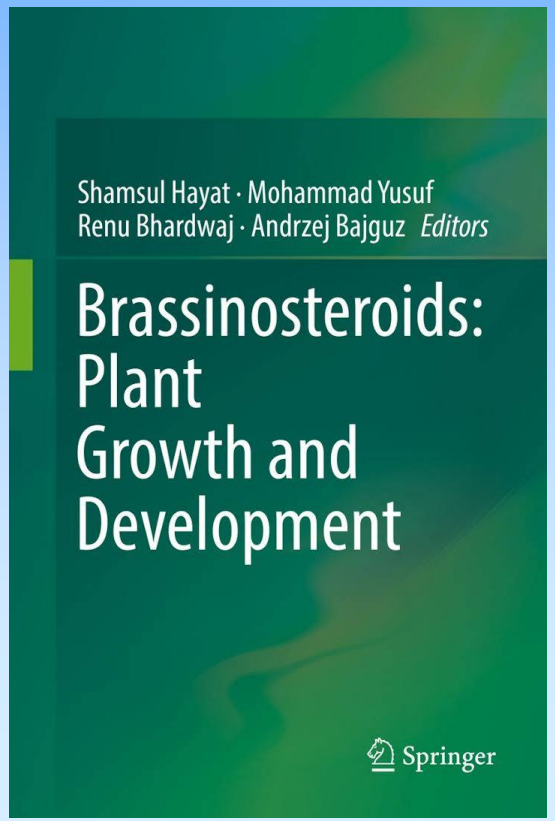
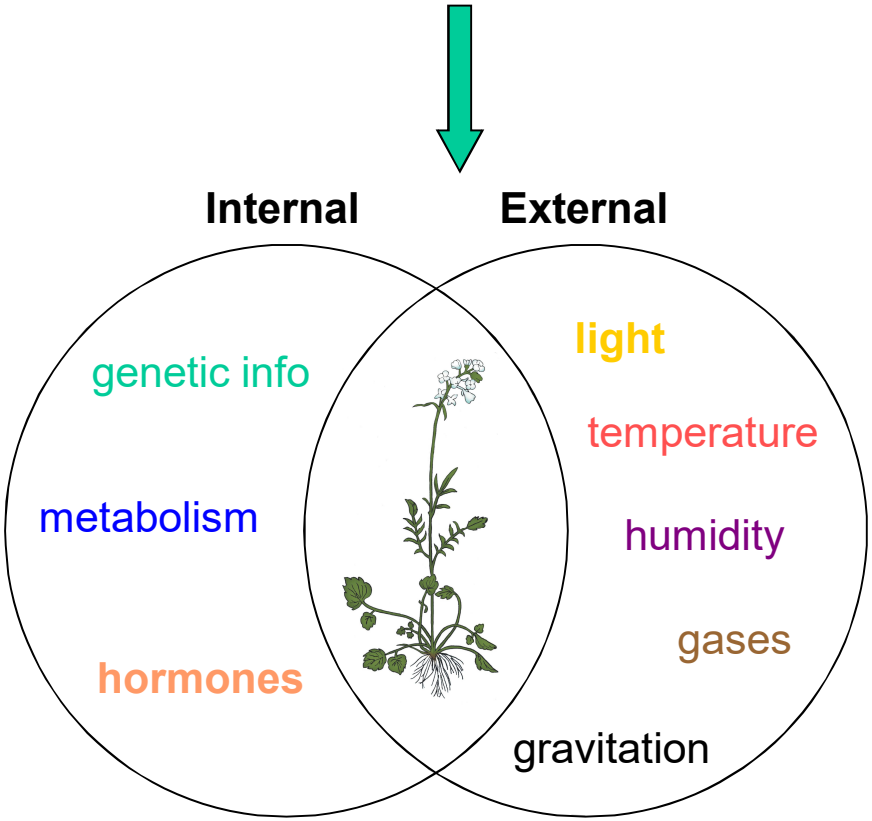
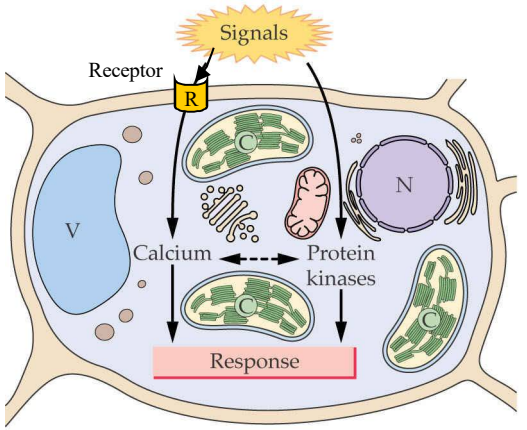
7) Plant hormones brassinosteroids and their role in plant growth and development



Hayat S, Yusuf M, Bhardwaj R, Bajguz A (2019) Brassinosteroids: A class of plant hormone. Springer, Berlin

Development of an organism is regulated by signals (factors)





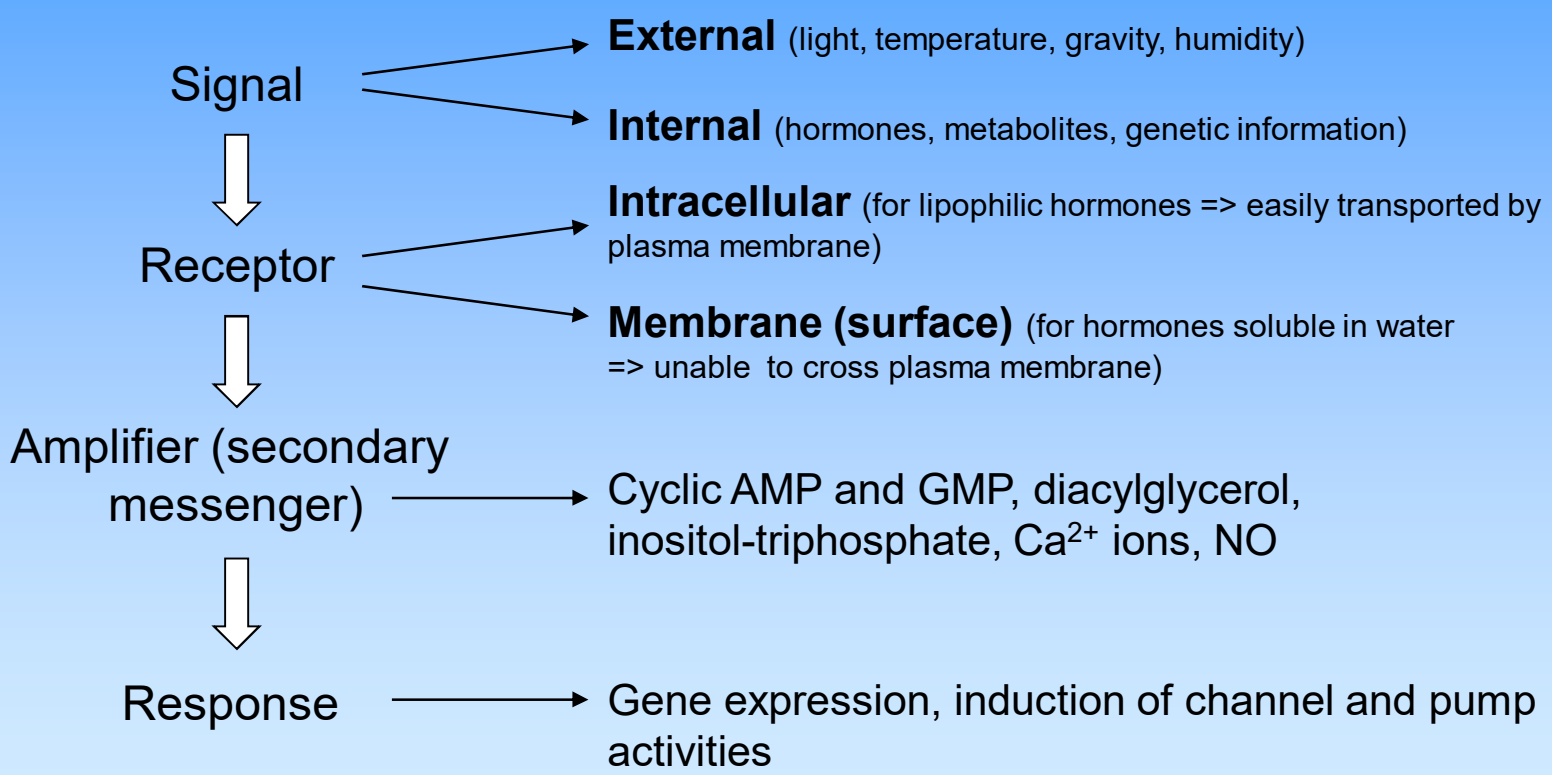
Signal

Receptor

Amplifier (secondary messenger)

Response
(e.g. gene expression)

Scheme of signal transduction in plants



Genetic studies - an effort to define molecular mechanisms of steroid hormone effects

Brassinosteroids - *Arabidopsis thaliana* (27 thousands of genes)

Detailed description of brassinosteroid biosynthesis

↓

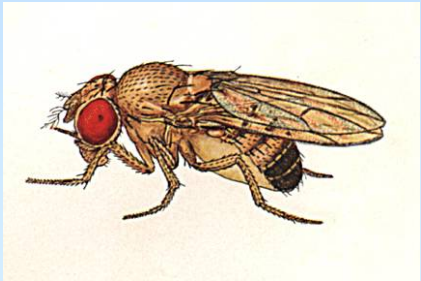
Knowledge of signaling pathways

Steroids – mammals, insect - *Drosophila melanogaster* (12 thousands of genes)

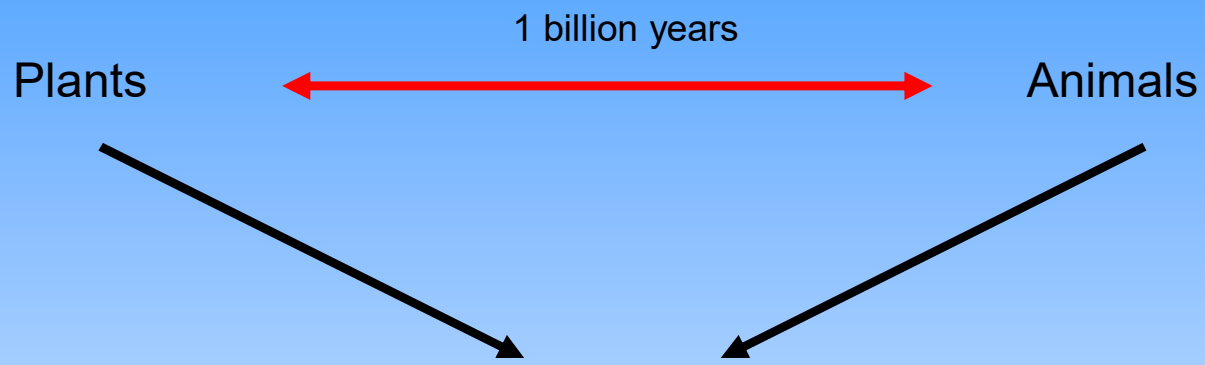
Detailed description of transcription of genes activated by 20E hydroxyecdysone

