Signalizace je vlastně komunikace

a komunikace je základem života….

- Přenos signálu
- Rotlinné hormony a jejich signální dráhy
  - Signální dráhy založené na regulované proteolýze
    - Komponenty proteolytických degradačních drah
    - Auxin
    - Giberellin
    - Jasmonát (?)
  - Signální dráhy založené na dvoukomponentní dráze
    - Cytokininy
    - Etylen
  - Brassinosteroidy a signalizace pomocí proteinkináz
  - ABA = mnoho funkcí a receptorů
  - Peptidové hormony
- Signalizace světlem

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  - ABA = mnoho funkcí a receptorů
  - Peptidové hormony
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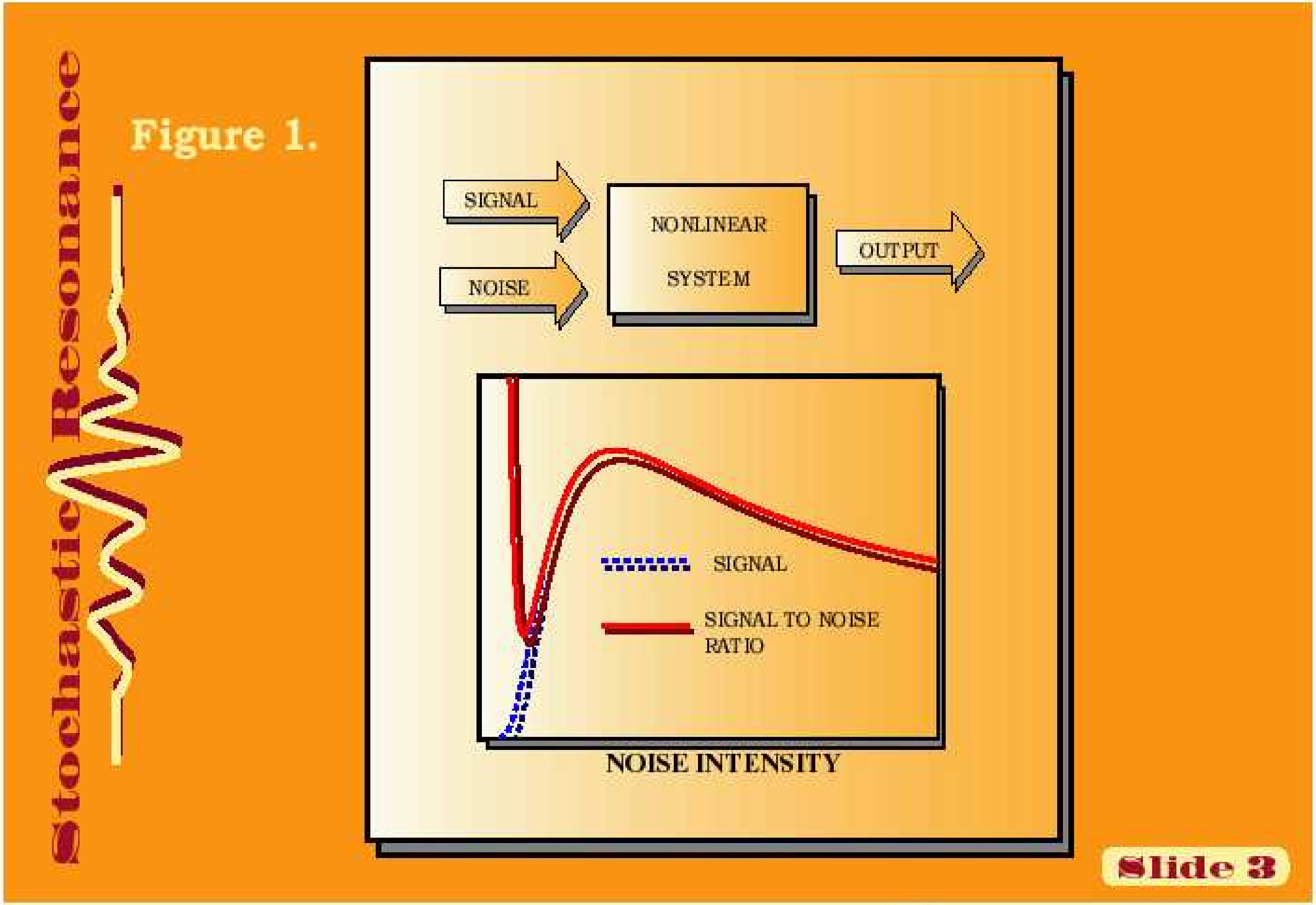
Signalizace, regulace, komunikace a integrace v buňce, pletivu a organizmu

Několik poznámek.

- Kvantitativně převažujícím prvkem regulace genové exprese je represe (a její odblokování).
- Většina bílkovin je polyfunkčních a jejich exprese a funkce je regulována na mnoha úrovních najednou.
- Signální dráhy se integrují na úrovni společného regulačního bílkovinného intermediátu (příklad fosf.), druhého posla, promotoru, procesu či struktury.
- Signál je zesilován, či zeslabován - při tom šum okolí může být pozitivně využit k zesílení signálu = **stochastická resonance**.



Stochastic Resonance

Figure 1.

SIGNAL

NOISE

NONLINEAR SYSTEM

OUTPUT

▪▪▪▪▪ SIGNAL

▬ SIGNAL TO NOISE RATIO

NOISE INTENSITY

http://www.lancs.ac.uk/depts/physics/research/condmatt/lng/srshow/srslide1.html

- Z toho, že organismus vládne buňkám (nejen buňky organizmu), také plyne, že také buňka a organizmus vládne signálním drahám a sítím. Buňka není jen výsledkem propletence procesů/struktur, které v ní probíhají/strukturují, ale také jejich tvůrcem.

Analogy view of cell signaling transduction pathway

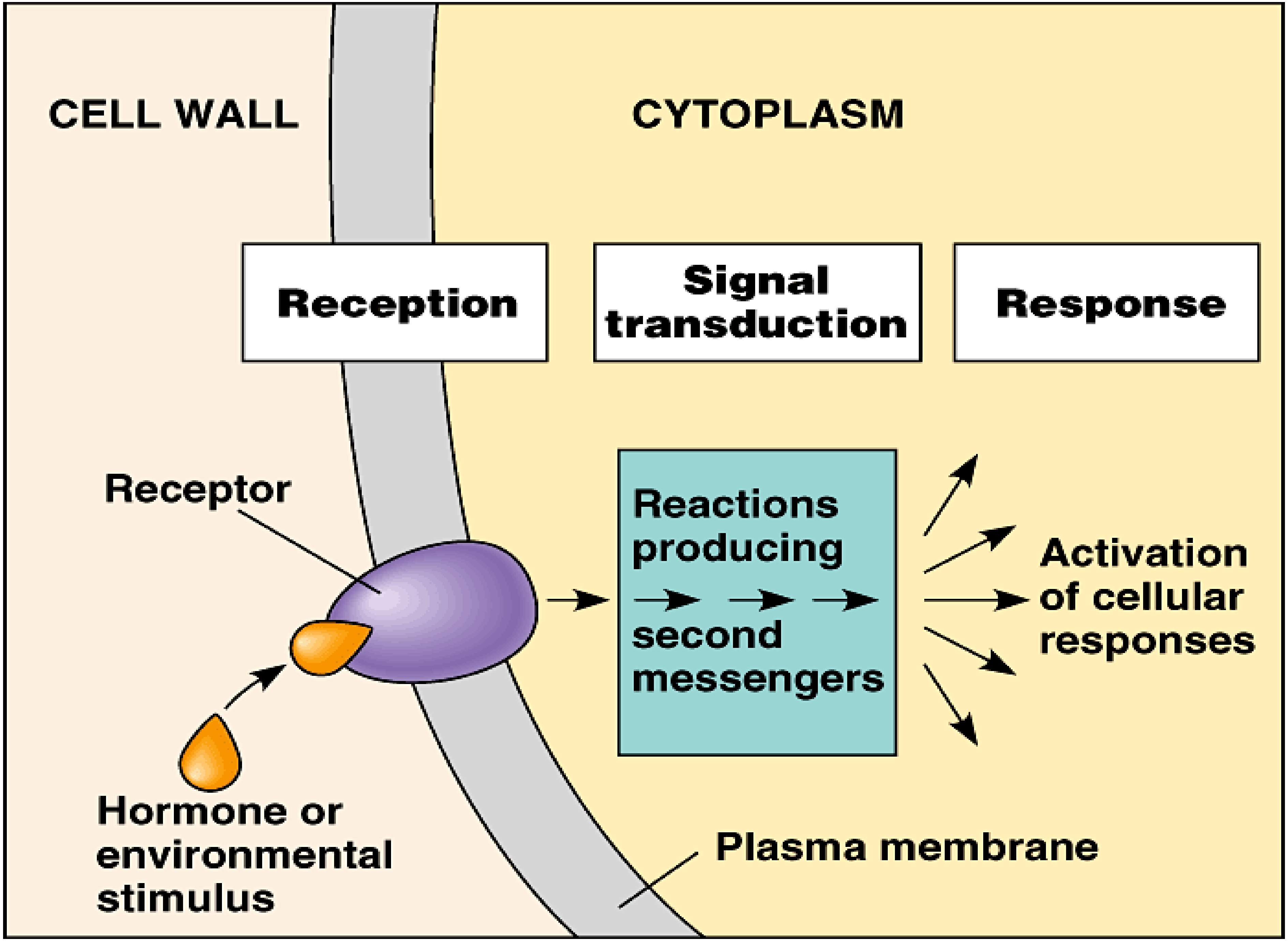
Input (command)
Keyboard
Signal perception
Endogenous:
phytohormone
Exogenous:
environmental cue

→

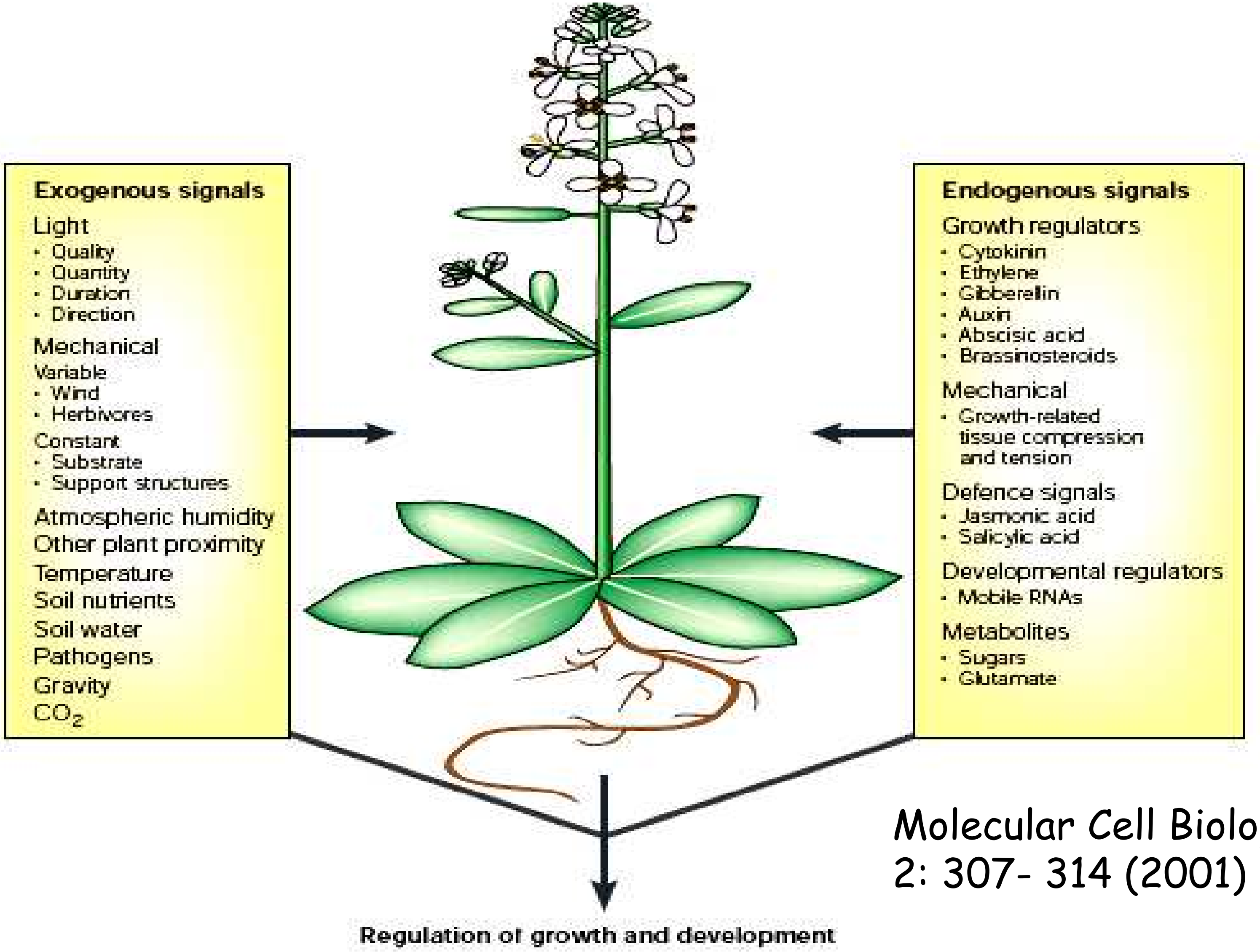
CPU (Central Processing Unit)
Chip
Signal Transduction
Network
Compiling,
Integration,
processing

→

Output (performance)
Printer
Signal Response
Morphogenesis change,
Growth development
differentiation


CELL WALL
CYTOPLASM
Reception
Signal transduction
Response
Receptor
Reactions producing second messengers
Activation of cellular responses
Hormone or environmental stimulus
Plasma membrane

A wide range of disparate *external and internal signals* is *monitored* by plants and *used to compute* appropriate developmental *responses*



Exogenous signals

Light
- Quality
- Quantity
- Duration
- Direction

Mechanical

Variable
- Wind
- Herbivores

Constant
- Substrate
- Support structures

Atmospheric humidity

Other plant proximity

Temperature

Soil nutrients

Soil water

Pathogens

Gravity

CO_2

Endogenous signals

Growth regulators
- Cytokinin
- Ethylene
- Gibberellin
- Auxin
- Abscisic acid
- Brassinosteroids

Mechanical
- Growth-related tissue compression and tension

Defence signals
- Jasmonic acid
- Salicylic acid

Developmental regulators
- Mobile RNAs

Metabolites
- Sugars
- Glutamate

Regulation of growth and development

Molecular Cell Biology
2: 307- 314 (2001)

- Přenos signálu
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- Signalizace světlem

Box 1. Plant hormones versus animal hormones

Plant hormones

- Small organic molecules
- Synthesised throughout the plant
- Affect local cells and tissues, or can be transported through the vascular system
- Wide-ranging effects
- ‘Decentralised’ regulation

Animal hormones

- Peptides/proteins/small organic molecules
- Synthesised in specialised glands
- Affect distant targets (often transported in the circulatory system)
- Specialised effects
- Regulated by the central nervous system

Rostlinné "hormony"

- Auxins
- Gibberellins
- Cytokinins
- Abscisic acid
- Ethylene
- Brassinosteroids
- JA, SA
- ………………….

TABLE 12.1 Plant Hormones: Structure and Effects

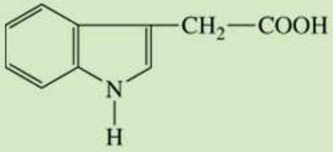
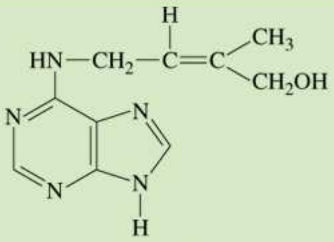
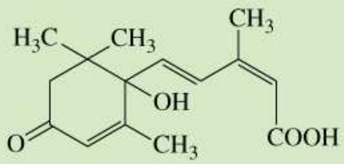
Plant Hormones	Chemical Structure	Functions
auxins	 indoleacetic acid (IAA)	Apical bud dominance (retards growth of lateral buds immediately below); mediate growth response to light direction; induce development of vascular tissue; promote activity of secondary meristems; induce formation of roots on cuttings; inhibit leaf and fruit drop; stimulate fruit development; stimulate ethylene synthesis
cytokinins	 zeatin	Promote cell division in shoot and root meristems; influence development of vascular tissues; delay leaf aging; promote development of shoots from undifferentiated tissue in lab culture
ethylene	$H_2C{=}CH_2$ ethylene	Promotes ripening of some fruits; promotes leaf and flower aging and leaf and fruit drop from plants; affects cell elongation and seed germination; helps plants perceive and respond to pathogen attack and mechanical stress
abscisic acid	 abscisic acid	Promotes transport of food from leaves to developing seeds; promotes dormancy in seeds and buds of some plants; helps plants respond to water stress emergencies; regulates gas exchange at the surfaces of leaves

TABLE 12.1 *continued*

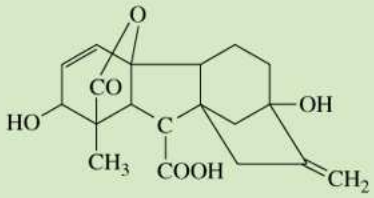
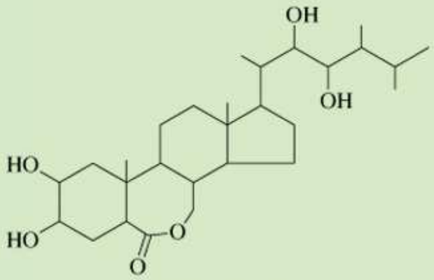
gibberellins	 gibberellic acid (GA)	Stimulate both cell division and cell enlargement during shoot elongation; promote seed germination; stimulate flowering in some plants
brassinosteroids	 brassinolide	Stimulate shoot elongation; reduce plant stress caused by heat, cold, drought, salt, and herbicide injury
salicylic acid	 salicylic acid	Helps plants perceive pathogen attack

TABLE 12.1 *continued*

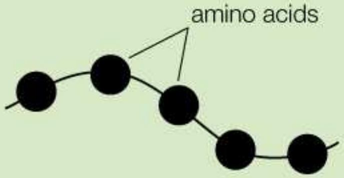
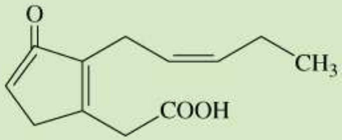
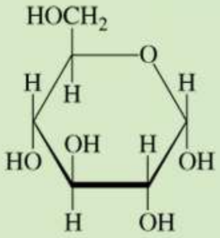
Plant Hormones	Chemical Structure	Functions
systemin	 systemin	Signals that wounding has occurred
jasmonic acid	 jasmonic acid	Helps plants resist fungal infection and other stresses; induces plant production of protective secondary compounds (alkaloids)
sugars	 glucose	Helps regulate amounts of chlorophyll and other photosynthetic components

Table 12.1 (part 3)   Plant Biology, 2/e

Main approaches used to study plant hormone perception, signal transduction biosynthesis and responses

1. **Molecular genetics approach**

   hormone biosynthetic mutants
   vs. hormone insensitive mutants

2. **Biochemistry approach**

   hormone binding protein
   (photo-affinity probe, affinity chromatography, immunopurification)

3. **Inhibitors approach**

   kinase/phosphatase, G-protein, PKC etc. inhibitors

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    - **Komponenty proteolytických degradačních drah**
    - Auxin
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    - Jasmonát (?)
  - Signální dráhy založené na dvoukomponentní dráze
    - Cytokininy
    - Etylen
  - Brassinosteroidy a signalizace pomocí proteinkináz
  - Peptidové hormony
- Signalizace světlem

Degradace bílkovin

je stejně důležitý regulační krok jako jejich syntéza. Signální dráhy často obsahují vysoce specifickou/regulovanou degradaci bílkoviny jako důležitý regulační krok. (Vzpomeňme na cykliny…)

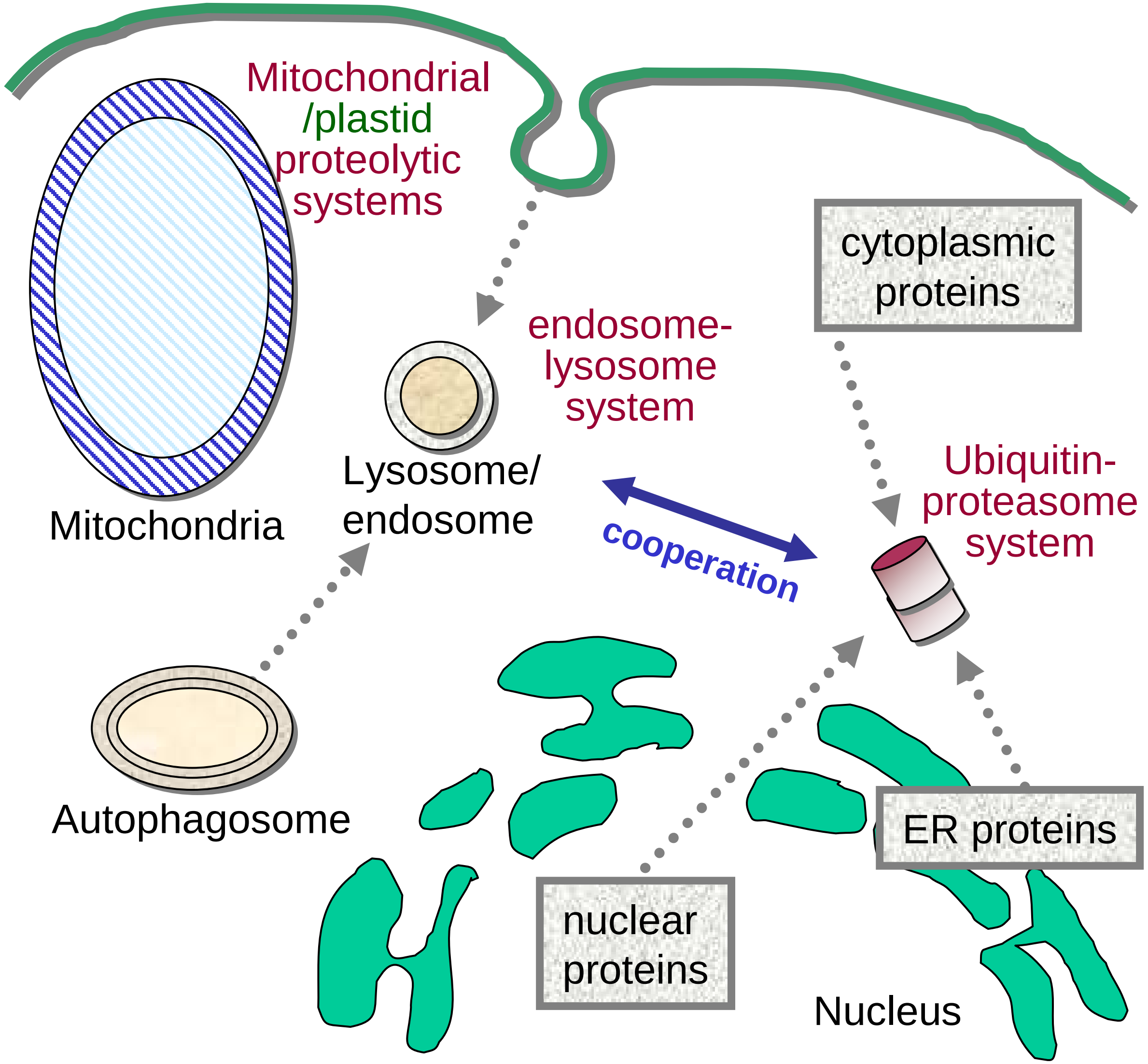
Proteolýza je ovšem také konstitutivní proces.
Až 30% translatovaných bílkovin je nefunkčních.

Degradace buněčných bílkovin

Proteolytické dráhy u eukaryot

- ***1. vakuolární/lysozomální***
- ***2. Na ubiquitinu-proteasomu závislá degradace***
- ***3. post-proteasomálni degradace : Tricorn, TPII?***
- ***4. Degradace membranových proteinů***

Hlavní proteolytické dráhy eukaryot



- endosome-lysosome pathway degrades extracellular and cell-surface proteins
- ubiquitin-proteasome pathway degrades proteins from the cytoplasm, nucleus and ER
- **mitochondria (and chloroplasts) have their own proteolytic system that are of bacterial origin**

Degradace membránových proteinů. ATP dep. membr. komplexy

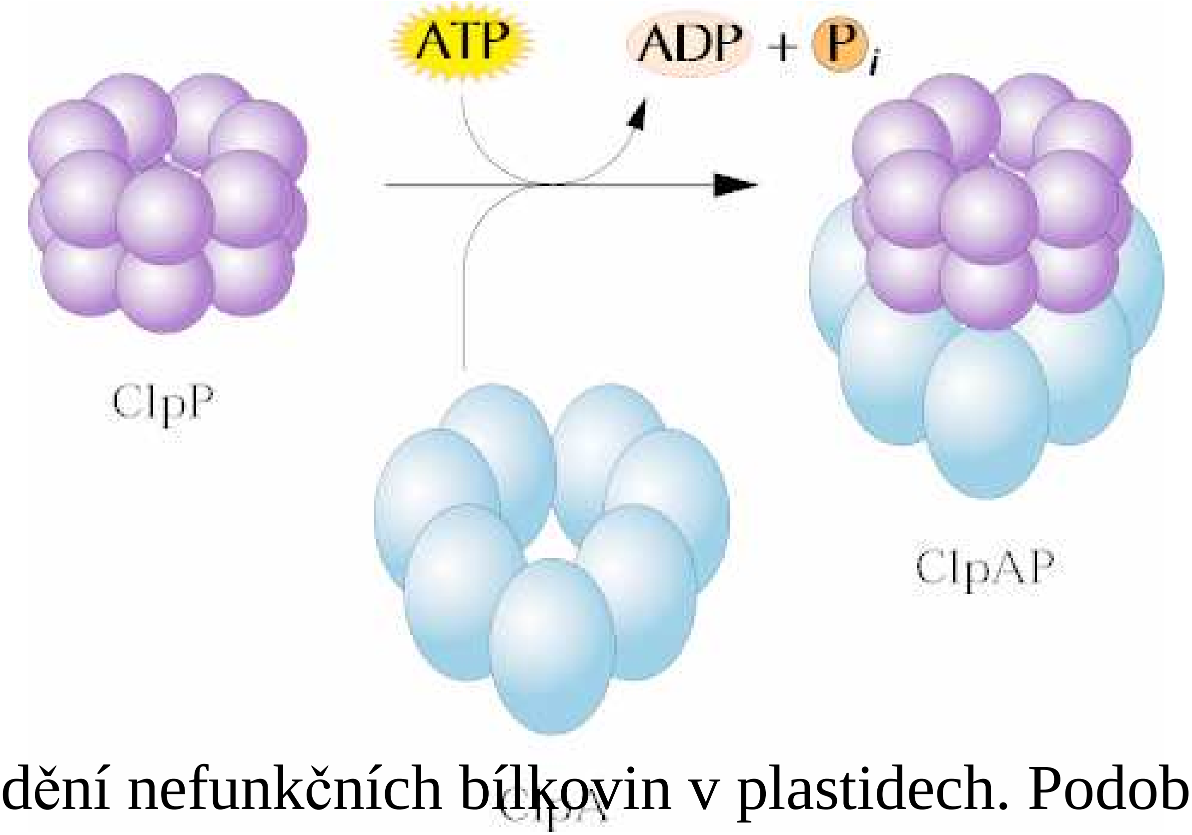
- **AAA proteases mediate** the degradation of membrane proteins in bacteria, mitochondria and chloroplasts (*i.e.*, compartments of eubacterial origin)
- combine proteolytic and chaperone activities in one system, acting as quality-control machineries

- model substrate polypeptides containing hydrophilic domains at *either* side of the membrane can be completely degraded by either of two AAA proteases found in mitochondria, if solvent-exposed domains are in an unfolded state

- a short protein tail protruding from the membrane surface is sufficient to allow the proteolytic attack of an AAA protease that facilitates domain unfolding at the opposite side

Leonhard *et al*. (2000) *Mol. Cell* 5, 629-638.

ClpAP je proteázový komplex aktivní v plastidech (homol. *E.coli*).



Brání hromadění nefunkčních bílkovin v plastidech. Podobně je tomu v mitochondriích.

Vakuolární/lysozomální degradace

- macroautophagy is the equivalent of forming intracellular endosomes (phagosomes) that fuse to the lysosome and result in the breakdown of its contents

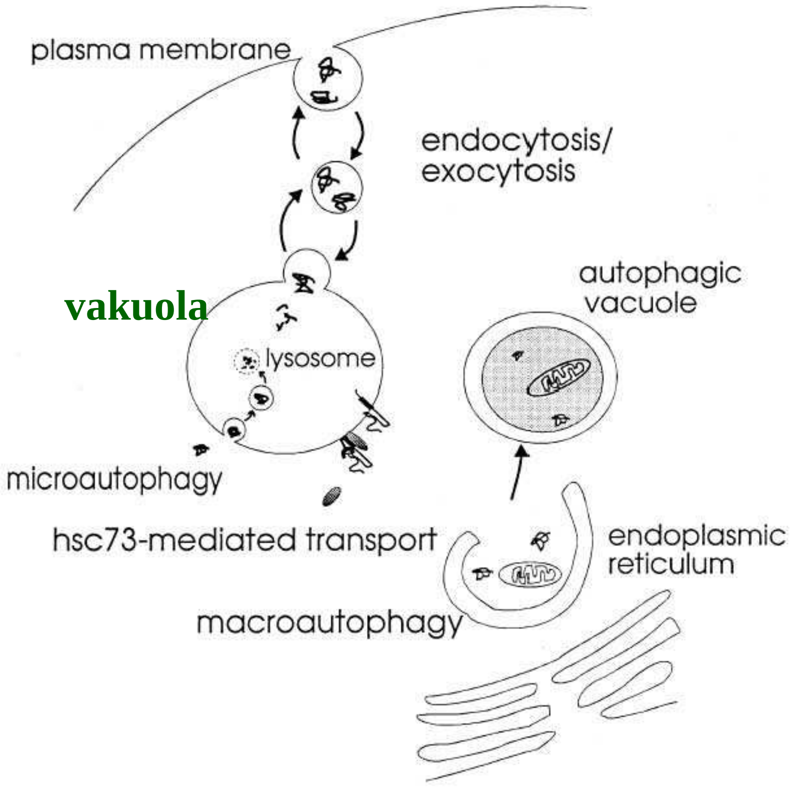


Fig. 1 Pathways of protein degradation in lysosomes. Lysosomes are able to degrade intra- and extracellular proteins following different mechanisms

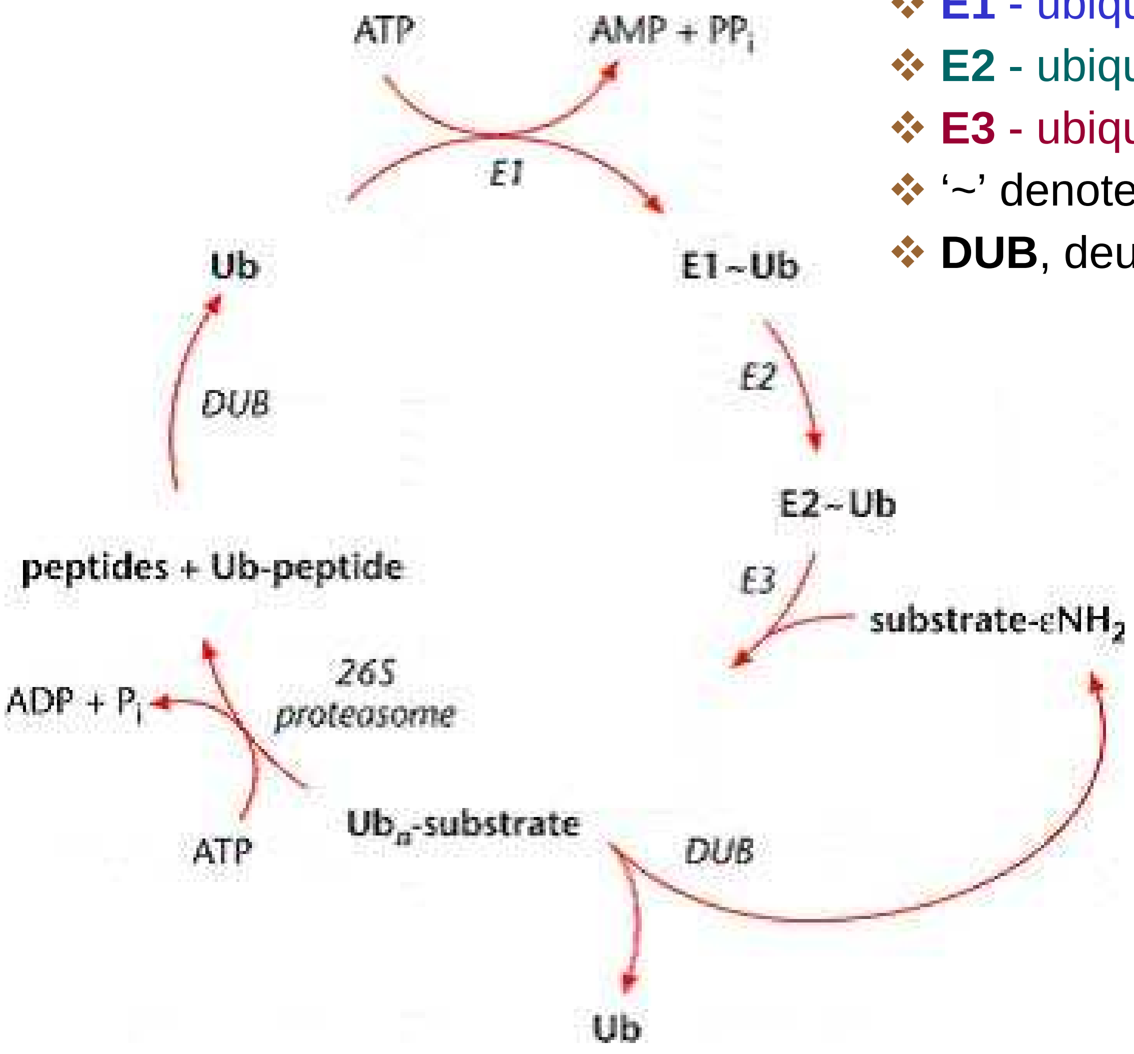
Cuervo and Dice (1998) *J. Mol. Med.* **76**, 6-12.

Bílkoviny určené k degradaci proteasomem jsou modifikovány ubiquitinem.

