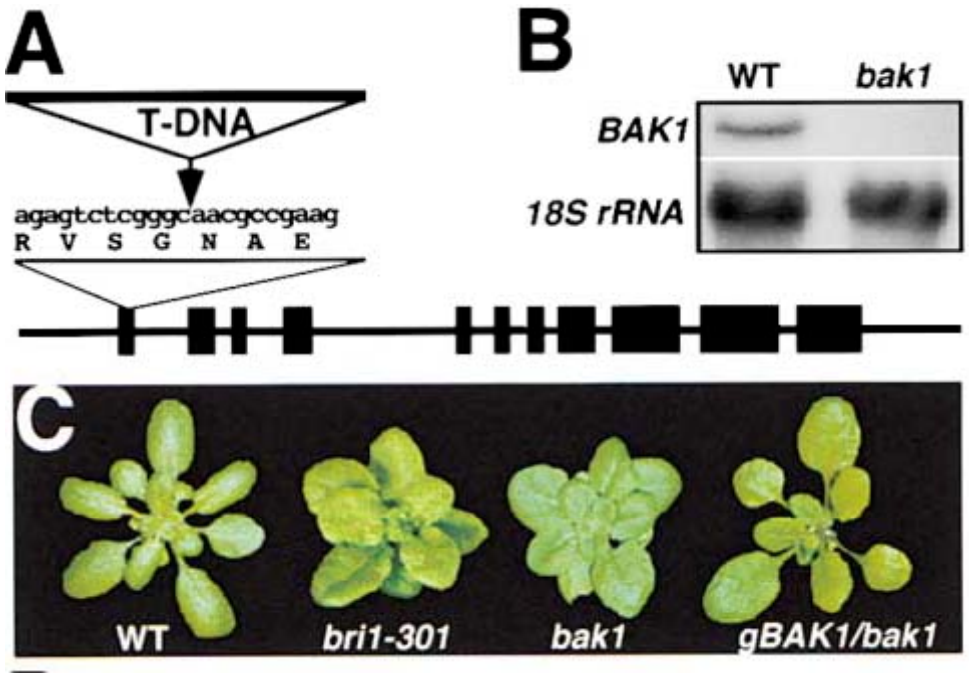
- **BRI1 becomes phosphorylated at multiple intracellular domains.**
  - ① Juxtamembrane region (JM)
  - ② Kinase
  - ③ C-terminal tail (CT)

- **Roles of phosphorylation sites in BRI1**
  - ① Receptor activation
  - ② Dissociation of BKI1 (BRI1's inhibitor)
    - BKI1 (BRI1-kinase inhibitor1)
  - ③ Interaction with BAK1 and BSK
    - BAK1 (BRI1-associated receptor kinase 1)
    - BSK (BR-signaling kinase)

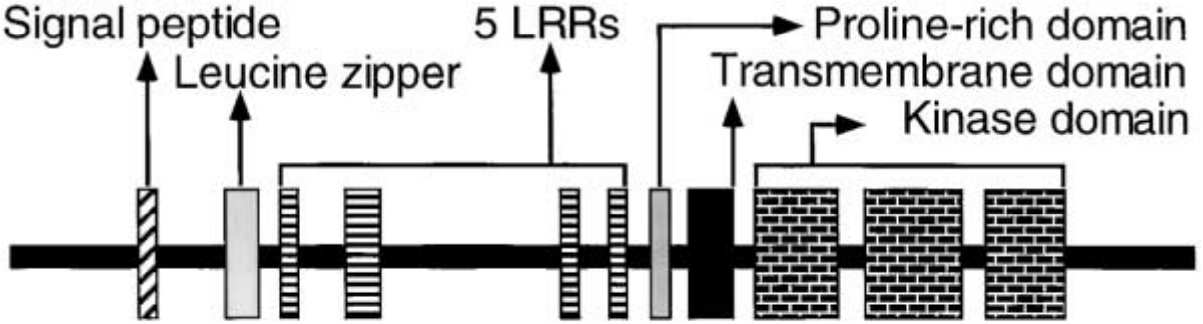
- **CT of BRI1 negatively regulates the receptor, this effect is nullified upon BR binding.**




a
N
β1
~60 Å
LRR
ID
17
18
19
20
21
22
C
90°
b
~30 Å
c
N
β1
LRR
ID
C

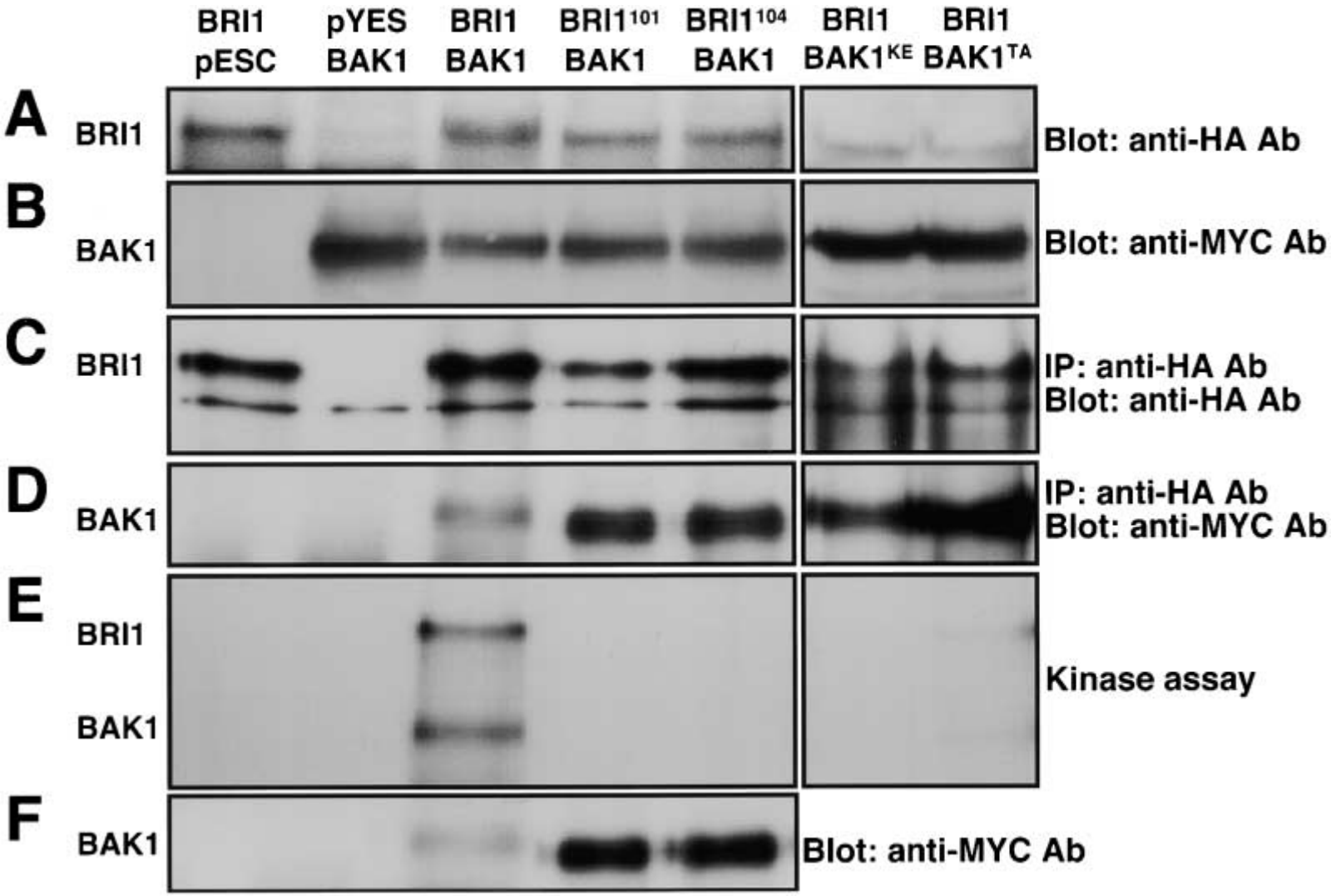

A
T-DNA
agagtctcgggcaacgccgaag
R V S G N A E
B
WT
bak1
BAK1
18S rRNA
C
WT
bri1-301
bak1
gBAK1/bak1