↓

Knowledge of ecdysteroid biosynthesis



Brassinosteroid (BR) biosynthesis and homeostasis

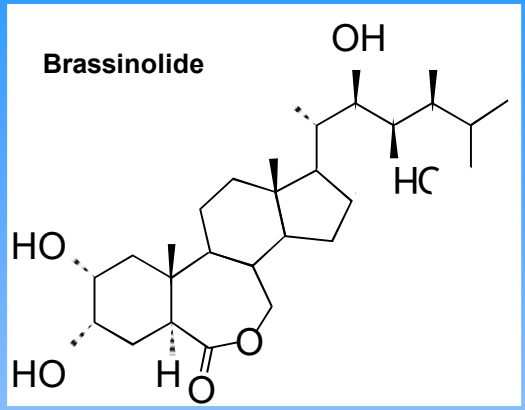


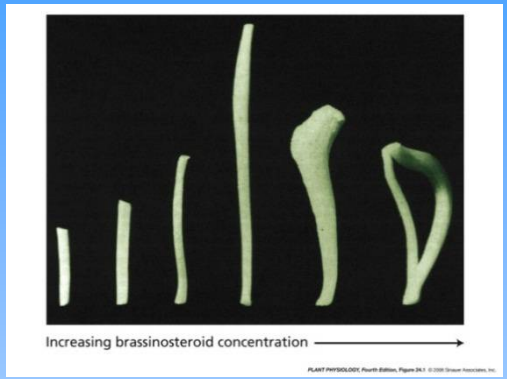
BR and steroids control:
- regulation of gene expression
- cell division
- cell expansion
- Cell differentiation
- PCD (programmed cell death)
- homeostasis

1979 – brassinolide (BL) – the end product of a biosynthetic pathway

- stimulates stem elongation
- stimulates root elongation (low concentrations)
- inhibits roo elongation (high concentrations)
- stimulates seed germination and leaf growth

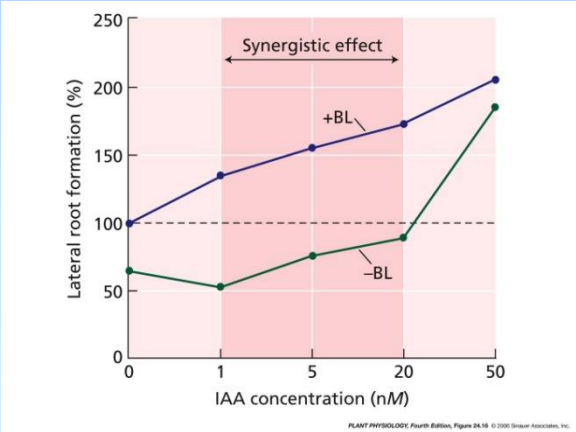
- stimulates stomata development
- stimulates tolerance to low temperature
- changes in CW expansion at abiotic stress
- influences stomata closure (?)
- stimulates pollen development (degradation of tapetal cells
- inhibits gravitropic growth of hypocotyl



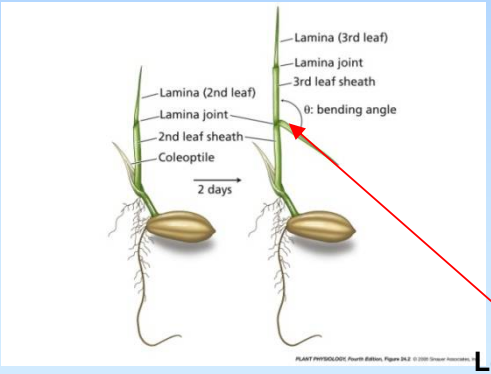


Elongation of 2nd stem internode in bean

Brassinosteroids and auxins have synergic effects. Stimulation of:



- lateral root development
- stem elongation
- pollen root growth
- leaf bending and epinasty
- protono pump activity !
- xylem differentiation



Leaf bending

Auxin – effect

Brassinolide – slow effect

Update 2010

Kang B et al. (2010) Molecular Plant 3: 260-268 *

Interaction of auxin and brassinosteroid signaling pathways

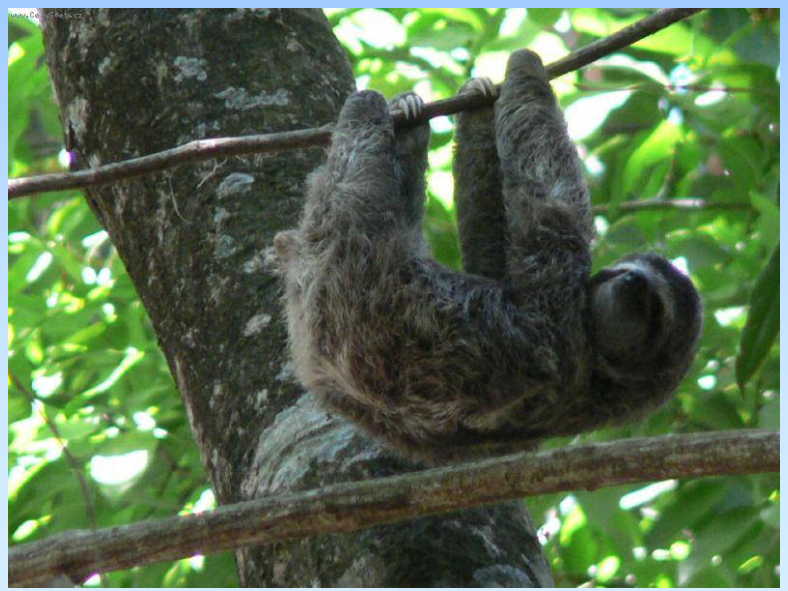
Brassinosteroid use in agriculture: - increasing yield

- stimulation of resistence to stress

Occurence: - in all tested tissues

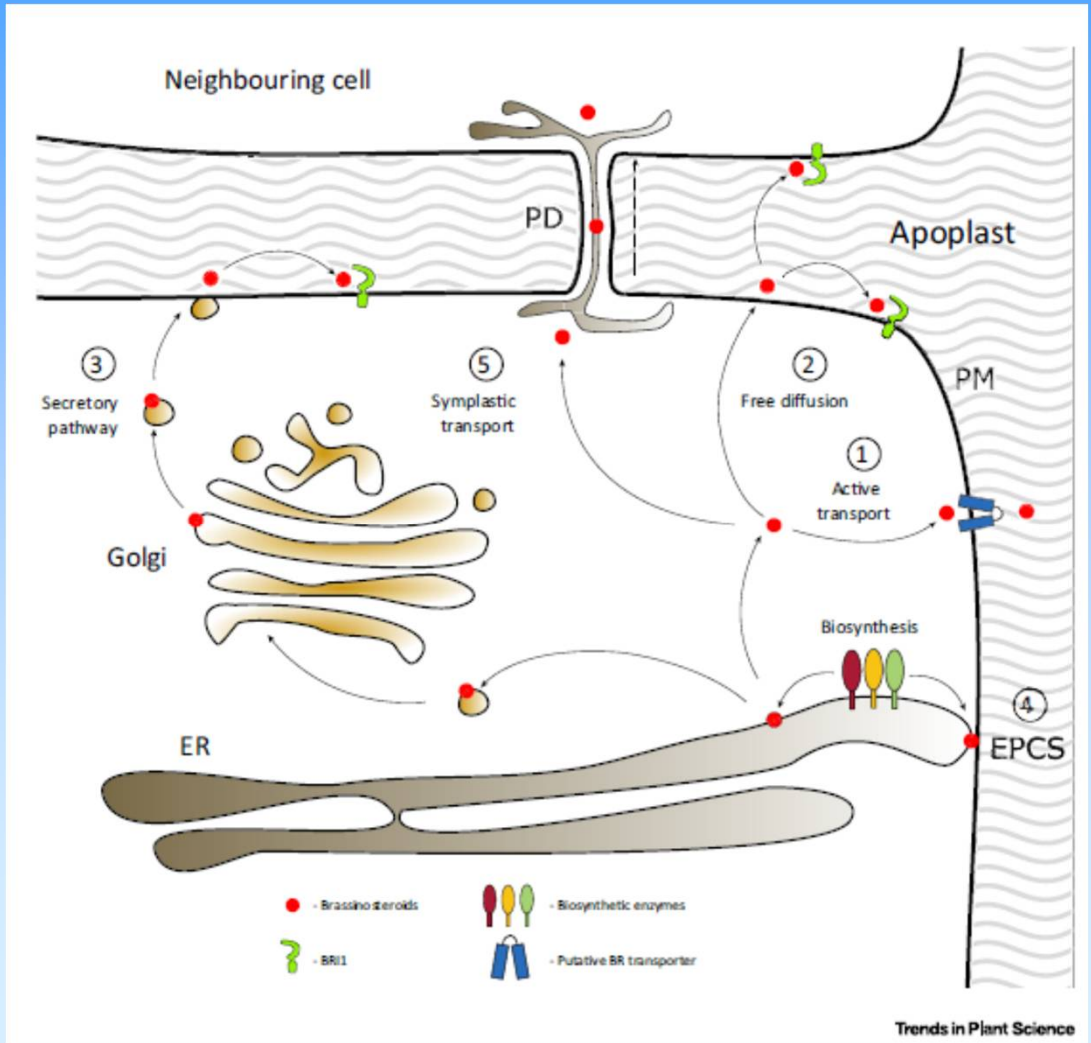
- the most in the apical part of stems

Brassinosteroids are not likely transported to long distances



Update 2018
Vukašinovic N and Russinova E (2018) TIPS 23: 285-292

The latest review on brassinosteroid transport in plants - BRexit



BR existence and biological activity – a large amount of literature

Functioning and signaling pathways - recently discovered

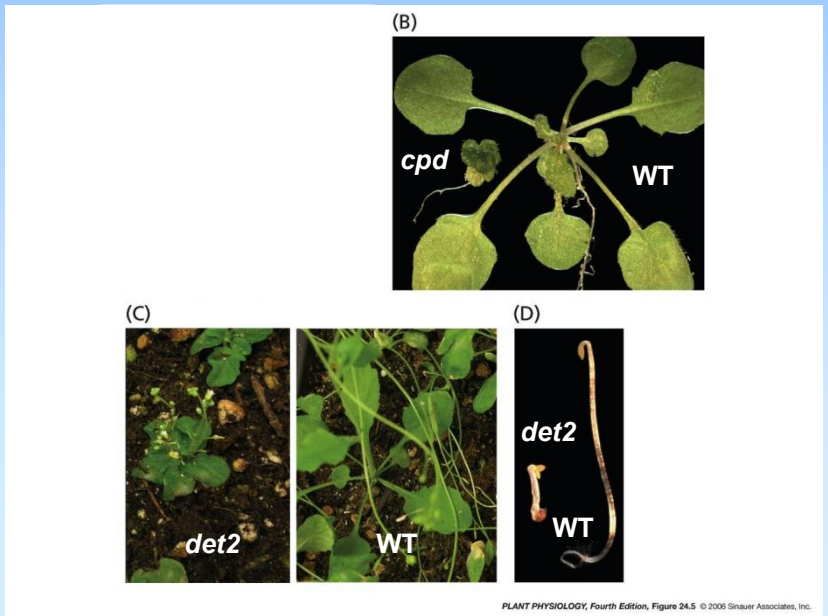
Genetic approach **– physiological, biochemical and molecular characterization of mutants**

Selection of mutants with reduced biosynthesis of BRs (tomato, pea, rice, *Arabidopsis*)

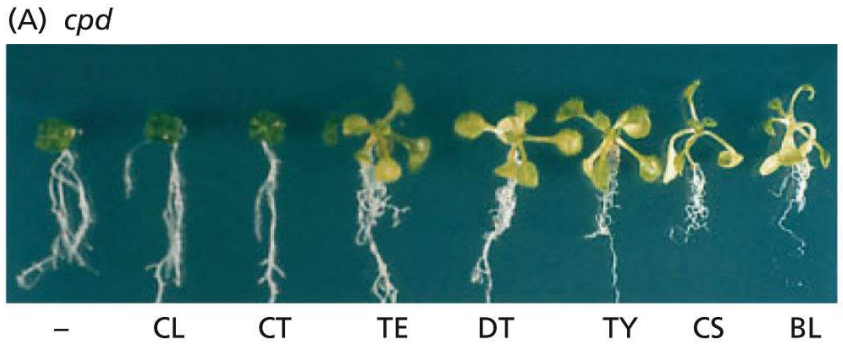
Distinct mutant phenotype; *Arabidopsis* – the pleiotropic effect of mutations

Darkness: short stature, thick hypocotyl, open enlarged cotyledons, presence of primary leaf buds

Light: dwarf growth, dark green color, reduced apical dominance, pollen sterility, impaired photoperiodic responses, delayed senescence of chloroplasts and leaves, reduced amount of xylem, changes in response to light



Application of exogenous BRs leads to normalization of mutant phenotypes



PLANT PHYSIOLOGY, Fourth Edition, **Figure 24.6** © 2006 Sinauer Associates, Inc.