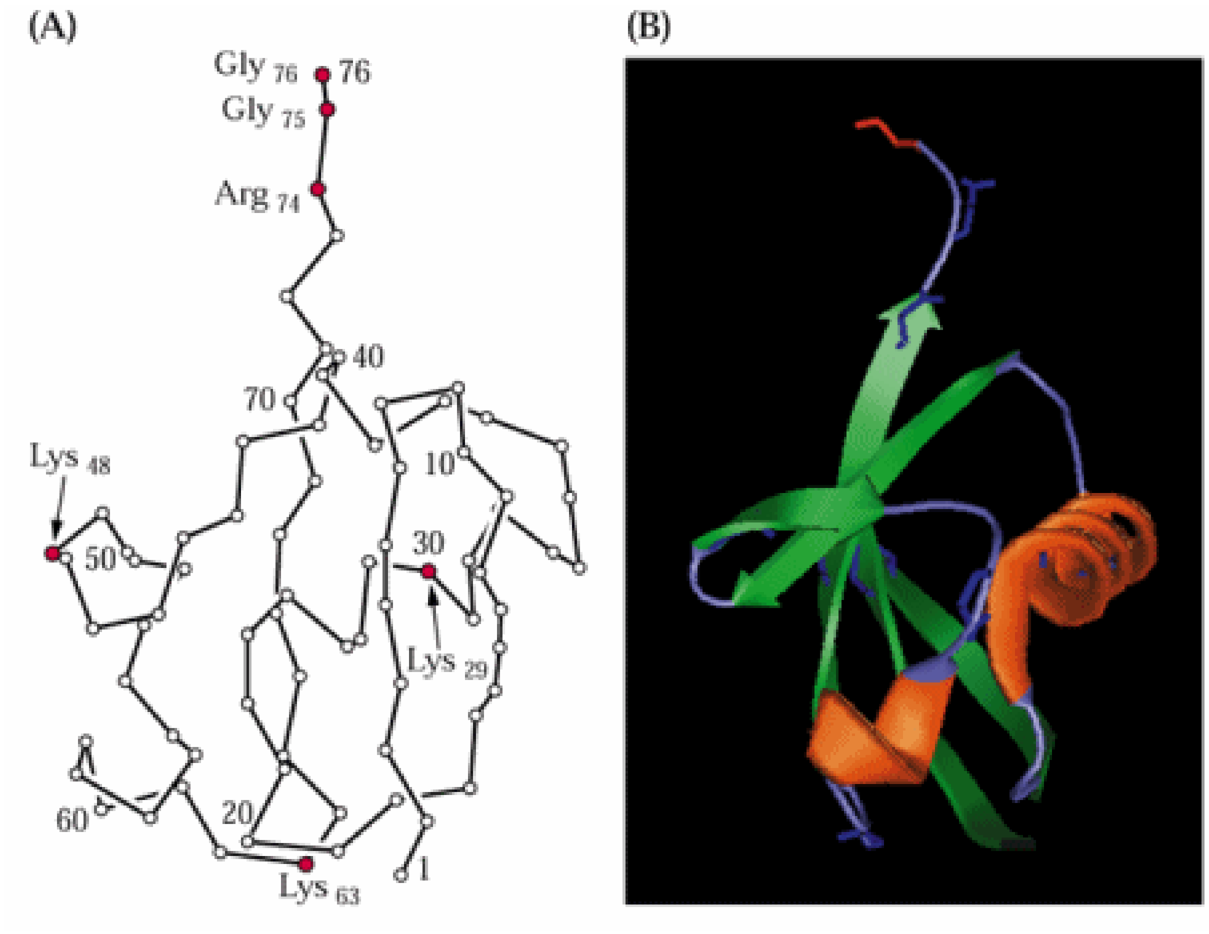
Prvním známým proteinem ubq. *in vivo* v aktivní formě byl u rostlin fytochrom.

Většina bílkovin je před ubiquitinací specificky fosforylována.

Ubiquitinová dráha



- **E1** - ubiquitin activating enzyme
- **E2** - ubiquitin conjugating enzyme
- **E3** - ubiquitin ligase
- ‘~’ denotes high-energy thioester bond
- **DUB**, deubiquinating enzyme

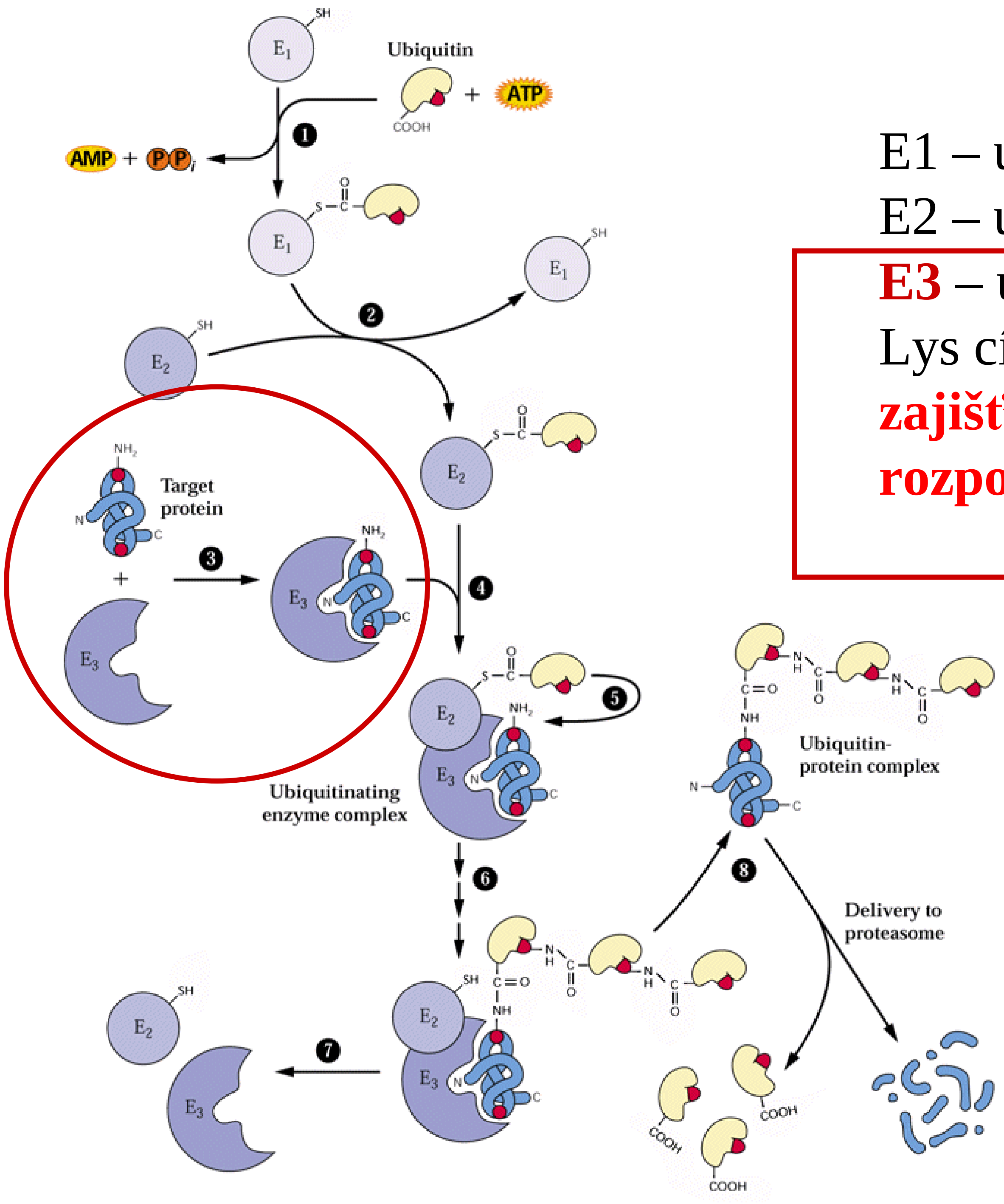


Syntet. z fusních tandemových prekursorů – 3 až 6.

Jsou štepeny deubiqutinačními enzymy/proteasami = **DUB**

76 AA – rostl. od kvasinek/živočichů se liší 2/3 AA.

Ubq. se kovalentně váže **na Lys** cílové bílkoviny C´-Gly.



E1 – ubq. Aktivace

E2 – ubq. Konjugace

E3 – ubq. Ligace ubq. na Lys cílové bílkoviny. E3 **zajišťuje specifitu rozpoznání**.

Na bílkovině je ubq. více Lys a to opakovaně = polyubiquitin.

Genes in the *Arabidopsis* Ubiquitin/26S Proteasome Pathway

Arabidopsis thaliana

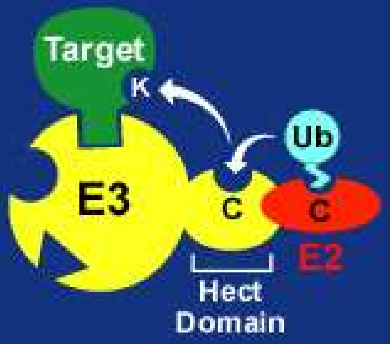
	No. of Genes
Ubiquitin	16
E1s	2
E2s	45
E3s	
HECT	7
F-Box	>694
Skp/Cullin/Rbx	33
APC	20?
Ring Finger	387
U-Box	37
UBPs	32
Proteasome	
20S CP	23
19S RP	31
● Estimated Total	>1327

~1200 E3 Genes!

5.1 % of PROTEOME !!!

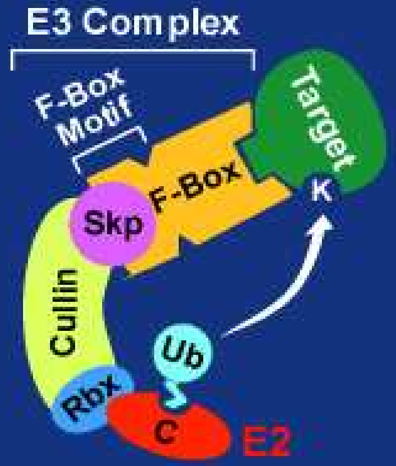
[Vierstra, 2003 *TIPS*]

E3 Types

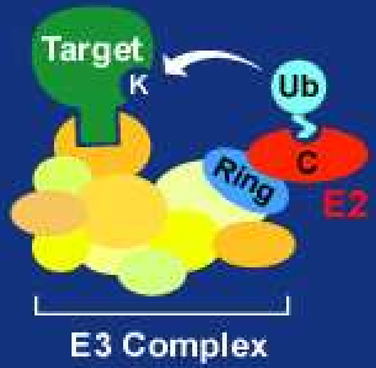


HECT

Ring/U-Box



SCF



APC

Predicted Diversity of E3s Among Eukaryotes

E3 type	Yeast	Drosophila	Caenorhabditis	Arabidopsis
HECT	7	13	9	7
SCF (F-Box)	14	24	337	>694 *
Ring/U-Box	47	105	154	455 *

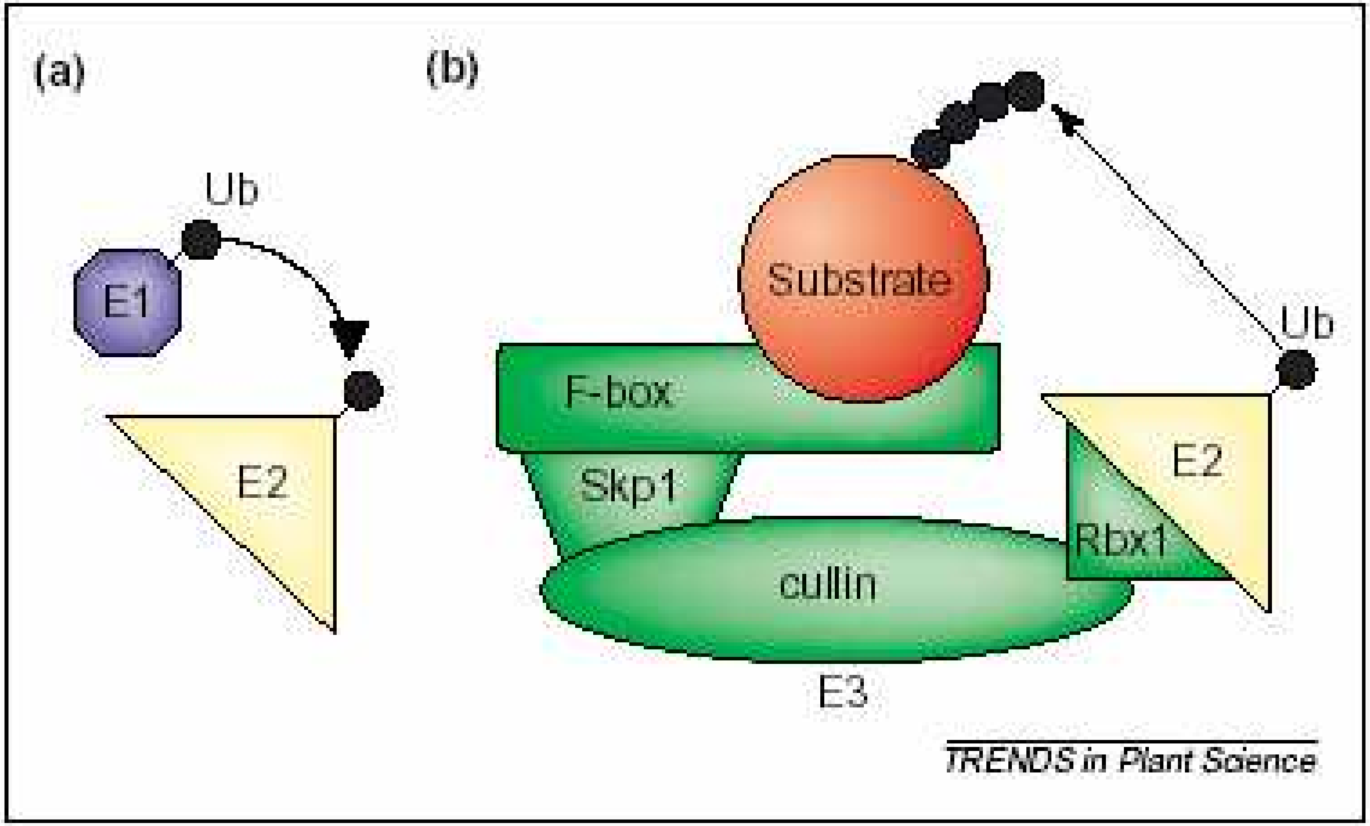


Figure 1. Key steps in the pathway of polyubiquitylation by SCF E3 ligase, which targets substrate protein and leads to degradation by the 26S proteasome. **(a)** Ubiquitin (Ub) is linked via a thioester bond to the ubiquitin-activating enzyme (E1). Ubiquitin is transferred from E1 to the cysteine of the ubiquitin-conjugating enzyme (E2). **(b)** The SCF E3 ubiquitin ligase (Skp1, cullin, F-box and Rbx1) catalyses the transfer of ubiquitin from E2 to a lysine residue on the substrate protein. Formation of a polyubiquitin chain on the substrate protein targets it for degradation by the 26S proteasome.

F-box odpovídá za specifitu interakce se substrátem.

Monoubiquitinace může sloužit jako regulační modifikace. Např. pro třídění do endocytotické dráhy a vakuoly/lysozomu či modifikuje např. transkripci TF-ubq.

Proteasom

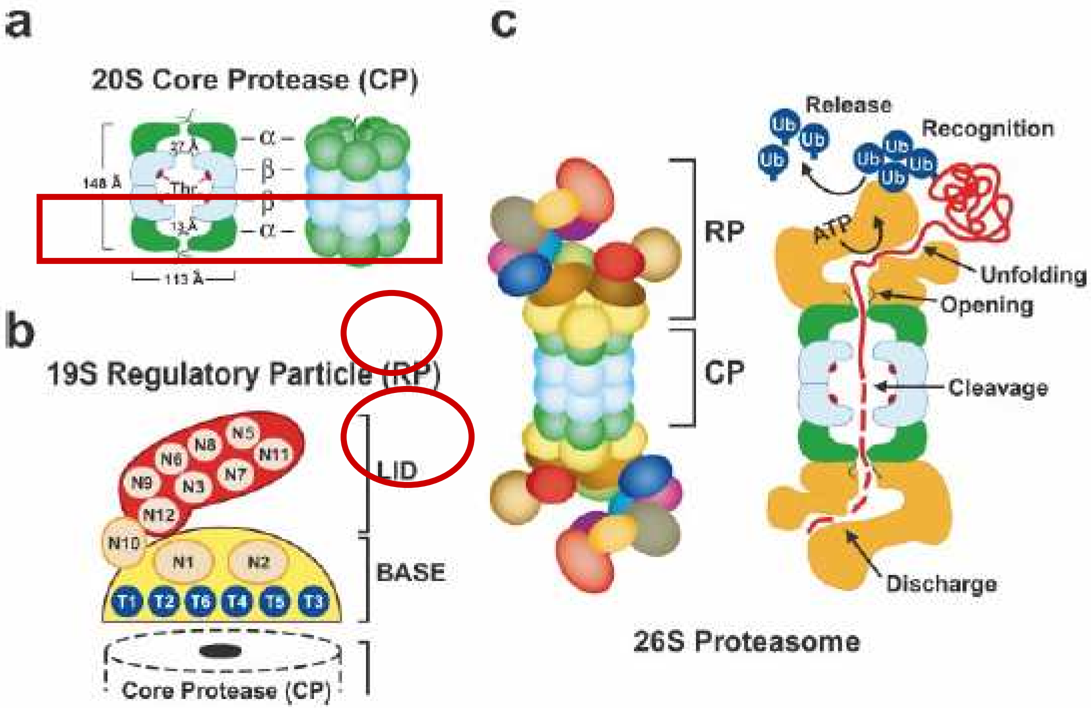
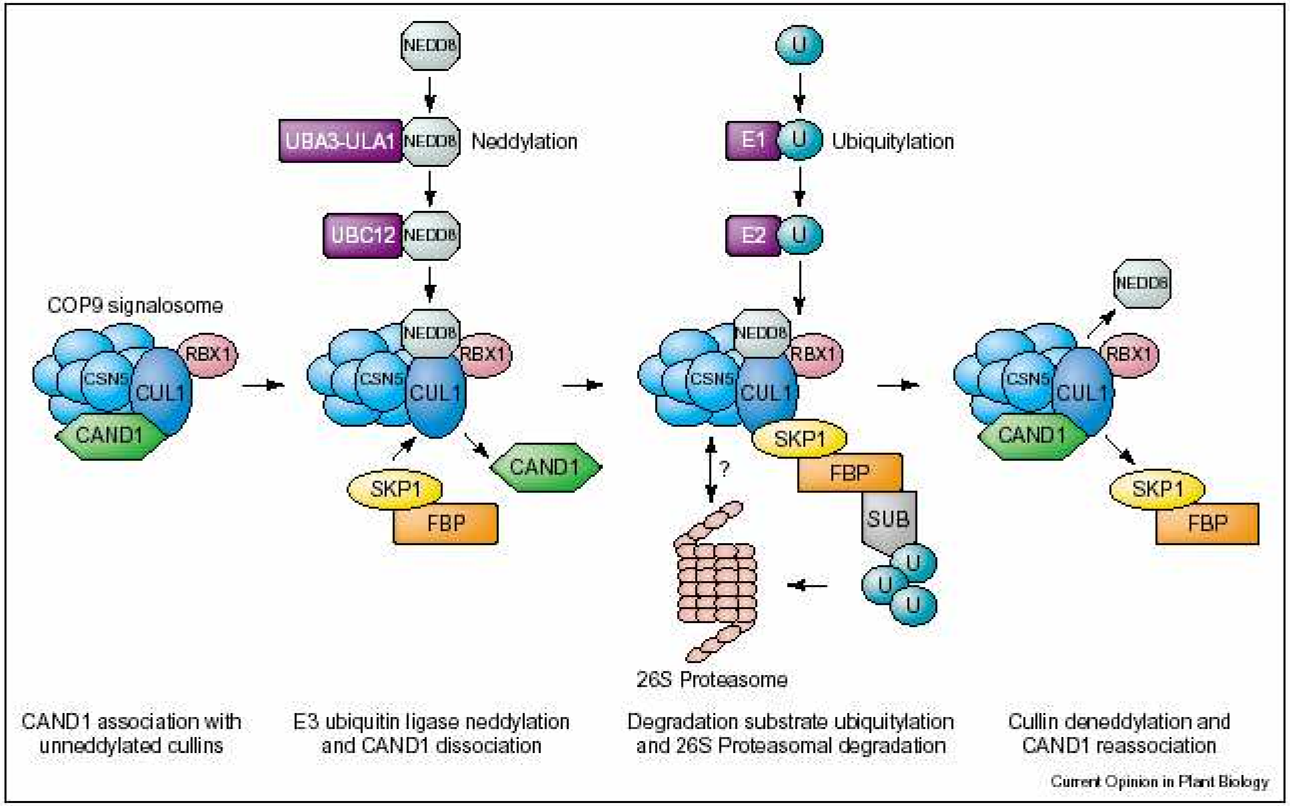


Figure 2 Organization and structure of the 26S proteasome. (*a*) Organization of the 20S core protease (CP) based on the crystal structure of the yeast particle (60). The positions of the active-site threonines are shown. (*b*) Predicted organization of the 19S regulatory particle (RP) based on its subunit interaction map with the Lid and Base shown in red and yellow, respectively (46). The RP AAA-ATPase (RPT) subunits are shown in blue. The RP non-ATPase subunits are shown in orange. (*c*) Diagram of the 26S proteasome combined with the predicted activities of the complex during the degradation of ubiquitinated proteins. Adapted from Reference 154.

Topologie proteasomu zajišťuje, že proteázovou aktivitou nebudou nespecificky zasaženy cytoplasmatické bílkoviny.

U Arabidopsis každá podjednotka = dva geny - to zn. ***různé subtypy proteasomu.***

CSN (COP9 / signalosom) komplex
byl poprvé
objeven u Arabidopsis (viz. dále =
světlo jako signál).
Jeho podjednotky a celková
organizace jsou homologní "víku" RP
proteasomu, ale funguje nezávisle,
jako **regulátor ubiquitinace a
aktivity proteazómu**.



Current Opinion in Plant Biology

General overview of the eukaryotic ubiquitin–proteasome system. Proteolysis substrates (SUB) are recognized by E3 ubiquitin (U) ligases (E3), exemplified here by an SCF-type E3 complex. Poly-ubiquitylation of the bound substrate also requires the activities of E1 ubiquitin-activating enzymes (E1) and E2 ubiquitin-conjugating enzymes (E2). Following poly-ubiquitylation, substrates are degraded in the 26S proteasome [1,3]. The E3 subunit cullin can be modified by NEDD8 conjugation (neddylation) [12]. At the biochemical level, ubiquitylation and neddylation are highly related processes. Cullin neddylation results in the dissociation of the cullin-interacting protein CAND1 [13,14,15•]. This process may allow the cullin–RBX1 complex to associate with specificity components of the E3, such as SKP1–F-box protein (FBP) heterodimers. The COP9 signalosome (CSN) is associated with unneddylated and neddylated cullins [16,17]. Its CSN5 subunit mediates cullin deneddylation and may therefore play a role in controlling E3 complex formation [16–18]. There is some evidence that CSN interacts with subunits of the 26S proteasome [25,74].

Abbreviations

ACS	1-aminocyclopropane-1-carboxylic acid synthase
APC/C	anaphase-promoting complex/cyclosome
BTB/POZ	Bric-a-Brac Tramtrack and Broad Complex/Pox virus and Zinc finger
CAND1	CULLIN-ASSOCIATED NEDDYLATION DISSOCIATED1
COP9	CONSTITUTIVELY PHOTOMORPHOGENIC9
CSN	COP9 signalosome
DCX	DDB1/cullin 4A/X-box
DDB1	DAMAGED DNA-BINDING PROTEIN1
DET1	DEETIOLATED1
E1	ubiquitin-activating enzyme
E2	ubiquitin-conjugating enzyme
E3	ubiquitin ligase
EBF	EIN3-BINDING F-BOX
EIN3	ETHYLENE INSENSITIVE3
EIL1	ETHYLENE INSENSITIVE3-LIKE1
eto2	*ethylene overproducer2*
GA	gibberellic acid
GAI	GIBBERELLIC ACID INSENSITIVE
HY5	LONG HYPOCOTYL5
HYH	LONG HYPOCOTYL5-LIKE
LAF1	LONG AFTER FAR-RED LIGHT1
NEDD8/RUB1	NEURAL PRECURSOR CELL EXPRESSED, DEVELOPMENTALLY DOWNREGULATED 8/ RELATED TO UBIQUITIN1
phyA	phytochrome A
RBX1	RING-BOX1
RGA	REPRESSOR OF *ga1-3*
SCF	SKP1/Cullin1/F-box protein
SKP1	SUPPRESSOR OF KINETOCHORE PROTEIN1
SLY1	SLEEPY1
SPA1	SUPPRESSOR OF PHYTOCHROME A1

CSN kontroluje aktivitu E3 SCF ligázy prostřednictvím neddylace či deneddylace (NEDD8 či RUB1 jsou peptidy podobné ubiquitinu)

a degradaci bílkovin interakcemi s proteasomem (alternativní „víko“).

- **Bílkoviny se mnohonásobně liší poločasem životnosti a ten se prudce mění s měnícím se diferenciačním/regulačním stavem buňky.**

- **Klíčové proteiny signálních drah (včetně transkripčních faktorů) bývají velmi labilní.**

- Přenos signálu
- **Rotlinné hormony a jejich signální dráhy**
  - Signální dráhy založené na regulované proteolýze
    - Komponenty proteolytických degradačních drah
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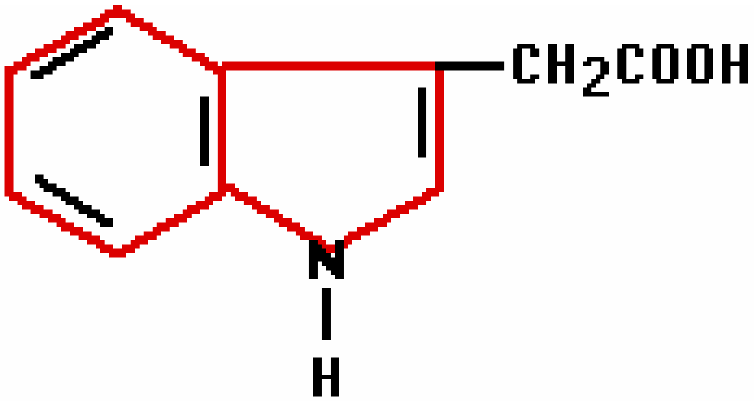
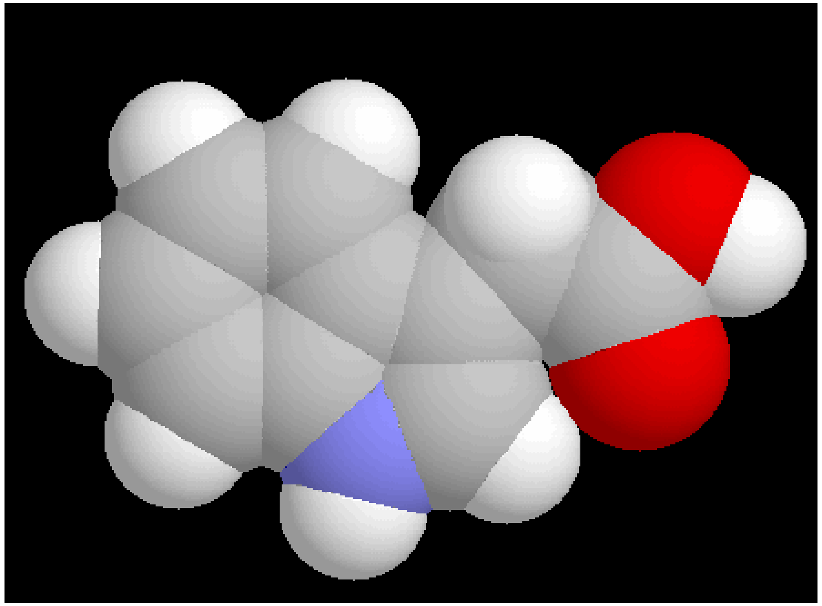
  - Signální dráhy založené na dvoukomponentní dráze
    - Cytokininy
    - Etylen
  - Brassinosteroidy a signalizace pomocí proteinkináz
  - Peptidové hormony
- Rotlinné hormony a jejich signální dráhy
- Signalizace světlem

Auxin



Indole-3-acetic acid (IAA)



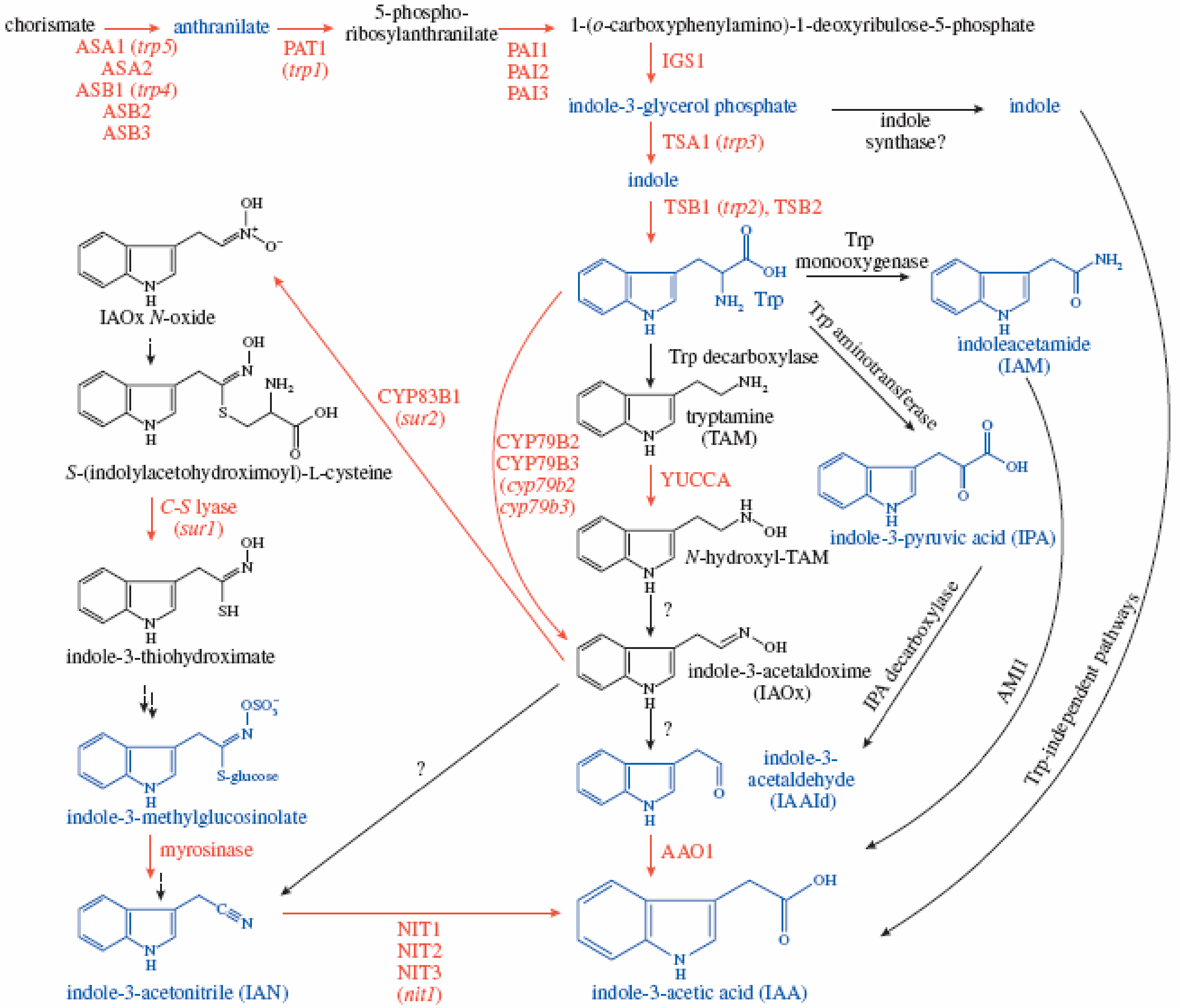


FIG. 2. Potential pathways of IAA biosynthesis in arabidopsis. *De novo* IAA biosynthetic pathways initiate from Trp or Trp precursors. Compounds quantified in arabidopsis are in blue, enzymes for which the arabidopsis genes are identified are in red, and arabidopsis mutants are in lower-case italics. Suggested conversions for which genes are not identified are indicated with question marks. Trp biosynthesis and the P450-catalysed conversion of Trp to IAOx are chloroplastic, whereas many Trp-dependent IAA biosynthetic enzymes are apparently cytoplasmic. See Table 1 for references.