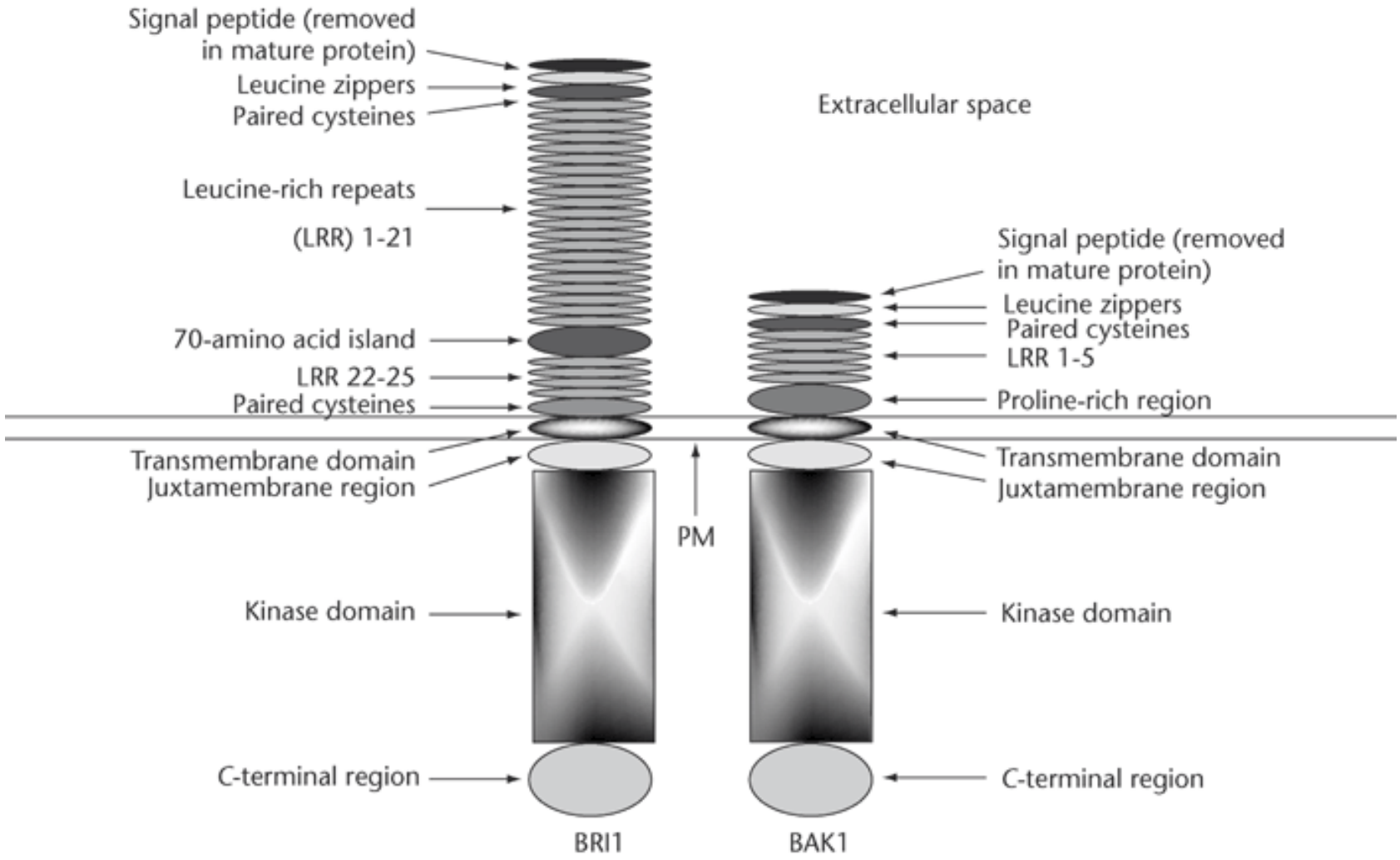
A



B

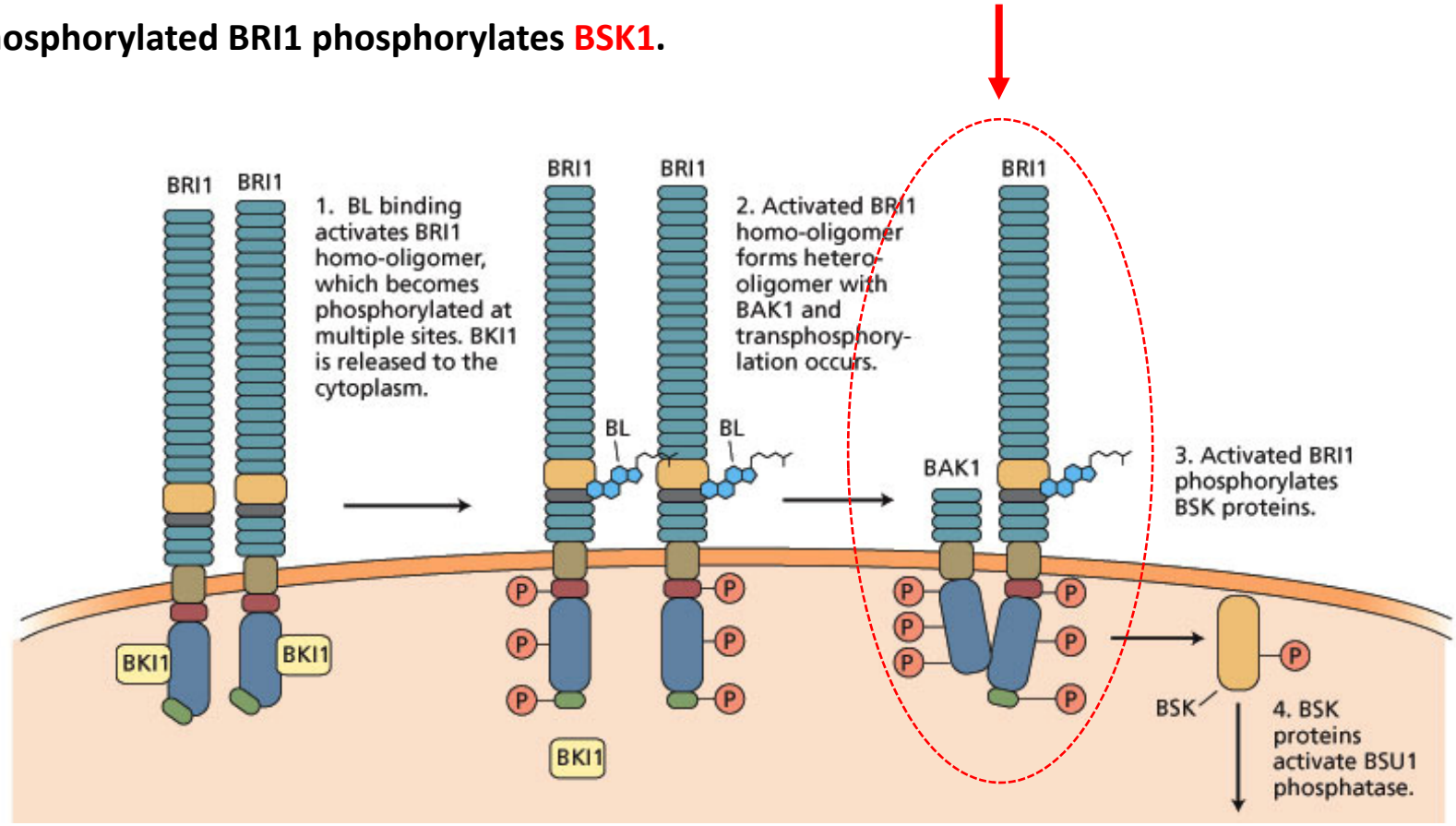
MERRLMIPCFFWLILVLDLVLRVSGNAEGDALSALKNSLADPN
KVLQSWDATLVTPCTWFHVTCNSDNSVTRVDLGNANLSGQLVM
QLGQLPNLQYLELYSNNITGTIPEQLGNLTELVSLDLYLNNLS
GPIPSTLGRLKKLRFLRLNNNSLSGEIPRSLTAVLTLQVLDLS
NNPLTGDIPVNGSFSLFTPISFANTKLTPLPASPPPPISPTPP
SPAGSNRITGAIAGGVAAGAALLFAVPAIALAWWRRKKPQDHF
FDVPAEEDPEVHLGQLKRFSLRELQVASDNFSNKNILGRGGFG
KVYKGRLADGTLVAVKRLKEERTQGGELQFQTEVEMISMAVHR
NLLRLRGFCMTPTERLLVYPYMANGSVASCLRERPESQPPLDW
PKRQRIALGSARGLAYLHDHCDPKIIHRDVKAANILLDEEFEA
VVGDFGLAKLMDYKDTHVTTAVRGTIGHIAPEYLSTGKSSEKT
DVFGYGVMLLELITGQRAFDLARLANDDDVMLLDWVKGLLKEK
KLEALVDVDLQGNYKDEEVEQLIQVALLCTQSSPMERPKMSEV
VRMLEGDGLAERWEEWQKEEMFRQDFNYPTHHPAVSGWIIGDS
TSQIENEYPSGPR

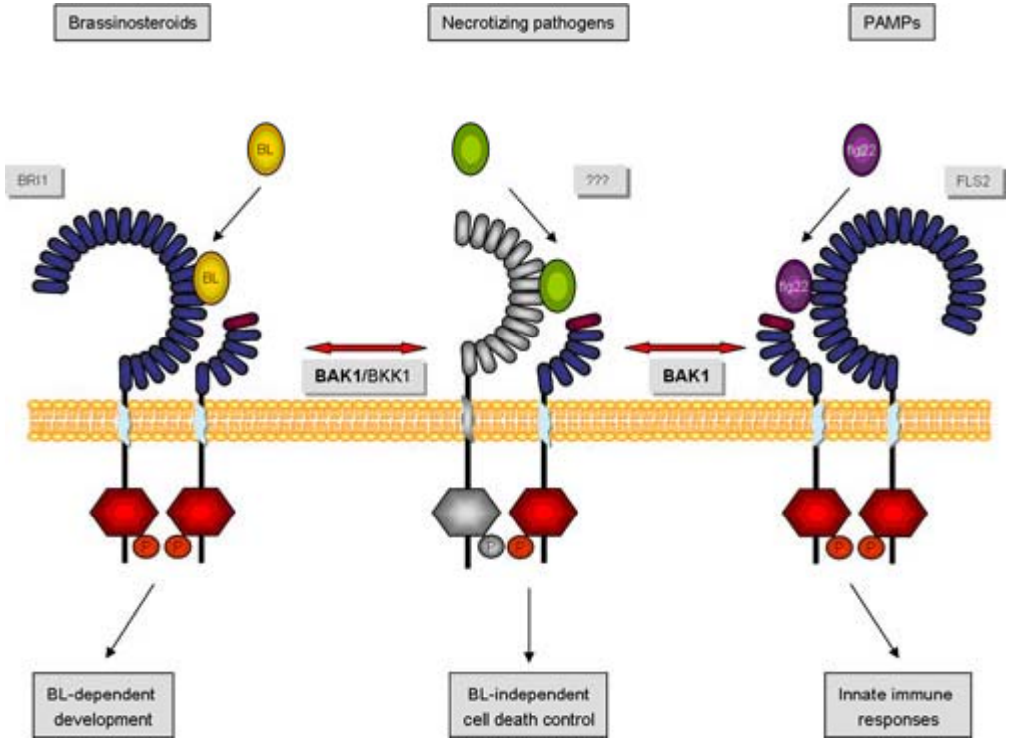

BRI1 pESC
pYES BAK1
BRI1 BAK1
BRI1101 BAK1
BRI1104 BAK1
BRI1 BAK1KE
BRI1 BAK1TA
A
BRI1
Blot: anti-HA Ab
B
BAK1
Blot: anti-MYC Ab
C
BRI1
IP: anti-HA Ab
Blot: anti-HA Ab
D
BAK1
IP: anti-HA Ab
Blot: anti-MYC Ab
E
BRI1
BAK1
Kinase assay
F
BAK1
Blot: anti-MYC Ab