CL - campesterol

CT - cathasterone

TE - teasterone

DT - dehydroteasterone

TY - typhasterol

CS - castasterone

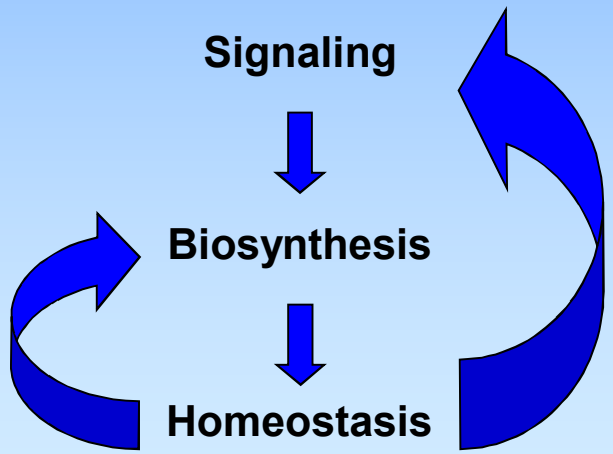
BL - brassinolide

Distinct mutant phenotype

Identification of genes involved in BR synthesis

Understanding homeostasis mechanisms

Understanding signaling mechanisms



Mutants in signaling pathways contain a higher level of endogenous hormones

BR-mutants

Cytokinin-mutants

Auxin-mutants

Control mechanisms regulating BR level:

- biosynthesis regulation
- BR inactivation
- feedback from the signaling pathway

Two biosynthetic BR pathways

- early C-6 oxidation
- late C-6 oxidation

Plants — **Campesterol** → → → → → **BR**

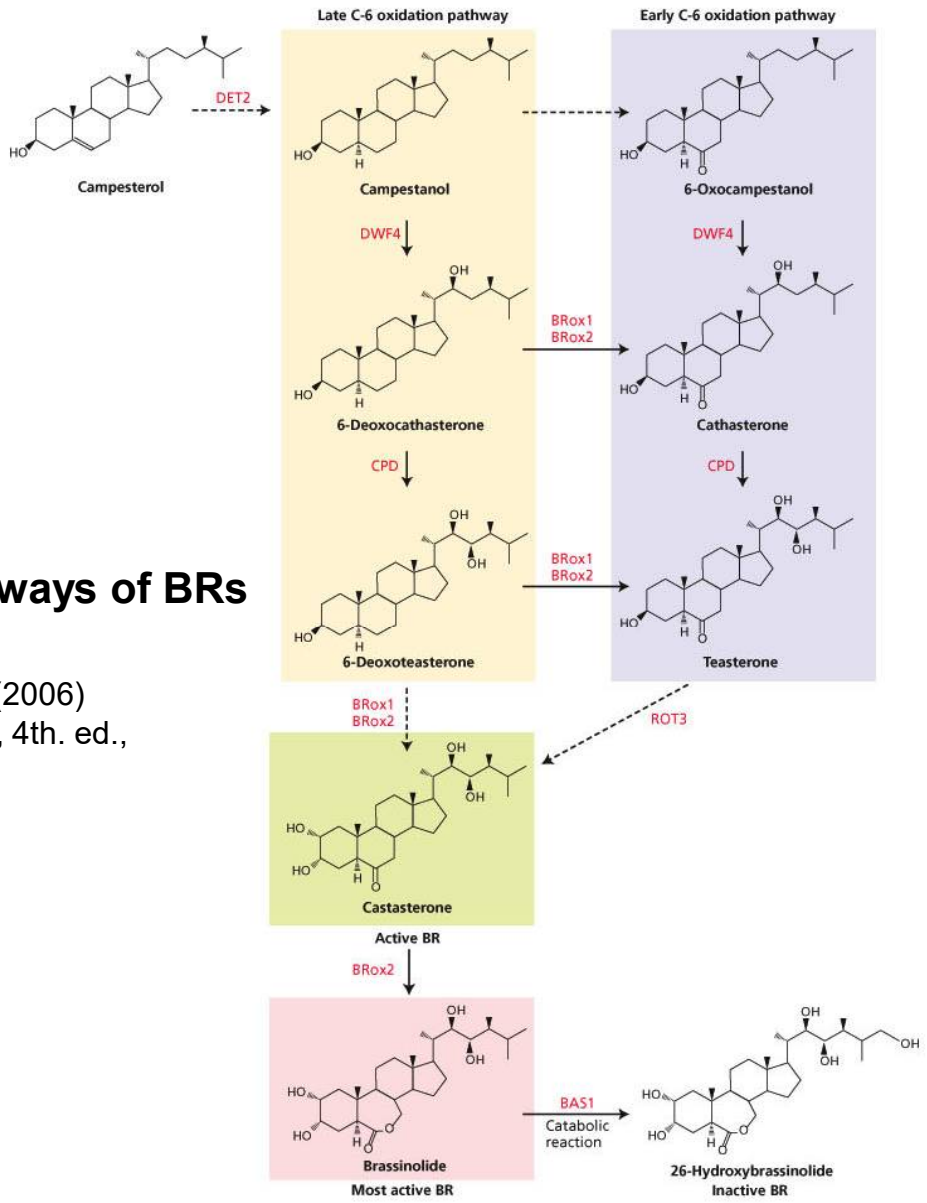
Animals — **Cholesterol** → → → → → **Steroids**

The key steps in the synthesis of BR and steroids are highly conserved – the presence of 5α-reductase

Insect – the presence of 5α-reductase has not yet been demonstrated

Biosynthetic pathways of BRs

Taiz L, Zeiger E (2006)
Plant Physiology, 4th. ed.,
str. 617 - 634



PLANT PHYSIOLOGY, Fourth Edition, **Figure 24.7** © 2006 Sinauer Associates, Inc.

Brassinosteroid signal transduction

Physiological studies → **Identification of BRs as growth regulators**

Genetic studies → **Identification of BRs as hormones**

Identification of BR receptor on plasma membrane + other pathways elements

A huge increase in the knowledge of mol. basics of BR effects

Enormous increase in publications about BR

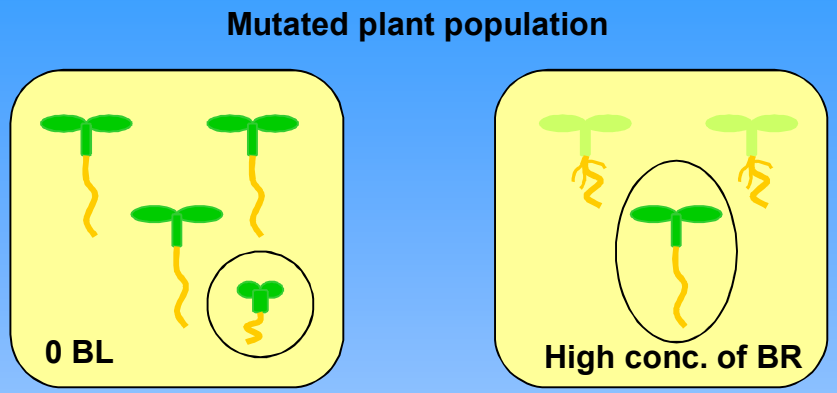
Update 2020

Hussain MA et al. (2020) J Plant Growth Regul 92: 141-156

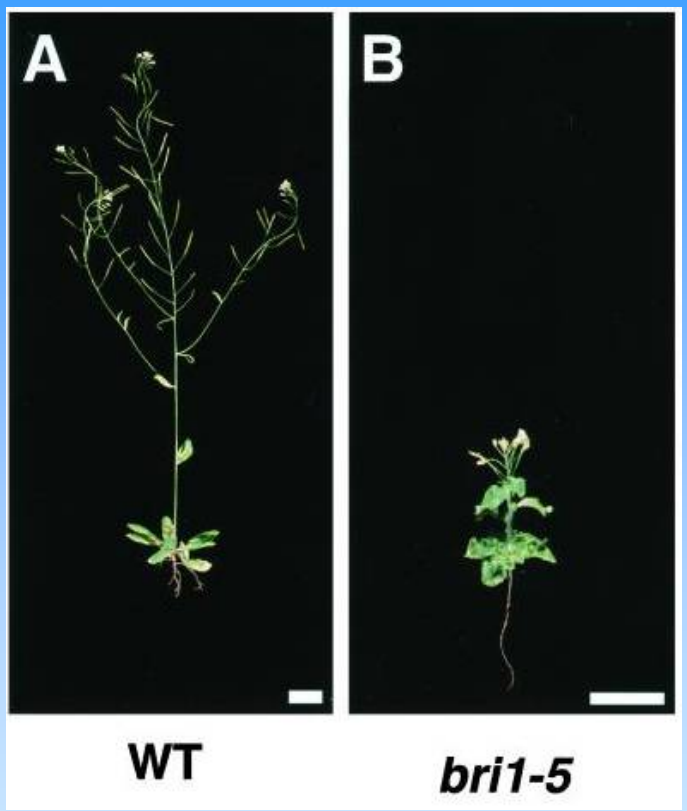
Kim E-J, Russinova E (2020) Current Biology 30: R287-R301