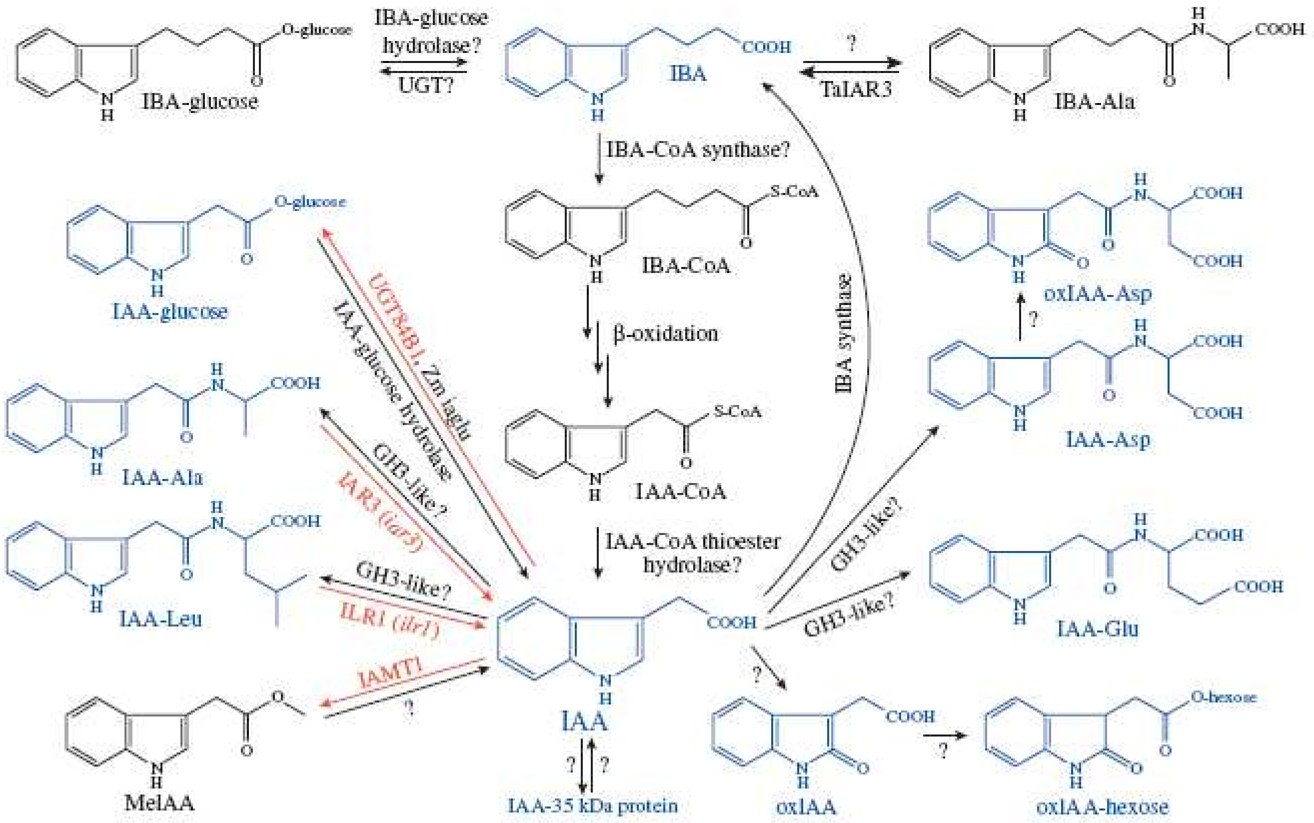
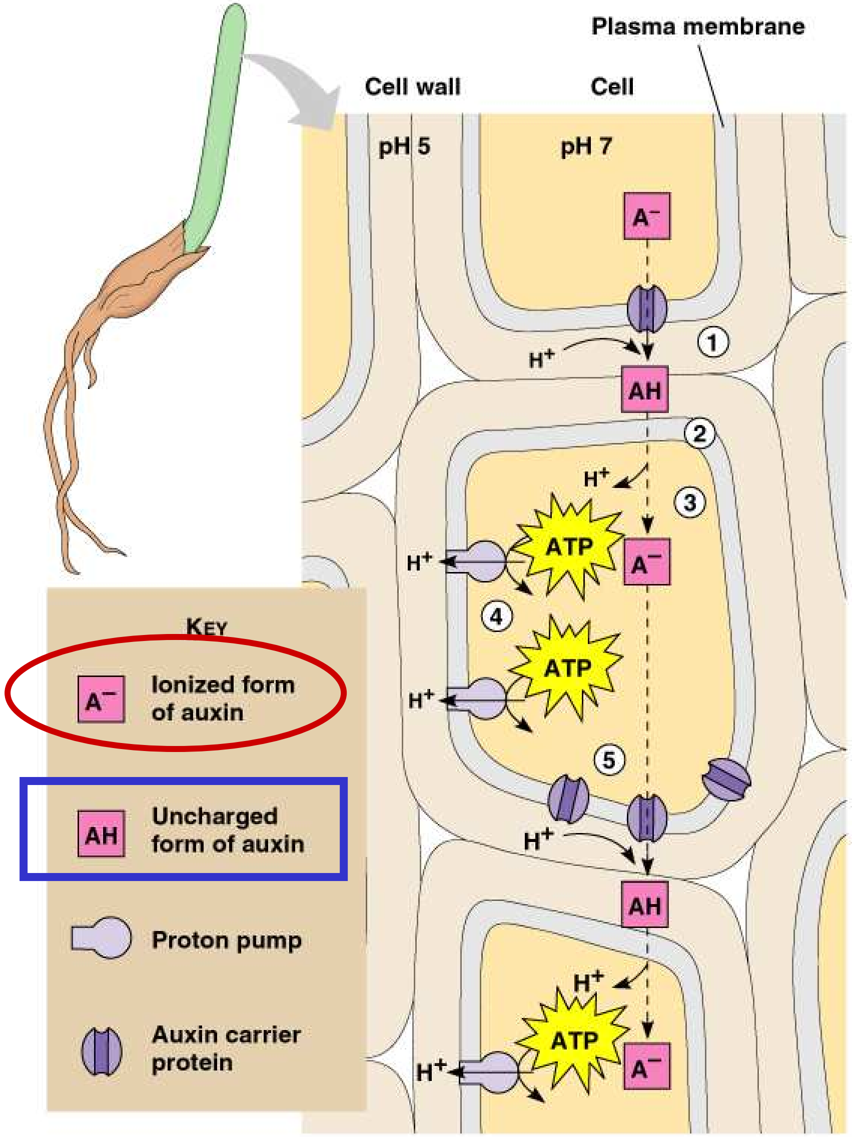


FIG. 3. Potential pathways of IAA metabolism. Compounds quantified in arabidopsis are in blue, enzymes for which the arabidopsis genes are cloned are in red, and arabidopsis mutants are in lower-case italics. Suggested conversions for which plant genes are not identified are indicated with question marks. A family of amidohydrolases that apparently resides in the ER lumen can release IAA from IAA conjugates. ILR1 has specificity for IAA–Leu (Bartel and Fink, 1995), whereas IAR3 prefers IAA–Ala (Davies *et al.*, 1999). Maize (Zm) iaglu and arabidopsis UGT84B1 esterify IAA to glucose (Szerszen *et al.*, 1994; Jackson *et al.*, 2001); the enzymes that form and hydrolyse IAA–peptides have not been identified. IBA is likely to be converted to IAA–CoA in a peroxisomal process that parallels fatty acid β-oxidation to acetyl-CoA (Bartel *et al.*, 2001). IAA can be inactivated by oxidation (oxIAA) or by formation of non-hydrolysable conjugates (IAA–Asp and IAA–Glu). IAA–amino acid conjugates can be formed by members of the GH3/JAR1 family (Staswick *et al.*, 2002, 2005). OxIAA can be conjugated to hexose, and IAA–Asp can be further oxidized (Östin *et al.*, 1998). IAMT1 can methylate IAA (Zubieta *et al.*, 2003), but whether this activates or inactivates IAA is not known. IBA and hydrolysable IAA conjugates are presumably derived from IAA; biosynthesis of these compounds may contribute to IAA inactivation. Formation and hydrolysis of IBA conjugates may also contribute to IAA homeostasis; the wheat (Ta) enzyme TaIAR3 hydrolyses IBA–Ala (Campanella *et al.*, 2004).

Hladina aktivního auxinu je regulována modifikacemi/konjugacemi - reverzibilními i ireverzibilními.

Polar transport of Auxin



Chemoosmotická teorie transportu auxinu potvrzena objevem AUX1 vtokového a PIN výtokových přenašečů a jejich polární lokalizace. Transport jde ovšem také pře ABC transportery (PGP, závislé na ATP) a je možná také sekrece z IAA naplněných váčků jako v případě neurotransmiterů.

Auxin - přenos signálu

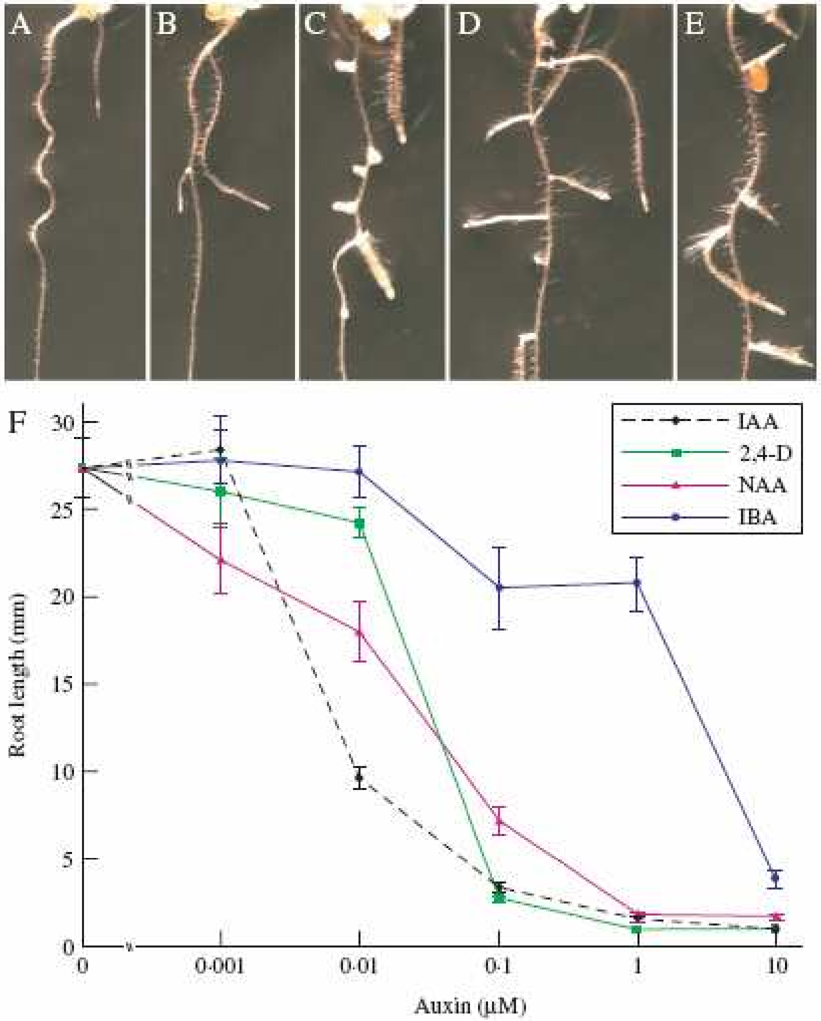
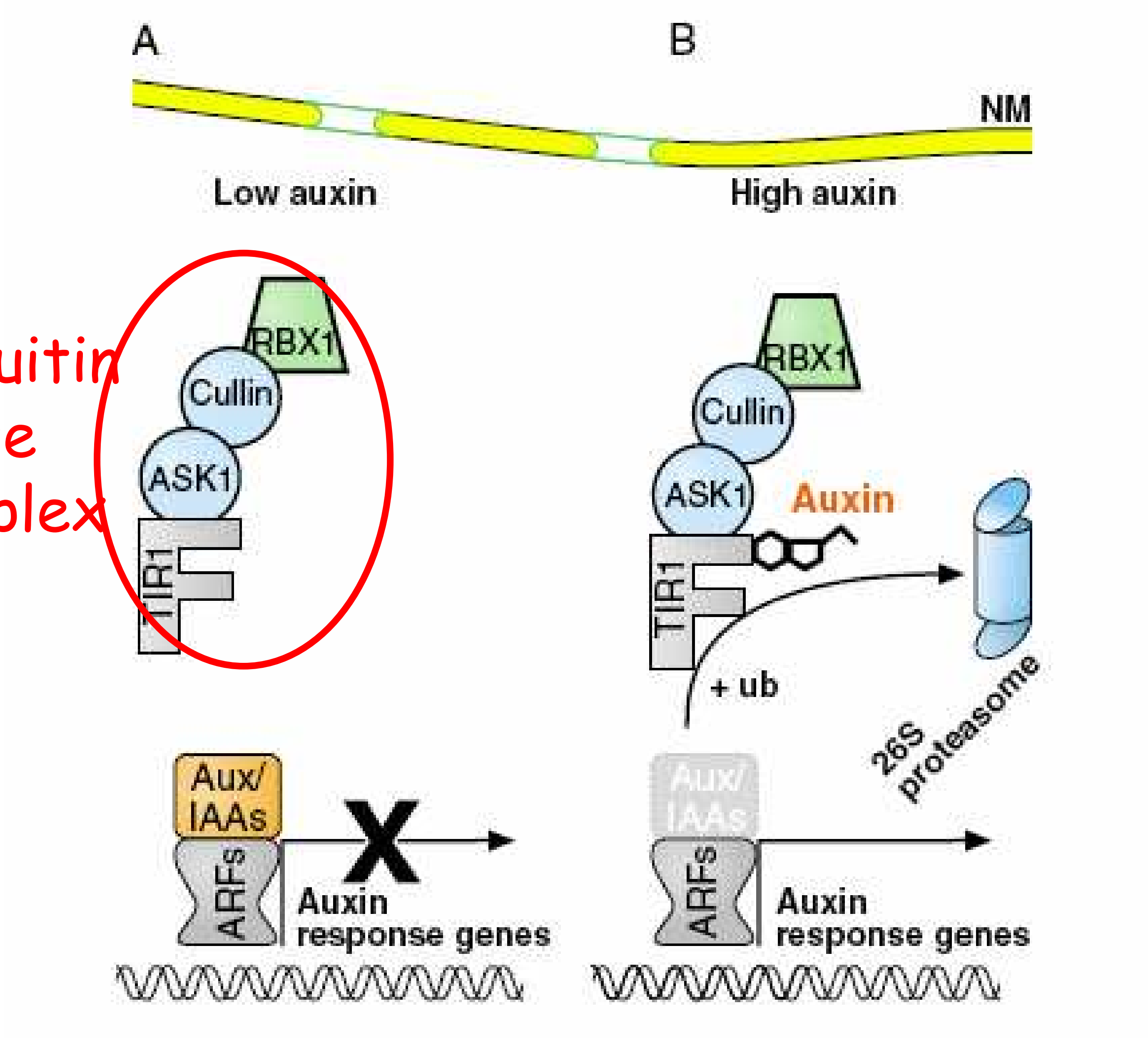


FIG. 1. Auxins promote lateral root formation and inhibit root elongation. *Arabidopsis thaliana* Col-0 ecotype plants were grown on unsupplemented medium (Haughn and Somerville, 1986) for 6 d, then transferred to unsupplemented medium (A) or medium supplemented with 10 nM IAA (B), 100 nM 2,4-D (C), 100 nM NAA (D) or 10 µM IBA (E) and grown for 6 additional days. (F) Plants were grown on various concentrations of natural and synthetic auxins for 8 d. Points represent means ± standard error, $n \geq 8$. All plants were grown at 22 °C under yellow light.

Fungují PINy jako senzory?
Co je receptorem auxinu???

- **F-BOX SCF-E3 LIG. KOMPLEXU TIR1 JE RECEPTOREM AUXINU.**

Auxin signal transduction



E3
ubiquitin
ligase
complex

Mutanti *monopteros(mp)* a *bodenlos(bdl)* nemají kořen.

- MP kóduje člena ARF (auxin response transcript. factors) rodiny transkripčních aktivátorů **ARF5**. (Prosím neplést s malými GTPázami!).

- BDL kóduje člena rodiny inhibitorů ARFů AUX/IAA, **IAA12**. Tyto bílkoviny se vyznačují minutovým poločasem životnosti. S ARFy tvoří heterodimery a tak blokují jejich aktivitu. Transkripce AUX/IAA genů je stimulována IAA (okamžitě) - tak byly objeveny.

ABP1

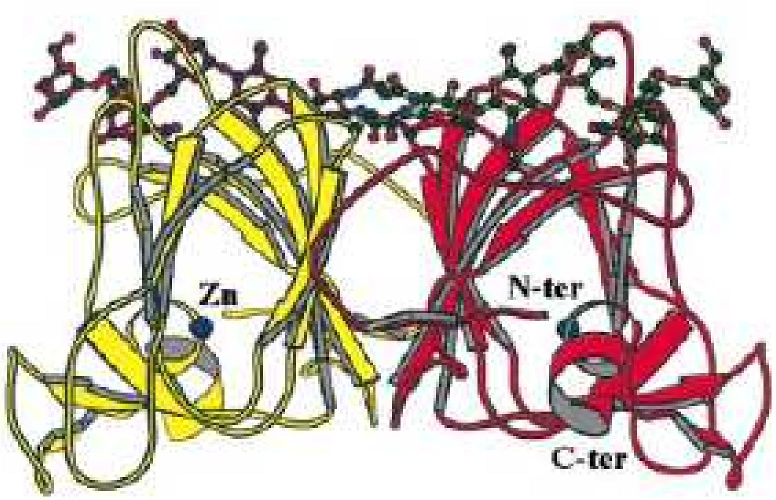
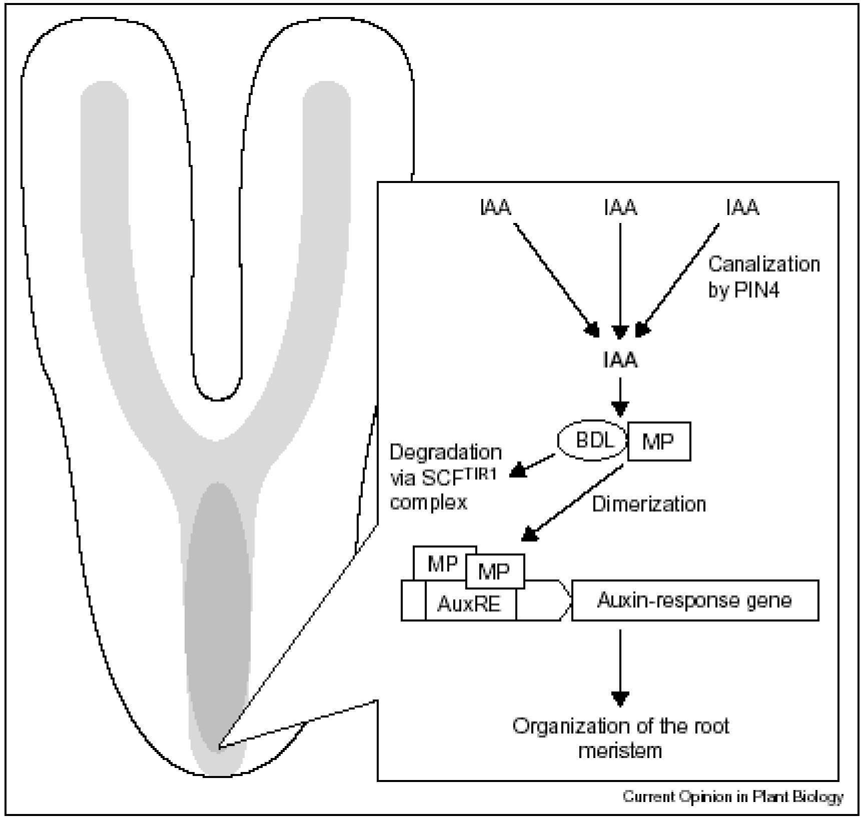


FIG. 4. Ribbon diagram showing the structure of an ABP1 dimer. The β-sheets are shown as broad arrows. ABP1 is *N*-glycosylated and some of the sugar residues are shown at the top of each monomer. Three C-terminal residues were not resolved and would extend the α-helices at the foot of the molecules. The zinc ion is shown in green. Reproduced from The EMBO Journal, Vol. 21 No. 12, pp. 2877–2885, 2002, with permission from Woo *et al.* (2002), Oxford University Press.

Auxin Binding Protein1 byl objeven biochemicky je lokalizován převážně do membrány ER, ale malá frakce "uniká" a je aktivní na povrchu buňky. Pokus s blokováním reakce protoplastů na IAA protilát. prokázal jeho podíl na reakci na IAA.

Řada mutantů s postiženým proteasomem (a také CSN) má auxinový fenotyp - bývají auxin rezistentní.

Model regulace ARFů AUX/IAA a jejich auxinem stimulovanou proteolýzou.



Current Opinion in Plant Biology

Aux/IAA and ARF proteins act together to mediate auxin responses in the embryo. Auxin is canalized in the embryonic root by PIN4. When a certain auxin concentration is reached BDL–MP heterodimers dissociate. BDL is then degraded via the SCF^{TIR1} (Skp1/Cullin/F-box–Toll/interleukin1 receptor) complex; whereas MP builds homodimers that bind to auxin-responsive elements (AuxRE) on the promoters of unknown auxin-responsive genes whose expression thus is turned 'on'. Correct BDL–MP signalling is necessary for the organization of the root meristem. The expression patterns of MP (light gray) and BDL (dark gray) are shown in a torpedo-stage embryo.

BDL a další AUX/IAA geny jsou také cílem transkripční stimulace.

De novo syntéza AUX/IAA represorů umožňuje **potlačení signálu** = atenuaci.

Podobně kinázy fungují jako přenašeče signálů jen díky proti-působícím fosfatázám.

a tak je to i s Ca2+ a dalšími….

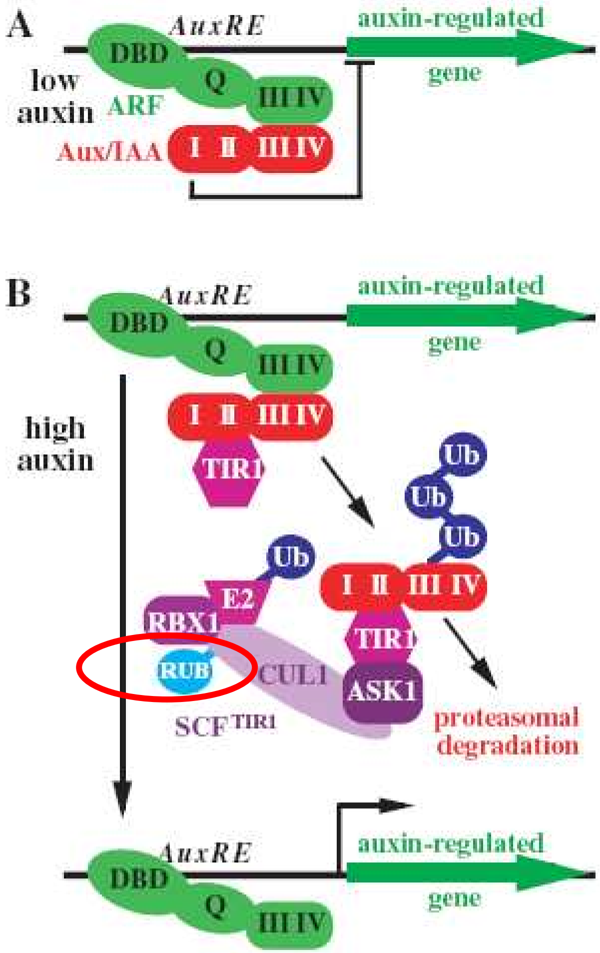
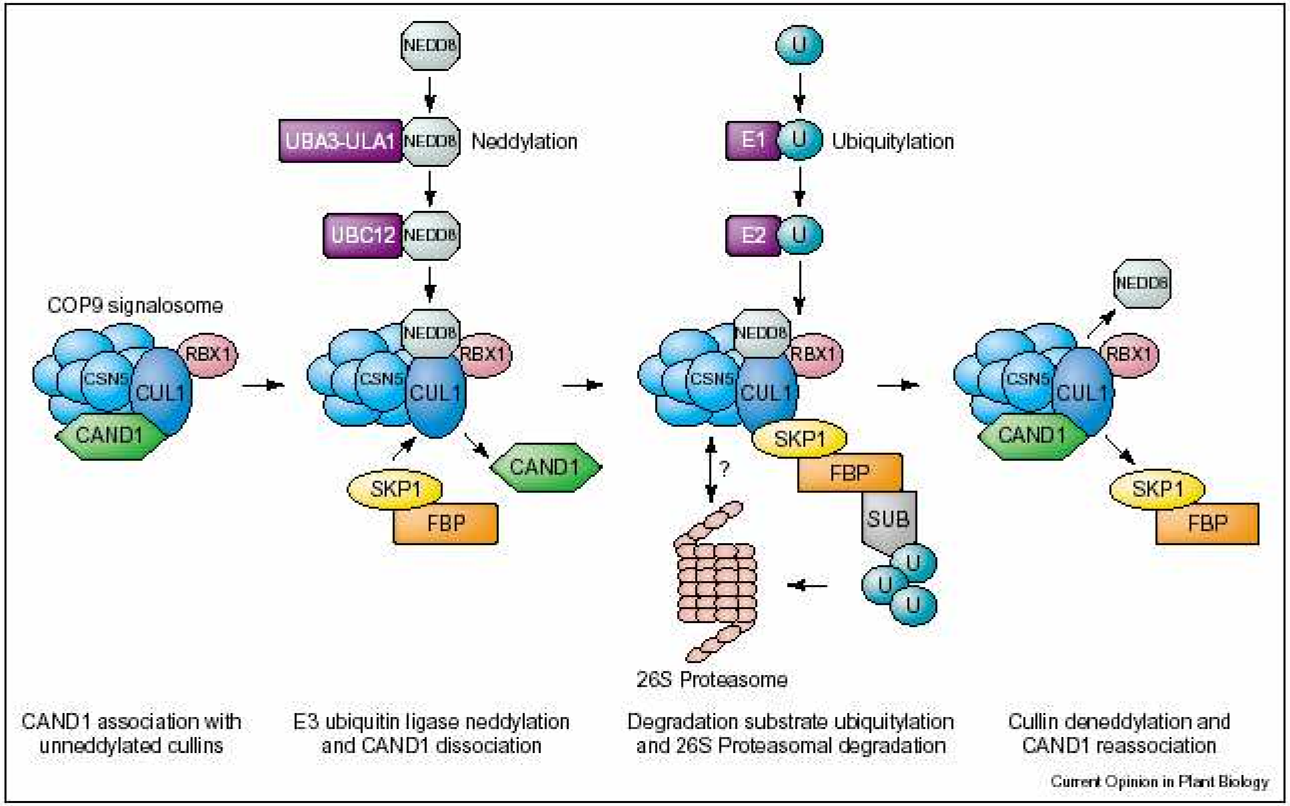


FIG. 4. The SCF^{TIR1} relieves Aux/IAA repression of activating ARFs. (A) An activating ARF protein (green) binds an *AuxRE* promoter element via an N-terminal DNA binding domain (DBD). Under low-auxin conditions, an Aux/IAA repressor (red) binds the activating ARF via heterodimerization between Aux/IAA and ARF domains III and IV. (B) Auxin promotes Aux/IAA domain II-TIR1 association, bringing the Aux/IAA protein to the SCF^{TIR1} complex (purple) for ubiquitination (Ub) and subsequent destruction by the 26S proteasome. The activating ARF, with a Gln-rich (Q) middle domain, is then freed to promote auxin-induced gene expression.

TIR1 a spol. jsou regulovány neddylací/RUB1 modifikací a tedy také CSN.



Current Opinion in Plant Biology

General overview of the eukaryotic ubiquitin–proteasome system. Proteolysis substrates (SUB) are recognized by E3 ubiquitin (U) ligases (E3), exemplified here by an SCF-type E3 complex. Poly-ubiquitylation of the bound substrate also requires the activities of E1 ubiquitin-activating enzymes (E1) and E2 ubiquitin-conjugating enzymes (E2). Following poly-ubiquitylation, substrates are degraded in the 26S proteasome [1,3]. The E3 subunit cullin can be modified by NEDD8 conjugation (neddylation) [12]. At the biochemical level, ubiquitylation and neddylation are highly related processes. Cullin neddylation results in the dissociation of the cullin-interacting protein CAND1 [13,14,15*]. This process may allow the cullin–RBX1 complex to associate with specificity components of the E3, such as SKP1–F-box protein (FBP) heterodimers. The COP9 signalosome (CSN) is associated with unneddylated and neddylated cullins [16,17]. Its CSN5 subunit mediates cullin deneddylation and may therefore play a role in controlling E3 complex formation [16–18]. There is some evidence that CSN interacts with subunits of the 26S proteasome [25,74].

NEDD8=RUB1

Abbreviations	
ACS	1-aminocyclopropane-1-carboxylic acid synthase
APC/C	anaphase-promoting complex/cyclosome
BTB/POZ	Bric-a-Brac Tramtrack and Broad Complex/Pox virus and Zinc finger
CAND1	CULLIN-ASSOCIATED NEDDYLATION DISSOCIATED1
COP9	CONSTITUTIVELY PHOTOMORPHOGENIC9
CSN	COP9 signalosome
DCX	DDB1/cullin 4A/X-box
DDB1	DAMAGED DNA-BINDING PROTEIN1
DET1	DEETIOLATED1
E1	ubiquitin-activating enzyme
E2	ubiquitin-conjugating enzyme
E3	ubiquitin ligase
EBF	EIN3-BINDING F-BOX
EIN3	ETHYLENE INSENSITIVE3
EIL1	ETHYLENE INSENSITIVE3-LIKE1
eto2	*ethylene overproducer2*
GA	gibberellic acid
GAI	GIBBERELLIC ACID INSENSITIVE
HY5	LONG HYPOCOTYL5
HYH	LONG HYPOCOTYL5-LIKE
LAF1	LONG AFTER FAR-RED LIGHT1
NEDD8/RUB1	NEURAL PRECURSOR CELL EXPRESSED, DEVELOPMENTALLY DOWNREGULATED 8/ RELATED TO UBIQUITIN1
phyA	phytochrome A
RBX1	RING-BOX1
RGA	REPRESSOR OF *ga1-3*
SCF	SKP1/Cullin1/F-box protein
SKP1	SUPPRESSOR OF KINETOCHORE PROTEIN1
SLY1	SLEEPY1
SPA1	SUPPRESSOR OF PHYTOCHROME A1

- Přenos signálu
- **Rotlinné hormony a jejich signální dráhy**
  - Signální dráhy založené na regulované proteolýze
    - Komponenty proteolytických degradačních drah
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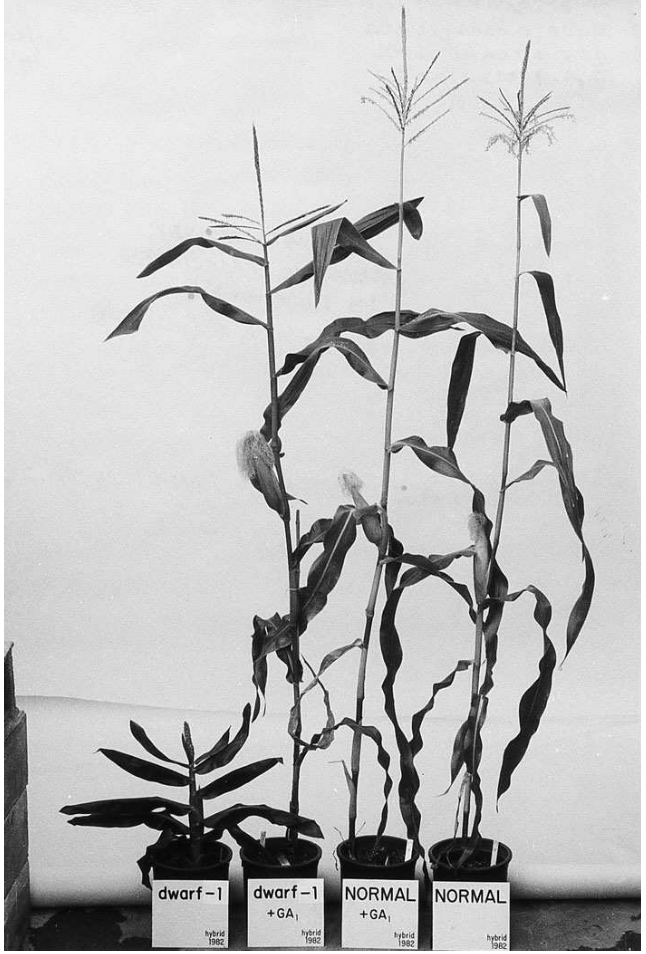
    - Giberellin
    - Jasmonát (?)
  - Signální dráhy založené na dvoukomponentní dráze
    - Cytokininy
    - Etylen
  - Brassinosteroidy a signalizace pomocí proteinkináz
  - ABA = mnoho funkcí a receptorů
  - Peptidové hormony
- Rotlinné hormony a jejich signální dráhy
- Signalizace světlem

Effects of Gibberellins

- General cell elongation.
- Breaking of dormancy in some plants which require stratification or light to induce germination.
- Promotion of flowering.
- Transport is non-polar, bidirectional producing general responses.

Discovered in association with Foolish disease of rice (*Gibberella fujikuroi)*

Effect of Gibberellins on growth and development

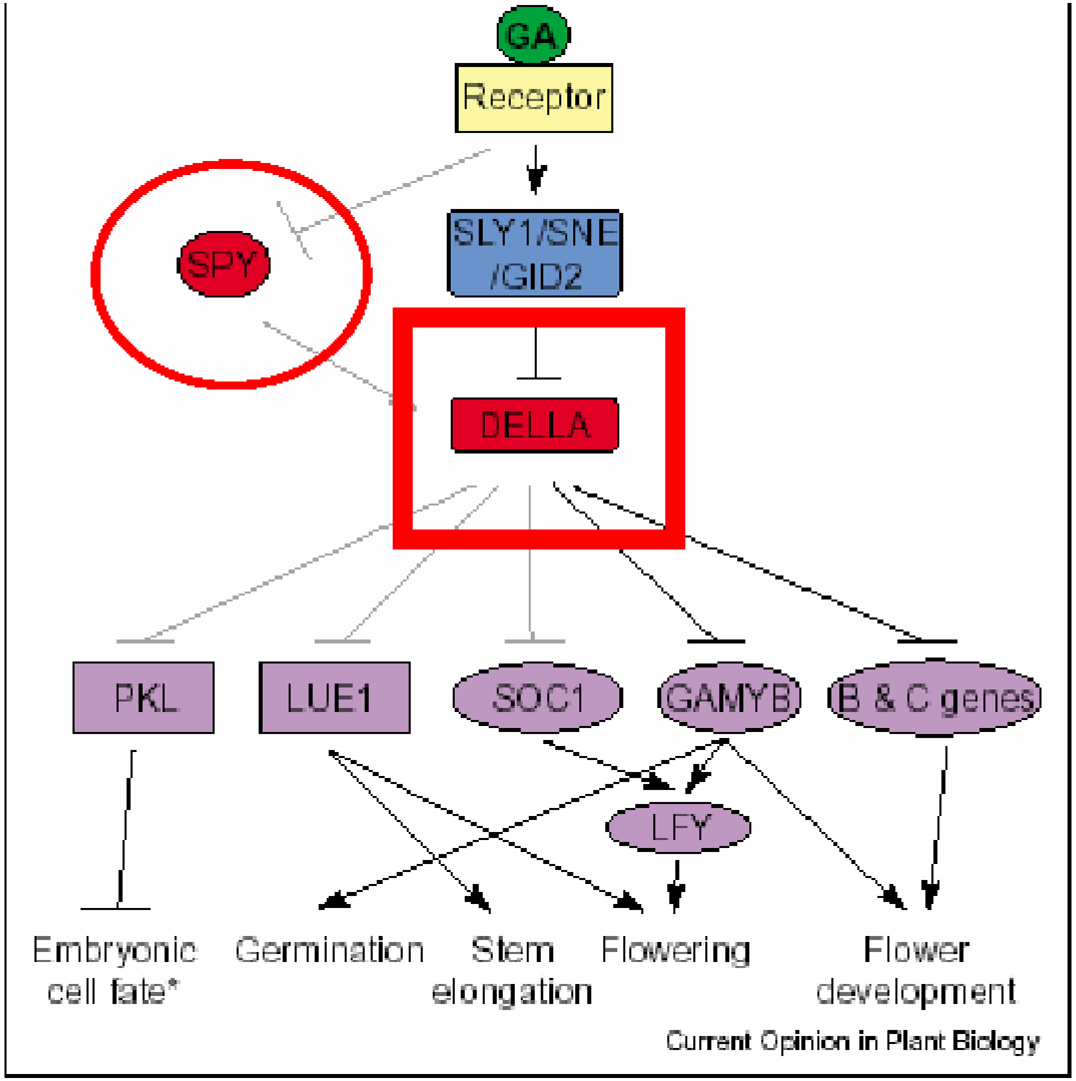


d1 *d1* WT WT
+GA_1 +GA_1

GA_1 stimulates stem elongation in corn dwarf mutant, but has no effect on tall WT

Cabbage, a long-day plant, remains a rosette under short day, but it can be induced to bolt and flower by applications of GAs

Signální dráha giberelinů



Model of the GA signaling pathway. Ovals represent transcription factors, gray lines indicate hypothesized interactions. Arrows and T-bars indicate direct or indirect activation and inhibition, respectively. B genes encode PI and AP3; the C gene in this pathway encodes AGAMOUS; DELLA includes RGA, GAI, RGLs, SLR1, SLN1, and other orthologs. *PKL inhibits embryonic cell fate during post-embryonic development.