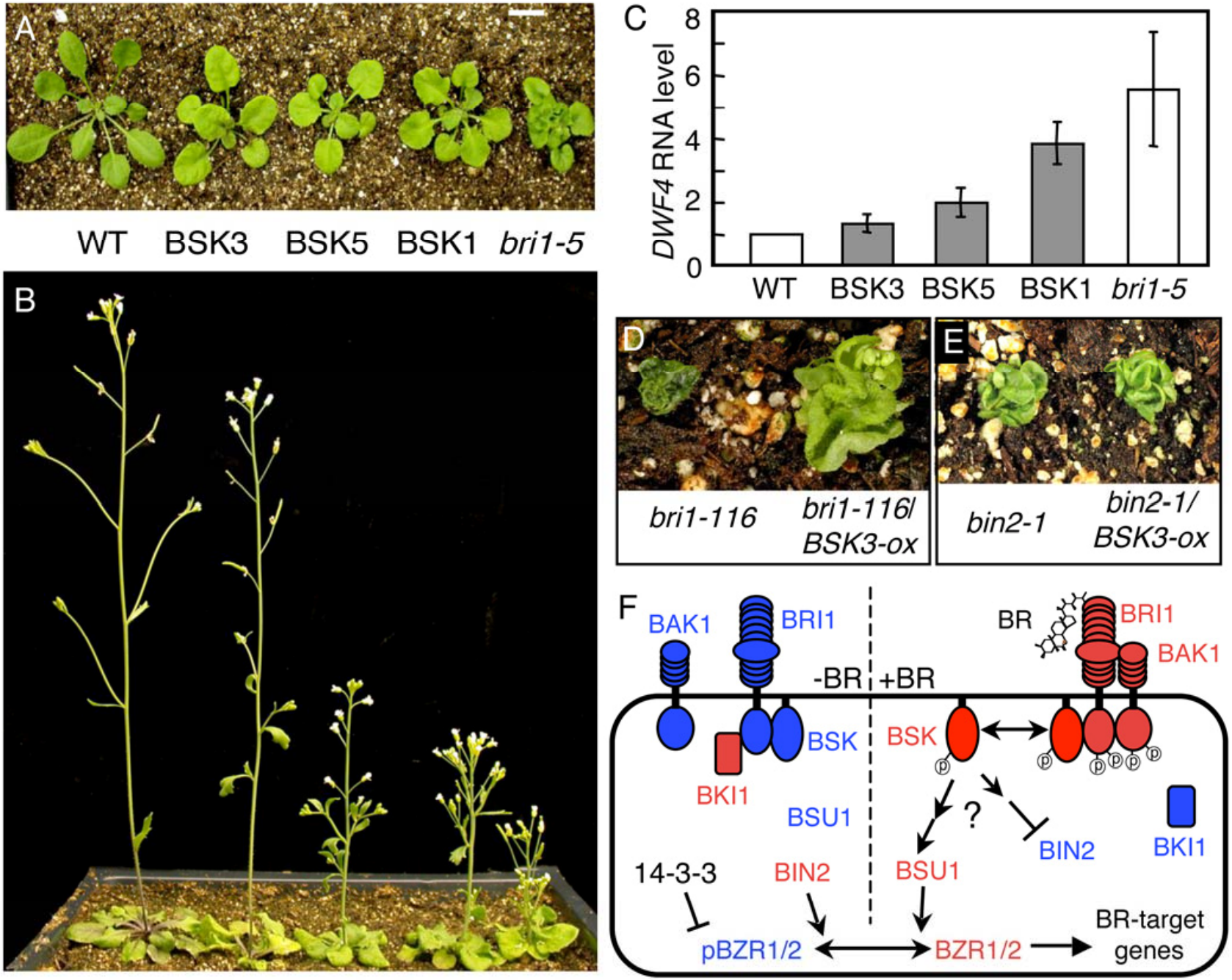

Signal peptide (removed in mature protein)
Leucine zippers
Paired cysteines
Leucine-rich repeats
(LRR) 1-21
70-amino acid island
LRR 22-25
Paired cysteines
Transmembrane domain
Juxtamembrane region
Kinase domain
C-terminal region
BRI1
Extracellular space
PM
Signal peptide (removed in mature protein)
Leucine zippers
Paired cysteines
LRR 1-5
Proline-rich region
Transmembrane domain
Juxtamembrane region
Kinase domain
C-terminal region
BAK1

BR signal transduction pathway (from BRI1 to BSK1)

- **BRI1 receptors normally function as homo-oligomers in the cell.**
- **Following BRI1's binding to and activation by BRs, phosphorylated-BRI1 forms a hetero-oligomer.**
  - ✓ BRI1/BRI1 homodimer → BRI1/BAK1 heterodimer
  - ✓ BAK1 : Second LRR kinase (with no TM domain)
- **Phosphorylated BRI1 trans-phosphorylates BAK1. → Activated form of receptor**
- **Phosphorylated BRI1 phosphorylates BSK1.**

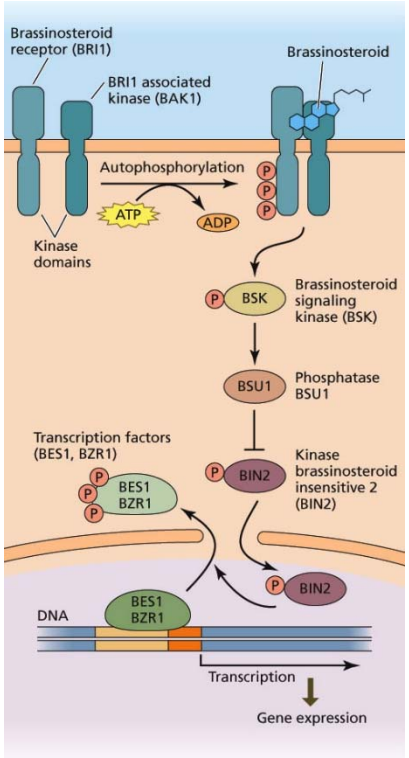



Brassinosteroids
Necrotizing pathogens
PAMPs
BRI1
BL
BL
???
flg22
flg22
FLS2
BAK1/BKK1
BAK1
BL-dependent development
BL-independent cell death control
Innate immune responses


A
WT
BSK3
BSK5
BSK1
bri1-5
B
C
DWF4 RNA level
0
2
4
6
8
WT
BSK3
BSK5
BSK1
bri1-5
D
bri1-116
bri1-116/
BSK3-ox
E
bin2-1
bin2-1/
BSK3-ox
F
BAK1
BRI1
-BR
+BR
BR
BRI1
BAK1
BSK
BKI1
BSU1
14-3-3
BIN2
pBZR1/2
BZR1/2
BR-target
genes
?
BIN2
BKI1

BR signal transduction pathway (BIN2)

- **BRI1/BAK1 heterodimer induces the BR response by inactivating a repressor called BIN2.**
  - ✓ BIN2 (brassinosteroid insensitive-2)
    - Plant GSK3(Glycogen synthase kinase3)-like Kinases, S/T kinases
    - Involves in a wide range of signaling pathways
    - Negative regulator of BR signaling : BIN2 inhibits BR-specific transcription factors.



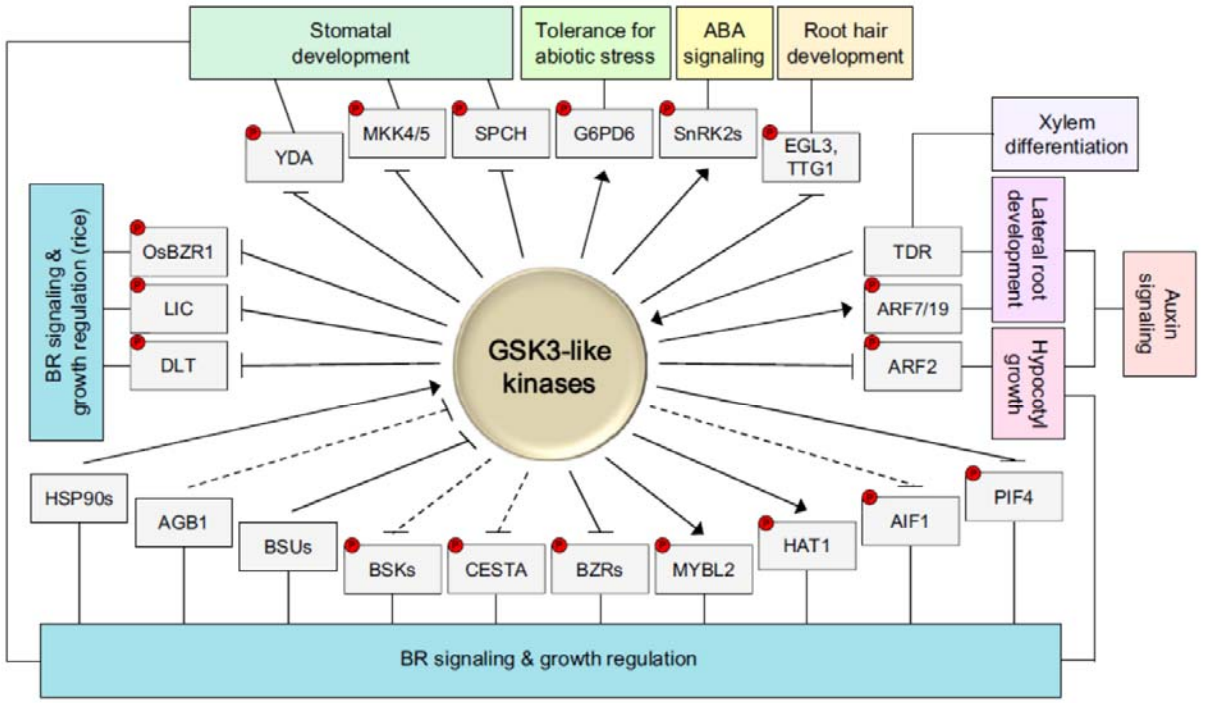
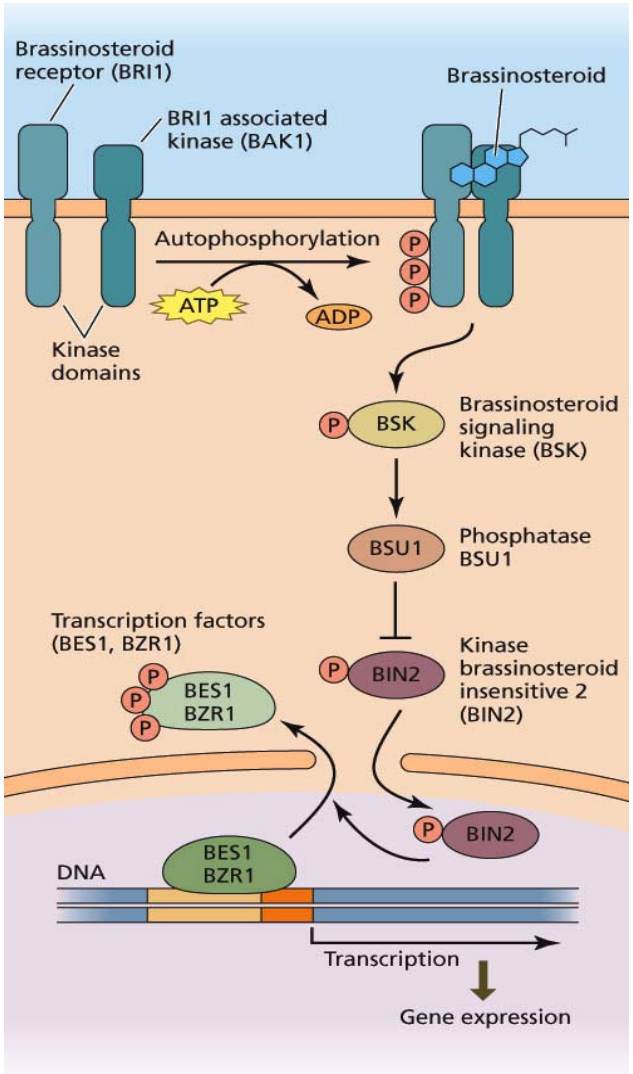


Figure 2. Signaling Network Mediated by GSK3-like Kinases in *Arabidopsis* and Rice.
Arrows and bar ends indicate activation and inhibitory action, respectively, while dotted lines indicate hypothetical regulation. Substrates phosphorylated by GSK3-like kinases are marked with red circles containing the letter P.