The latest review on the role of brassinosteroids

Identification of BR-insensitive mutants



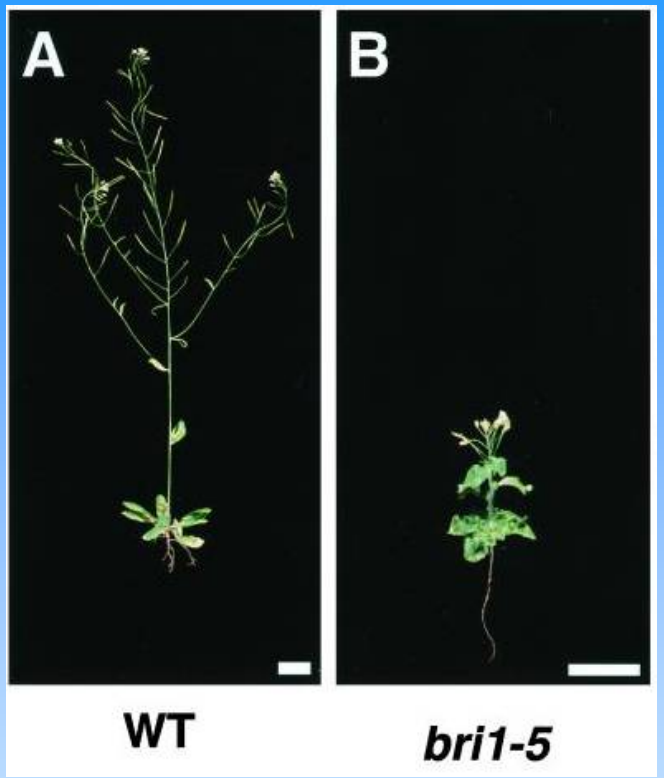
BR-insensitive mutants

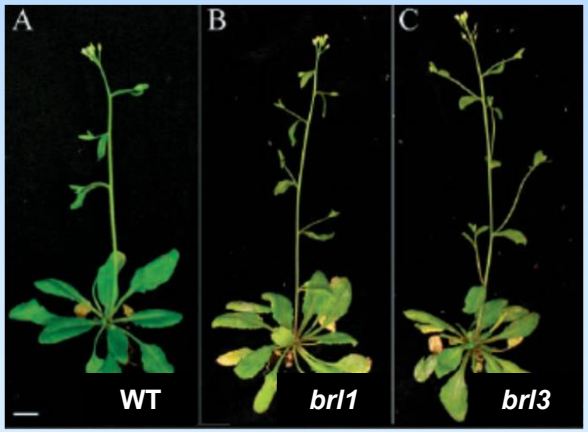


Mutant *bri1*
(*brassinosteroid-insensitive1*)

Wild type and *brd2* mutant in rice

BR-insensitive mutants are similar to mutants defective in the biosynthesis of BR. However, their phenotype cannot be changed by exogenous BRs.





Laboratory of J. Chory – identification of other dwarf alleles *bri1*

Fully functioning BRI1 is a positive regulator of BR signaling

Other genes of this family: *BRL1, BRL2* a *BRL3*

BRL1 **and** ***BRL3*** **bind BR. Ectopic expression of these genes driven by the BRI1 promoter is able to eliminate the effect of the mutation *bri1*.**

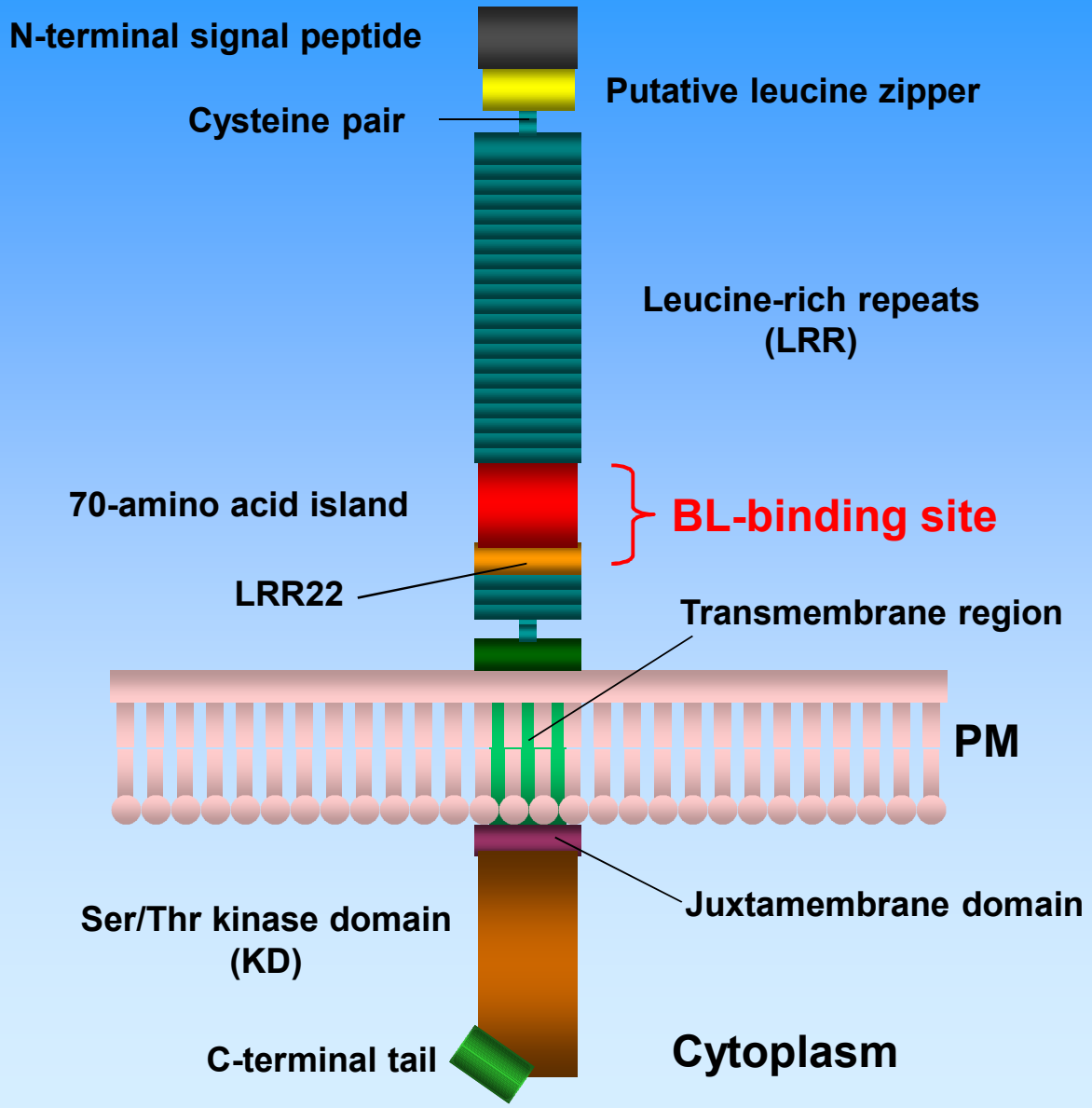
BRL1 **and** ***BRL3*** **are specifically expressed in vacular system => mutations *brl1* and *brl3* result in abnormal ratio in the differentiation of phloem and xylem.**

Triple mutant *bri1brl1brl3* - amplified dwarf growth and vascular phenotype.

Binding study: high specificity of BL binding to BRI1 protein

BRI1 is brassinosteroid receptor.

Structure of BRI1 receptor

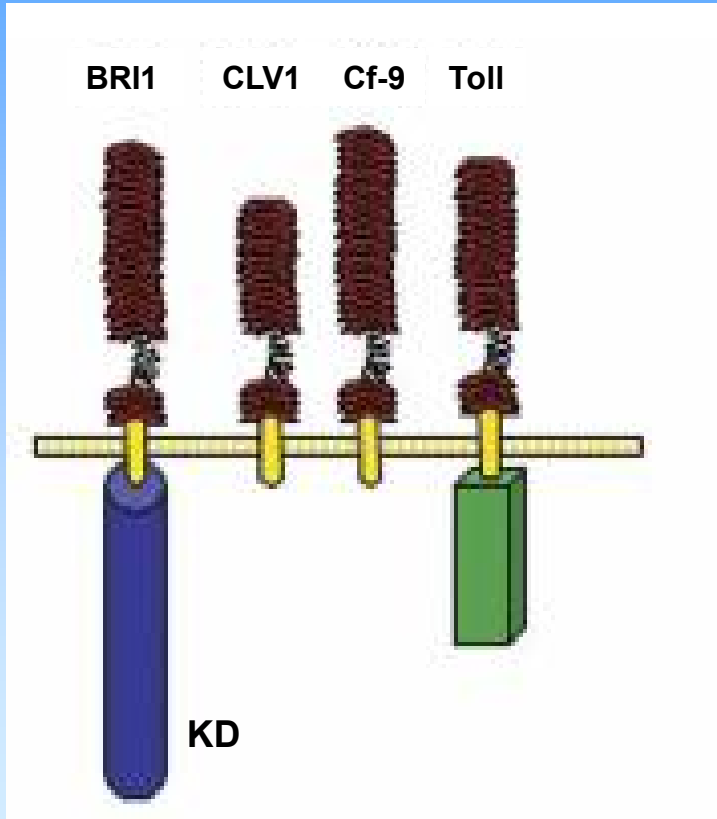


BRI1 codes for leucin-rich repeat (LRR) transmembrane receptor-like kinase (RLK) (LRR-RLK) (positional cloning)

Localization: plasma membrane (expresion of BRI1-GFP)

LRR-LRK – largest group of receptors in *Arabidopsis* genome: ~230 members

70 amino+LRR – general protein motif, which occurs in a family of proteins called LRR-R (LRR-Receptor-like) with very different functions in all kinds of organisms.



The unique feature of BRI1: it contains 70 amino acids island and at the same time the kinase domain (KD)

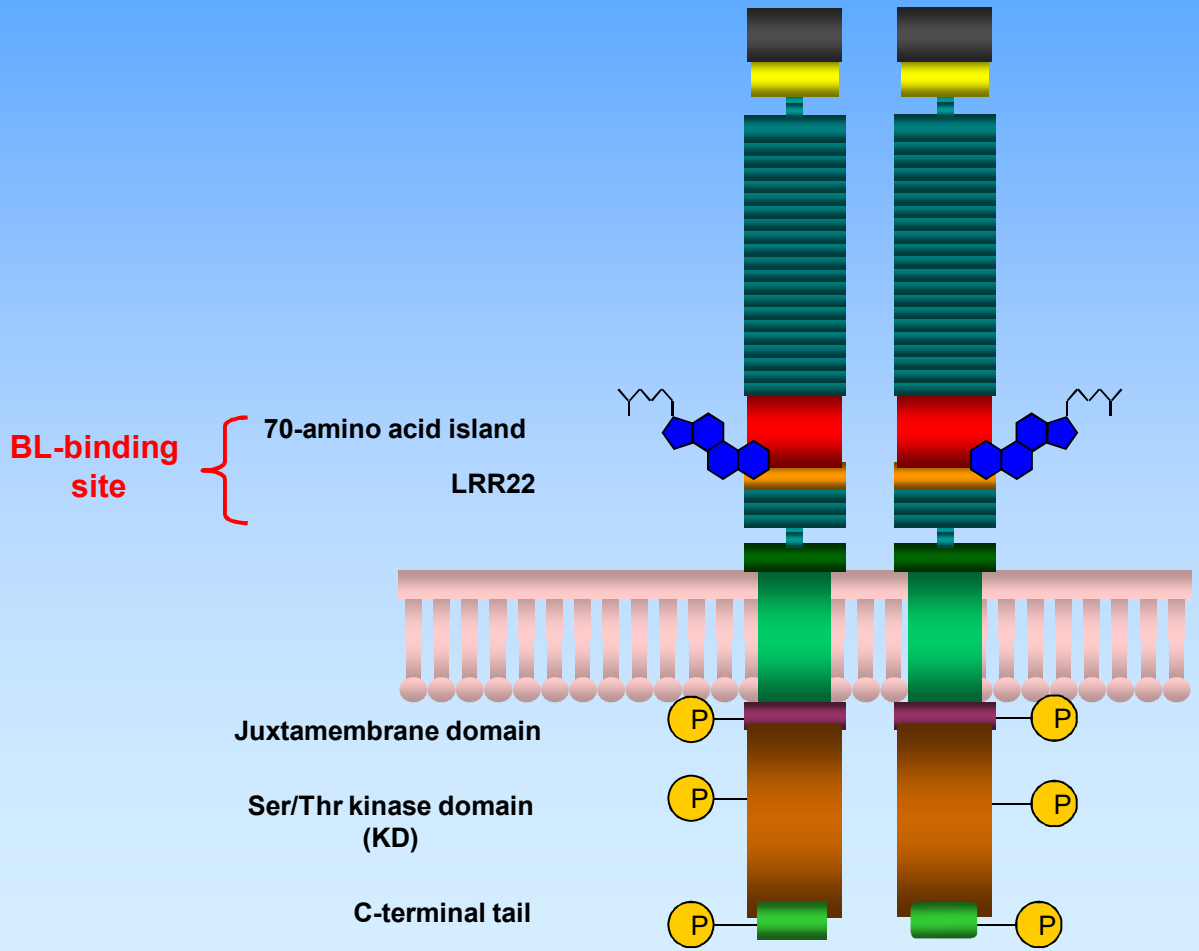
Other LRR-Rs do not contain the KD and transmit the signal via intracellular protein-protein interactions (CLV1, Cf-9).