DELLA – represory GIBBERELINové odpovědi

GA SIGNALING C-1

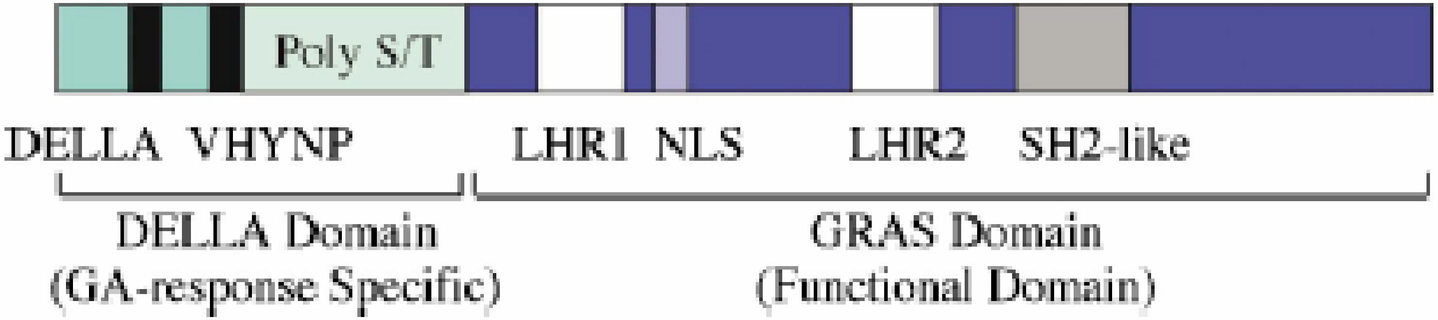


Figure 1 The DELLA subfamily of GRAS proteins. The unique N-terminal "DELLA domain" of the DELLA proteins contains two highly conserved motifs (named DELLA and VHYNP) and a Poly S/T region. The C-terminal region is conserved among all GRAS family members. Poly S/T, polymeric Ser and Thr; LHR, Leu heptad repeat; NLS, nuclear localization signal.

nemají zřetelnou DNA vazebnou doménu a interagují tedy pravděpodobně s DNA prostřednictvím jiných bílkovin. Arabidopsis = RGA, GAI a 3xRGL

Rýže = **SLR1**

SPY negativní regulátor

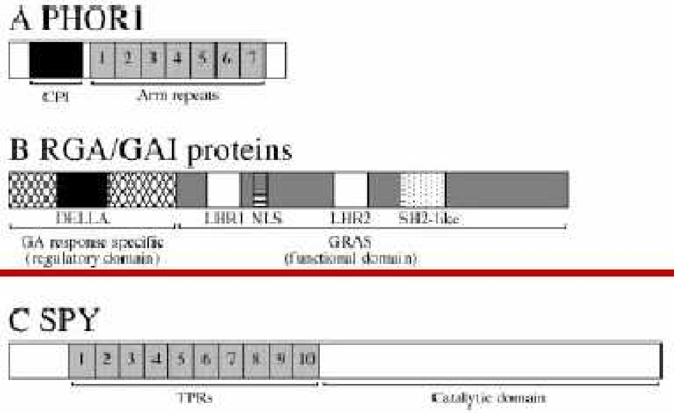


Figure 2. Schemes of PHOR1, RGA/GAI, and SPY.

(A) PHOR1 contains a CPI domain, which is a GA-repressible cytosolic retention signal, and the armadillo repeats, which function in nuclear localization and may allow it to interact with transcription factors.

(B) RGA/GAI proteins contain a highly conserved C-terminal region that is shared among all GRAS family members. The nuclear localization signal is absent in some of the GRAS members (Pysh et al., 1999; Schumacher et al., 1999; Bolle et al., 2000). RGA/GAI and other nuclear localization signal–containing GRAS family members are likely to function as transcriptional regulators. The N-terminal region of the RGA/GAI proteins is required for the inactivation of these proteins by the GA signal.

(C) SPY proteins contain two conserved domains: the TPR domain, which is believed to interact with other proteins; and the catalytic domain, which post-translationally modifies proteins with GlcNAc. Arm, armadillo; LHR, Leu heptad repeat; NLS, nuclear localization signal.

SPY (SPINDLY,Arabidopsis)=Ser/Thr O-N-acetylglukosamin (GlcNAc) transferáza je **negativním** regulátorem GA signálu. **Připojením GlcNAc na Ser či Thr interferuje s fosforylací, která předchází ubiquitinaci – stabilizuje inhibitory GA signálu.**

Patří mezi geny, které šlechtitelé nevědomky modifikovali při šlechtění na zkrácení stébla = **Zelená revoluc** 60 léta 20 století.

Green revolution

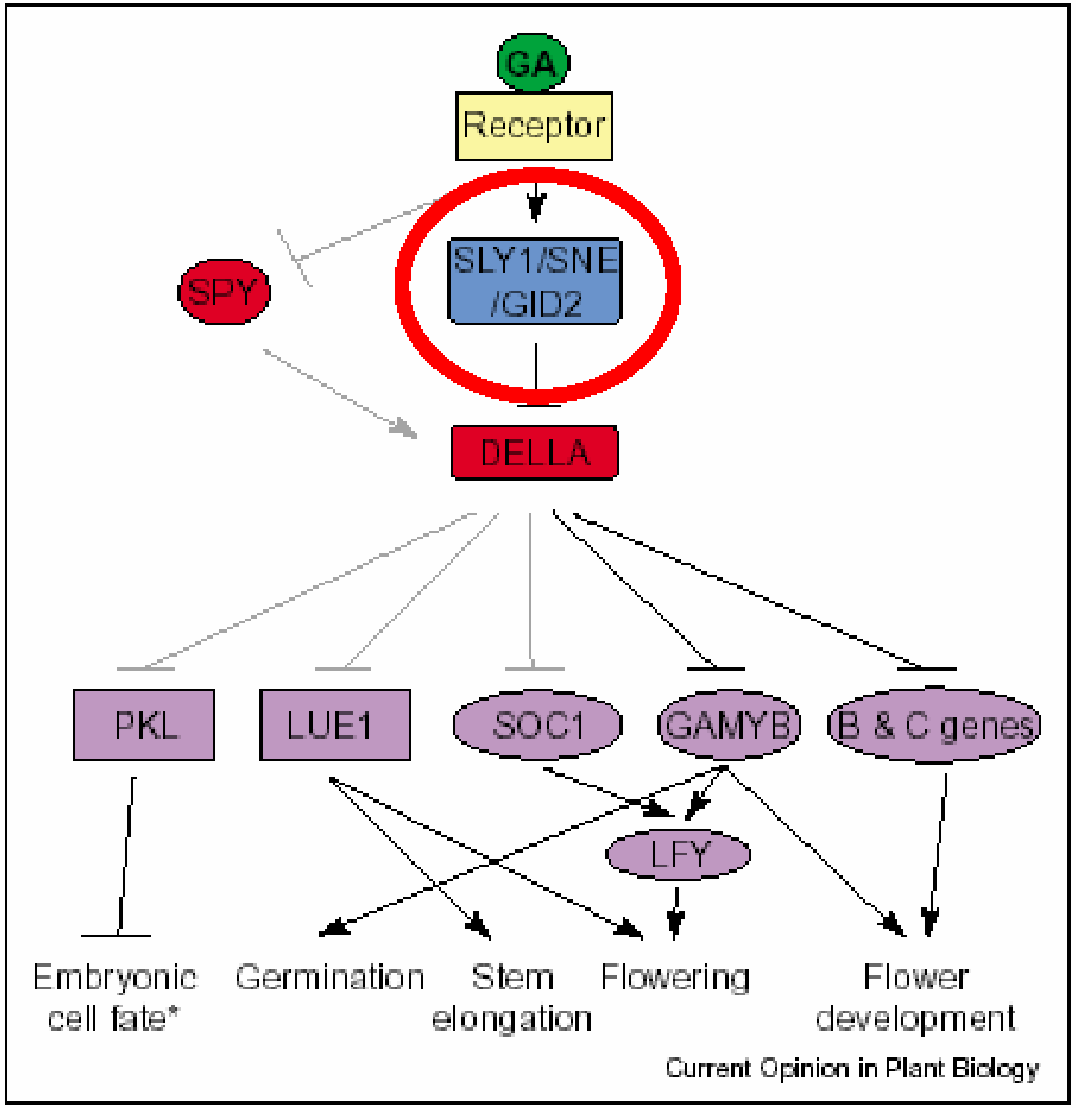
Fig. 1. Semi-dwarf rice cultivars and their tall isogenic lines. From left to right: Dee-geo-woo-gen (dwarf *indica* cultivar), woo-gen (tall equivalent), Calrose 76 (dwarf *japonica* cultivar), Calrose (tall equivalent).

Norman Borlaugh

***Indica* and *japonica* rice:** Subspecies of rice (*Oryza sativa*). *Indica* is grown mostly in tropical and subtropical regions. It tends to be tall and, therefore, prone to lodging. *Japonica* rice is grown typically in temperate regions and is shorter than *indica* rice.

Semi-dwarf: A mutant variety that is moderately reduced in height relative to the normal plant. It has been defined as having a height that lies between 50–100% that of the normal plant, whereas a dwarf is <50% the height of the normal plant [26].

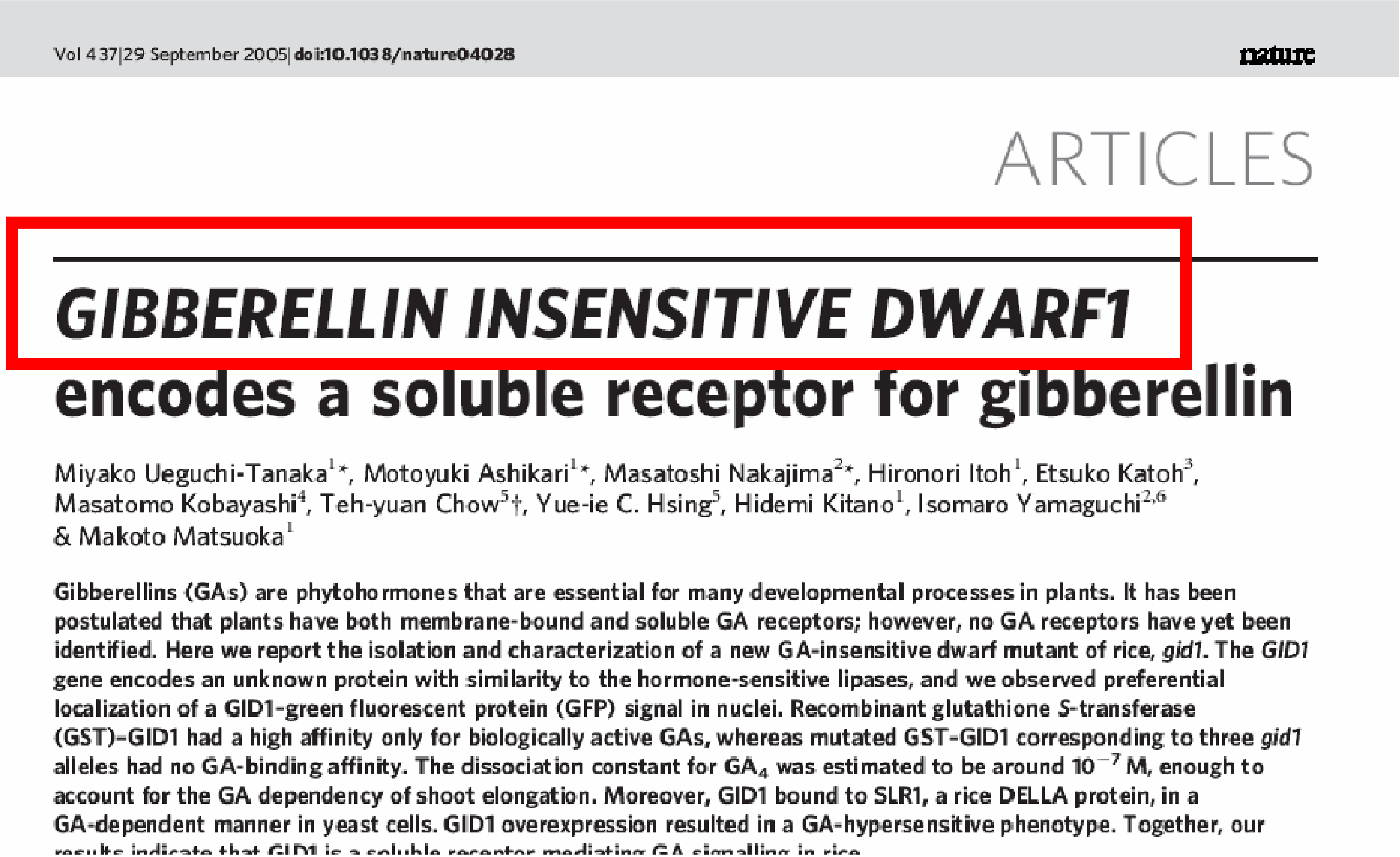
Pozitivními regulátory GA signálu jsou GID2(GA insensitive dwarf2) u rýže a SLY1 (SLEEPY1) u Arabidopsis



Current Opinion in Plant Biology

Oba kodují vysoce homologní **Fbox podjednotku SCF E3** ligázového komplexu.

Receptorem GA v cytoplasmě je GID1 – nový typ bílkovin příb. lipázám objeven u rýže.



Vol 437|29 September 2005| **doi:10.1038/nature04028**

nature

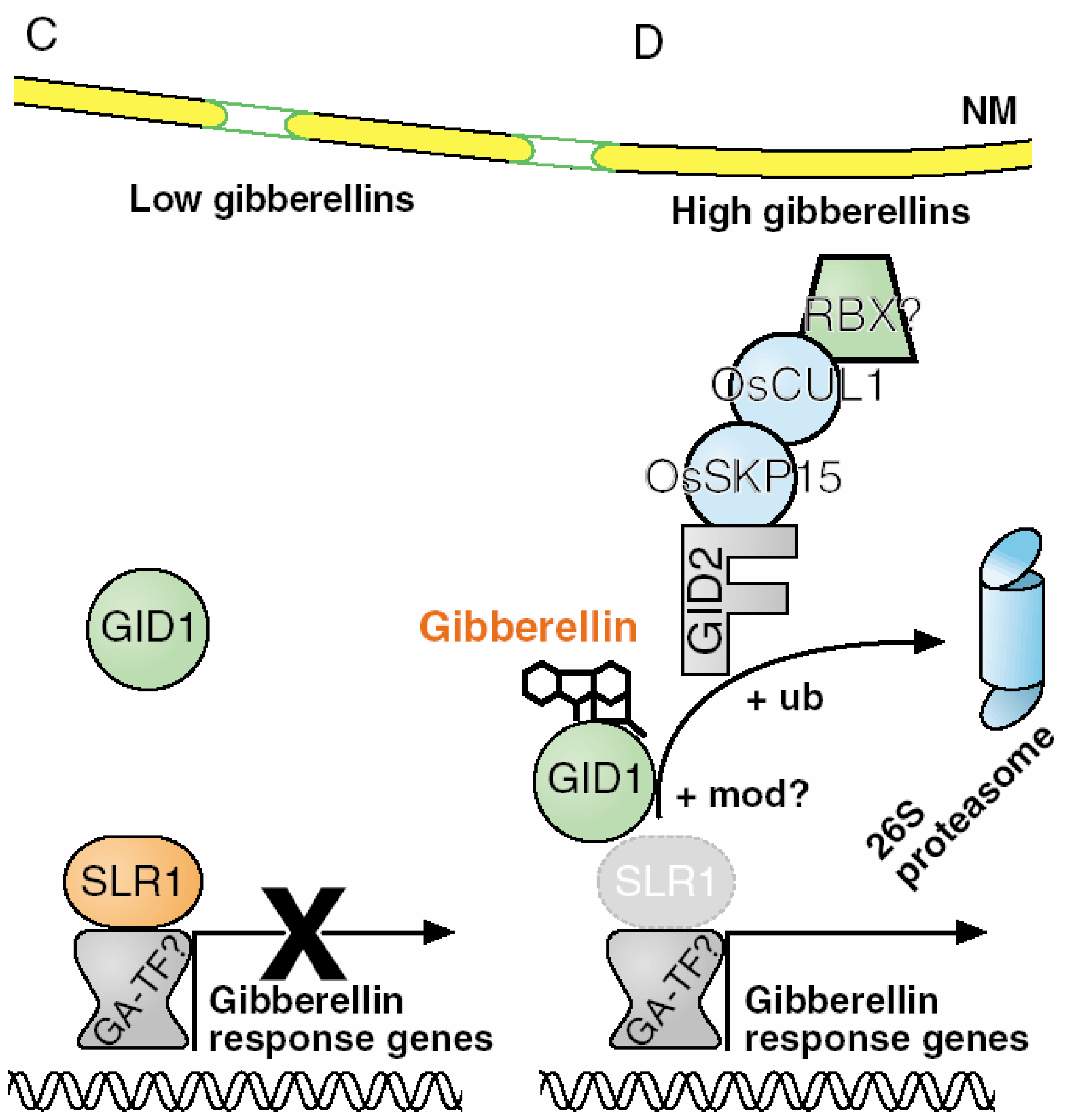
ARTICLES

GIBBERELLIN INSENSITIVE DWARF1 encodes a soluble receptor for gibberellin

Miyako Ueguchi-Tanaka[1]*, Motoyuki Ashikari[1]*, Masatoshi Nakajima[2]*, Hironori Itoh[1], Etsuko Katoh[3], Masatomo Kobayashi[4], Teh-yuan Chow[5]†, Yue-ie C. Hsing[5], Hidemi Kitano[1], Isomaro Yamaguchi[2,6] & Makoto Matsuoka[1]

Gibberellins (GAs) are phytohormones that are essential for many developmental processes in plants. It has been postulated that plants have both membrane-bound and soluble GA receptors; however, no GA receptors have yet been identified. Here we report the isolation and characterization of a new GA-insensitive dwarf mutant of rice, *gid1*. The *GID1* gene encodes an unknown protein with similarity to the hormone-sensitive lipases, and we observed preferential localization of a GID1-green fluorescent protein (GFP) signal in nuclei. Recombinant glutathione *S*-transferase (GST)-GID1 had a high affinity only for biologically active GAs, whereas mutated GST-GID1 corresponding to three *gid1* alleles had no GA-binding affinity. The dissociation constant for GA_4 was estimated to be around 10^{-7} M, enough to account for the GA dependency of shoot elongation. Moreover, GID1 bound to SLR1, a rice DELLA protein, in a GA-dependent manner in yeast cells. GID1 overexpression resulted in a GA-hypersensitive phenotype. Together, our results indicate that GID1 is a soluble receptor mediating GA signalling in rice.

Signální dráha GA - analogická k auxinu



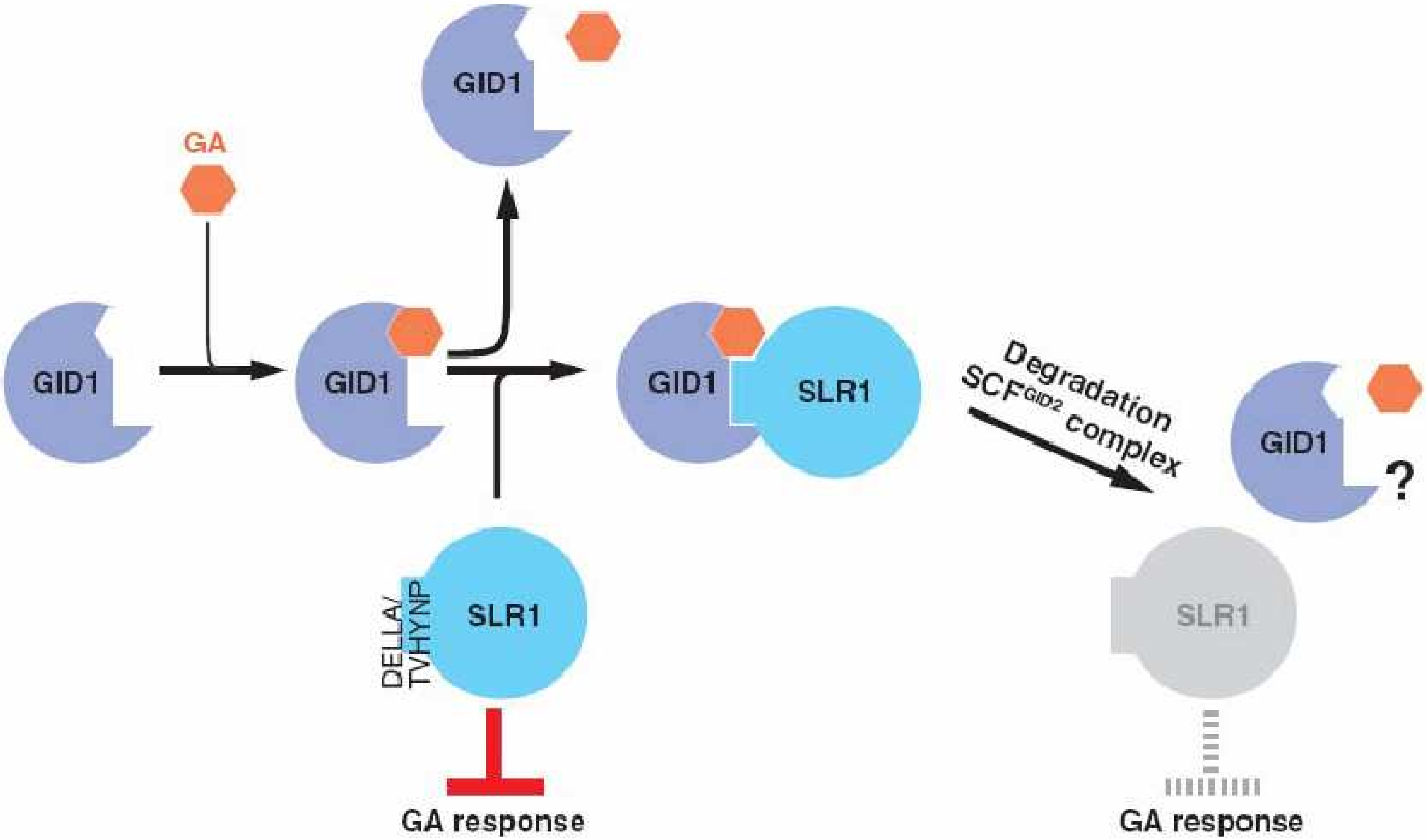


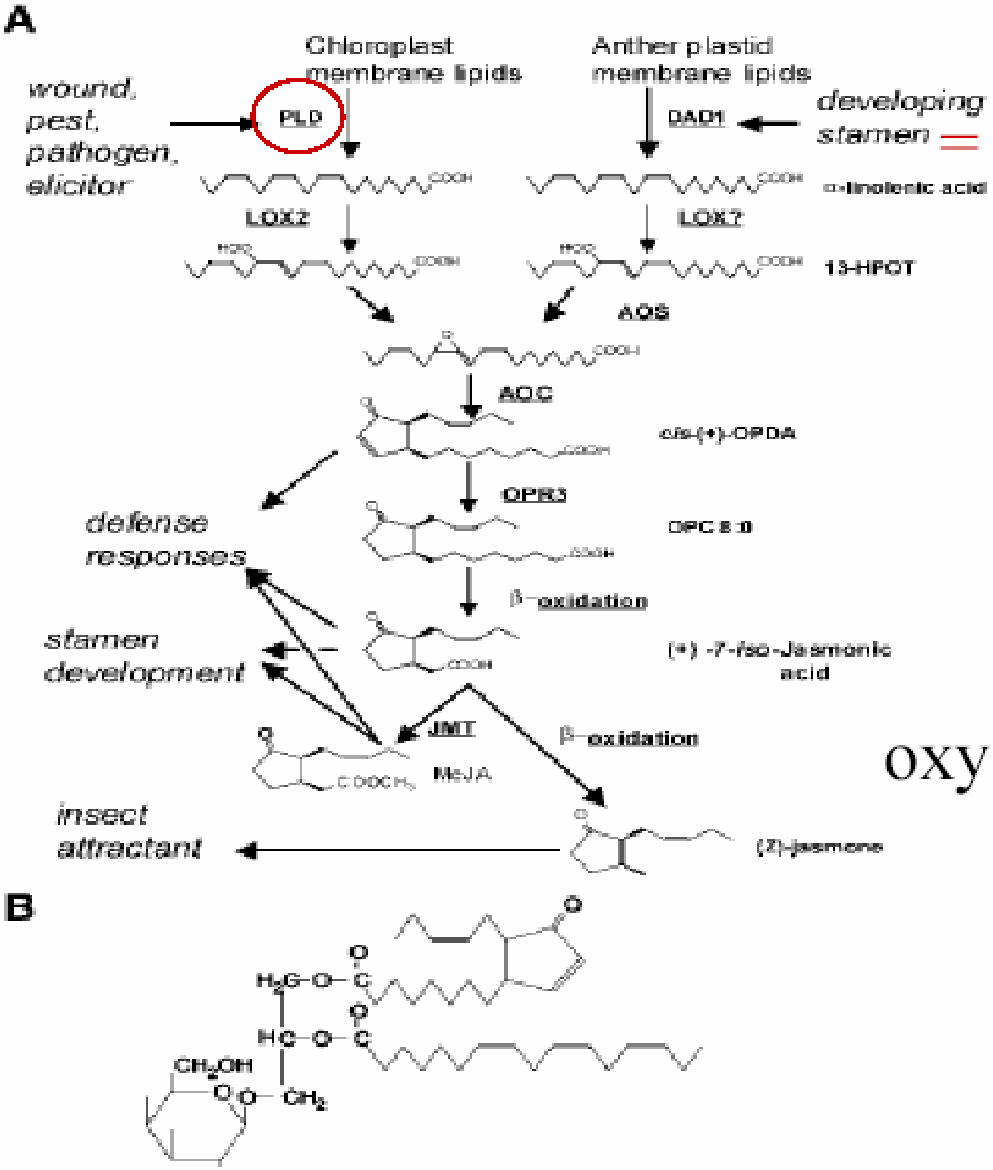
Figure 1

Model of gibberellin signaling in rice. Under low GA concentrations, SLR1 represses the GA responses. Under high GA concentrations, a soluble receptor, GID1, binds to GA; however, the binding is unstable and easily dissociates from the other. The GID1-GA complex specifically interacts with SLR1 at the site of DELLA and TVHYNP domains. The triple complex composed of GID1-GA-SLR1 is stable and does not easily dissociate. The triple complex is in turn targeted by the SCF^{GID2} complex and the SLR1 protein is degraded by the 26S proteosome, which releases the repressive state of GA responses.

(met)JA

kyselina (met)jasmonová

Reakce na poranění a interakce s patogeny (často v souhře s kys. salicylovo - SA)

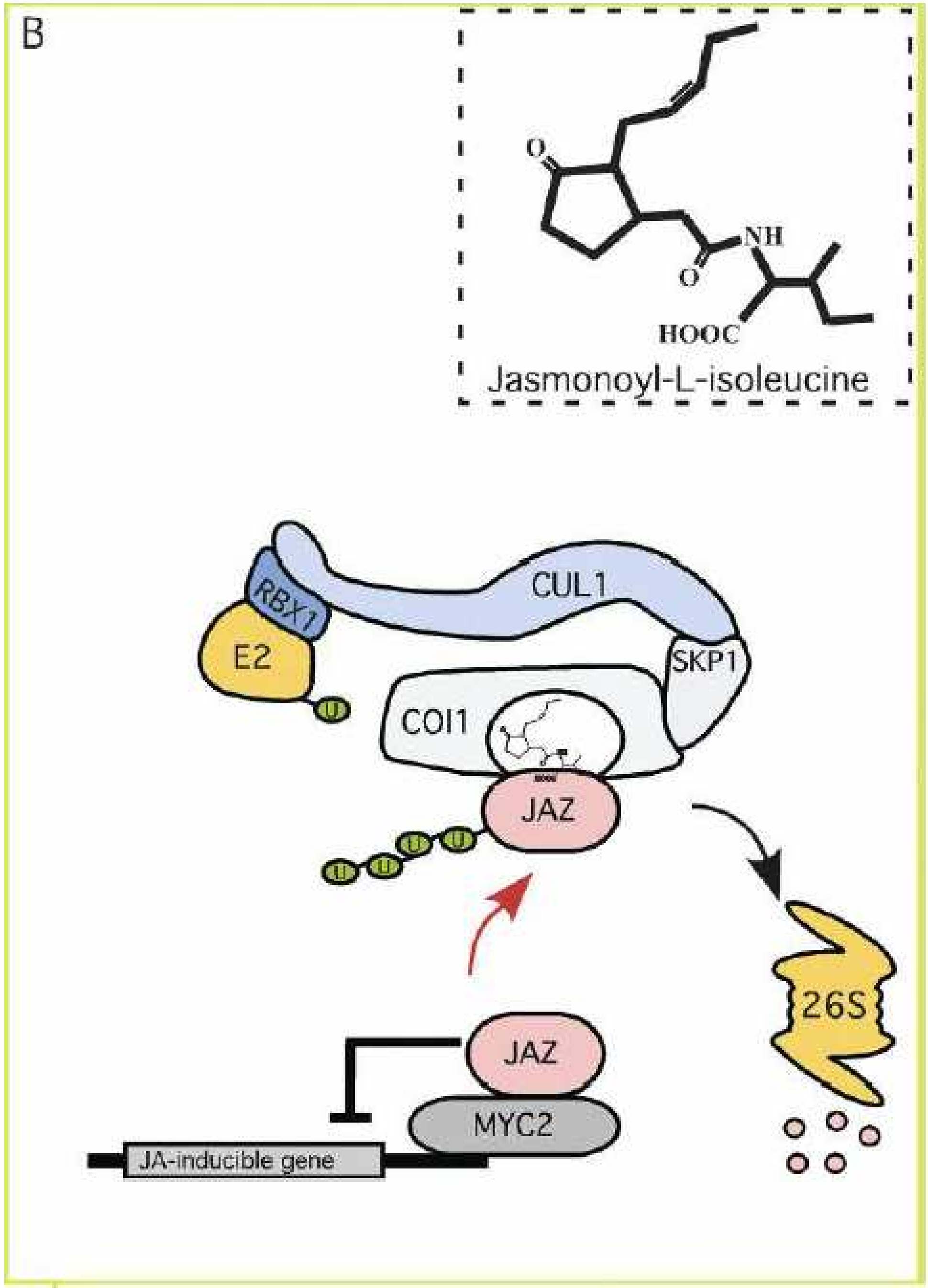


= PLA

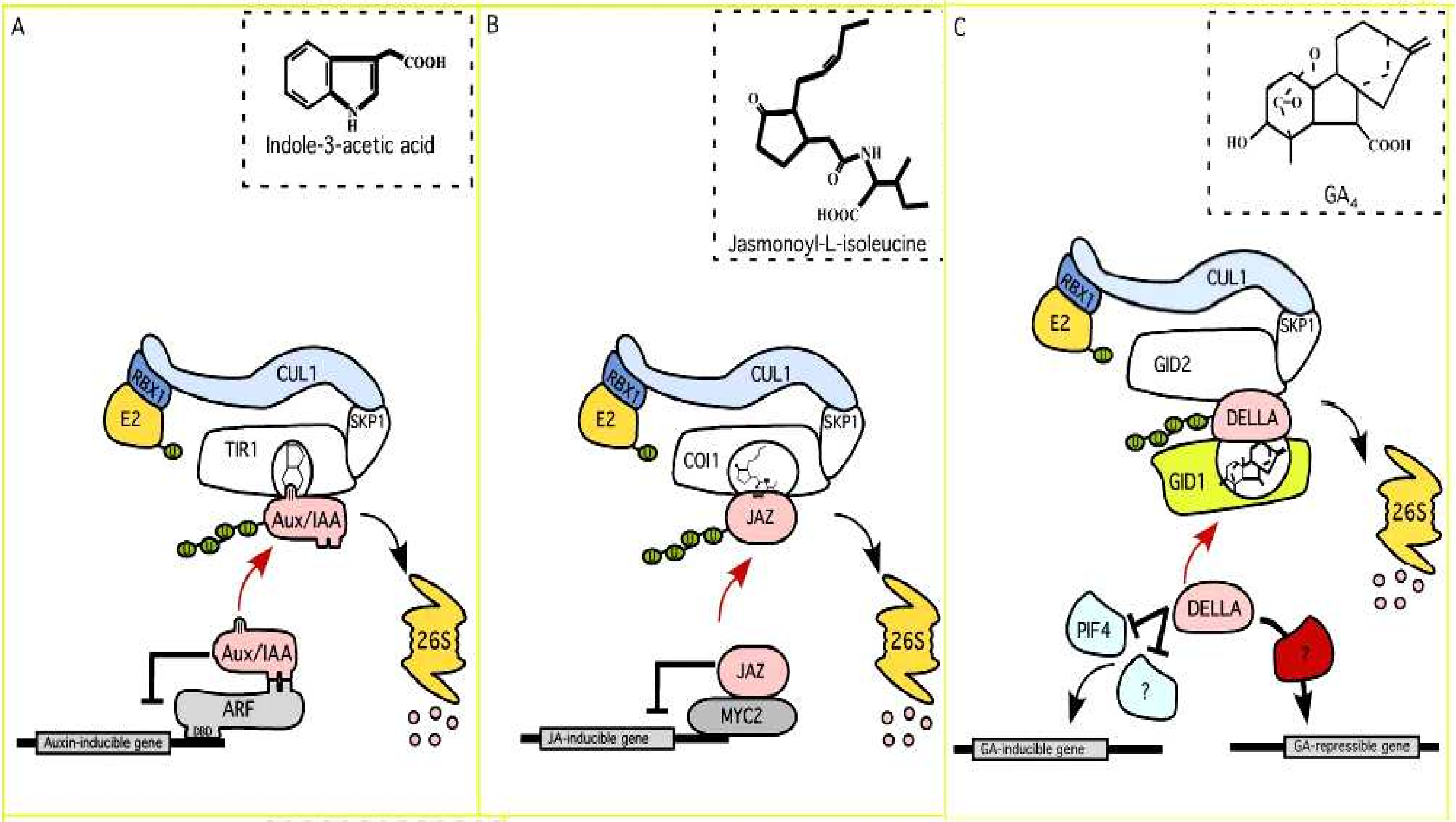
LOX=lipoxygenáza

oxylipiny

Signální dráha JA?