Youn et al., 2015

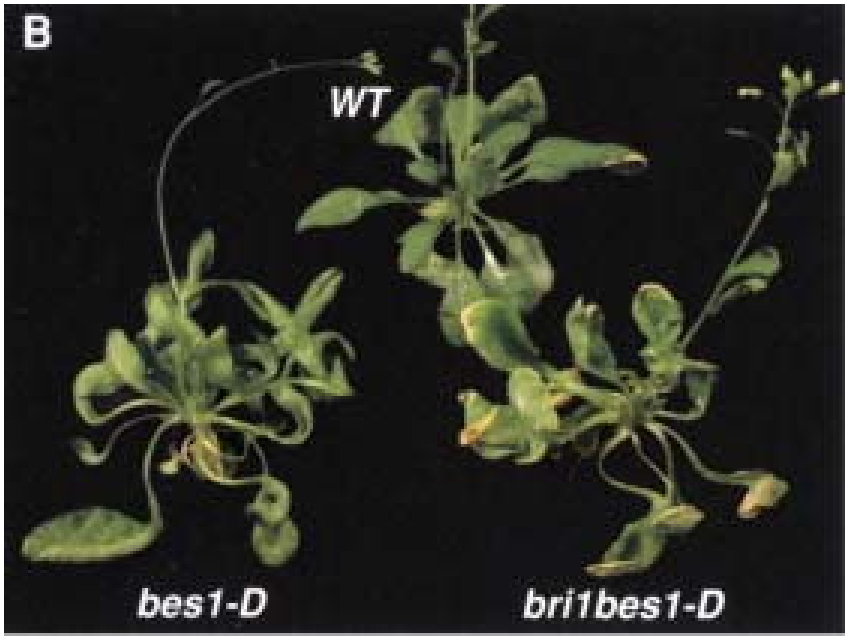
BR signal transduction pathway (BIN2)

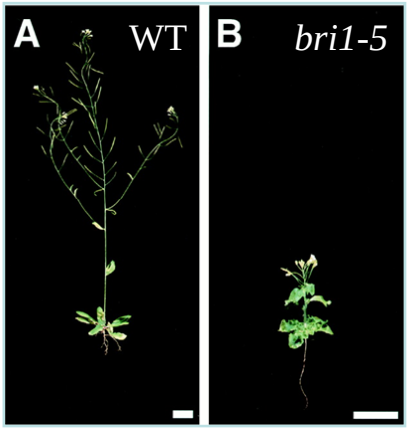
- **In the absence of BRs,**
  - ✓ BIN2 → Phosphorylated BIN2 (Active form) → Acts as a negative regulator of BR signaling
  - ✓ BIN2 phosphorylates BR-specific TFs (BES1 and BZR1).
    - BES1 (bri1-EMS-suppressor 1)
    - BZR1 (brassinazole-resistant 1)
    - Sharing 90% identity in a.a. sequences
  - ✓ Activity of phosphorylated BES1/BZR1 by BIN2 are inhibited by several mechanisms.
    - ① Blocking binding to a target promoter
    - ② Shuttling (from nucleus to cytoplasm)
      - by 14-3-3 proteins
    - ③ Degradation by 26S proteasome



- **In the presence of BRs,**
  - ① BRI1/BAK1 heterodimer
  - ② BSK1 activation (BSK1 → ⓟ-BSK1)
  - ③ BSU1 activation (BSU1 → ⓟ-BSU1)
    - BSU1 (bri1 suppressor 1) : S/T phosphatase
  - ④ BIN2 inactivation (ⓟ-BIN2 → BIN2)
  - ⑤ Accumulation of BES1/BZR1 in nucleus (ⓟ-BES1/BZR1 → BES1/BZR1)

Cell (2002), 109(2), 181-191

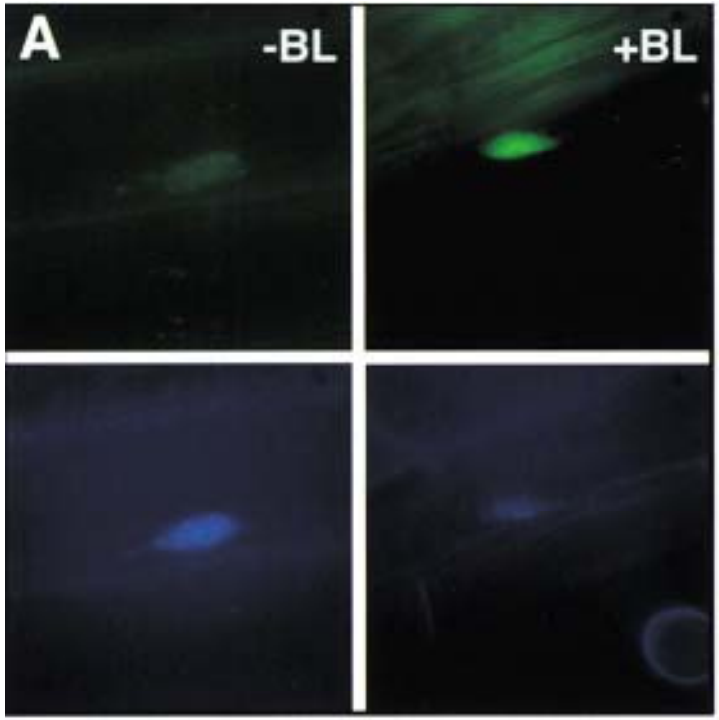




Phenotypes of the *bes1-D* Mutant

(B) *bes1-D* Suppresses *bri1* Phenotypes

Cell (2002), 109(2), 181-191



BES1 Accumulation in the Nucleus in Response to BL Treatment

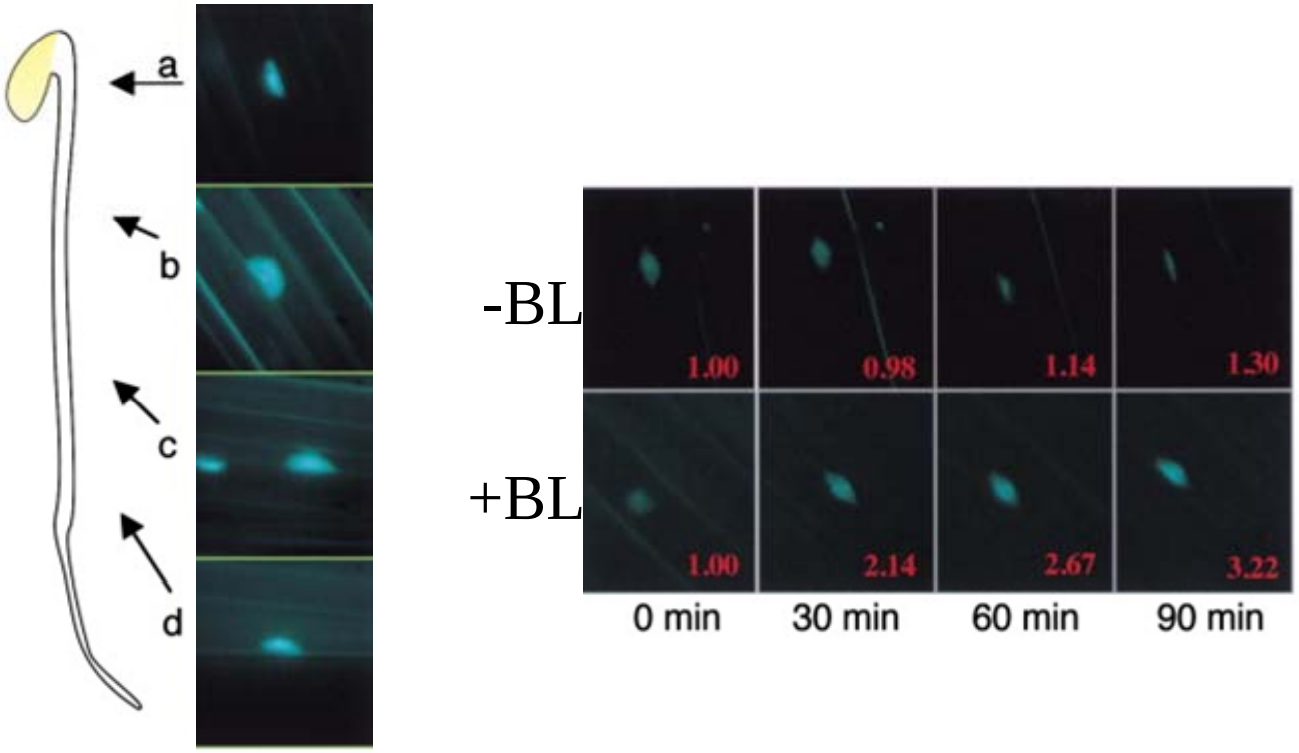
Wild-type BES1::GFP is present in the nucleus at relatively low levels
in dark-grown hypocotyl cells without BL treatment (upper-left).
BL treatment for 24 hr increased the BES1-GFP nuclear accumulation (upper-right).
Lower panels show nuclei stained with DAPI

Developmental Cell (2002), 2(4), 505-513

Phenotypes of the *bzr1-1D* Mutant

(D) Wild-type and *bzr1-1D* mutant grown in long days (16 hr light, 8 hr dark) for 26 days.
(E) Wild-type and *bzr1-1D* plants grown in short days (9 hr light/15 hr dark) for 26 days

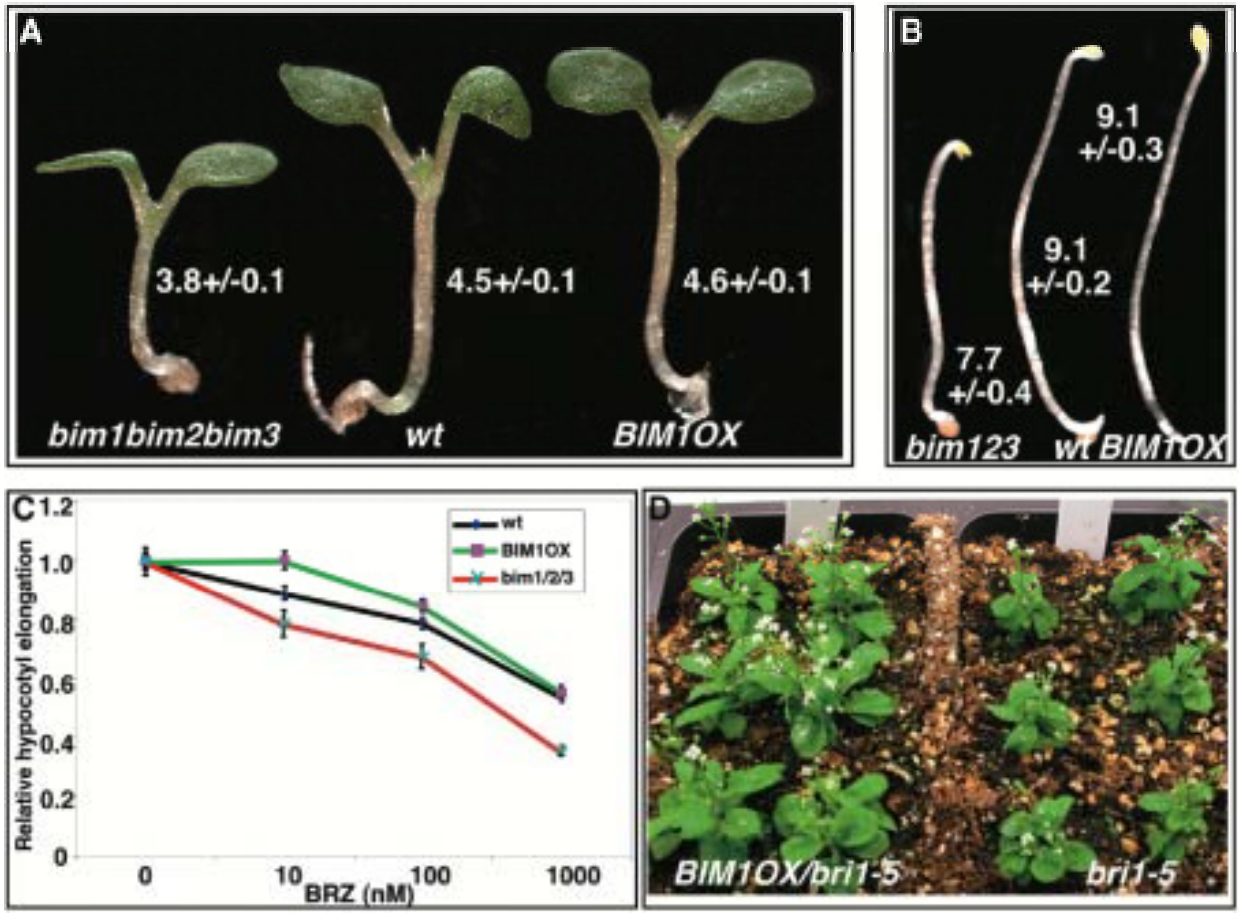
Developmental Cell (2002), 2(4), 505-513