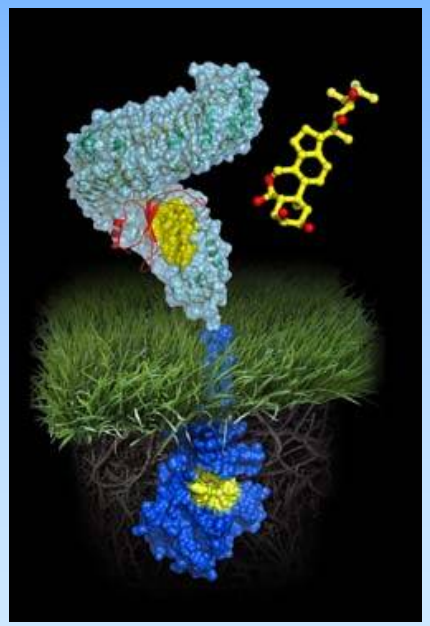
The Xa21 receptor involved in resistance to pathogens has KD but does not contain 70 amino acid island.

The Toll receptor in *Drosophila* contains intracellular protein (interleukin domain), which binds the protein after activation of the receptor similar to KD.

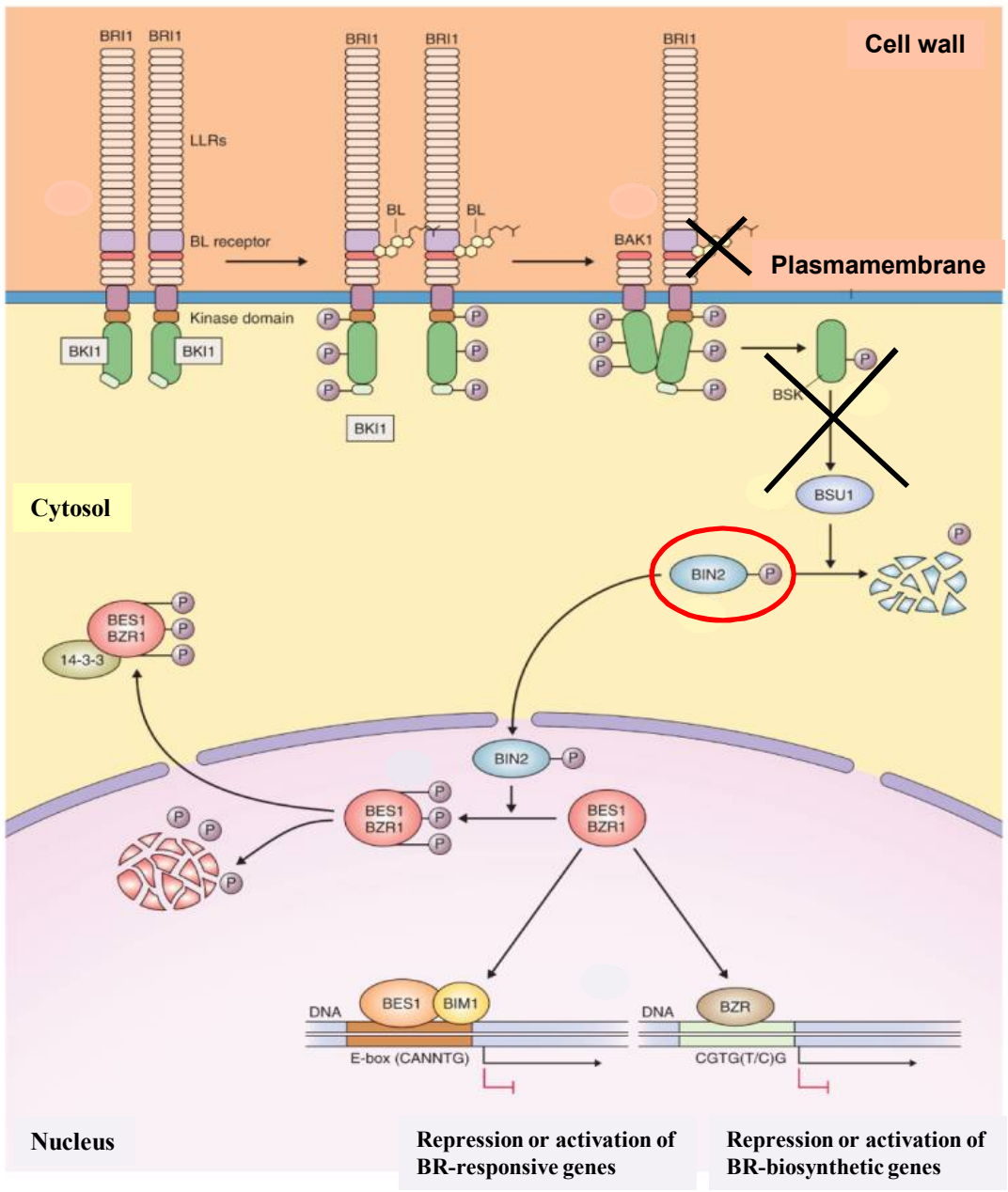
Phosphorylation of BRI1 receptor

Analysis of mutants with defects in BRI1 → Identification of domains needed for signal transduction





Hothorn M et al. (2011) Nature 474: 467- 471



Absence of BR:

BRI1/BAK1 heterodimer is not active

↓

BIN2 (**B**rassinosteroid-**IN**sensitive**2**) remains phosphorylated - active

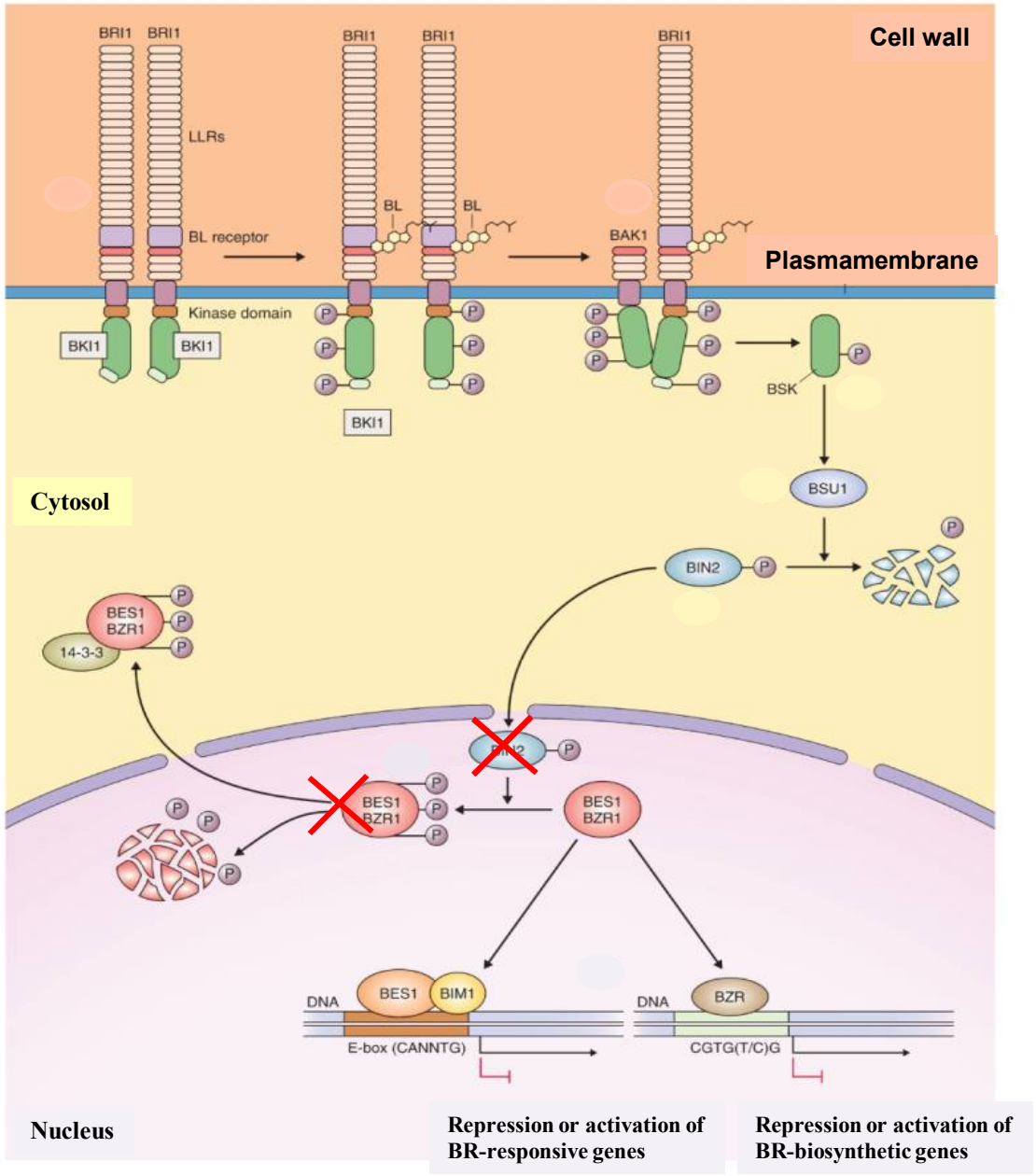
↓

Phosphorylation of proteins BES1 and BZR1 constitutively localized in nucleus.

Phosphorylated BES1 and BZR1 cannot bind to DNA and are degraded.

↓

BR-induced genes are not expressed.