SHRNUTÍ - Ubiquitin-ligase based receptors (auxin, jasmonates and gibberellin)



GENES & DEVELOPMENT 22:2139–2148 (2008)

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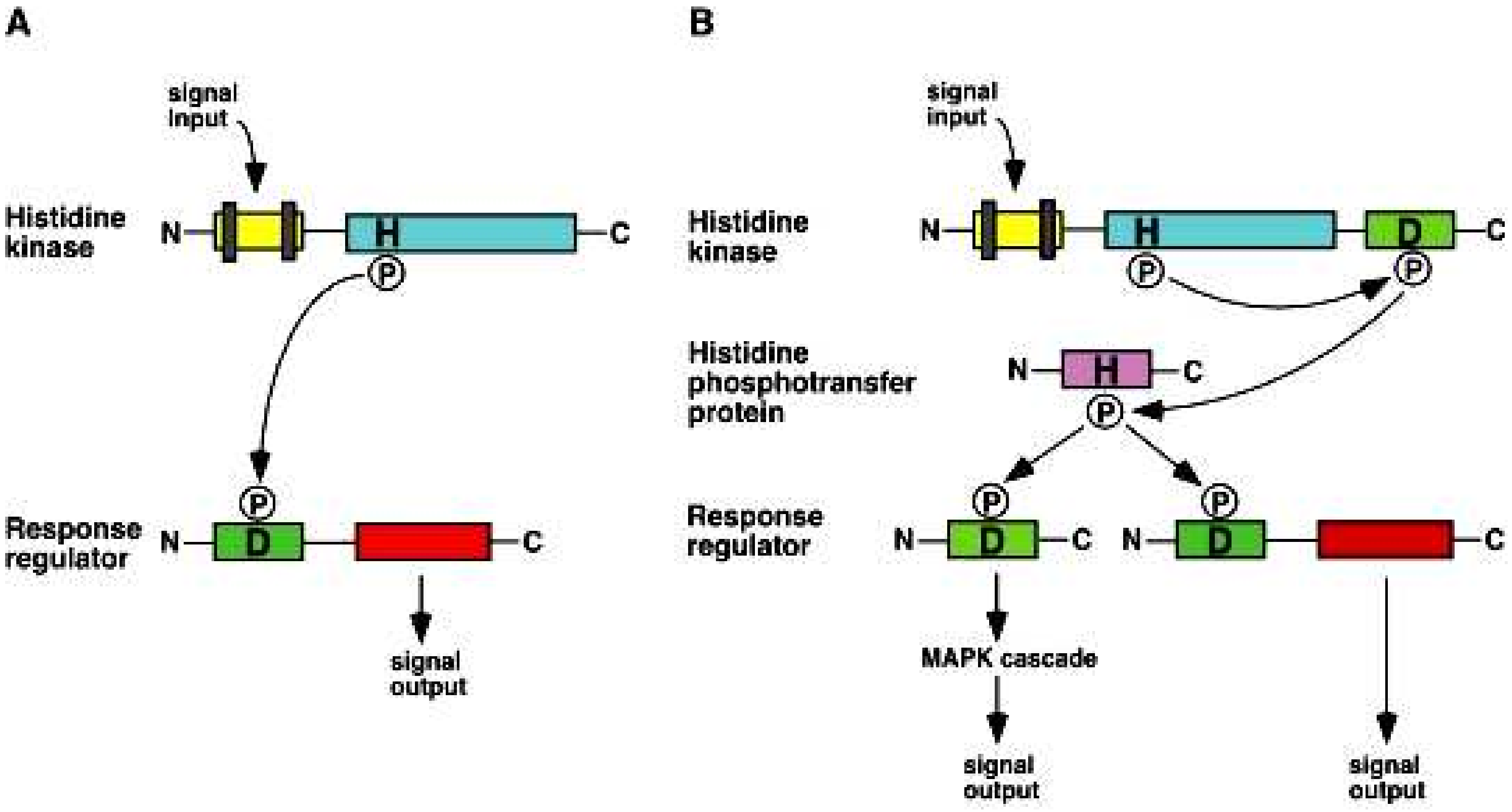
  - ABA = mnoho funkcí a receptorů
  - Peptidové hormony
- Rotlinné hormony a jejich signální dráhy
- Signalizace světlem

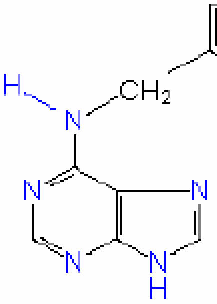
Two-component or phospho-relay circuitry



Prokaryota

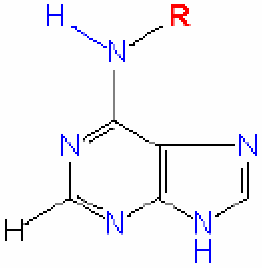
Eukaryota

Cytokininy

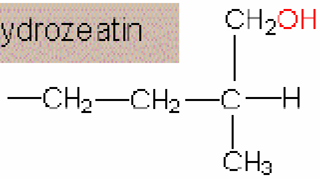


kinetin:
6 - (2 - furfuryl -
7 - amino purine)

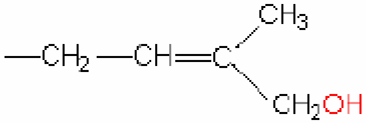
cytokinin
(basic structure)



dihydrozeatin

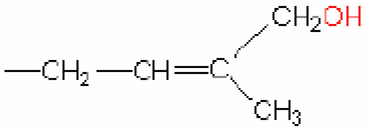


$$-CH_2-CH_2-\overset{\displaystyle CH_2OH}{\underset{\displaystyle CH_3}{C}}-H$$



$$-CH_2-CH=C\begin{array}{l}CH_3\\CH_2OH\end{array}$$

zeatin



$$-CH_2-CH=C\begin{array}{l}CH_2OH\\CH_3\end{array}$$

Function of cytokinins

- Promotes cell division.
- Morphogenesis.
- Lateral bud development.
- Delay of senescence.
- Stomatal opening.
- Rapid transport in xylem stream.

cre1/wol chybí v kořeni floém a tvoří se jen xylém.

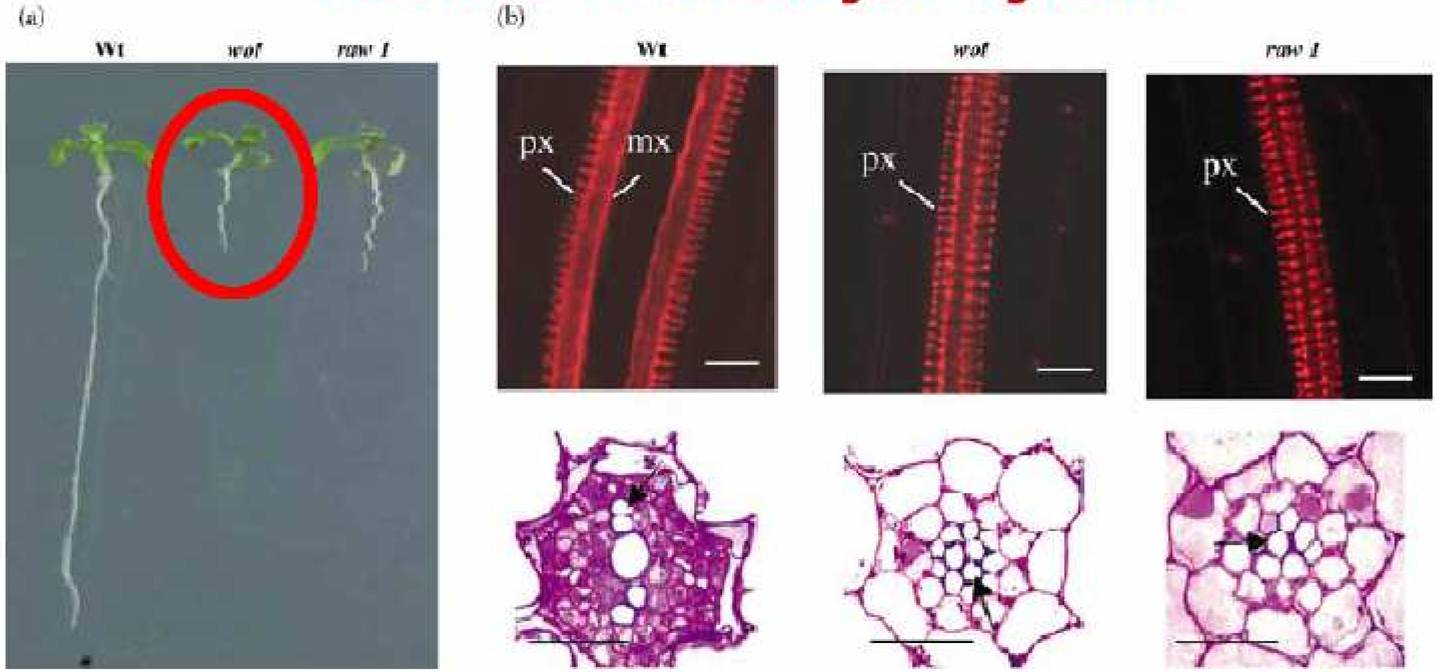
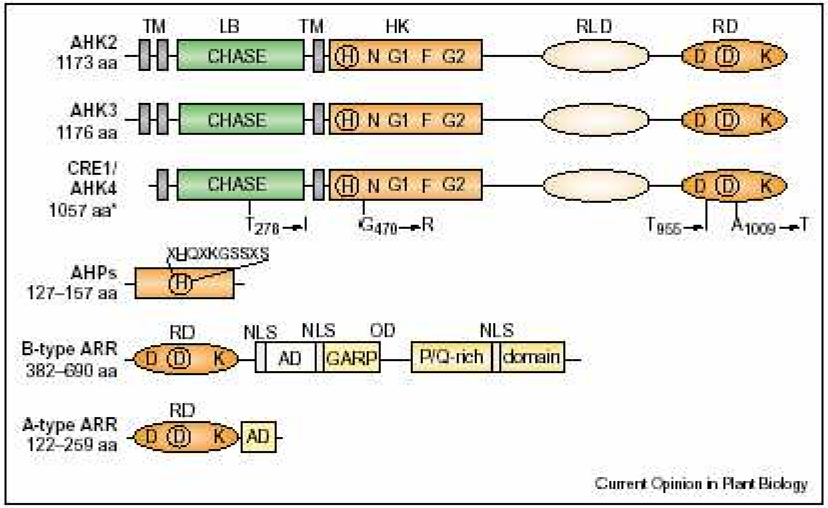


Figure 1. Short-root phenotype of the *raw* mutant.
(a) *raw1* mutant seedlings were grown for 7 days on vertical plates, with the wild type (Wt) and the *wol* mutant.
(b) Upper panel: Confocal images of protoxylem (px) and metaxylem (mx) cells stained with basic fuchsin in the main root in Wt, *wol* and *raw1* mutant. Scale bars, 30 µm. Lower panel: Transverse sections of the primary root stained with Toluidine Blue. Vascular cylinder of Wt, *wol* and *raw1* mutants are shown; arrows indicate protoxylem tissues. Scale bars, 20 µm.

WOODEN LEG kóduje jeden z receptorů cytokininů.



Current Opinion in Plant Biology

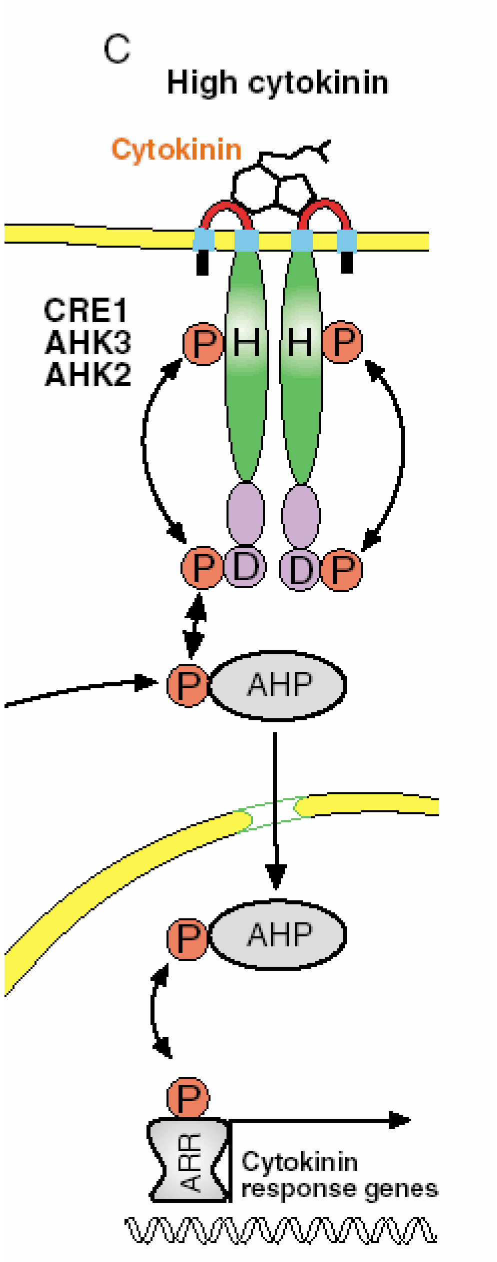
také WOL (wooden leg)

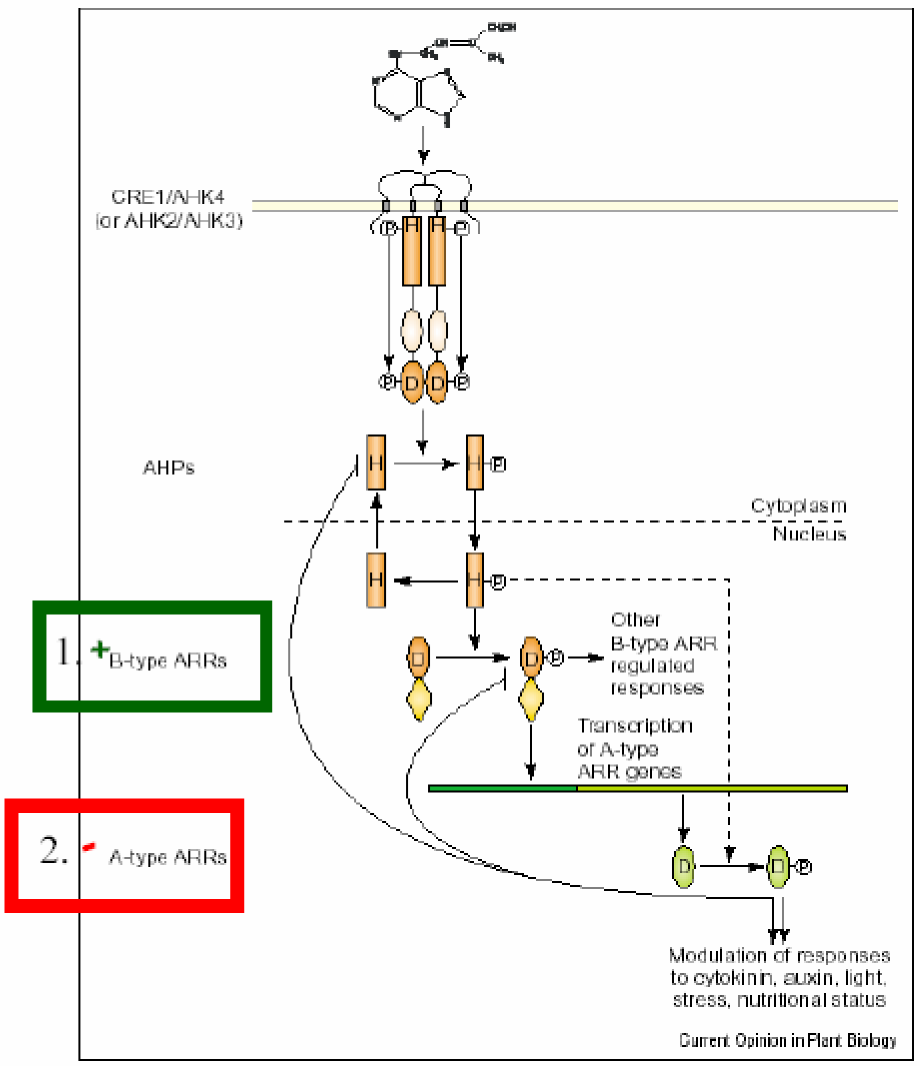
Structures of cytokinin receptors and other proteins of the cytokinin signalling pathway. Amino acids that participate in the phosphorelay are circled. Other characteristic consensus motifs are also indicated. Mutations that lead to loss of function in CRE1/AHK4 are shown below the CRE1/AHK4 structure [17**,20**]. Abbreviations: aa, amino acids; AD, acidic domain; CHASE, cyclases/histidine kinases associated sensory extracellular; GARP, DNA-binding motif; HK, histidine kinase; LB, putative ligand binding domain; NLS, nuclear localisation signal; OD, output domain; RD, receiver domain; RLD, receiver-like domain; TM, transmembrane domain. Domains are according to [12,27,34**,38]. *A longer open reading frame of *CRE1* coding for additional 23 amino acids at the N-terminal end was also identified [7**].

Abbreviations

AHK	*Arabidopsis* histidine kinase
AHP	*Arabidopsis* histidine phosphotransfer protein
ARR	*Arabidopsis* response regulator
CHASE	cyclases/histidine-kinase-associated sensory extracellular
CK	cytokinin
CKI	CYTOKININ INDEPENDENT
CRE1	CYTOKININ RESPONSE 1
Hpt	histidine phosphotransfer protein
nCI	mitochondrial respiratory chain complex I
TCS	two-component system

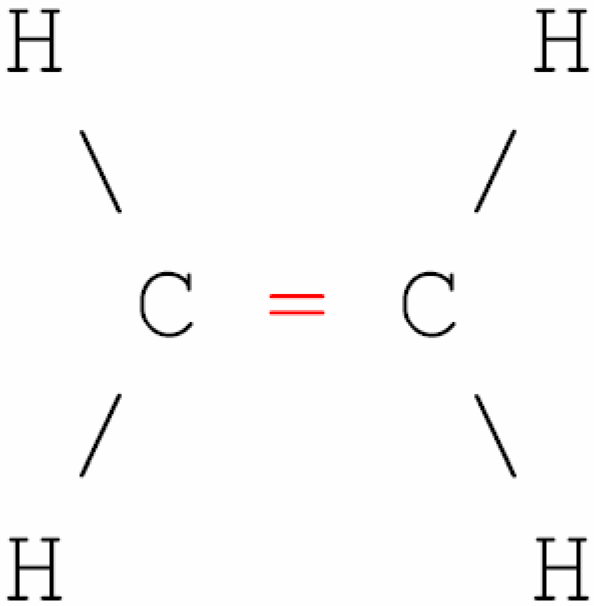
cre1/wol chybí v kořeni
floém a tvoří se jen xylém.





ARR A-typu se podílejí na negativně zpětnovazebném potlačení odpovědi na cytokininy.

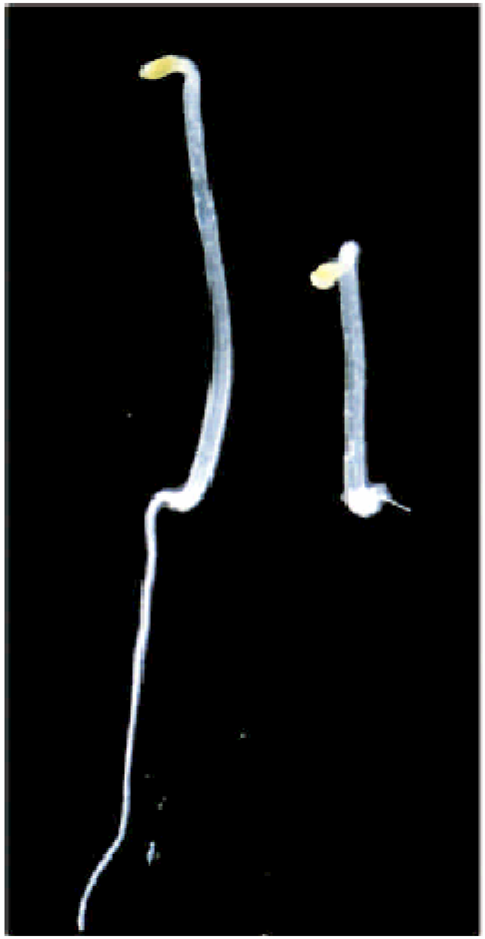
Ethylen



Functions of ethylene

- Gaseous in form.
- Rapid diffusion.
- Affects adjacent individuals.
- Fruit ripening.
- Senescence and abscission.
- Interference with auxin transport.
- Initiation of stem elongation and bud development.

Triple response of etiolated seedlings with ethylene



- short hypocotyl
- thick radial growth
- apical hook formation

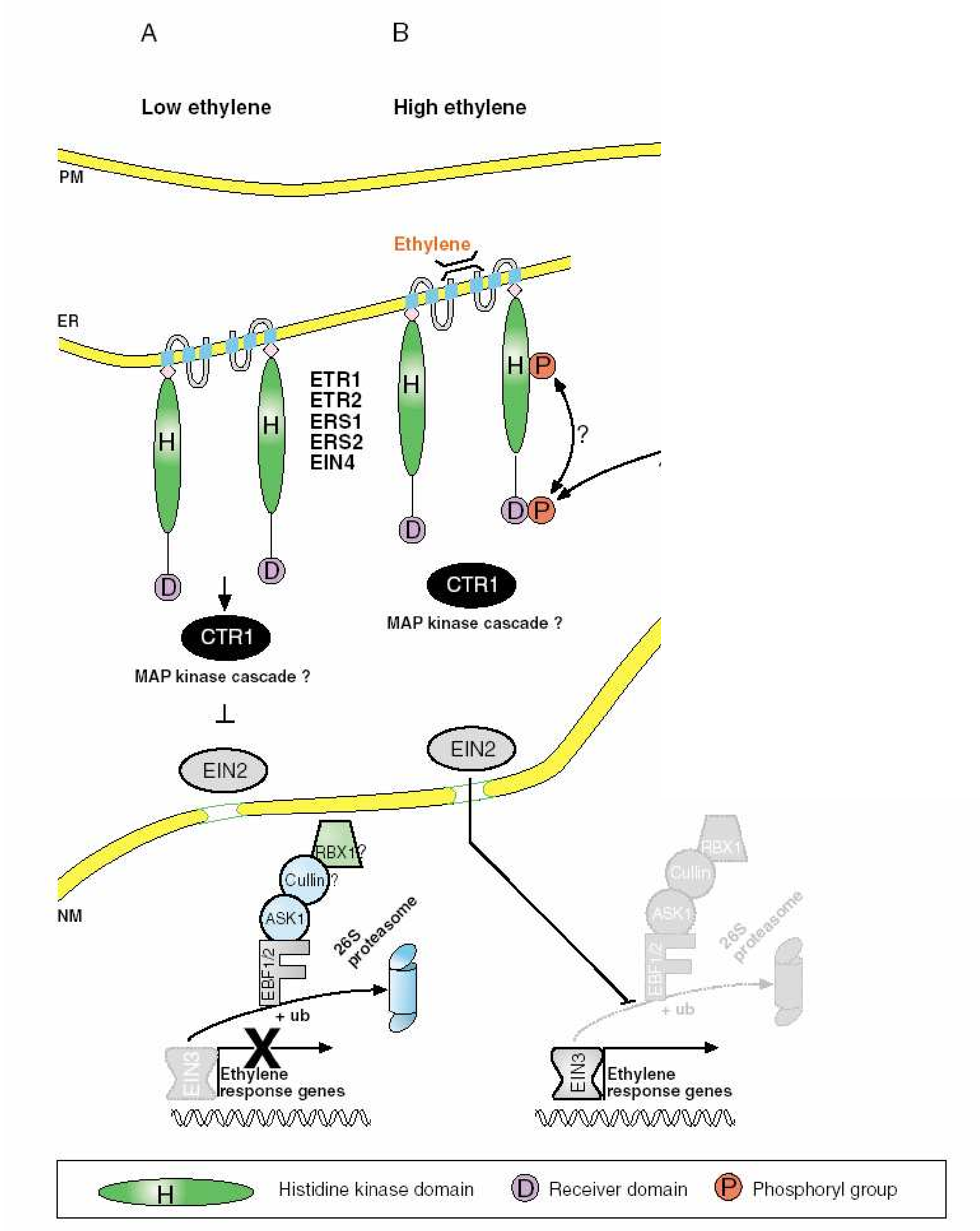
Screening of ethylene response mutants

Constitutive ethylene response in air (CER)

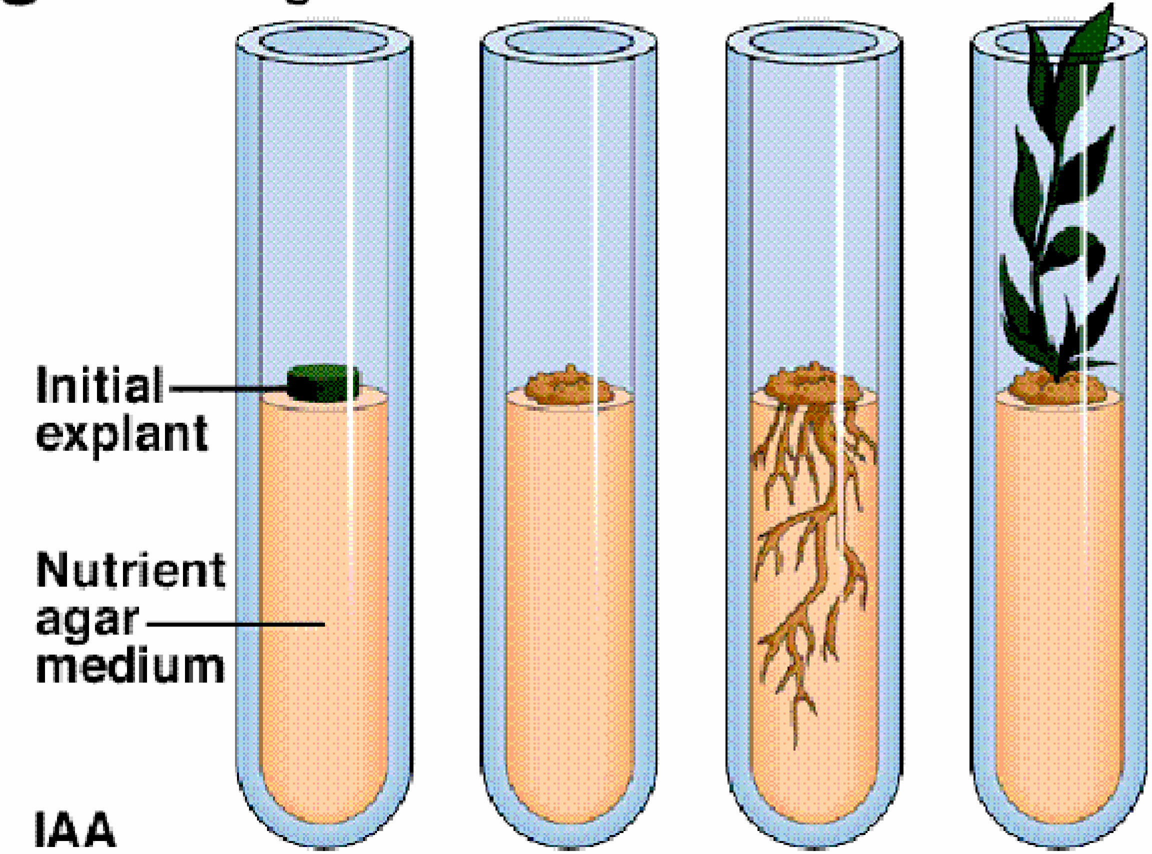
- ETO
- CTR

Ethylene resistant/insensitive in ethylene (ETR/EIN)

Signální dráha etylenu



Responses of Plant Tissue Culture to Kinetin and Auxin



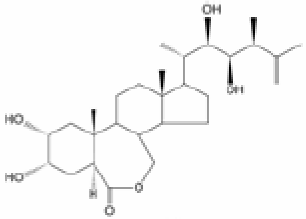
		No growth	Callus	Roots	Shoots
auxin	IAA (mg per liter)	none	2	2 (high)	0.02 (low)
cytokinin	Kinetin (mg per liter)	0.2	0.2	0.02 (low)	1 (high)

- Přenos signálu
- **Rotlinné hormony a jejich signální dráhy**
  - Signální dráhy založené na regulované proteolýze
    - Komponenty proteolytických degradačních drah
    - Auxin
    - Giberellin
    - Jasmonát (?)
  - **Signální dráhy založené na dvoukomponentní dráze**
    - Cytokininy
    - Etylen
  - Brassinosteroidy a signalizace pomocí proteinkináz
  - ABA = mnoho funkcí a receptorů
  - Peptidové hormony
- Rotlinné hormony a jejich signální dráhy
- Signalizace světlem

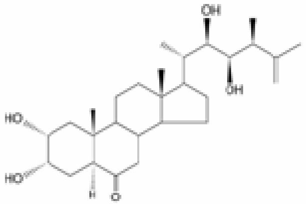
Brassinosteroidy

Brassinosteroids

- A steroidal compound found in both vascular and non-vascular plants
- Effects include:
    - Increase stem and cell elongation
    - Unrolling and bending of grasses
    - H^+ activation
    - Ethylene production
    - Photomorphogenesis?
- Mutants show altered growth and sensitivity to light

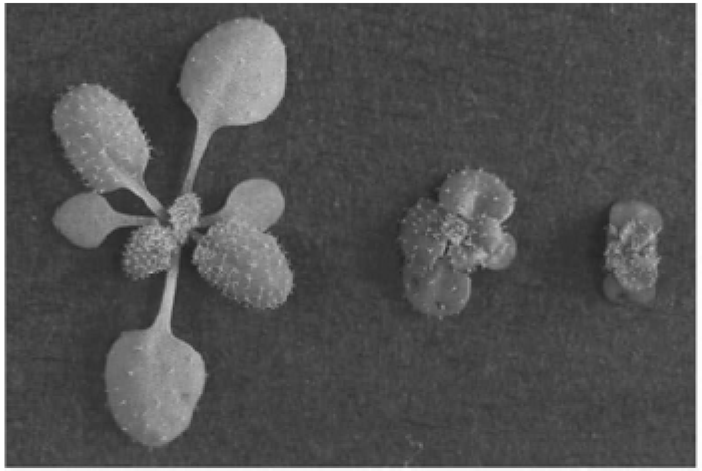


Brassinolide



Castasterone

Brassinosteroid-deficient mutants



BRI1 je Receptor Like Kinase (RLK), která po aktivaci interaguje s dalšími kinázami. Pro další přenos signálu jsou klíčové fosfatázy.