BZR1 Accumulation in the Nucleus in Response to BL Treatment

Using *BZR1* native promoter-driven BZR1-CFP transgenic lines, BZR1 levels were monitored in different regions of elongating hypocotyls.

BL treatment increases BZR1-CFP protein nuclear accumulation


A
3.8+/-0.1
4.5+/-0.1
4.6+/-0.1
bim1bim2bim3
wt
BIM1OX
B
9.1
+/-0.3
9.1
+/-0.2
7.7
+/-0.4
bim123
wt
BIM1OX
C
Relative hypocotyl elongation
1.2
1.0
0.8
0.6
0.4
0.2
0
0
10
100
1000
BRZ (nM)
wt
BIM1OX
bim1/2/3
D
BIM1OX/bri1-5
bri1-5

BR signal transduction pathway (BES1/BZR1)

- **BES1 and BZR1 regulate different subsets of genes in Arabidopsis.**
- **BES1 enhances the expression of BR-stimulated genes by interacting with <u>other proteins</u>.**

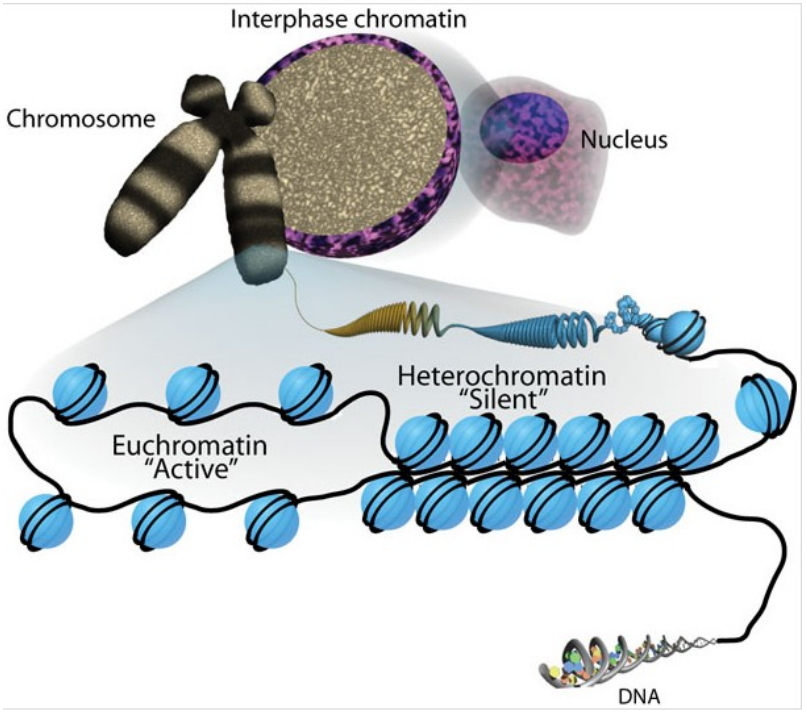
  ✓ (ex) TFs of distinct familes

  : BIM1 (BES1-interacting Myc-like 1)

  - BES1/BIM1 heterodimers bind to E-box (CANNTG) and activate transcription

  ✓ (ex) Factors involved in chromatin remodeling

  ① IWS1 (RNAPII recruitment)
  ② Jumonji-domain proteins, REF6 and ELF6 (Histone modification)
  ③ SDG725 (H3K36 methyltransferase)

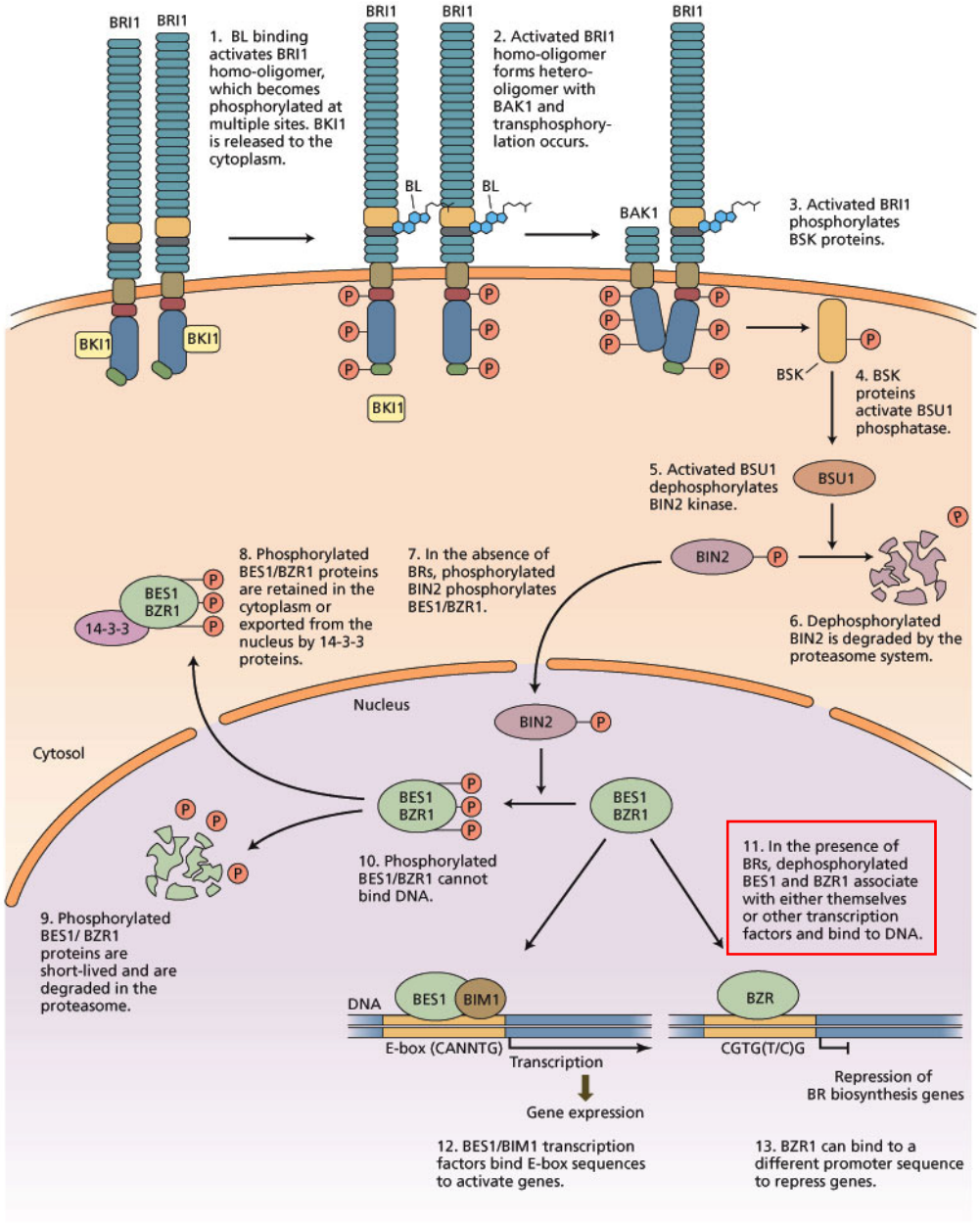


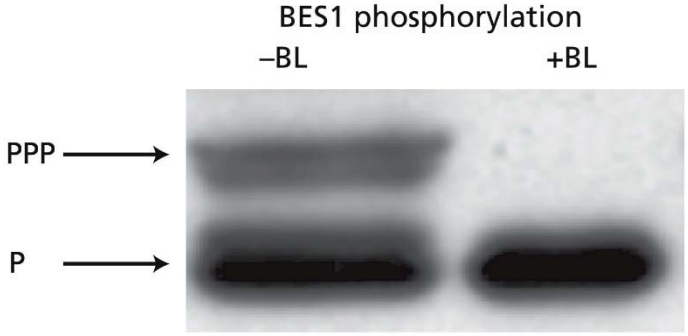
- **BZR1 acts as a repressor of BR biosynthesis.**

  ✓ BZR1 directly binds to BRRE (CGTG(T/C)G).

  ✓ BZR1 plays key role in the negative feedback regulation of the BR biosynthesis.

BR signal transduction pathway





PLANT PHYSIOLOGY, 5e, Figure 24.9

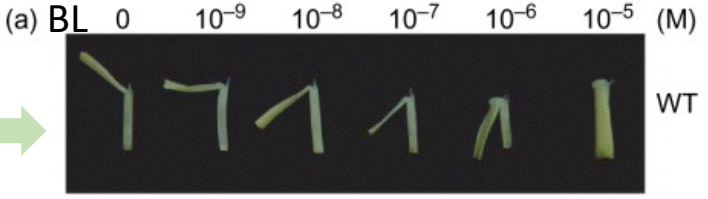
BRs promote both cell expansion and cell division in shoots.

- **The growth-promoting effects of BRs**

  ✓ BR-induced cell expansion

  (ex) Rice lamina inclination assay

  [+BR] Upper (adaxial) surface of the leaf near the joint expand more than the cells on the lower(abaxial) surface.