Presence of BL = binding BL to receptor

Activated BRI1/BAK1 heterodimer inhibits the activity of BIN2 kinase Through **BSK** (**B**rassinosteroid-**S**ignaling **K**inases) and **BSU1** (**Bri1 SU**ppressor **1**)

+

Direct activity of BSU1

↓

Accumulation of dephosphorylated form of BES1 and BZR1 in nucleus

BES1 (bri1-EMS-suppressor 1)

BZR1 (brassinazole-resistant 1)

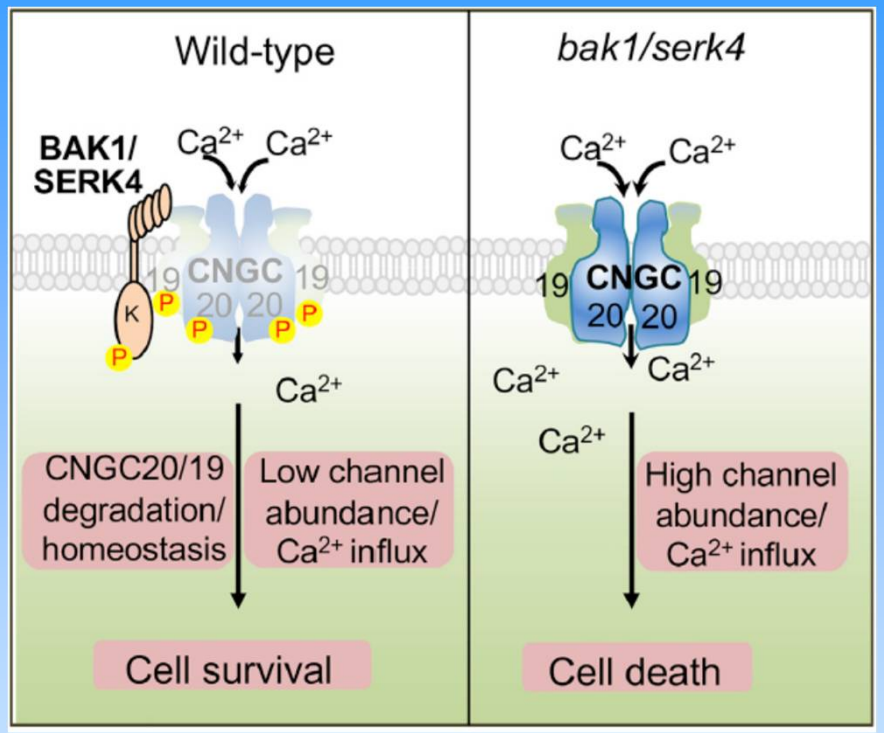
BES1 and BZR1 – transcription factors of BR-induced genes; short vitality; degradation in 26S proteasome

↓

Activation or suppression of gene expression

Update 2019

Yu X et al. (2019) Current Biology 29: 1-13

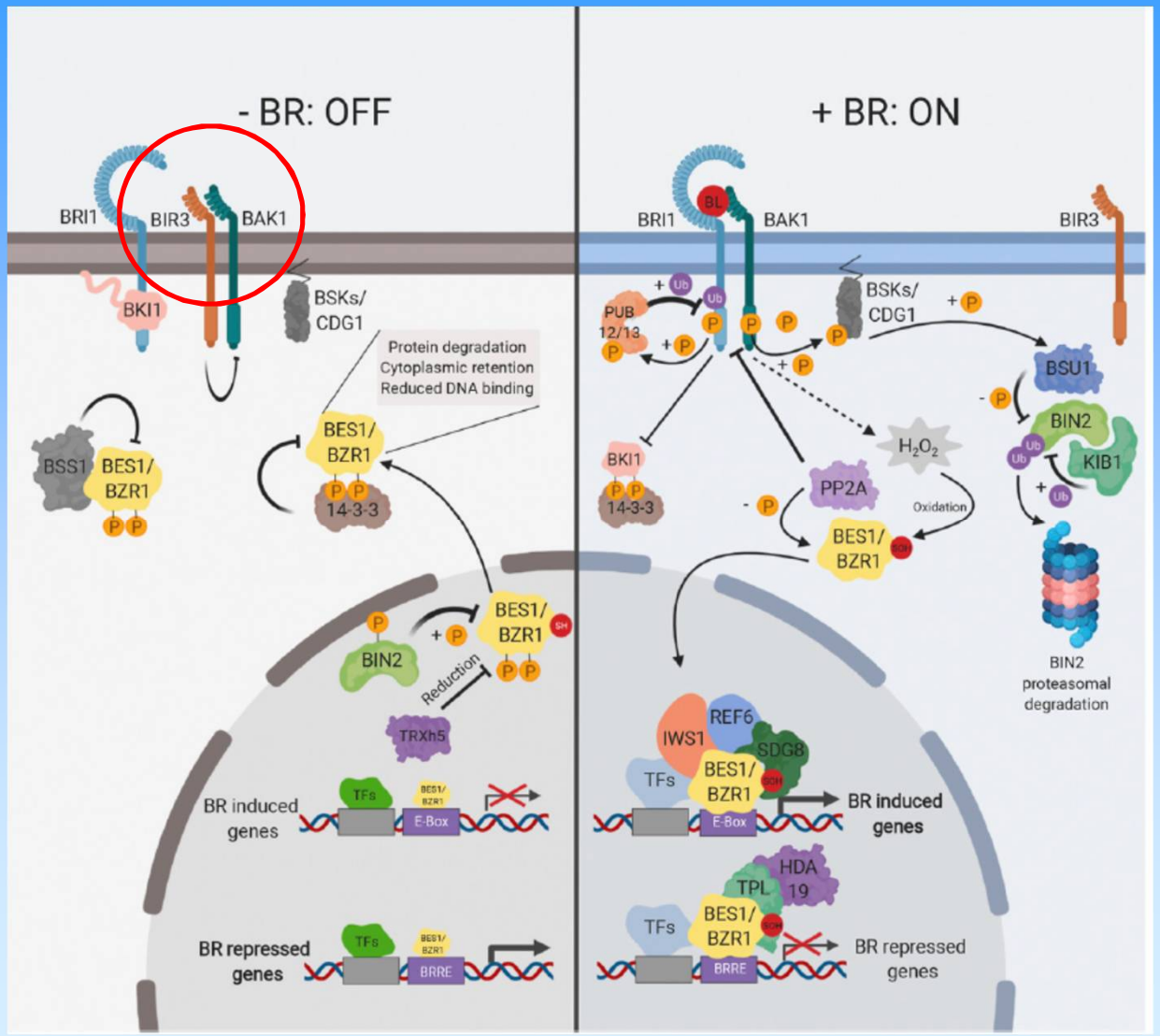


BAK1 (**B**RI1-**A**ssociated Receptor **Kinase1)** functions as a co-receptor for BRI1, FLS2 and some other LRR-RLKs, and regulates a wide range of physiological responses independently of brassinosteroids.

BAK1 and its homologue SERK4 block Ca^{2+} channel CNGC19 and CNGC20 and thus regulate cell fate.

If BAK1/SERK4 is missing during membrane hyperpolarization (= in the *bak1/serk4* mutant), Ca^{2+} channels are activated. Ca^{2+} is transported into the cell, thereby reducing the hyperpolarization. Subsequently, the programmed cell death appears.

BIR3 - negative regulator of BRI1-BAK1 interaction at the absence of brassinosteroids



Nolan TM et al. (2020)

BIR3 - leucine-rich repeat receptor kinase

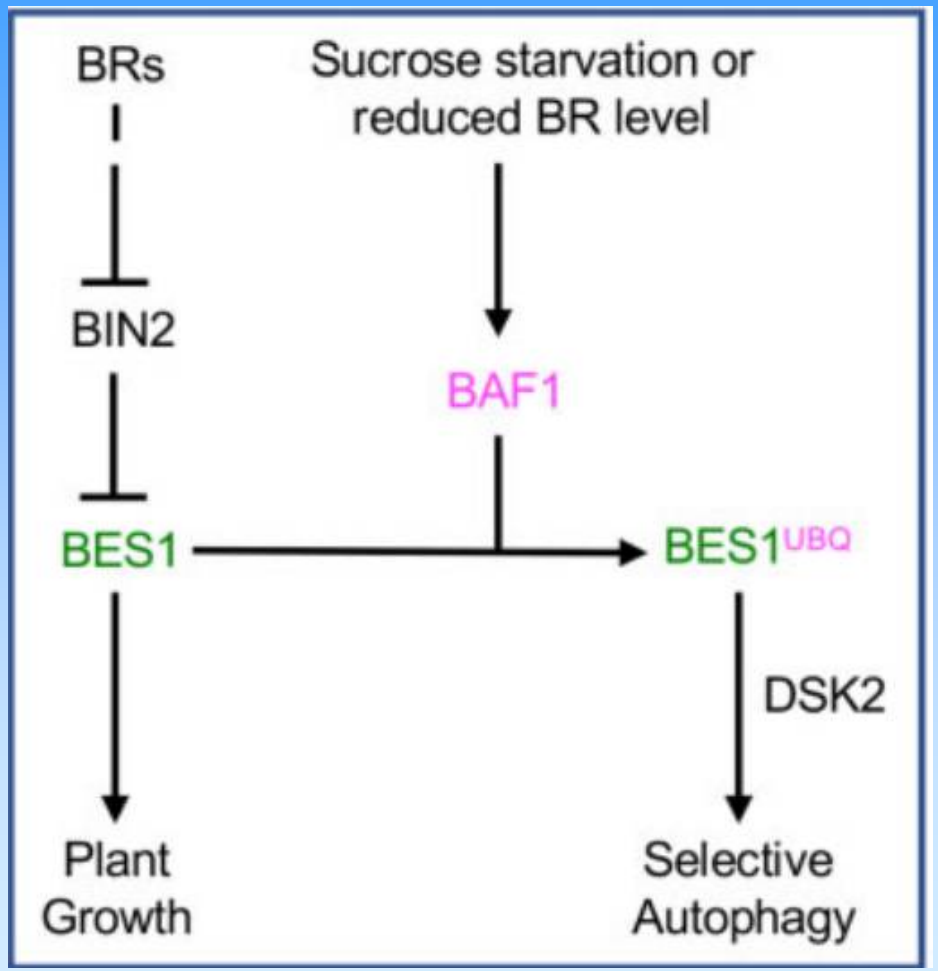
In the absence of brassinosteroids, BIR3 binds to BRI1 or BAK1. BIR3 and BRI1 share the same interaction site with BAK1.

Update 2017

Imkampe J et al. (2017) Plant Cell 29: 2285-2303

BAF1 - F-box E3 ubiquitin ligase – mediates degradation of the BES1 transcription factor under brassinosteroid deficiency or energy deficiency.

Update 2021
Wang P et al. (2021)
Plant Cell 33: 3532-3554



BAF1 - ubiquitinates BES1 and condemning it to degradation through selective autophagy.