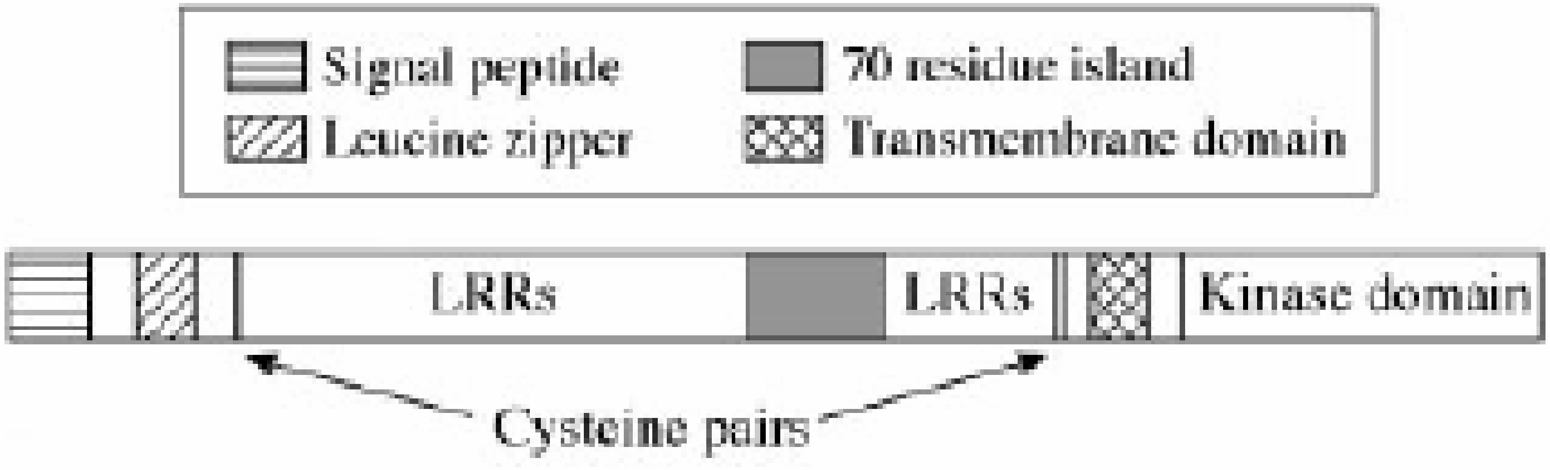
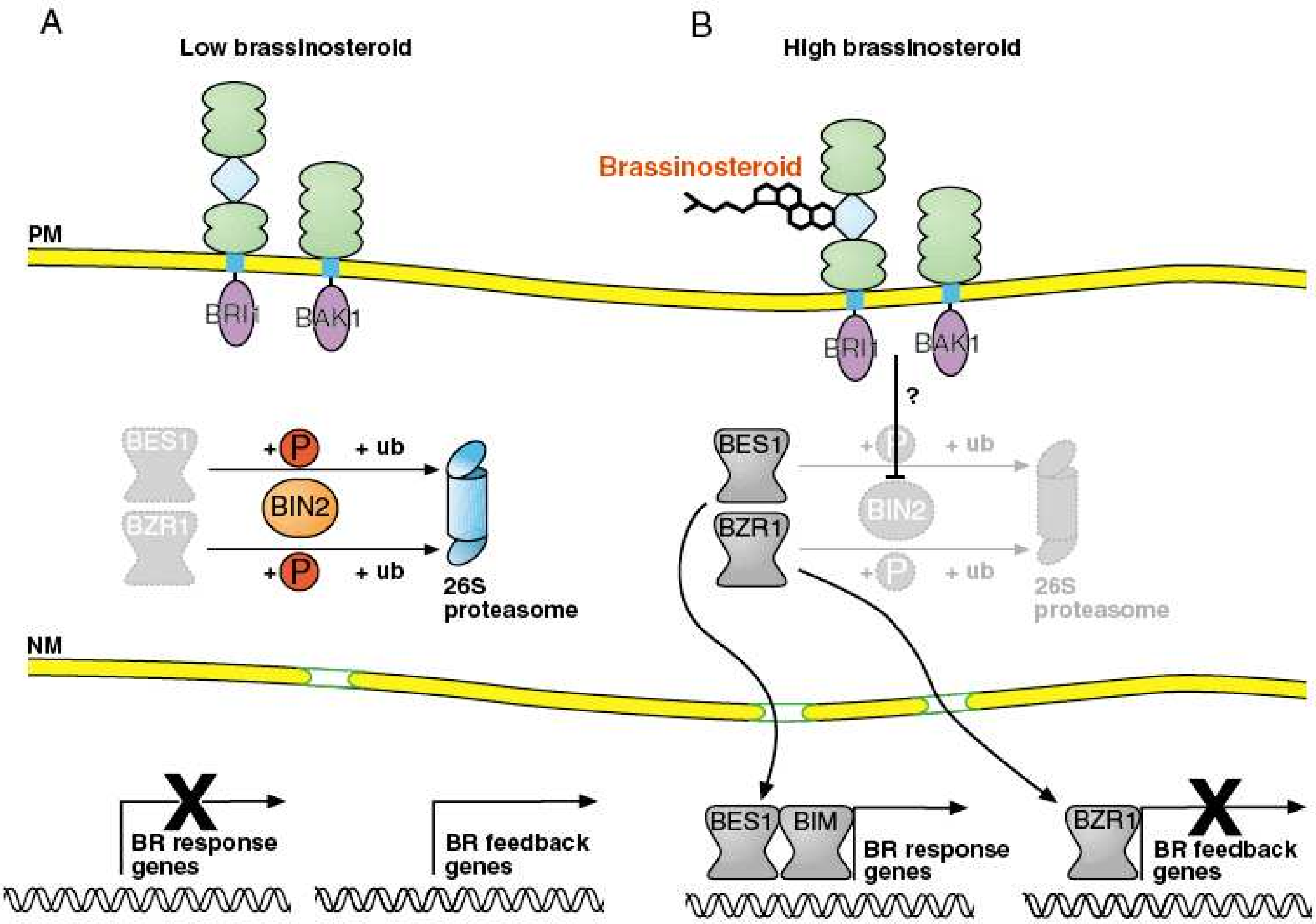
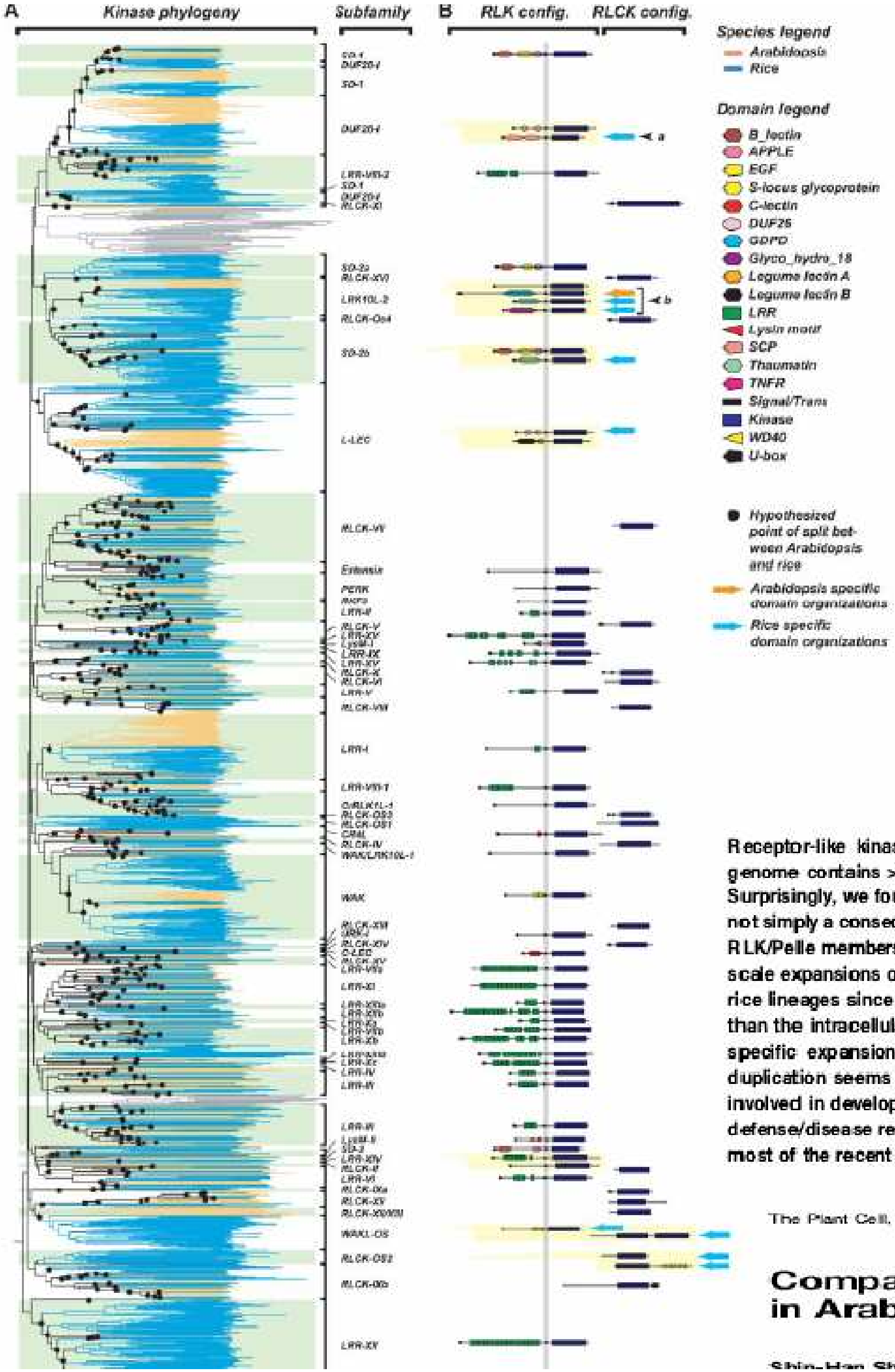


FIG. 3. Representation of BRI1.

Brassinosteroid signaling pathway



Development 133, 1857-1869 (2006)



Arabidopsis má více než **600** RLKs a **rýže** přes **1000**.

Multiplikace RLKs je pravděpodobně spojena především s obranou proti fytopatogenům.

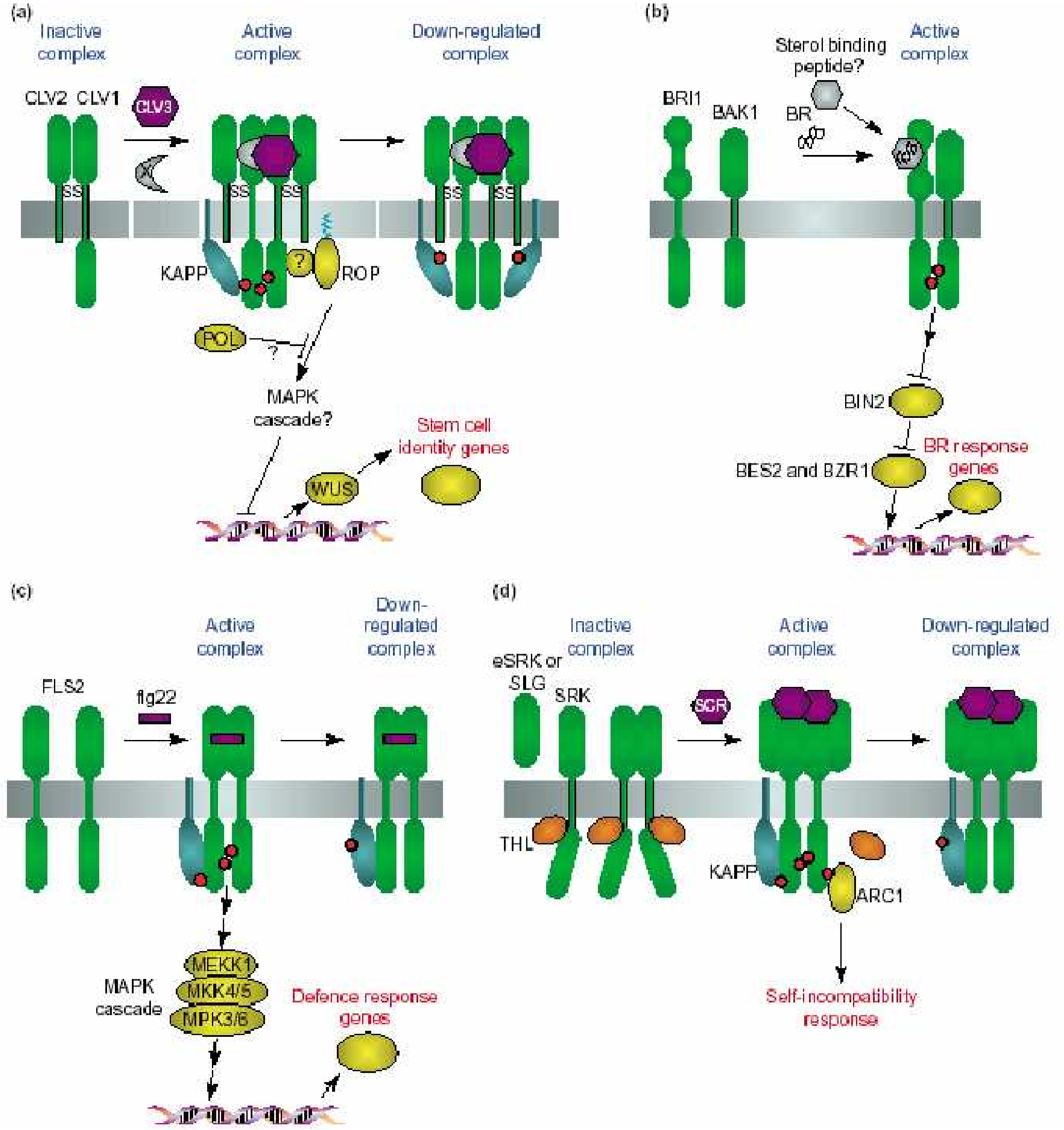
Receptor-like kinases (RLKs) belong to the large RLK/Pelle gene family, and it is known that the *Arabidopsis thaliana* genome contains >600 such members, which play important roles in plant growth, development, and defense responses. Surprisingly, we found that rice (*Oryza sativa*) has nearly twice as many RLK/Pelle members as Arabidopsis does, and it is not simply a consequence of a larger predicted gene number in rice. From the inferred phylogeny of all Arabidopsis and rice RLK/Pelle members, we estimated that the common ancestor of Arabidopsis and rice had >440 RLK/Pelles and that large-scale expansions of certain RLK/Pelle members and fusions of novel domains have occurred in both the Arabidopsis and rice lineages since their divergence. In addition, the extracellular domains have higher nonsynonymous substitution rates than the intracellular domains, consistent with the role of extracellular domains in sensing diverse signals. The lineage-specific expansions in Arabidopsis can be attributed to both tandem and large-scale duplications, whereas tandem duplication seems to be the major mechanism for recent expansions in rice. Interestingly, although the RLKs that are involved in development seem to have rarely been duplicated after the Arabidopsis-rice split, those that are involved in defense/disease resistance apparently have undergone many duplication events. These findings led us to hypothesize that most of the recent expansions of the RLK/Pelle family have involved defense/resistance-related genes.

The Plant Cell, Vol. 16, 1220–1234, May 2004, www.plantcell.org © 2004 American Society of Plant Biologists

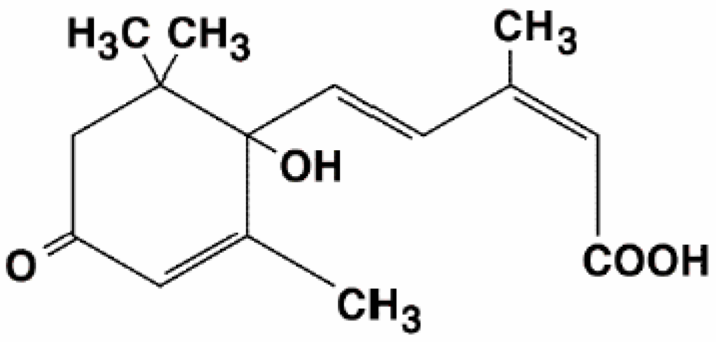
Comparative Analysis of the Receptor-Like Kinase Famil in Arabidopsis and Rice[W]

Shin-Han Shiu,a Wojciech M. Karlowski,b Runsun Pan,a,d Yun-Huei Tzeng,a,c Klaus F. X. Mayer,b

Příklady signalizací pomocí RLK



ABA

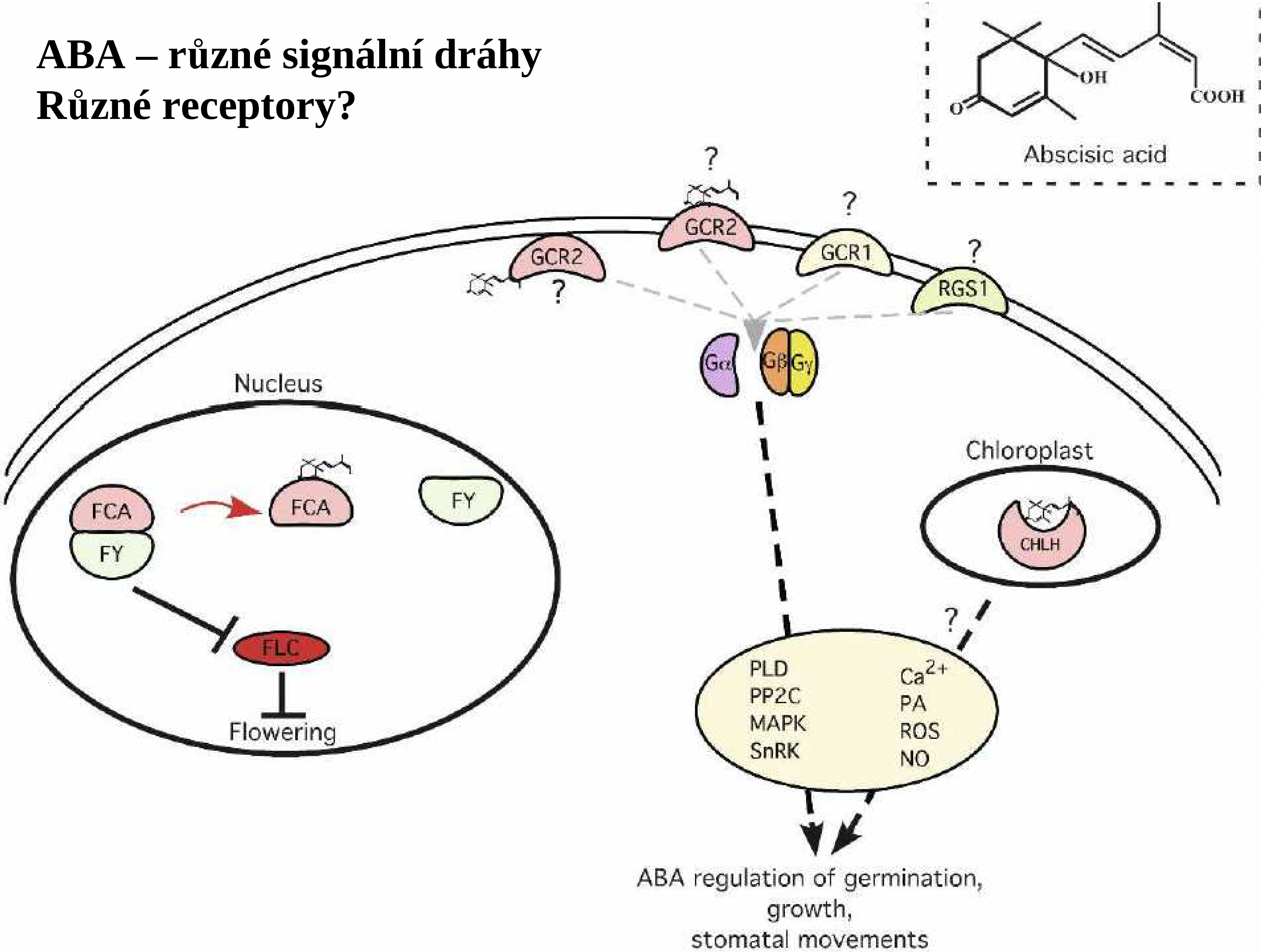


Structure of Abscisic Acid (ABA)

Functions of abscisic acid

- General growth inhibitor.
- Causes stomatal closure.
- Inhibits stomatal opening.
- Readily translocated.
- Produced in response to stress.

ABA – různé signální dráhy
Různé receptory?



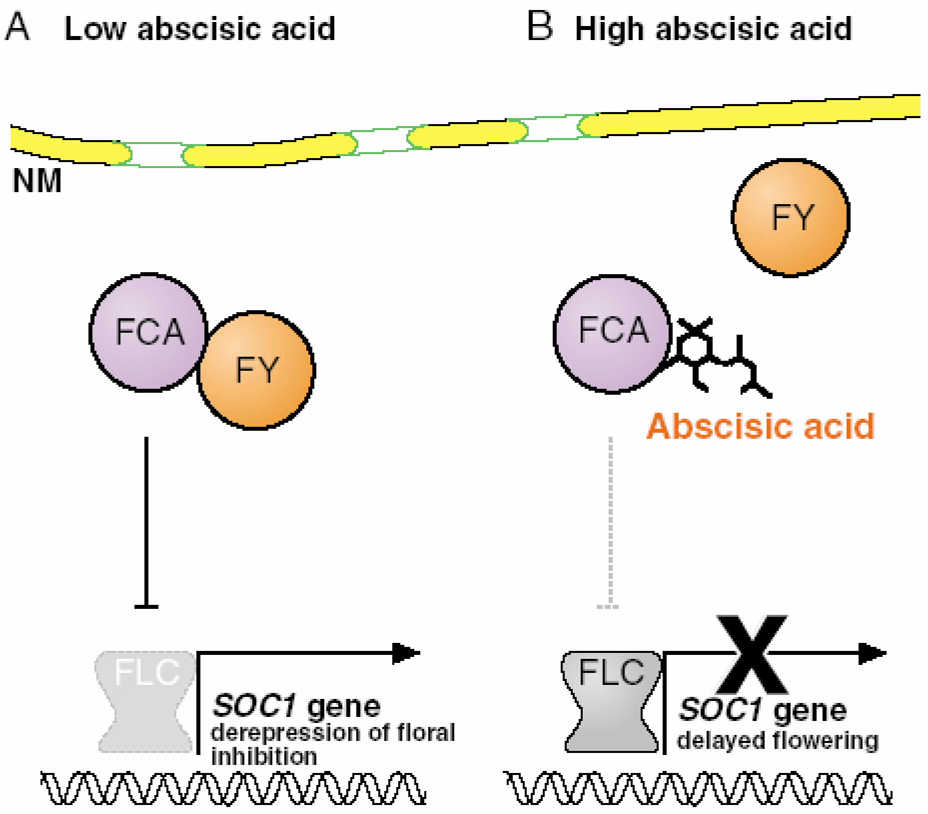


Fig. 7. The abscisic acid (ABA) signalling pathway that controls the regulation of flowering time. (**A**) At low ABA concentrations, FCA and FY interact and together prevent the accumulation of *FLC* mRNA. FLC is a potent inhibitor of flowering, as several pathways converge on it to block the expression of the floral integration gene *SOC1* by directly binding to its promoter (Hepworth et al., 2002). (**B**) ABA binds to the C-terminal part of FCA, close to its interaction site with FY, disrupting the association of these two proteins in vitro and leading to the accumulation of *FLC* in vivo, which delays flowering. Transcription of *SOC1* only occurs in plants with low *FLC* levels; additional cues are required for the transition to flowering.

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  - Brassinosteroidy a signalizace pomocí proteinkináz
  - ABA = mnoho funkcí a receptorů
  - Peptidové hormony
- Signalizace světlem

PEPTIDY jako hormony

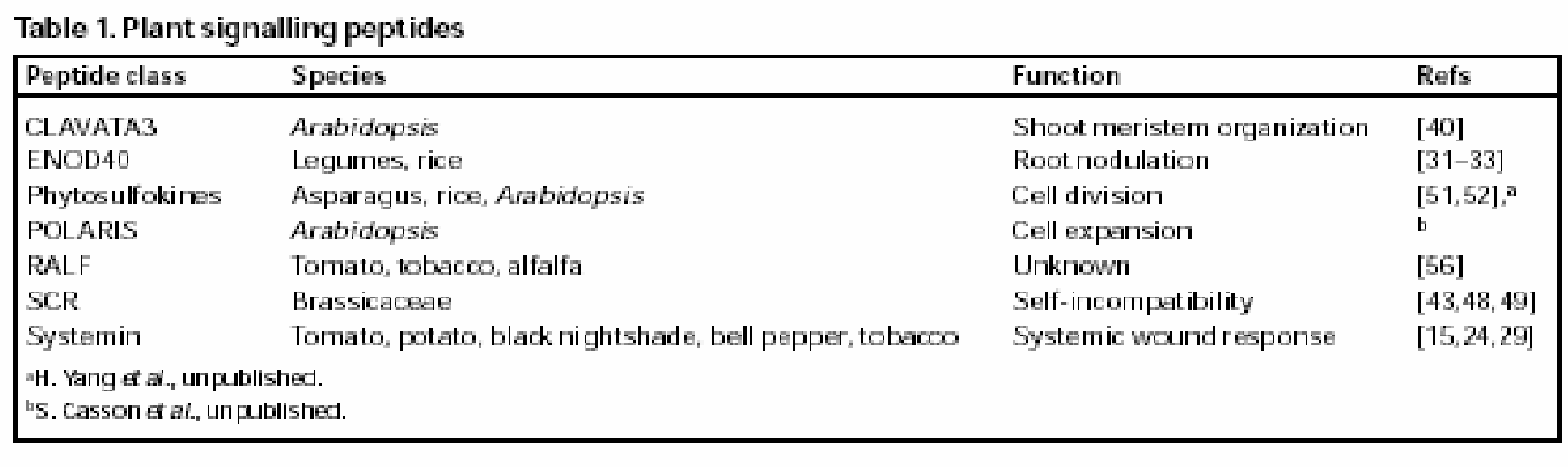


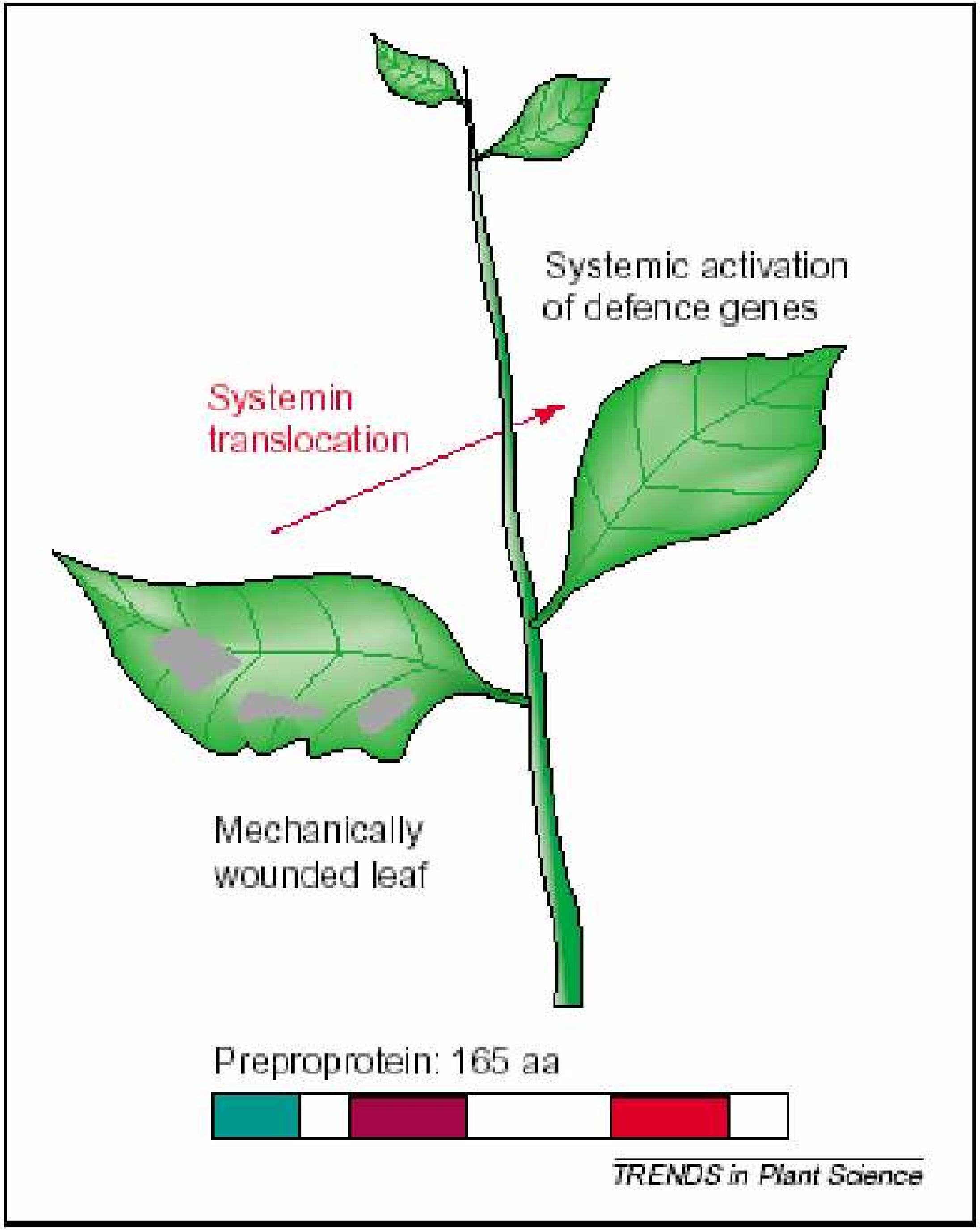
Table 1. Plant signalling peptides

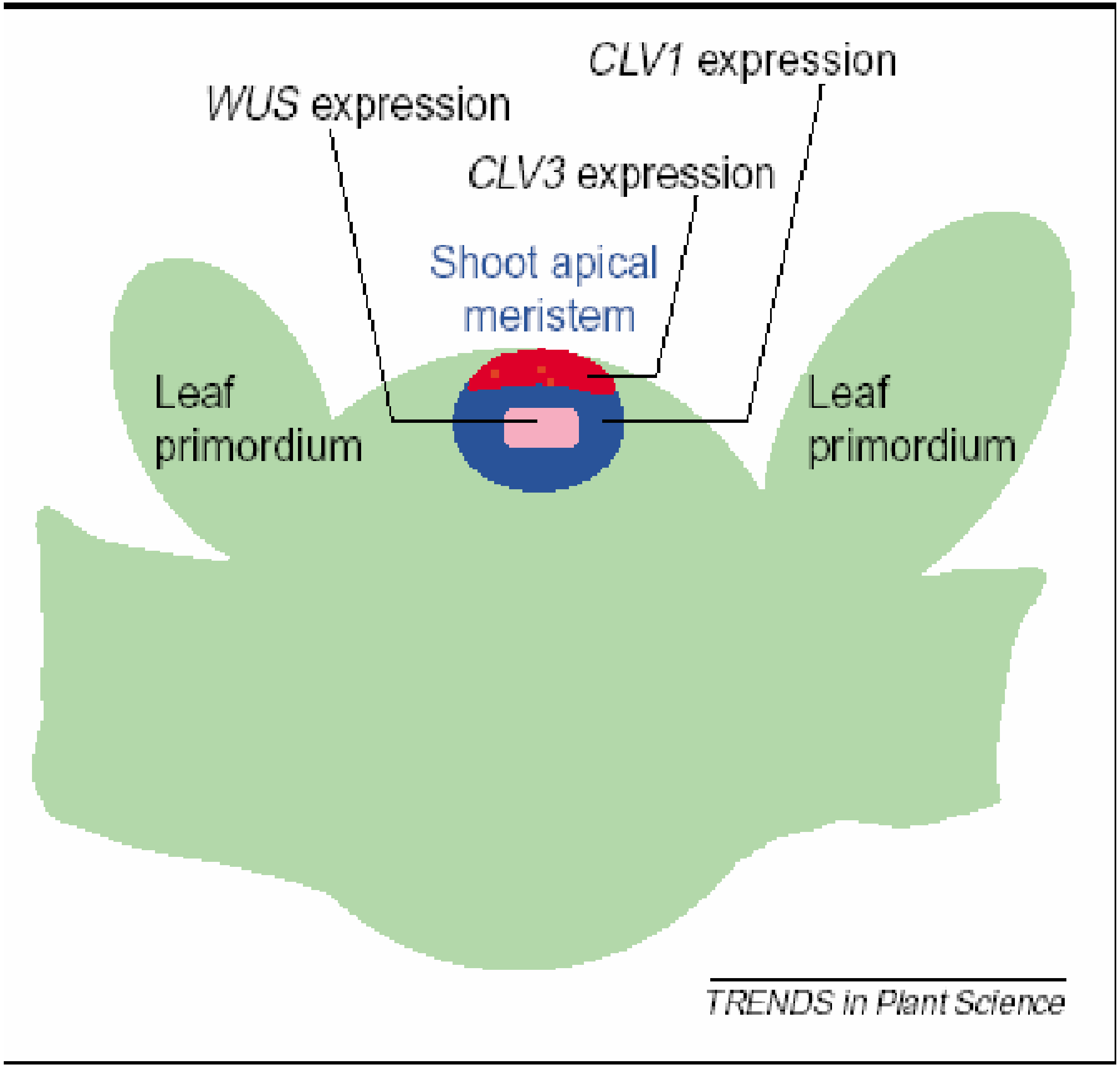
Peptide class	Species	Function	Refs
CLAVATA3	*Arabidopsis*	Shoot meristem organization	[40]
ENOD40	Legumes, rice	Root nodulation	[31–33]
Phytosulfokines	Asparagus, rice, *Arabidopsis*	Cell division	[51,52],[a]
POLARIS	*Arabidopsis*	Cell expansion	[b]
RALF	Tomato, tobacco, alfalfa	Unknown	[56]
SCR	Brassicaceae	Self-incompatibility	[43,48,49]
Systemin	Tomato, potato, black nightshade, bell pepper, tobacco	Systemic wound response	[15,24,29]

[a] H. Yang *et al.*, unpublished.

[b] S. Casson *et al.*, unpublished.

Fig. 1. The systemic activation of defence-related genes, as occurs in solanaceous species, is mediated by a transmissible signal from the damaged leaf to other leaves. The mobile 18-amino acid (18 aa) signalling peptide systemin has been identified in several species, including tomato, potato and bell pepper, and is generated by the cleavage of the 200-aa proprotein prosystemin. In tobacco, a 165-aa preproprotein encodes two distinct 18-aa peptides, TobSys I (dark red) and TobSys II (light red). The signal sequence is represented in green.

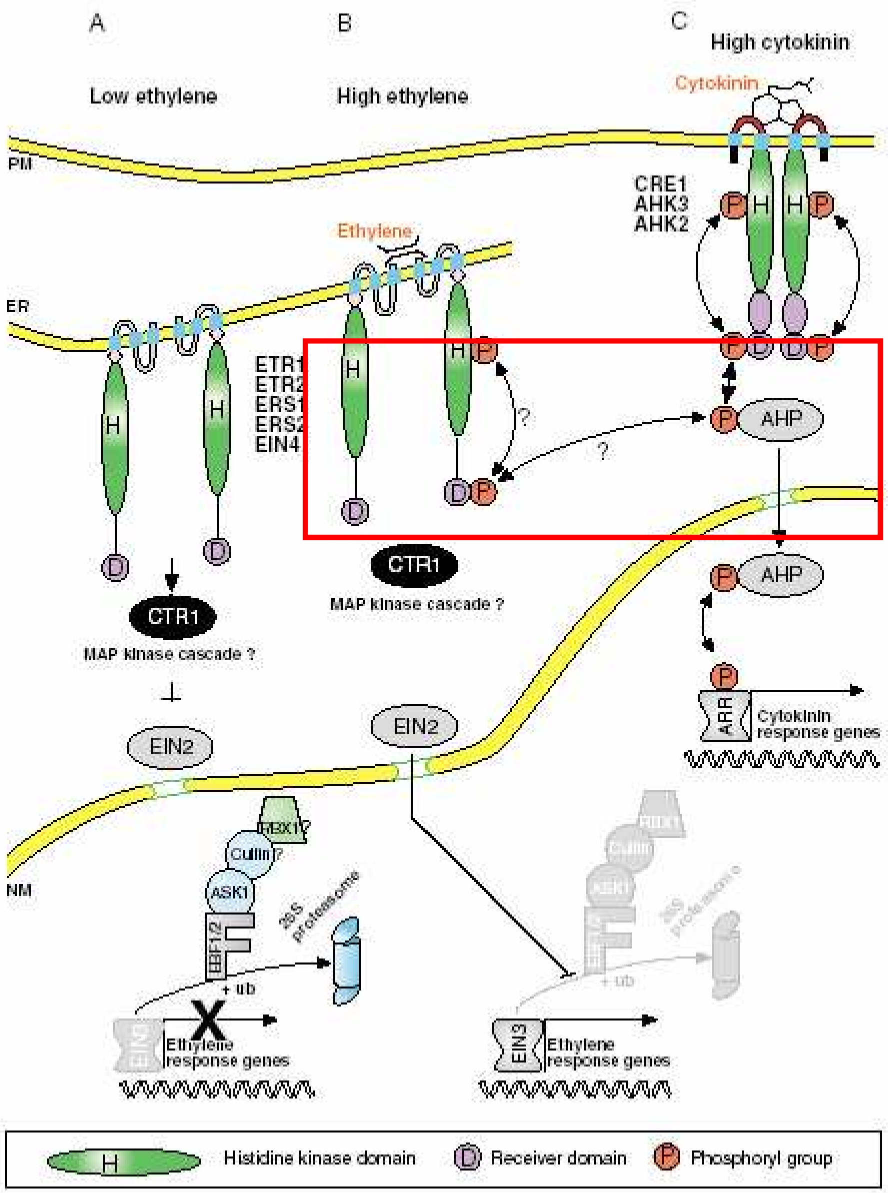


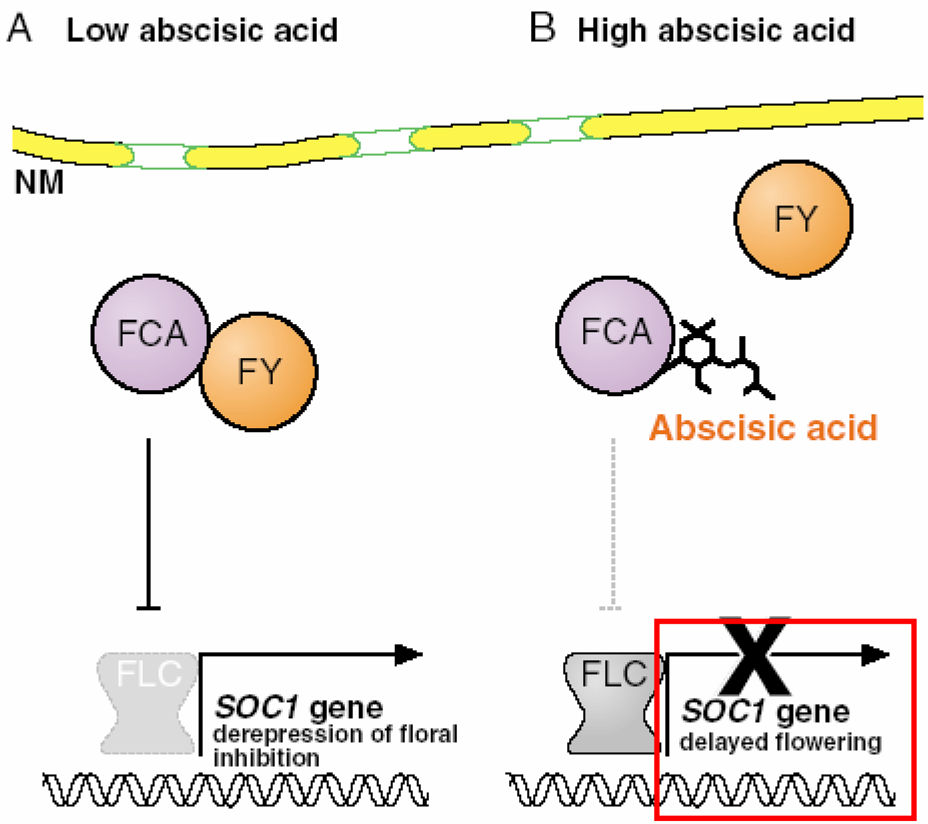


TRENDS in Plant Science

Fig. 3. *CLAVATA1* (*CLV1*) and *CLAVATA3* (*CLV3*) are both expressed in the central zone of the shoot apical meristem (SAM) and prevent the overproliferation of undifferentiated stem cells at that position. *CLV3* encodes a peptide produced by the stem cells at the SAM apex, which might be the ligand for the CLV1 receptor-kinase-like protein produced in cells deeper in the central zone. The *WUSCHEL* (*WUS*) gene encodes a homeodomain protein that promotes stem cell identity and is negatively regulated by CLV signalling in a feedback system that regulates the balance of stem cell production and division activity at the SAM [63,64].