  ✓ Acceleration of both cell elongation and expansion

  (ex) Bean second internode assay

  (ex) Cell size and number in BR-deficient mutants

  Nakaya et al., 2002

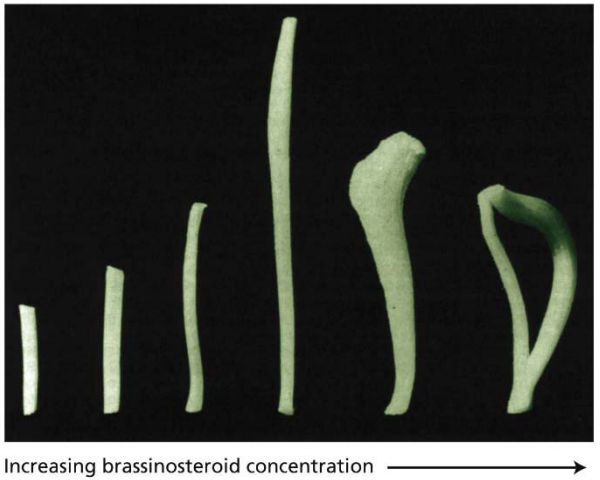


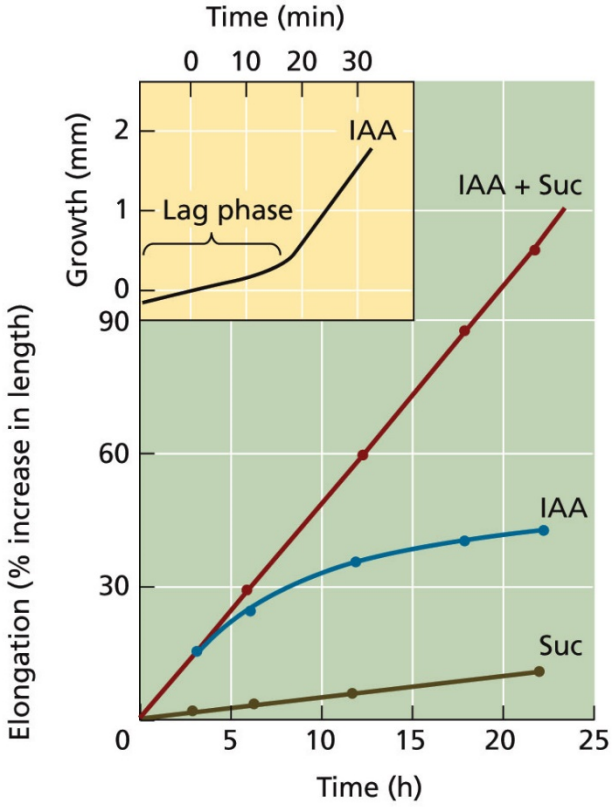
Table 2 Dimensions and numbers of cells in the uppermost layer of the palisade tissue of the fifth foliage leaves of wild-type and mutant Arabidopsis plants

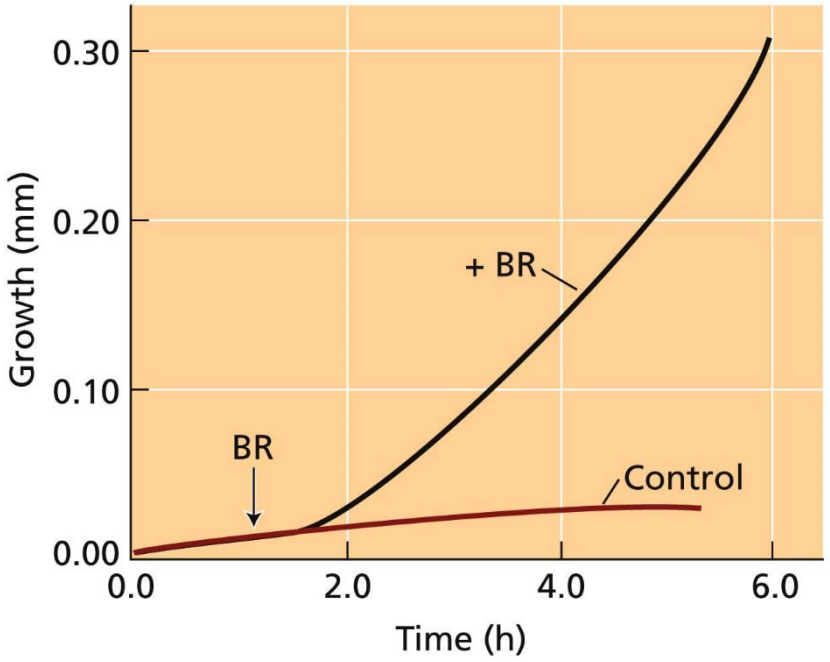
Strain	Cell size (μm) (number of cells examined)			Cell numbers in longitudinal or transverse sections [a]			
	Leaf-length	Leaf-width	Leaf-thickness	In a longitudinal section		In half a transverse section	
				Palisade cells	Mesophyll cells	Palisade cells	Mesophyll cells
Wild type	40.3± 6.1 (66)	40.7± 5.9 (43)	37.5±11.5 (109)	332±23.1	1,008±10.6	110±11.0	339±35.0
dwf1-5	22.4± 6.3 [b] (128)	26.3± 6.8 [b] (78)	37.5±12.8 (206)	218± 8.5 [b]	620±18.7 [b]	101±13.6	378±33.1
det2-13	32.1± 9.3 [b] (117)	35.0±11.0 [b] (67)	41.4±12.6 [b] (194)	197± 7.6 [b]	605±23.6 [b]	115±18.0	360±54.9
det2-18	34.3±10.1 [b] (95)	31.6± 8.5 [b] (95)	47.8±16.5 [b] (190)	160±12.9 [b]	495±29.0 [b]	93±11.2 [b]	301±19.7

Each value is given as a mean ± standard deviation and is based on data from more than three individual plants.
[a] Number of palisade cells in the uppermost layer and total mesophyll cells counted in the longitudinal section along the midrib (left two columns),

BRs promote both cell expansion and cell division in shoots.

- **Growth response to BRs may involve a slower pathway involving gene transcription, whereas the rapid response to auxin may not require gene transcription.**





The kinetics of cell expansion in response to IAAs

- ✓ 15-minute lag period
- ✓ Reaches maximum rate within 45 minutes

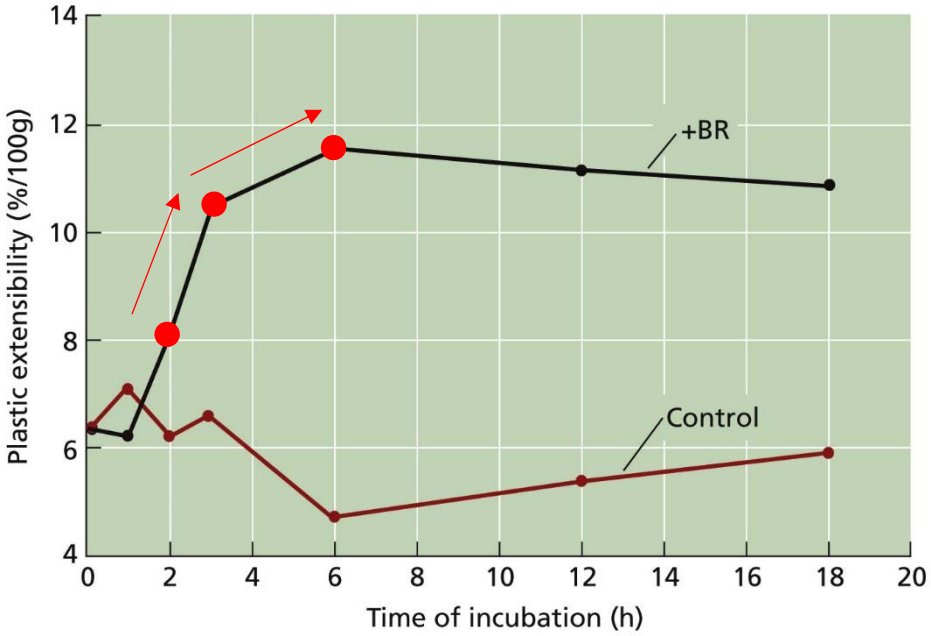
The kinetics of cell expansion in response to BRs

- ✓ 45-minute lag period
- ✓ Reaches maximum rate after several hours

BRs promote both cell expansion and cell division in shoots.

- **Plant cells expand in 3 steps**
  - ① Osmotic uptake of water across the membrane
  - ② Turgor pressure builds up because of the rigidity of the cell wall
  - ③ Biochemical wall loosening occurs

- **Each of these steps is likely to be modulated by BRs.**
  - ① BRs increase the uptake of water through aquaporins.
  - ② Induce the expression of wall modifying enzymes and enhance the cell wall loosening
    - Xyloglucan endotransglycosylase/hydrolase (XTHs)
    - Expansins



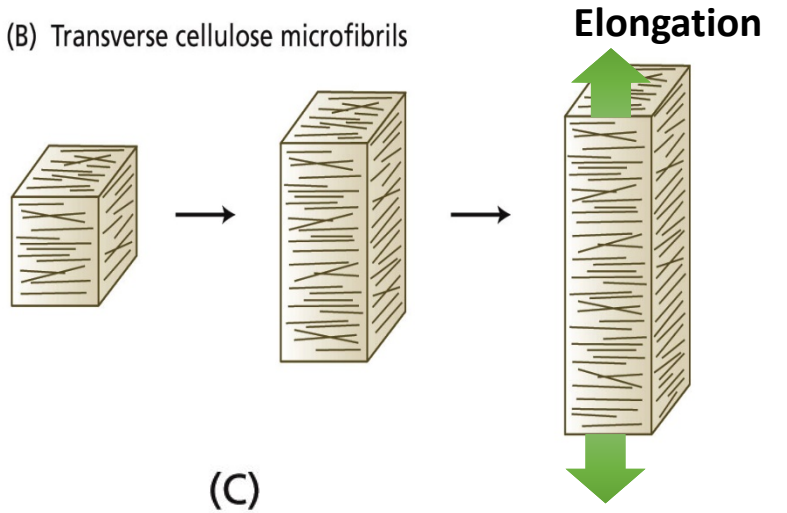
The increase in wall plasticity in the presence of BR indicates that BR has induced cell wall loosening, which is required for cell expansion.