Regulation of gene expression by transcription factors BES1 and BZR1

Sequencing of *Arabidopsis* genome → **Various methods of study of gene expression - microarray**

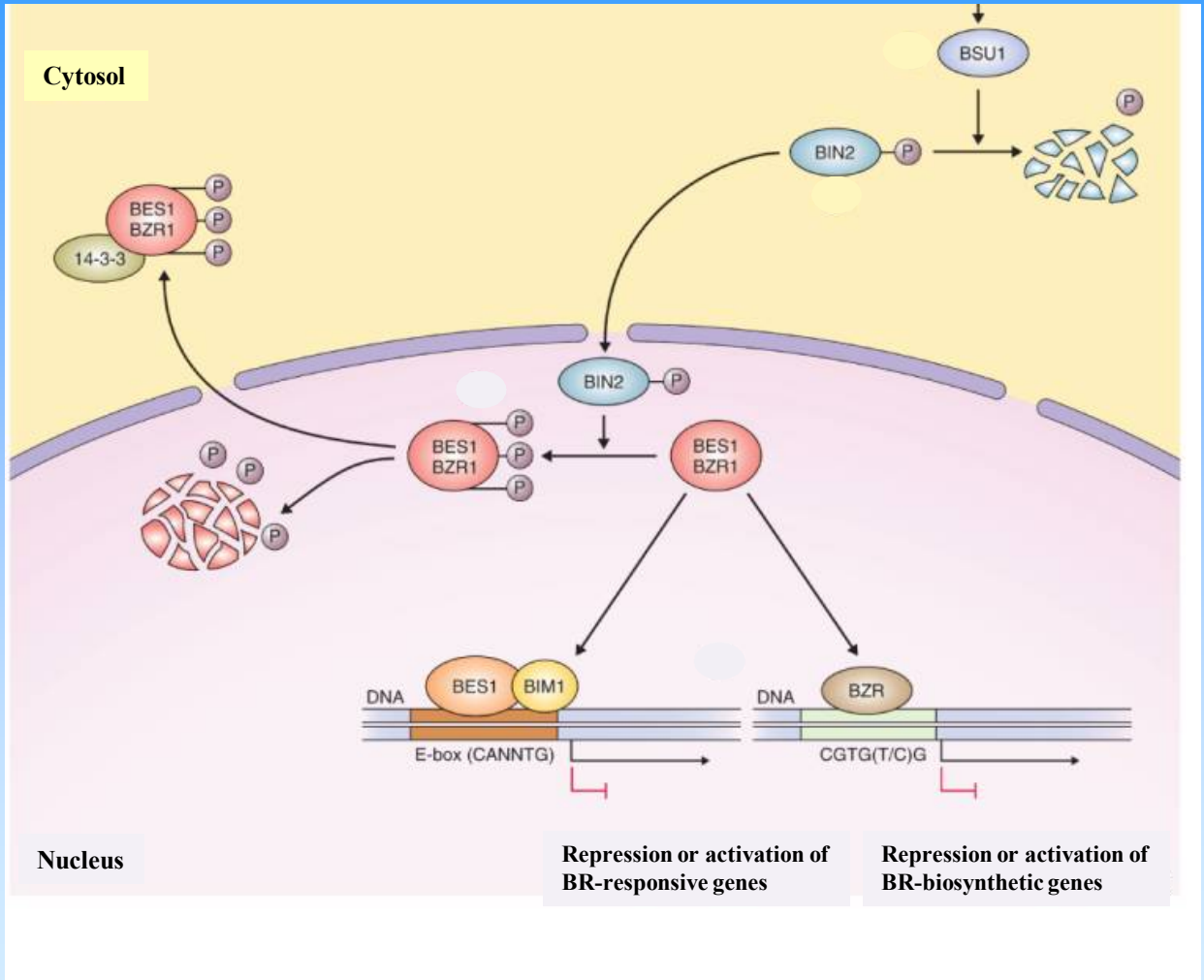
Identification of hundreds of BR-induced genes

Up-regulated and down-regulated genes probably involved in:

- synthesis and modification of cell wall
- cytoskeleton formation
- BR biosynthesis
- Signaling pathways
- BR transport

BES1 and BZR1 are 90% identical but regulate genes of various groups.

Identification of novel transcription factors interacting with BES1



BES1

+

BIM1 (BES1-interacting Myc-like 1)

BES1/BIM1 heterodimer

Activation of gene expression by binding to E-box

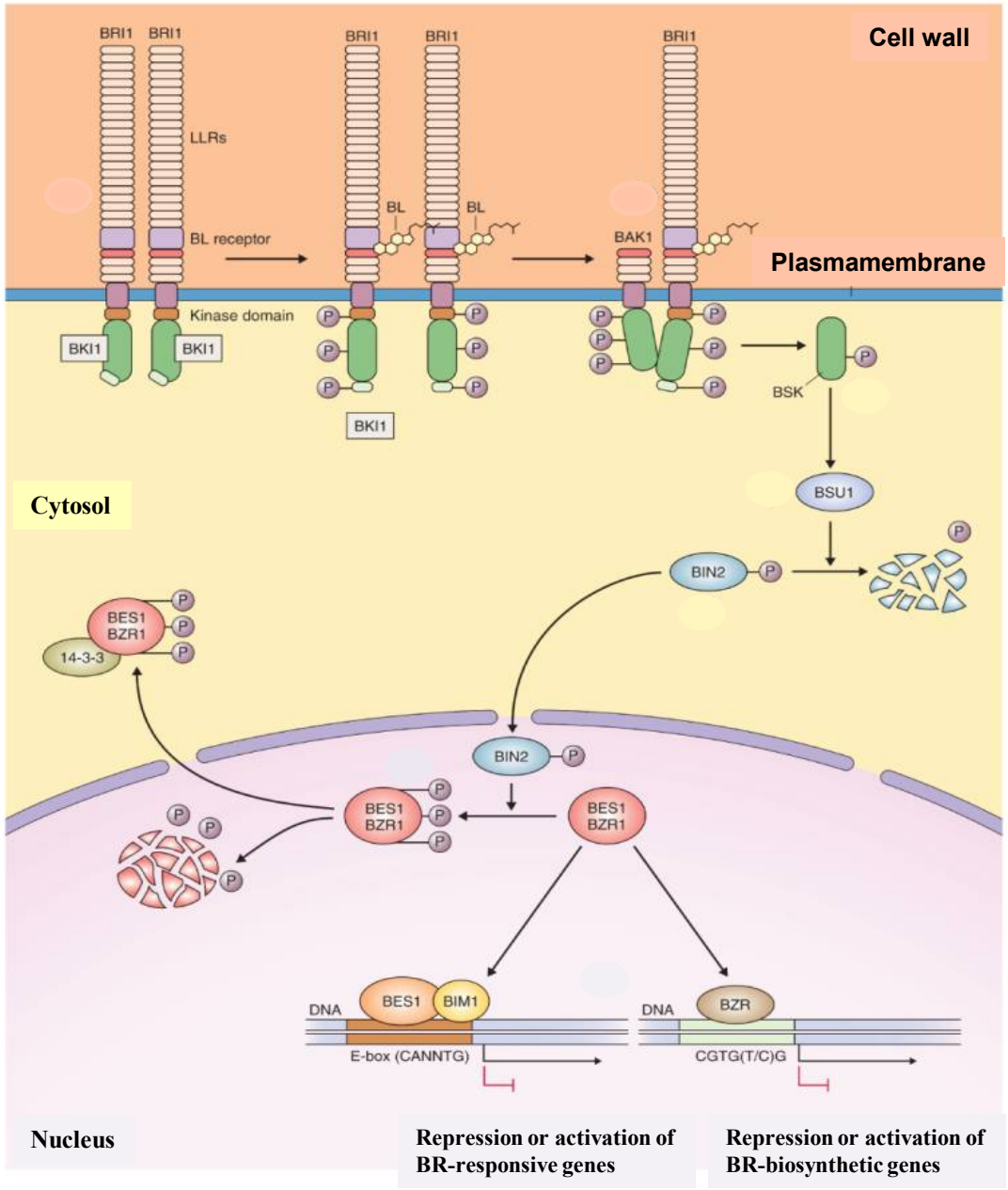
Update 2011

Yu X et al. (2011) Plant Journal 65: 634-646

Identification of 1609 genes that are regulated transcription factor BES1

For example:

- Stimulation of phloem and xylem differentiation(2018)
- BZR2 stimulates responses to drought (2019)



BZR1 – binding to BRRE (BR-responsive element)

↓

Inhibition of expression of BR-biosynthetic genes

↓

Feedback regulation of BR biosynthetic pathway

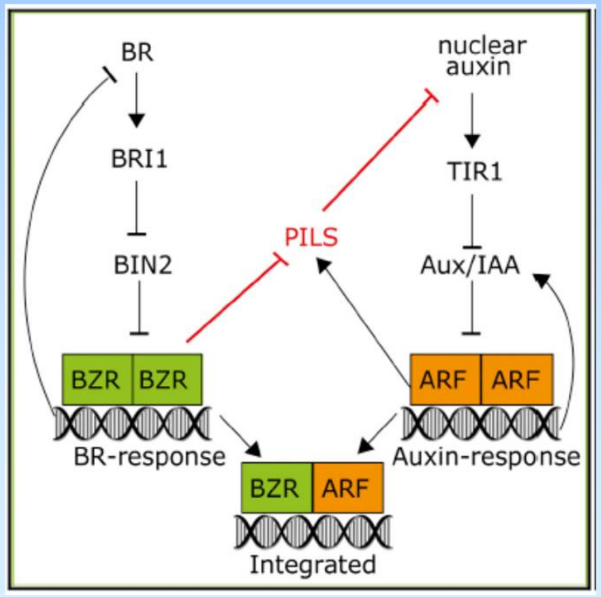
Update 2020
Sun L et al. (2020) Current Biology 30: 1579-1588

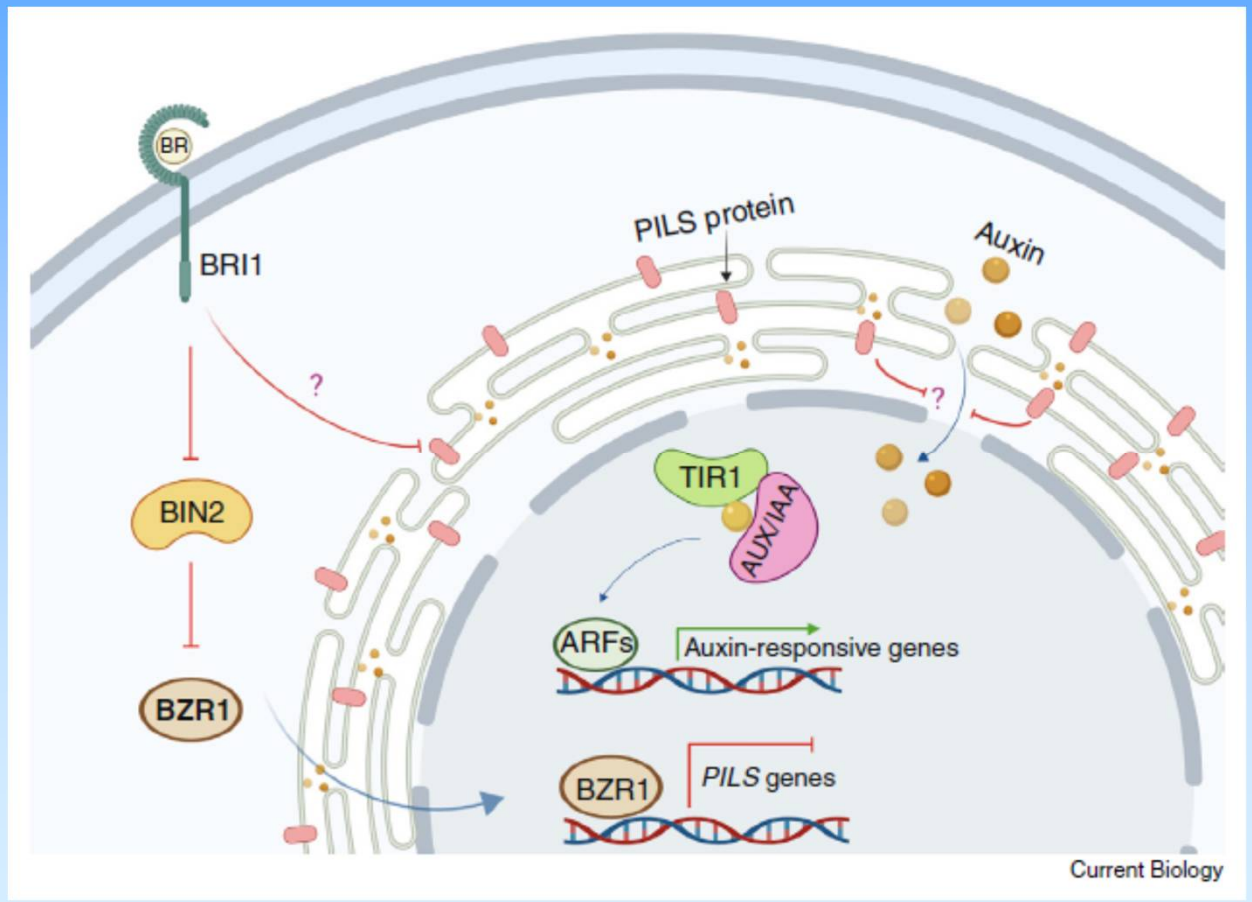
* Link from the slide 6

BRs signaling modulates the amount of auxin in the nucleus and thereby influences its effects on plant growth and development.