KONVERGENCE SIGNÁLNÍCH DRAH


A
B
C
Low ethylene
High ethylene
High cytokinin
Cytokinin
Ethylene
PM
ER
NM
ETR1
ETR2
ERS1
ERS2
EIN4
CRE1
AHK3
AHK2
H
D
P
?
AHP
CTR1
MAP kinase cascade ?
EIN2
RBX1?
Cullin?
ASK1
EBF1/2
26S proteasome
+ ub
EIN3
Ethylene response genes
ARR
Cytokinin response genes
H Histidine kinase domain
D Receiver domain
P Phosphoryl group



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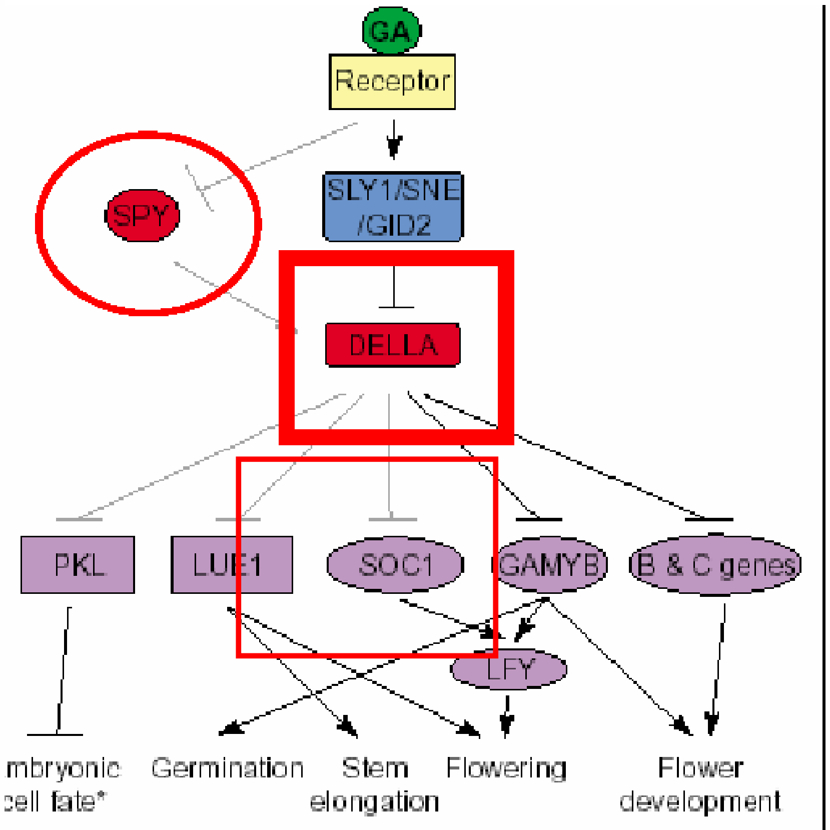
ABA



Current Opinion in Plant Biology

del of the GA signaling pathway. Ovals represent transcription
tors, gray lines indicate hypothesized interactions. Arrows and
ars indicate direct or indirect activation and inhibition, respectively.
enes encode PI and AP3; the C gene in this pathway encodes
AMOUS; DELLA includes RGA, GAI, RGLs, SLR1, SLN1, and
er orthologs. *PKL inhibits embryonic cell fate during
t-embryonic development.

GA

SVĚTLO

jako

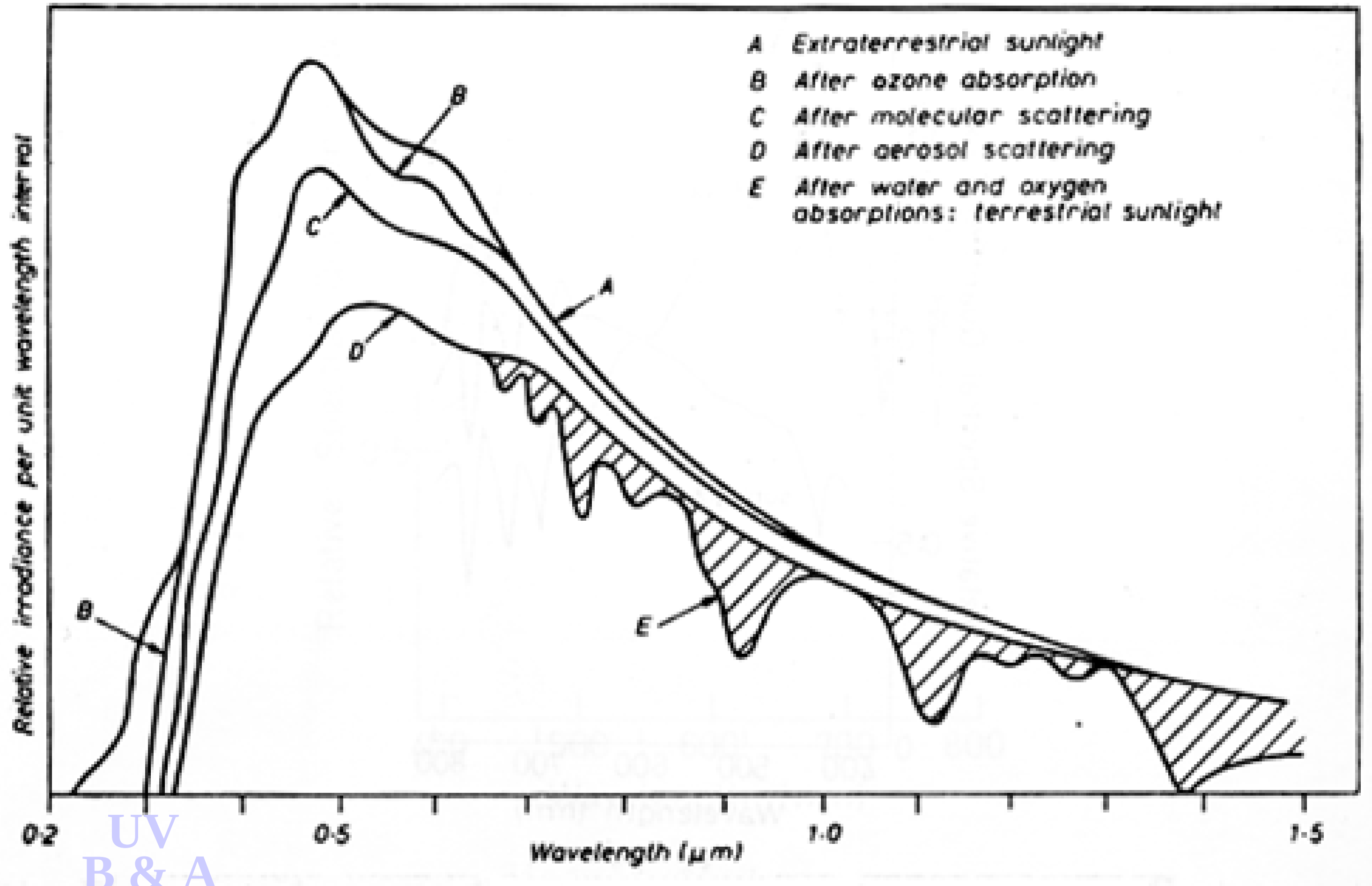
SIGNÁL

Světlo

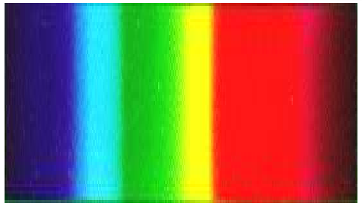
- Kvalita
- Kvantita
- Směr
- Trvání/Perioda

- **UV–B 280-320nm**
- **UV-A 320-380nm**
- **Modré s. 380-500nm**
- **Červené s. 620-700nm**
- **Dl. Červ. S. 700-800nm**

Light environment: sunlight

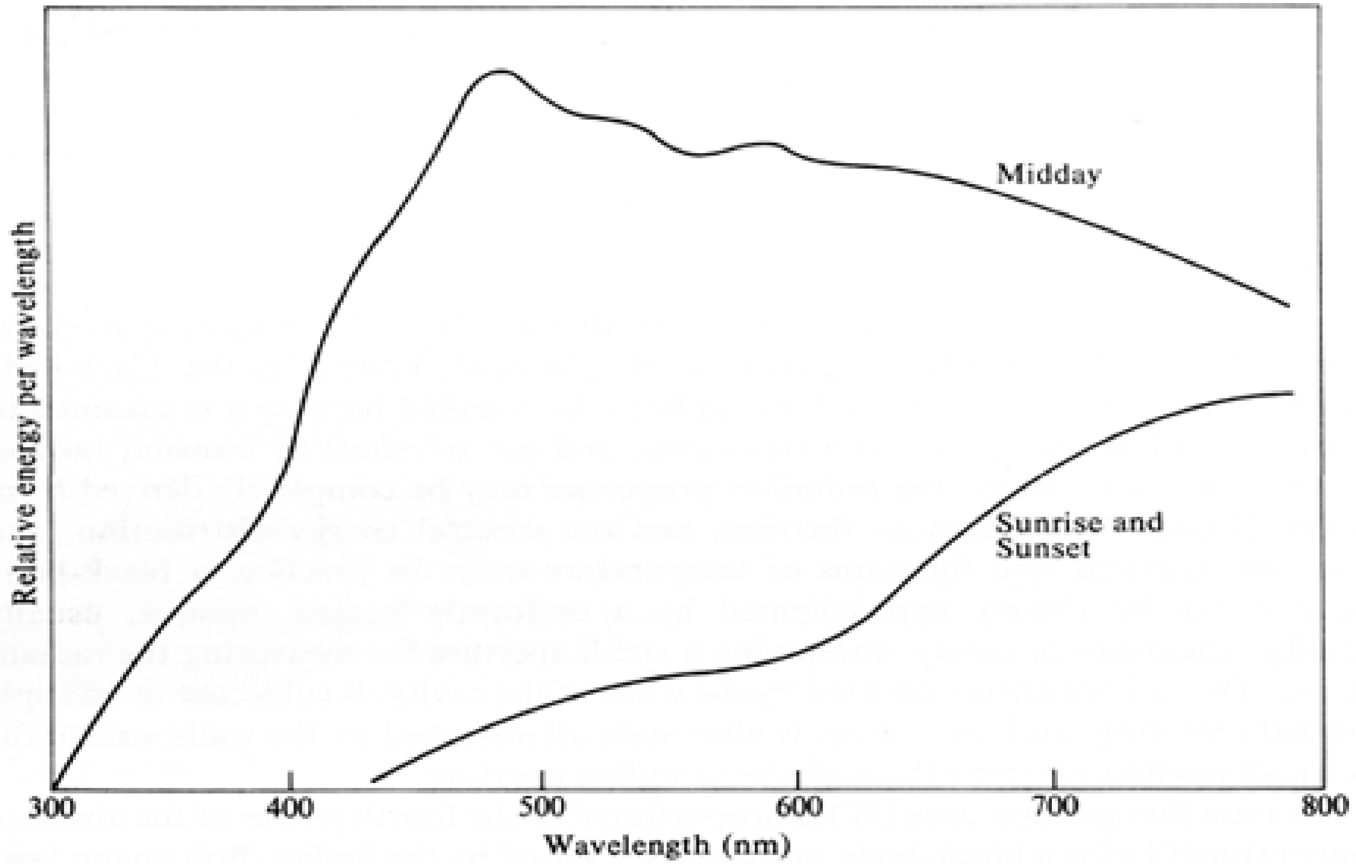


sunlight during penetration of the atmosphere

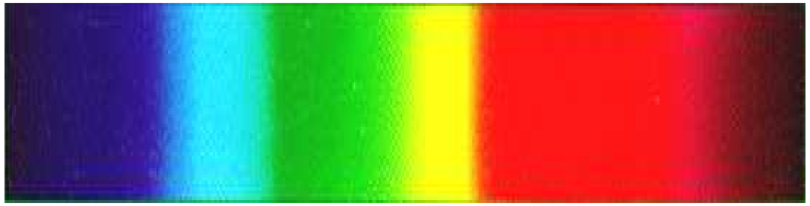


Visible light: 400-700 nm
or 0.4 -0.7 µm

Light quality variation during the day



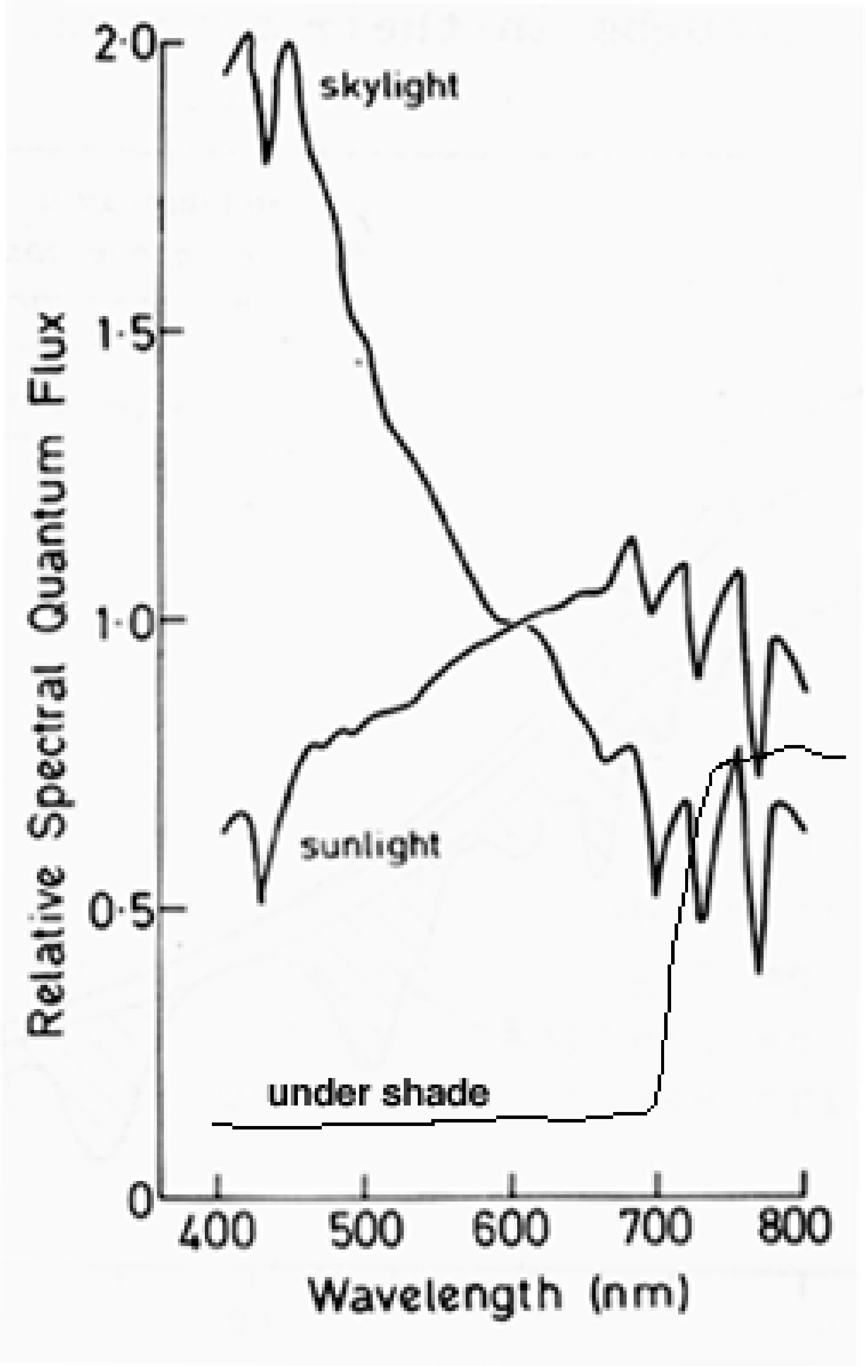
The spectral distribution of radiant energy in direct sunlight at midday and at sunrise and sunset



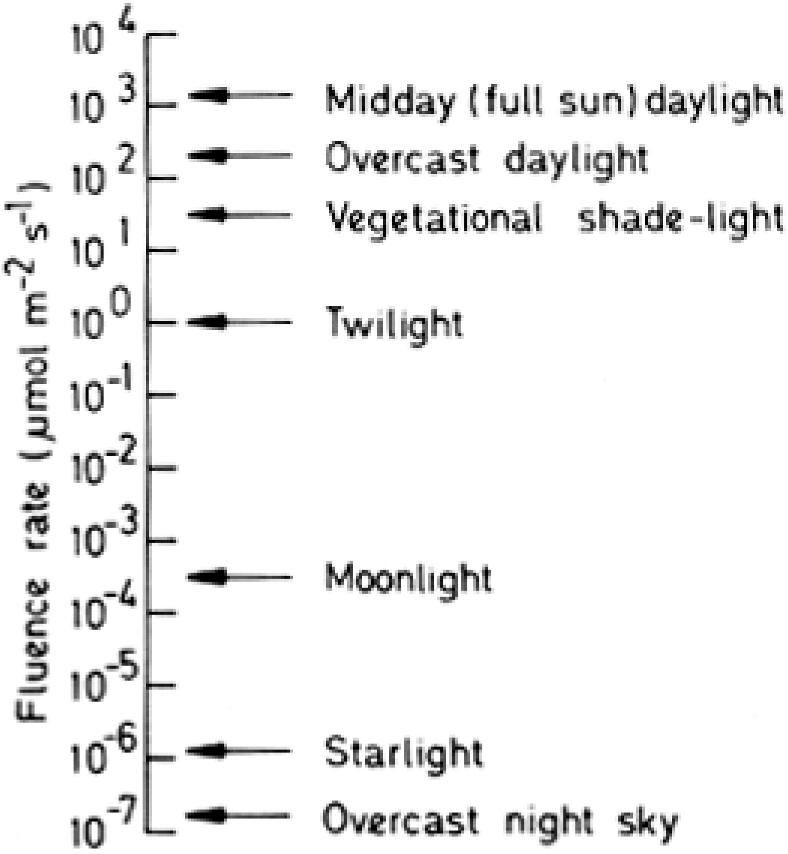
Light quality variation due to location

The light quality alters drastically depending on the situation.

- Direct sunlight
- Skylight (blue sky!)
- Under shade

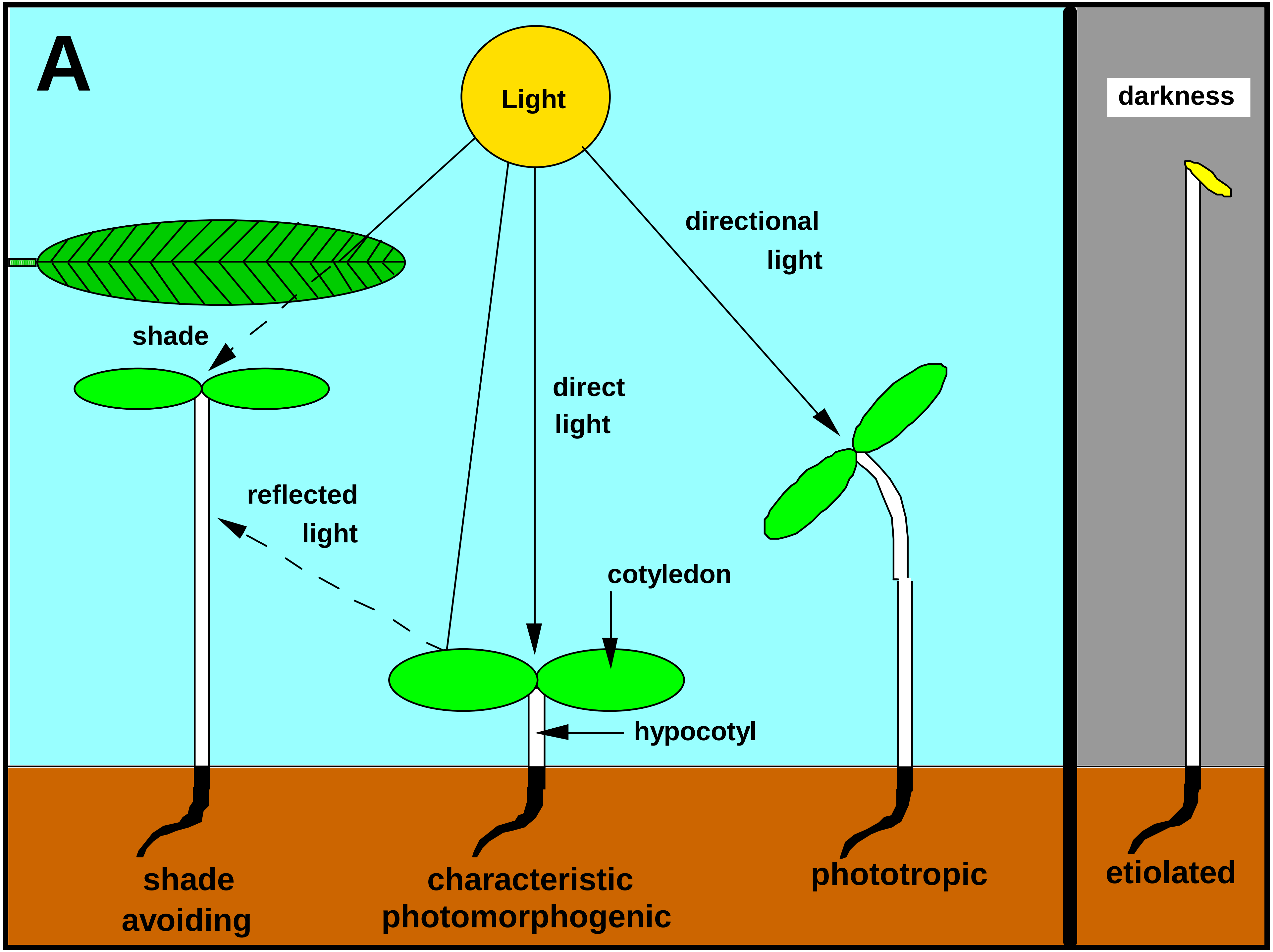


Light quantity variation in nature



Typical photon fluence rates (400–700nm) for natural radiation

The light intensity in nature varies at least ten orders of magnitude


A
Light
darkness
directional
light
shade
direct
light
reflected
light
cotyledon
hypocotyl
shade
avoiding
characteristic
photomorphogenic
phototropic
etiolated

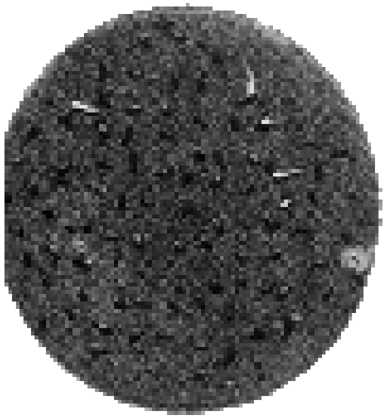
Borthwick’s Experiment in Grand Rapids Lettuce (1954)

R

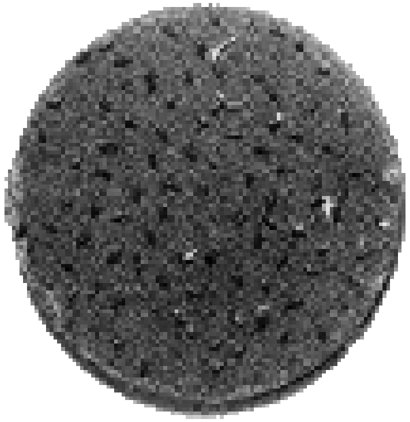
R,Fr,R

R,Fr,R,Fr,R

R,Fr,R,Fr,R,Fr,R

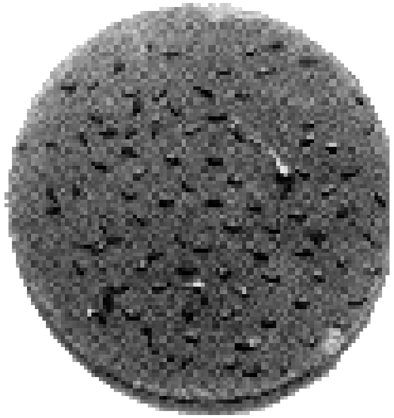


R,Fr



R,Fr,R,Fr

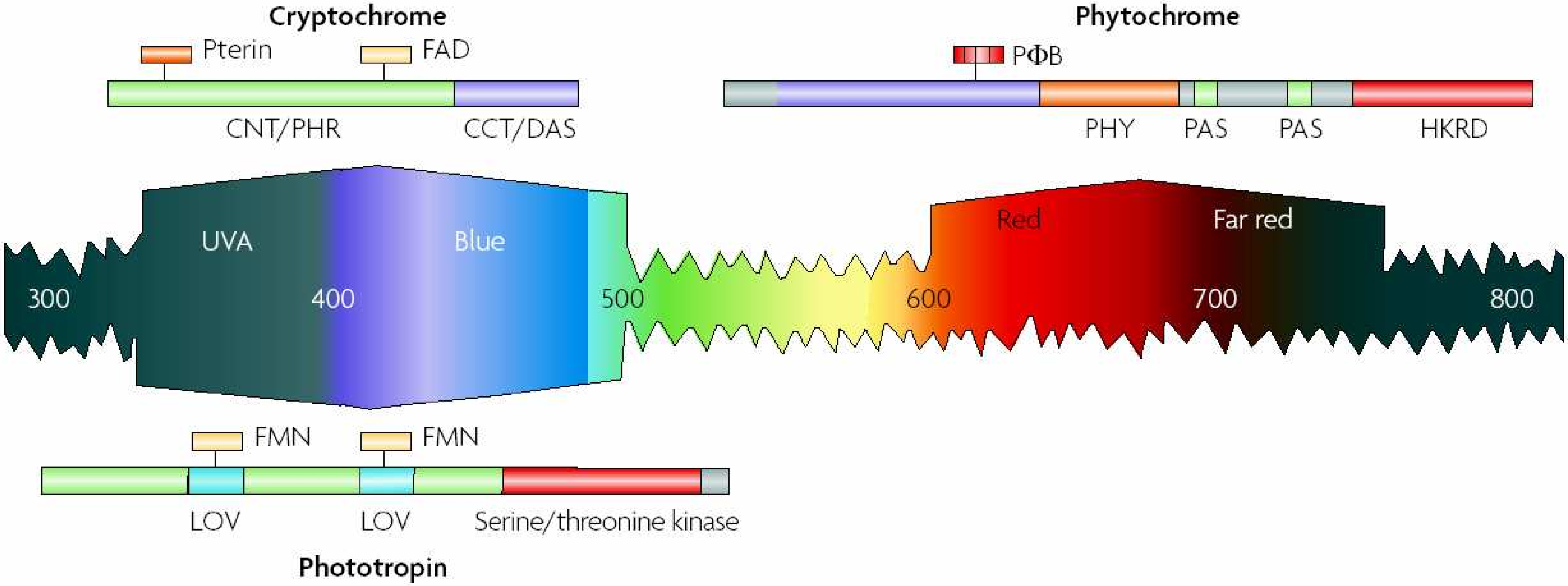
R,Fr,R,Fr,R,Fr



R,Fr,R,Fr,R,Fr,R,Fr

Fotoreceptory

- Fytochromy
- Kryptochromy
- Fototropiny (NPH1)
- Neznámý rec. UV-B

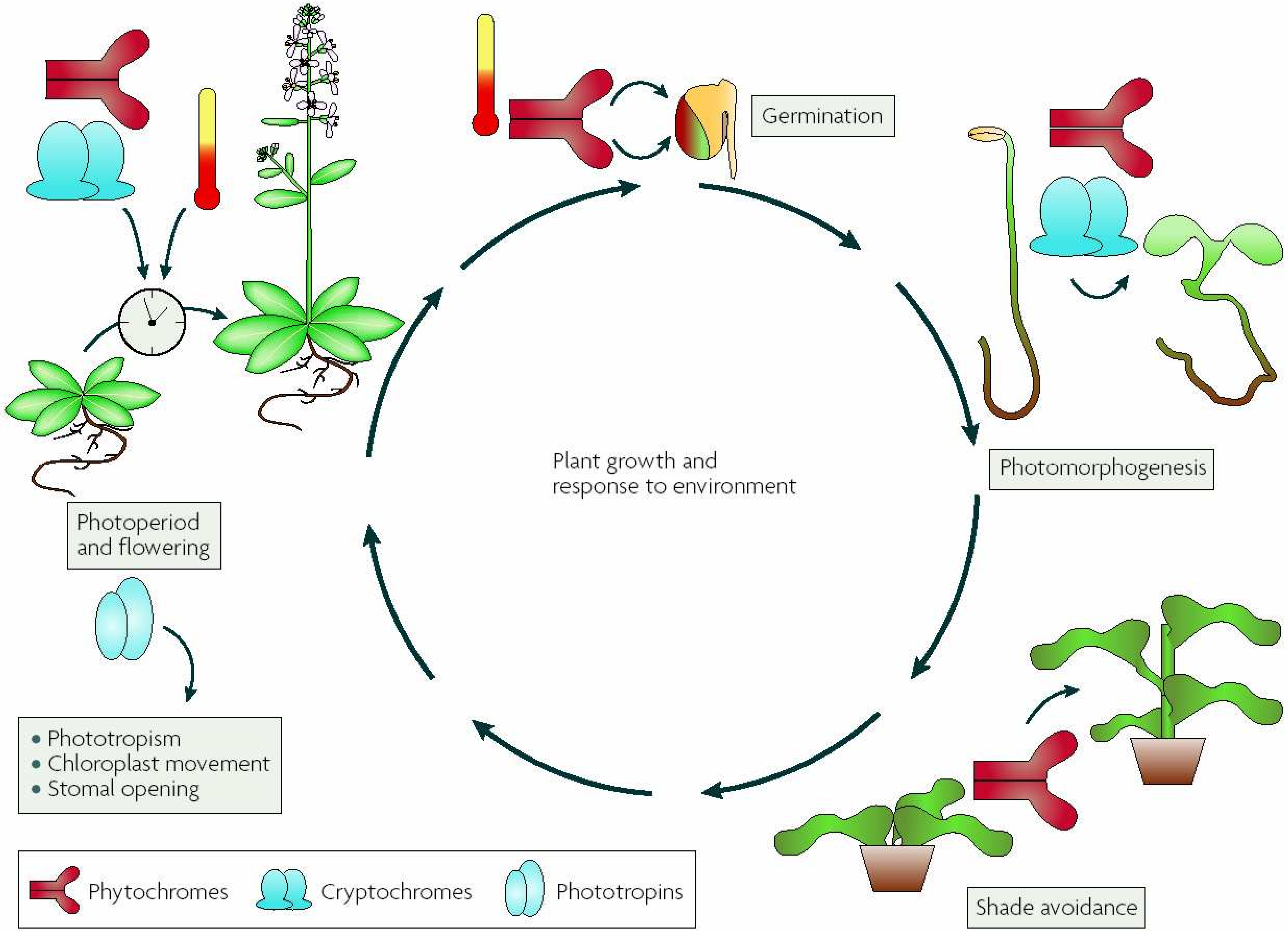


To monitor the light environment, plants have evolved a series of photoreceptors. Cryptochromes and phototropins perceive blue and ultraviolet A (UVA) wavelengths. Phytochromes predominately absorb the far-red and red wavelengths, and an unidentified photoreceptor, or photoreceptors, absorbs UVB.

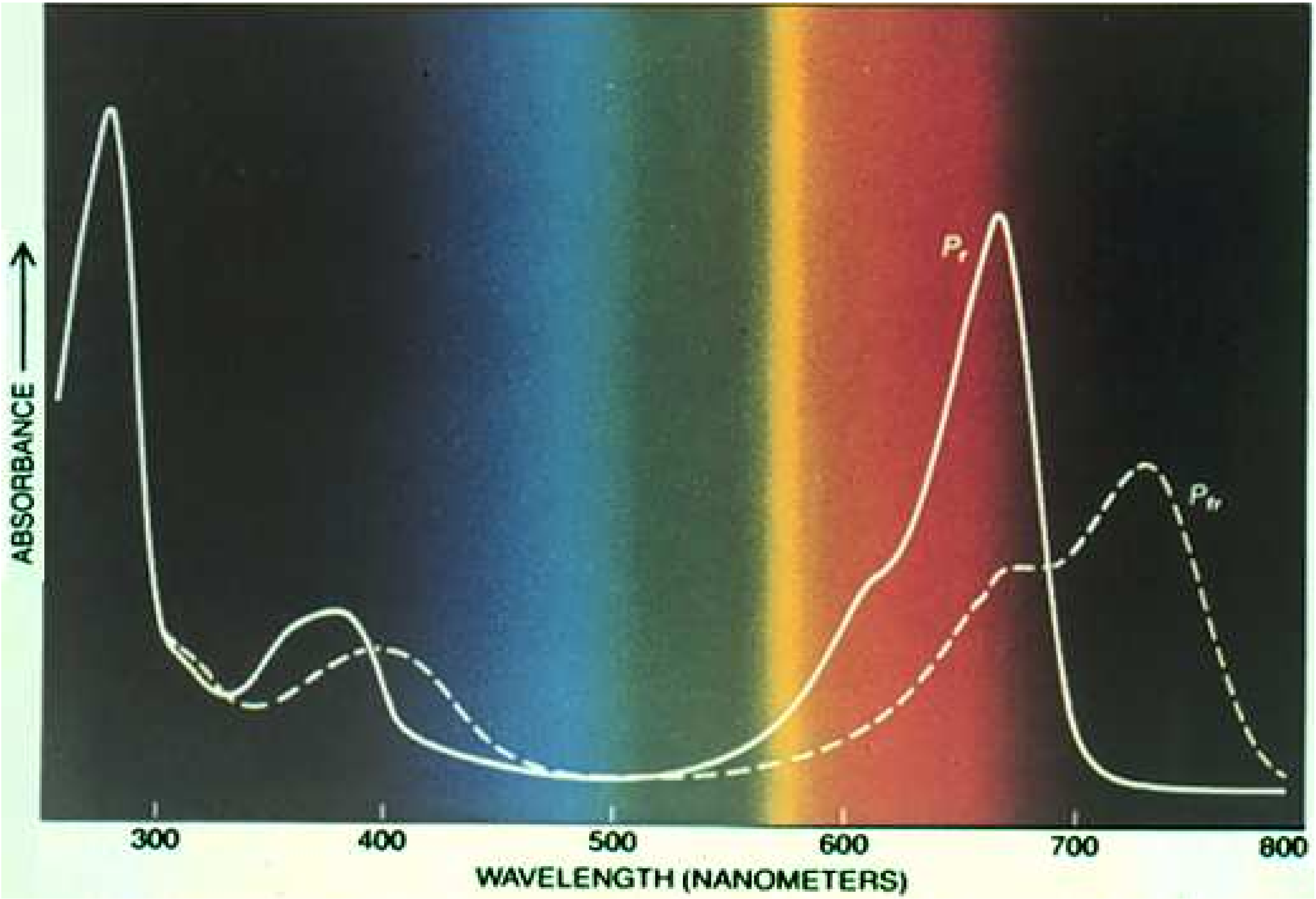
In higher plants, phytochromes form a small family, which further evolved independently in dicots[138]. There are five phytochromes (PHYA to PHYE) in *Arabidopsis thaliana*. PHYA is a type I phytochrome, which is most abundant in the dark and degrades rapidly after light exposure. All other phytochromes are relatively stable in the light and are classified as type II (REF. 139). The phytochromes are dimeric chromoproteins. Each polypeptide consists of an N-terminal photosensory domain that covalently binds a single bilin chromophore (PΦB), followed by a C-terminal domain that contains several motifs and functions in dimerization, light-dependent nuclear localization and, possibly, regulation of signalling[140].