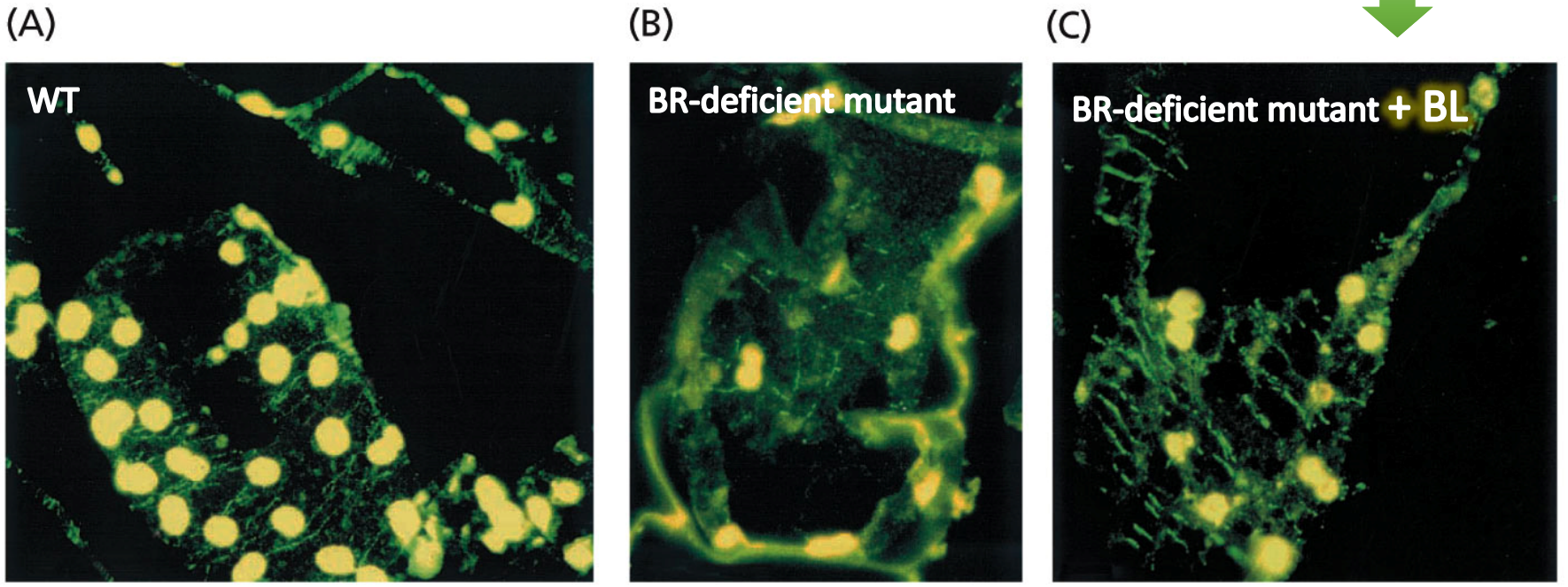
Effect of BR on microtubule organization

- **An additional requirement for normal elongation is the control of microtubule organization.**

  ✓ BR-deficient mutant cells contain very few microtubules and that those present lack organization.

  → [+BR] Restoration of normal microtubule abundance and organization





- Microtubules (green)
- Chloroplast (yellow)

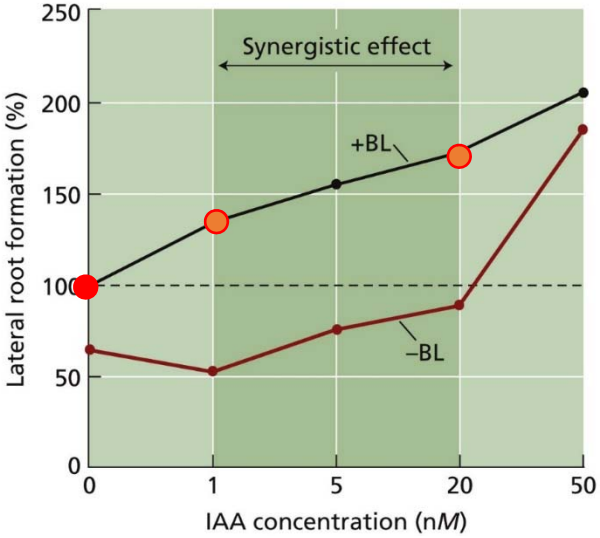
BRs both promote and inhibit root growth.

- **BR exerts strong effects on overall root morphology, influencing both the elongation rate and branching habit.**

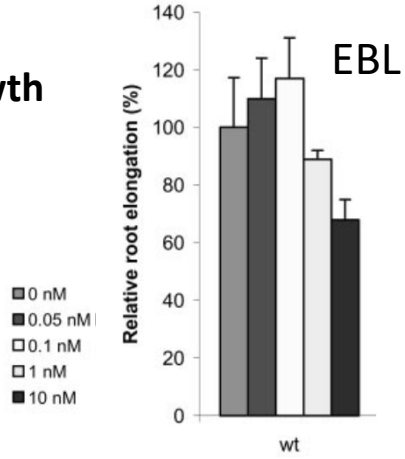
- **The phenotypes of BR-deficient mutants, which typically exhibit reduced root growth.**
  → BRs are required for normal root elongation.

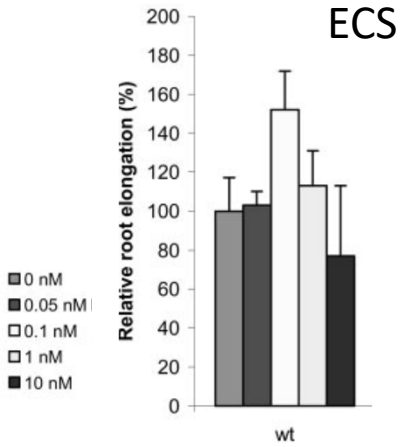
- **Exogenously applied BRs may have positive or negative effects on root growth depending on the concentration.**
  → High concentrations of BR, like auxin, stimulate ethylene production.

- **At low concentrations, BRs can also induce the formation of lateral roots by influencing polar auxin transport.**



BL and IAA act synergistically to promote lateral root development





BRs and cytokinins appear to regulate the cell cycle via similar mechanism

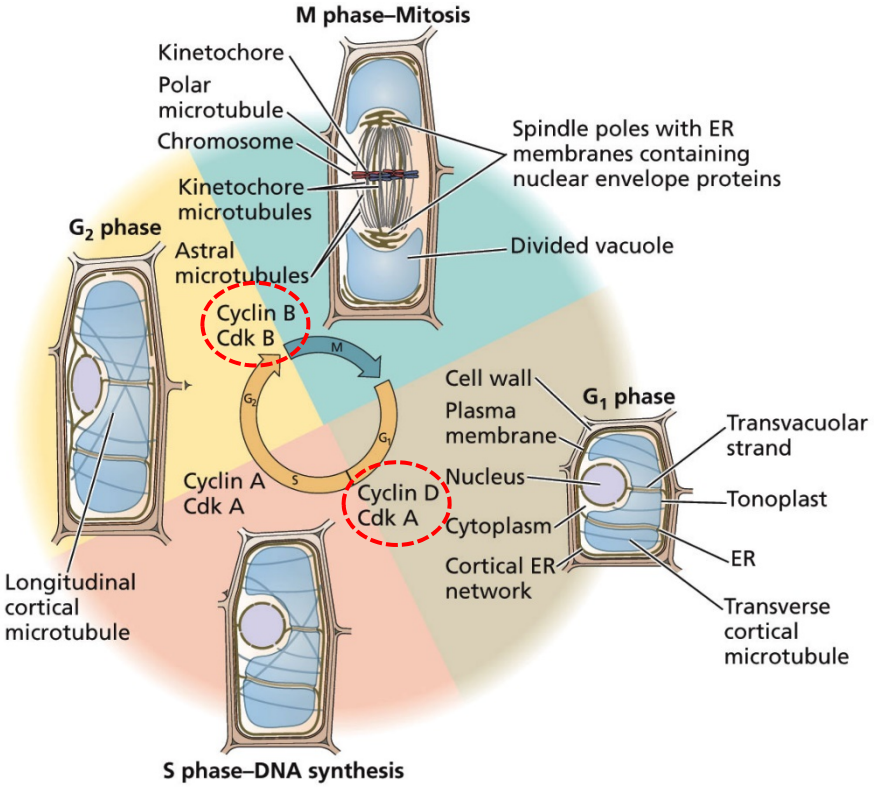
Interphase ← → Mitosis

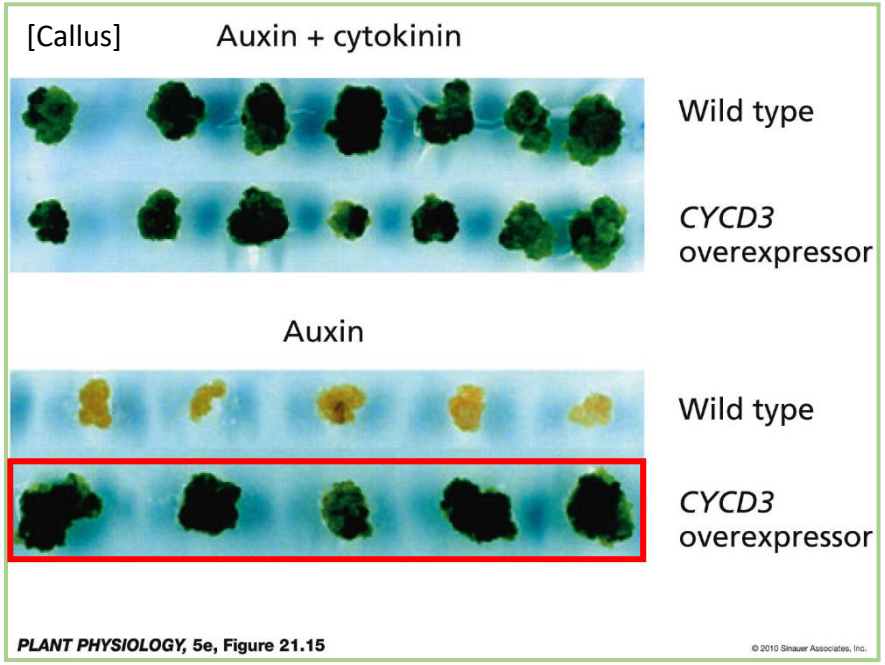
- **Cell cycle consists of 4 phases : G1, S, G2, M**

① CDC2 (G2 → M) : major CDK (Auxin → expression induction/CK → activation)

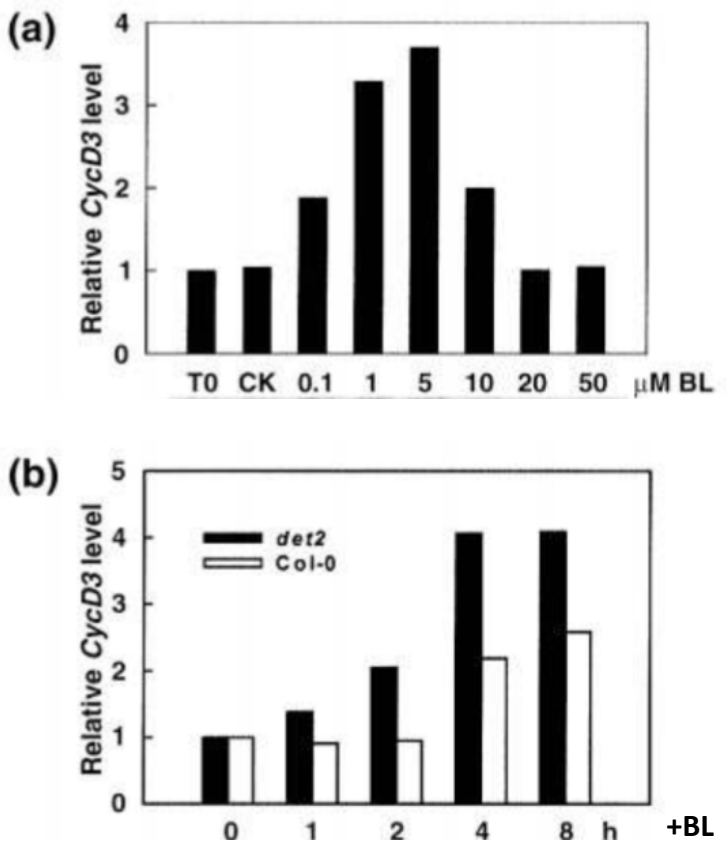
② CYCD3 (G1 → S) : D-type cyclin
- ✓ (CK → expression induction)
- ✓ Overexpression of *CYCD3* can bypass the cytokinin.