PILS (PIN-LIKES) = homologue of auxin transporter PIN

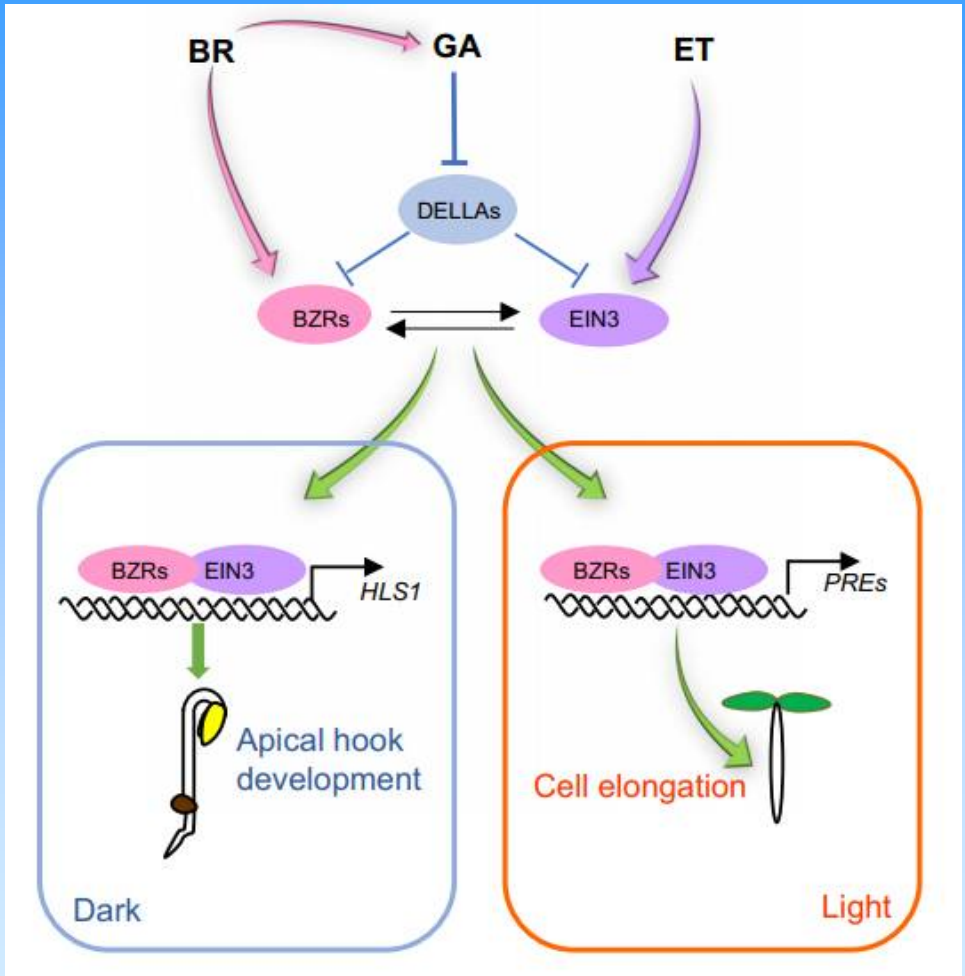
PILS proteins limit auxin availability in the nucleus by trapping auxin in the ER.





Rana S and Hardtke CS (2020)

Involvement of BZR1 in interaction with gibberellins and ethylene in plant development and growth



Brassinosteroids (BR) induce the biosynthesis of gibberellins (GA) and stimulate the activity of the transcription factor BZR1.

Ethylene (ET) stimulates the activity of transcription factor EIN3.

DELLA proteins interact with BZR1 and EIN3 proteins and inhibit their ability to bind to DNA. Gibberellins suppress the activity of DELLA proteins.

Inhibition of DELLA proteins leads to restoration of BZR1 and EIN3 activity. This drives the expression of HLS1 and PREs genes involved in apical hook development in the dark and hypocotyl elongation in the light.

HLS1 = HOOKLESS1

PREs = PACLOBUTRAZOL RESISTANCE FACTORS

Update 2021

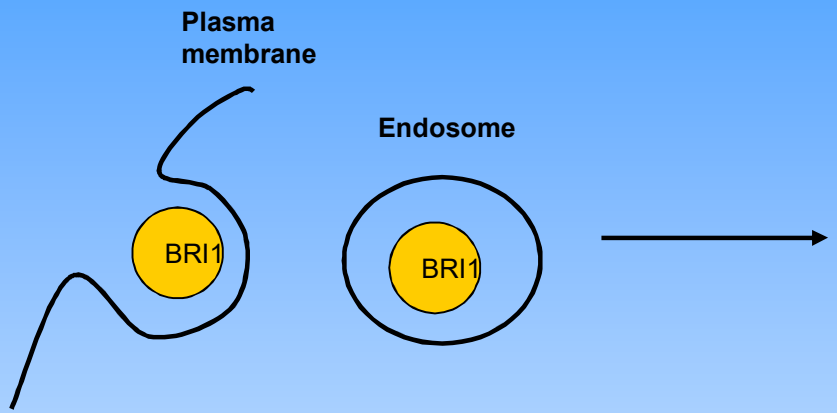
Zhao N et al. (2021) New Phytologist 232: 2308-2323

Update 2007 Geldner et al. (2007) Genes and Development 21: 1598-1602
Gendron and Wang (2007) Current Opinion in Plant Biology 10: 436-441

Endosomal localization of BRI1

Endocytosis common in animals and yeast

- inactivation of receptor (e.g. BOR1)
- signaling activation



Transport to destination or transport to the lysosome and degradation

- the distribution of BRI1 in the PM or endosomes is not altered in BR-deficient mutants or after affecting BR => endocytosis is not dependent on the activation state of BRI1

- endosomal BRI1 is constitutively active

Reasons for BRI1 signaling pathway via endosomes:

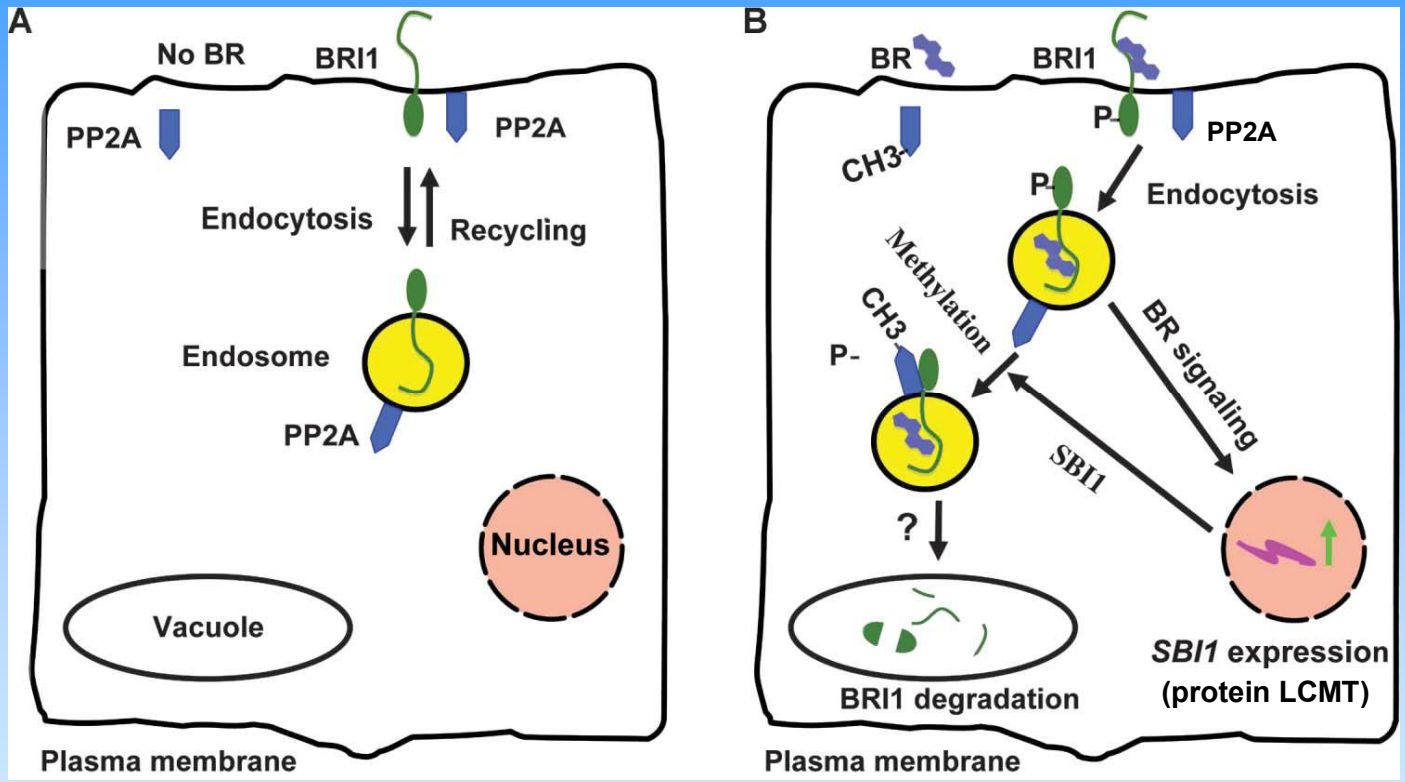
- supplementary to PM BRI1 (lack of space for PM)
- degradation of BRI1 – attenuation of the signaling pathway

Brassinosteroidy control own signaling.

Update 2011

Wu G et al. (2011) Science Signaling 4: ra29

Di Rubbo S et al. (2011) Science Signaling 4: pe25



The BRI1 receptor cycles between the membrane and the endosome. When BRI1 is activated = phosphorylated, it stimulates the expression of SBI1 (Suppressor of BRI1) in endosomal form. SBI1 encodes a leucine carboxylmethyltransferase (LCMT) and therefore methylates the protein phosphatase PP2A. This facilitates the association of PP2A with active BRI1. This leads to dephosphorylation and degradation of the BRI1 receptor. This inhibits the signal. Brassinosteroids thus control their own signaling.


JiriSebek.com