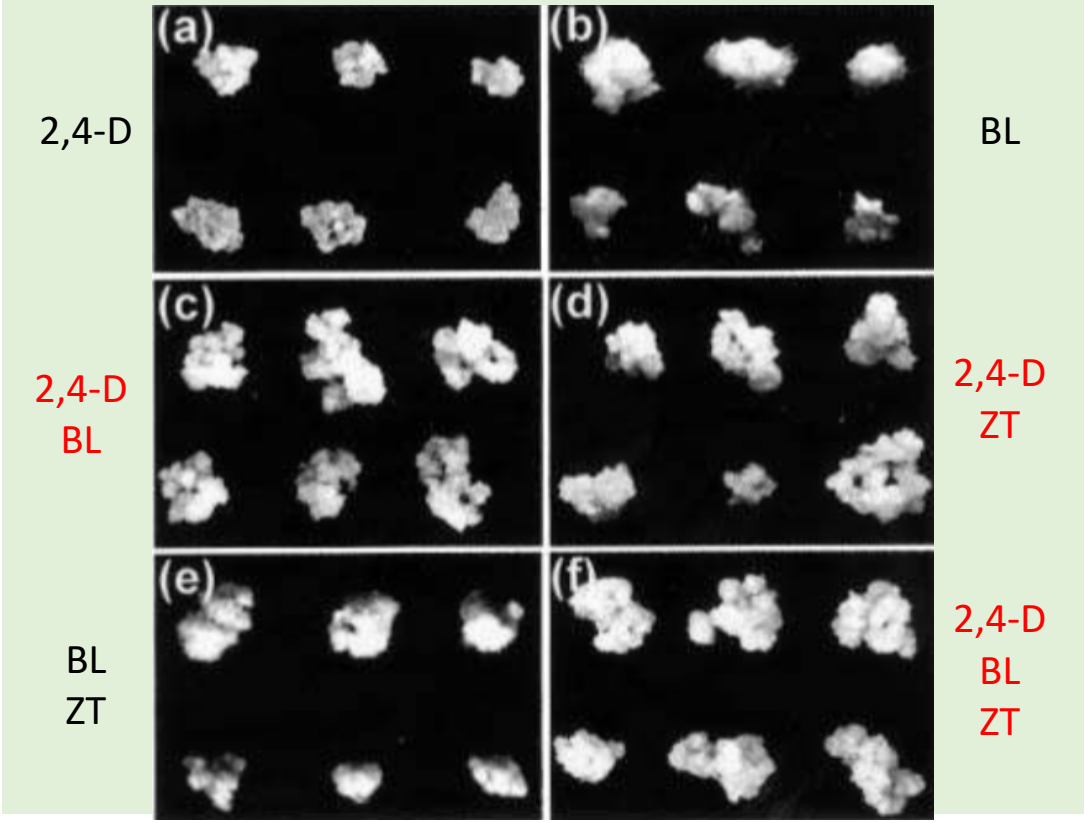
- BRs increase CYCD3 gene expression.
- BRs can substitute for zeatin in the growth of callus





BRs also stimulate cell proliferation via CYCD3 expression.

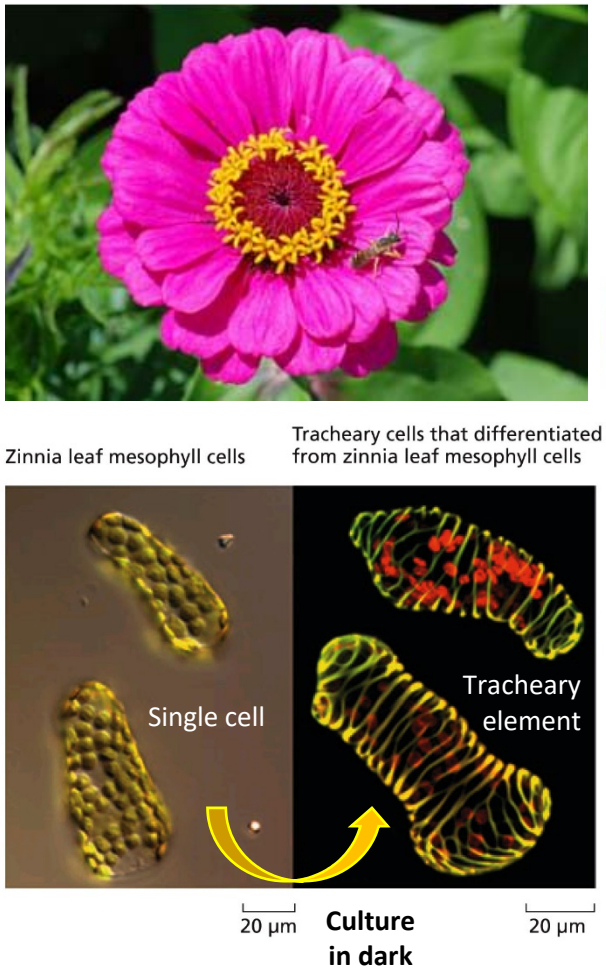




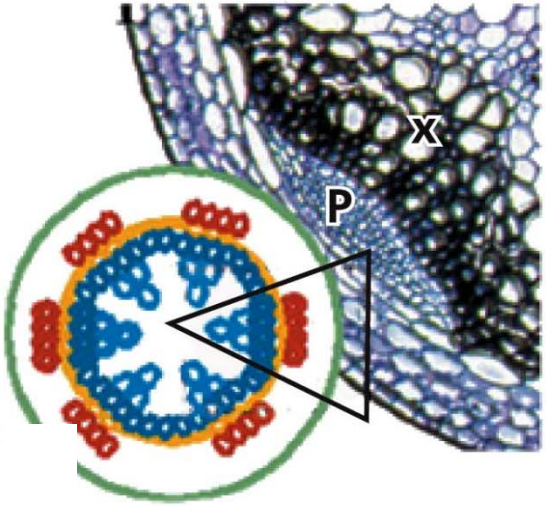
Hu et al., 2000

BRs promote xylem differentiation during vascular development.

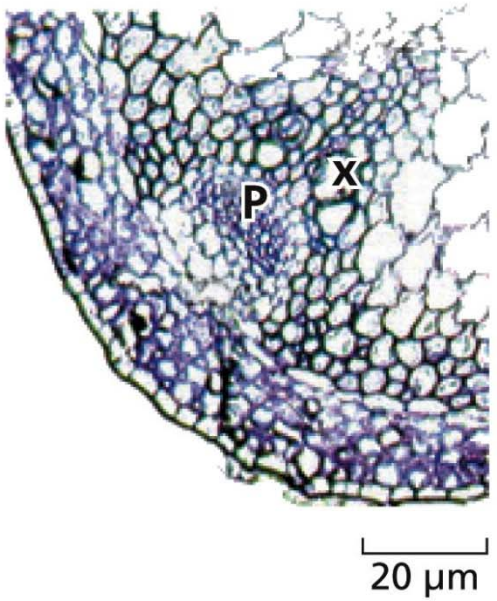
- **BRs play an important role in vascular development, by promoting differentiation of the xylem and suppressing that of the phloem.**



(A) Wild-type Arabidopsis stem cross section



(B) Arabidopsis *det2* mutant stem cross section

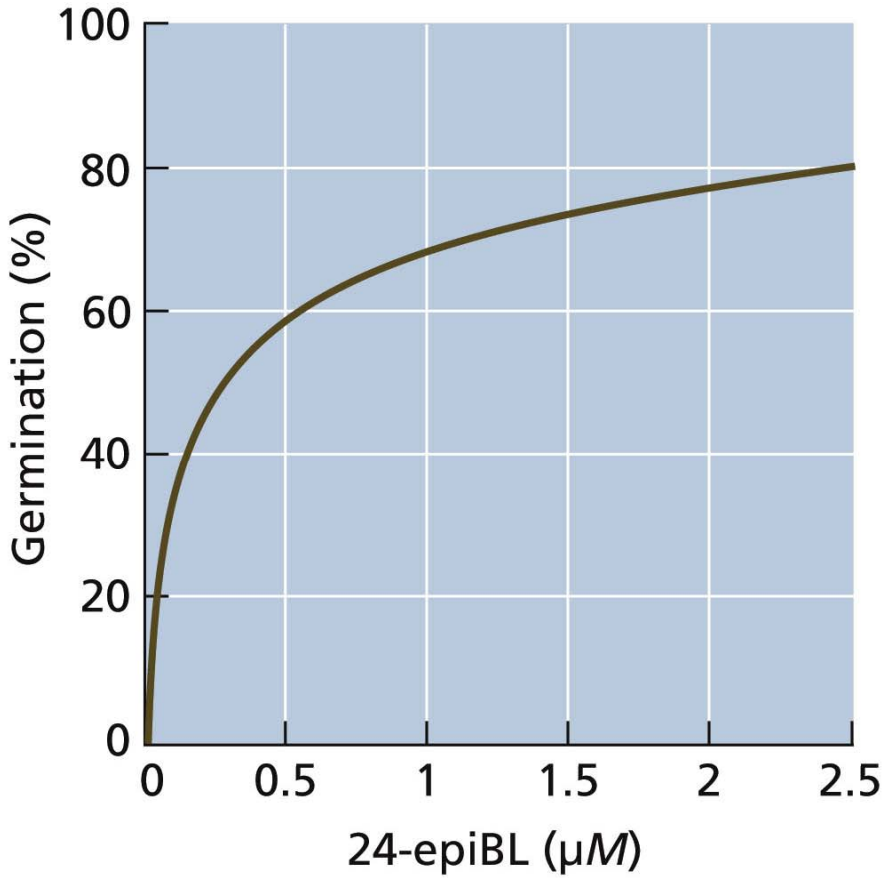


Higher **phloem**-to-xylem ratio than wild-type

- ***Zinnia elegans* (백일홍)**
  - ✓ In vitro xylem differentiation study system

BRs promote seed germination.

- Seeds, contain very high levels of BRs.
- BRs promote seed germination as well.



PLANT PHYSIOLOGY, 5e, Figure 24.22

Summary

Brassinosteroids : Regulators of Cell Expansion and Development

- Biosynthesis, Metabolism and Transport of BRs
- BR Signaling Pathway
- Effects on Growth and Development
  - ① Shoot growth
  - ② Root growth
  - ③ Xylem differentiation
  - ④ Germination