There are two well-characterized cryptochromes in *A. thaliana*[5], CRY1 and CRY2, and a more divergent CRY3 (REFS 7,141). CRY1 and CRY2 have an N-terminal photolysase-related (PHR) domain (CNT) and a less-conserved, intrinsically unstructured C-terminal DAS domain (CCT), which is not present in CRY3 (REFS 141,142). The PHR domain non-covalently binds to two chromophores, a flavin adenine dinucleotide (FAD), and a pterin. CCT mediates a constitutive light response through direct interaction with CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) (REFS 143–145).

Phototropins are plant-specific blue light receptors, which have a photosensory N-terminal half and a C-terminal half with serine/threonine kinase function[146]. The N terminus contains two flavin mononucleotide (FMN) chromophore-binding LOV domains (LOV1 and LOV2).


Germination
Photomorphogenesis
Shade avoidance
Plant growth and response to environment
Photoperiod and flowering
• Phototropism
• Chloroplast movement
• Stomal opening
Phytochromes
Cryptochromes
Phototropins

Absorption spectra of the two forms of Phytochrome



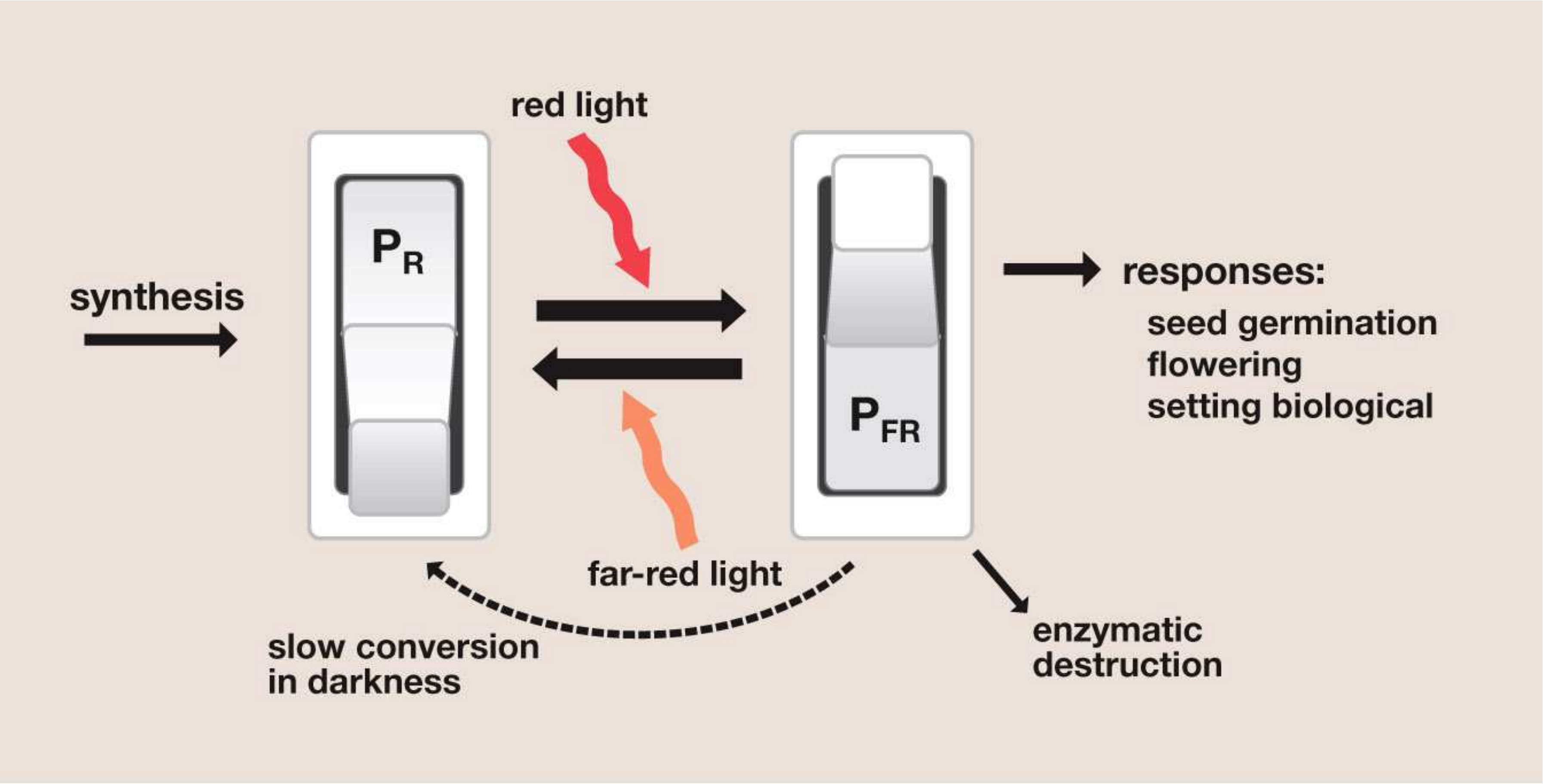


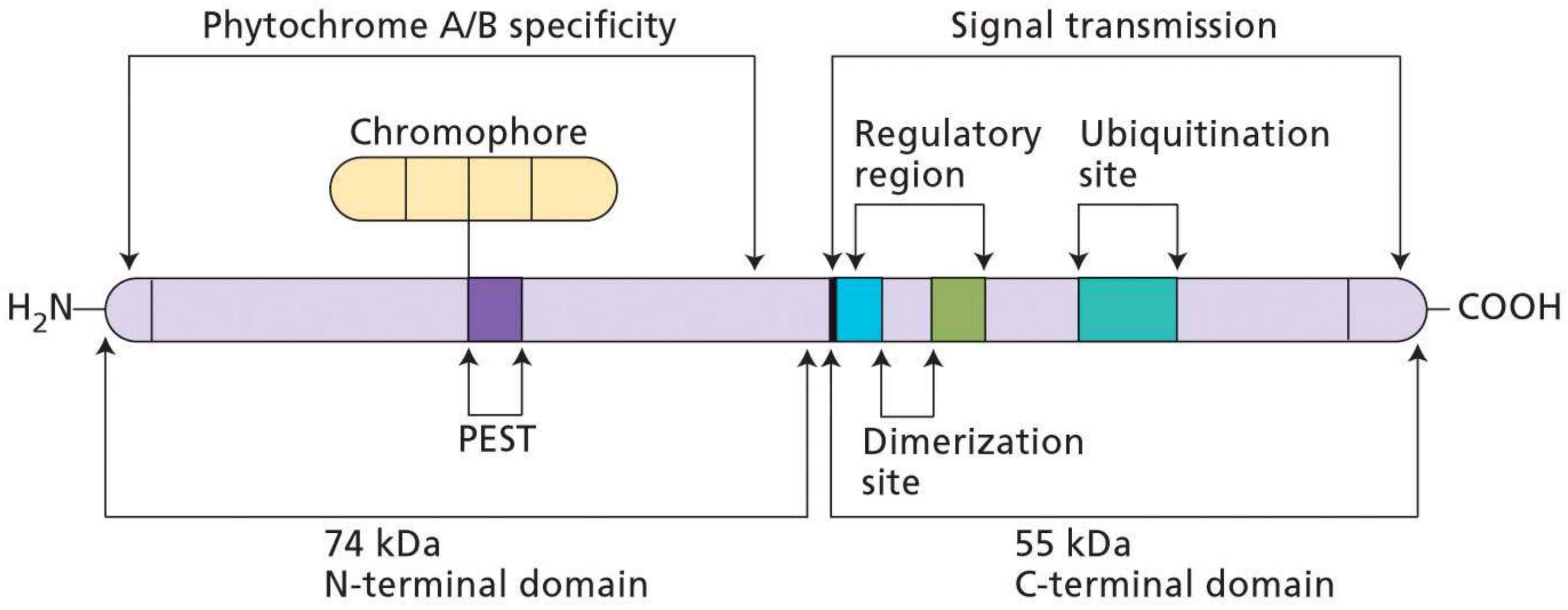
Figure 12.9 Plant Biology, 2/e

Fytochromy

- 5 genů v Arabidopsis (PhyA - PhyE)
- Všechny exprimovány +/- všude –> post-translační regulace
- PhyA a PhyB dominantní a nejvíce studovány

- PhyA je foto-labilní
- Phy B,C,D,E jsou foto-stabilní

- PhyA je nejen nestabilní na světle (poločas rozpadu PfrA ~ 1h), ale dochází také k poklesu jeho transkripce zprostředkovaného fytochromovým systémem



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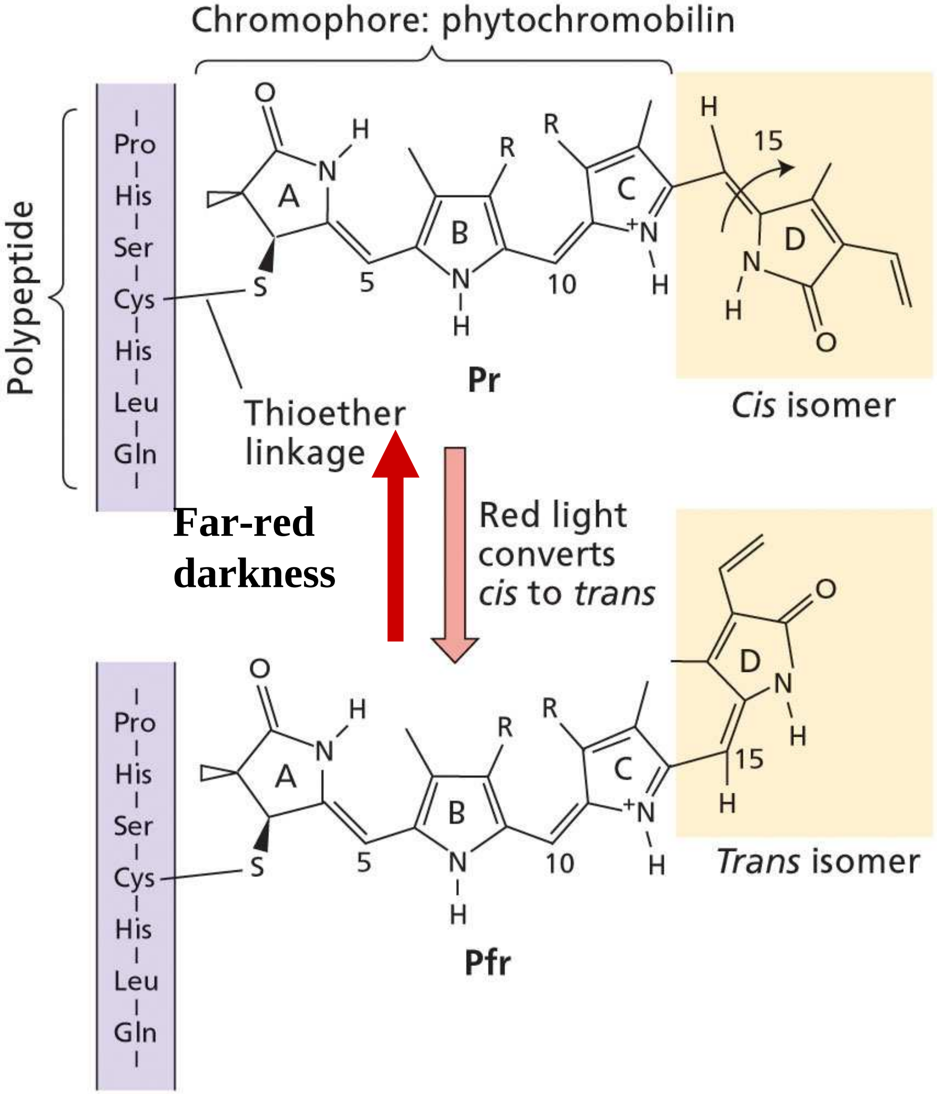
- Phytochromes consist of a polypeptide (~125 kDa, 1200 aa) and a covalently linked tetrapyrrole chromophore in the N-terminal part.
- Phytochromes are homodimers through the C-terminal dimerization (heterodimer?)
- All phytochromes seem to have identical chromophore
- The protein part of phytochrome is encoded by multigene family (phytochrome gene family) in all higher plants examined



Far-red darkness

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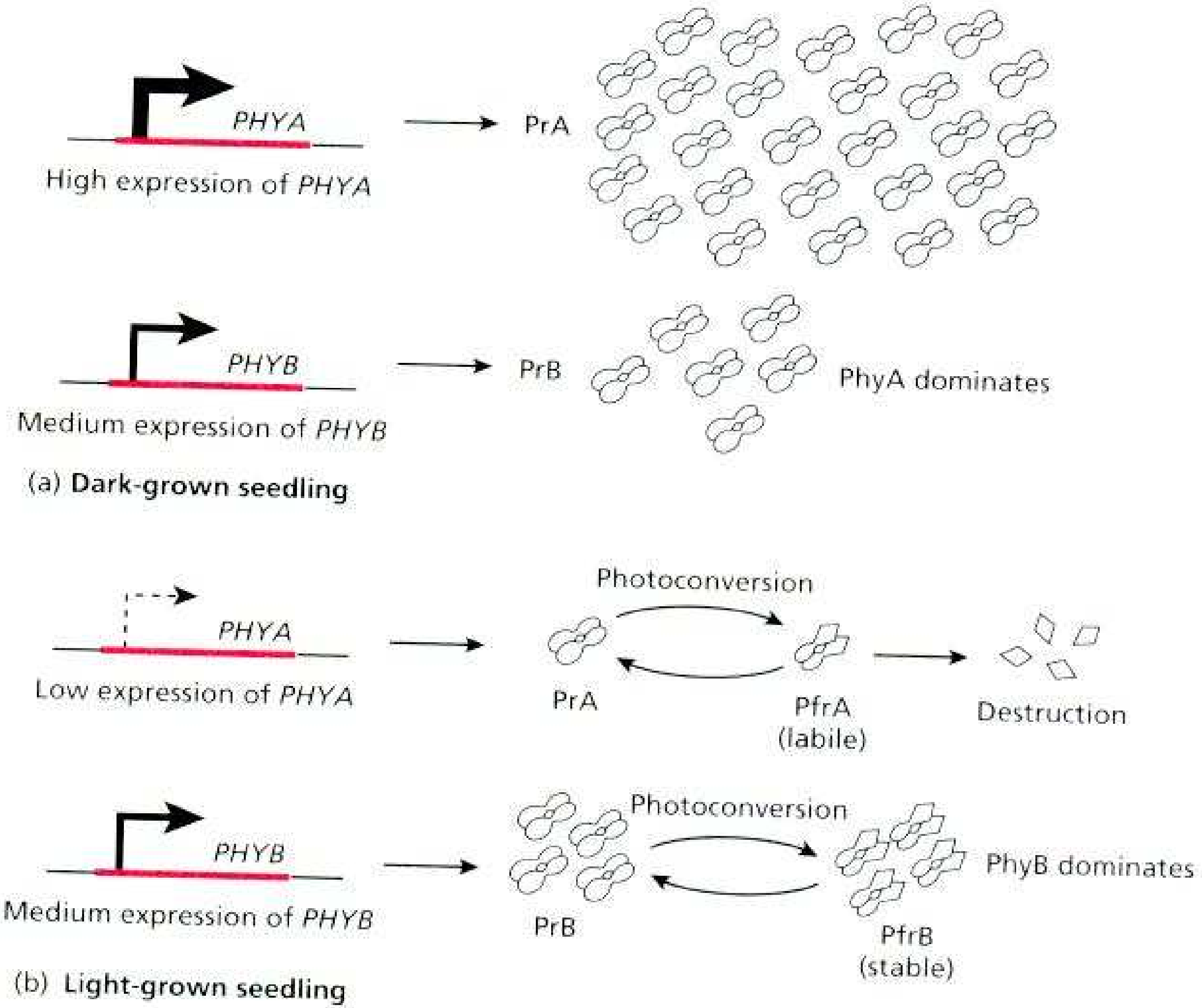
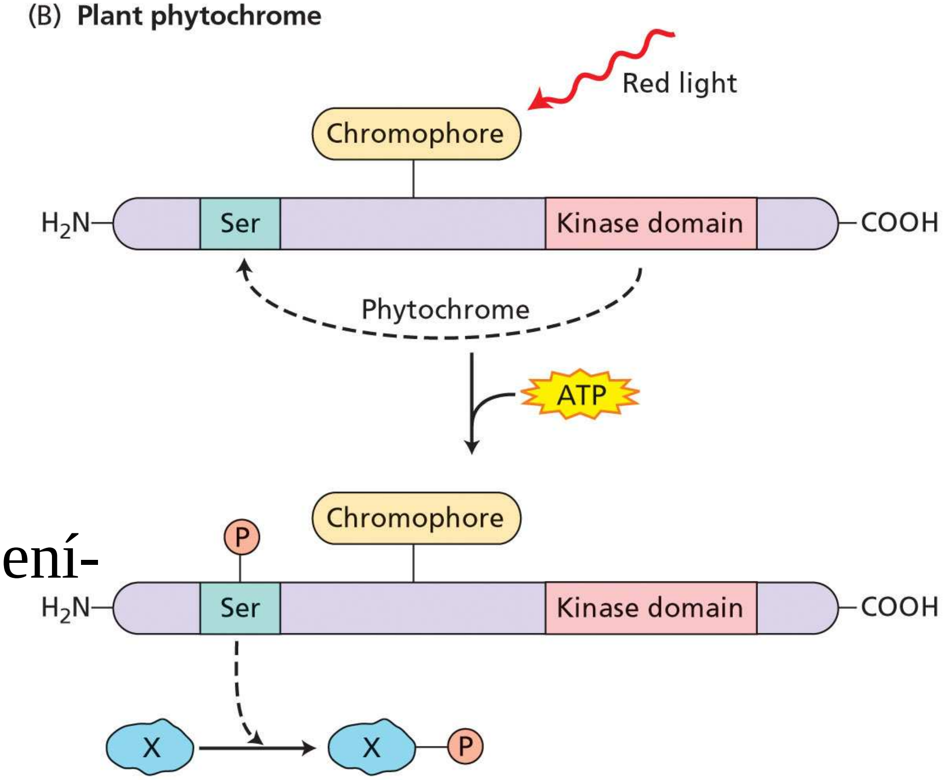


Fig. 7.2 The balance between phyA and phyB in dark- versus light-grown seedlings. (a) In dark-grown seedlings, the *PHYA* gene is highly expressed resulting in the accumulation of large amounts of phyA in the Pr form (PrA). The *PHYB* gene is expressed at a lower level. (b) The concentration of phyA declines rapidly when dark-grown seedlings are transferred to the light because of light-induced inhibition of transcription from the *PHYA* gene, and the rapid destruction of PfrA. In contrast, the *PHYB* gene is transcribed at a similar rate in both light and dark conditions, and PfrB is stable. Consequently, whereas phyA dominates in dark-grown seedlings, phyB dominates in light-grown seedlings.

Phy v Pfr formě jsou kinázy

- Autofosforylace, fosf. regulatory (Aux/IAA, TFs), kryptochromy
- Jen PfrB jde do jádra (FR to inhibuje)– kin. hodiny.
- PhyA obě formy v jádře po osvětlení- kin. 15min.



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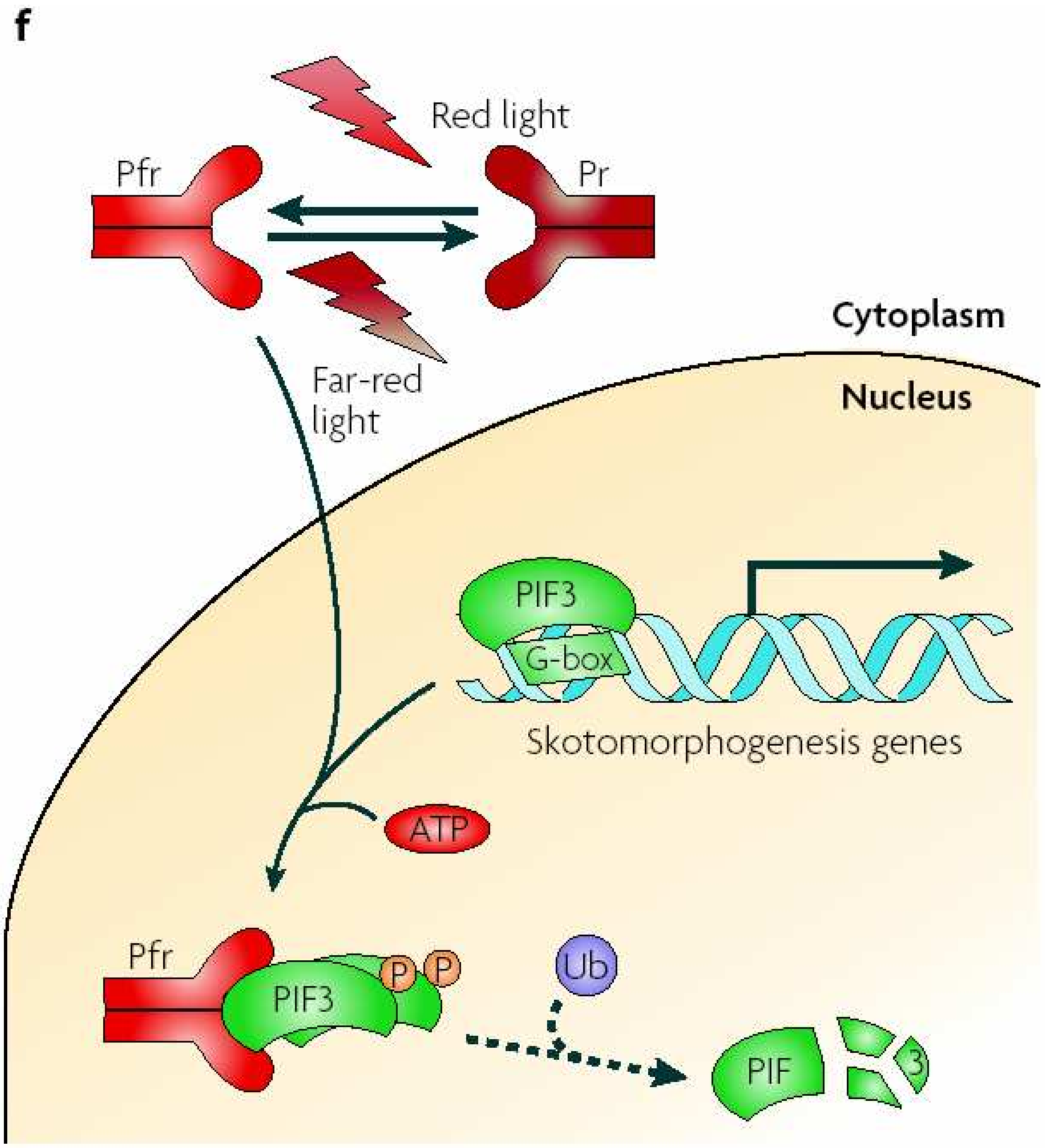
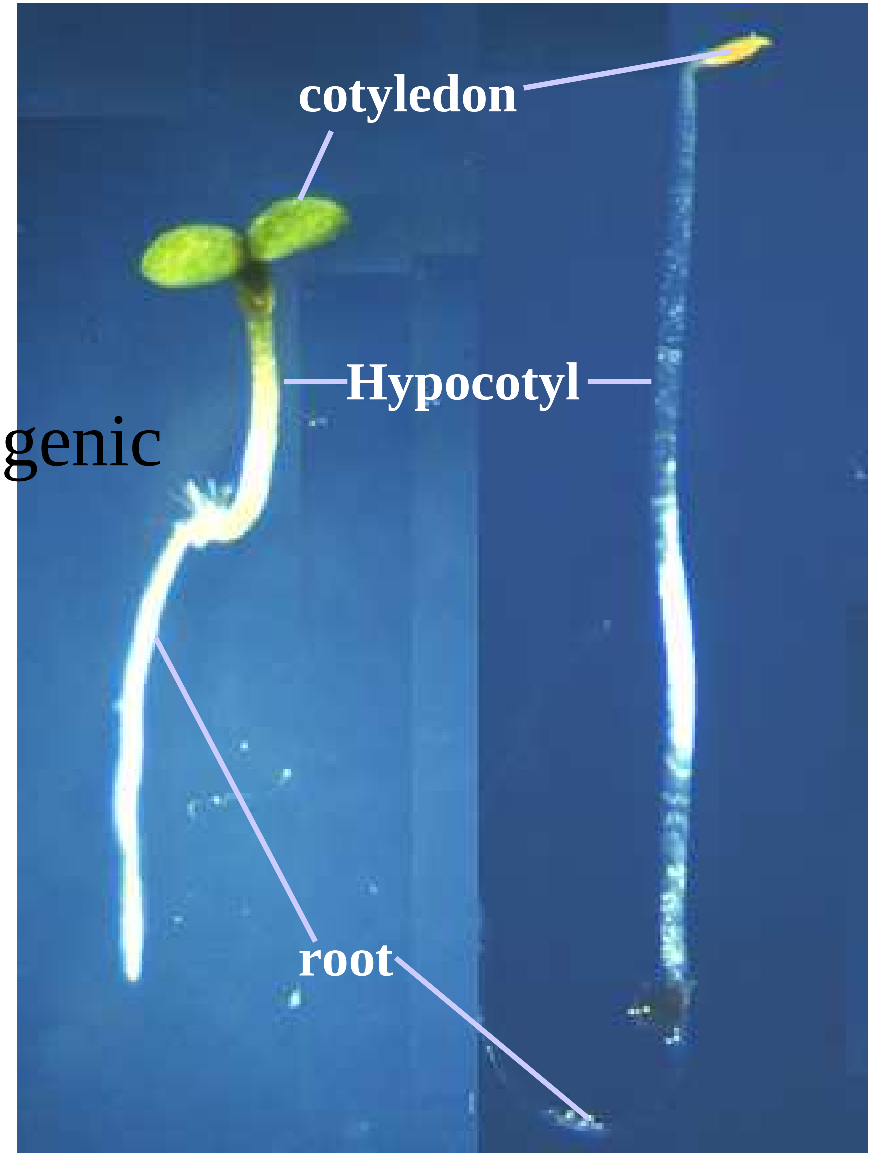

f
Red light
Pfr
Pr
Far-red light
Cytoplasm
Nucleus
PIF3
G-box
Skotomorphogenesis genes
ATP
Pfr
PIF3
P
P
Ub
PIF
3

Fytochromoví mutanti

Jak je hledat?

Jsou i na světle částečně slepí - a tedy vypadají jako ve tmě.



Photomorphogenic
development

Skotomorphogenic
development or
Etiolation

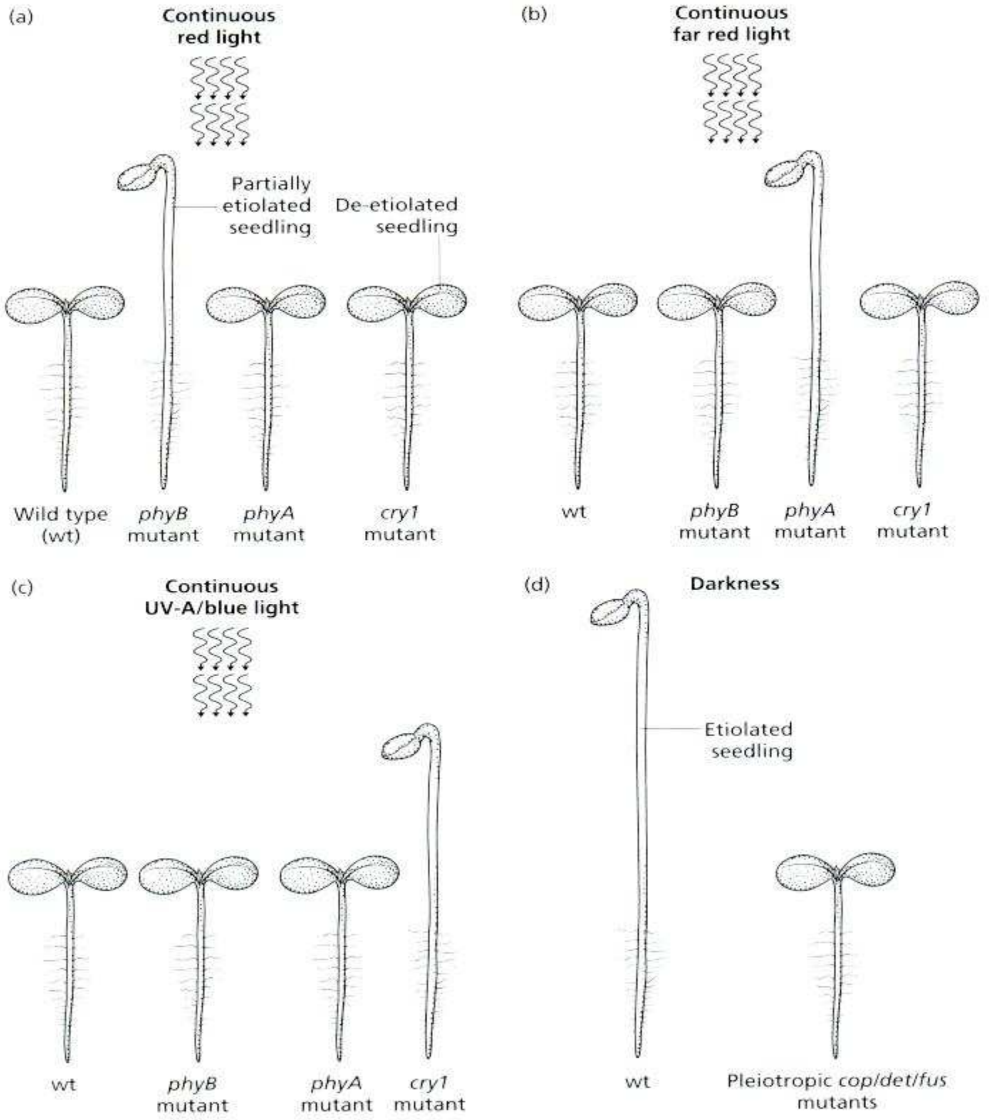
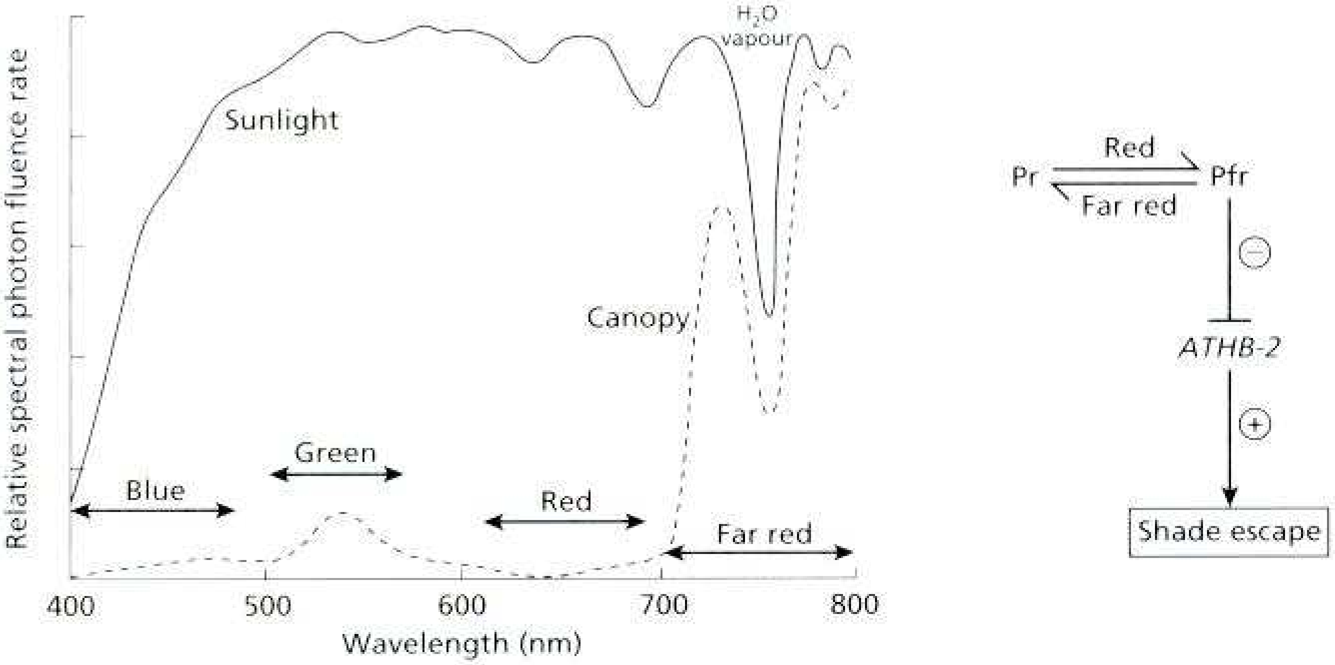


Fig. 7.6 Positive and negative regulation of photomorphogenesis in *Arabidopsis* revealed by mutants. Photomorphogenesis is promoted by continuous red light, continuous far red light and continuous UV-A/blue light in wild-type seedlings. The phenotypes of photoreceptor mutants indicate that the response to red light requires phyB (a); the response to far red light requires phyA (b); and the response to UV-A/blue light requires cry1 (c). (d) In darkness, wild-type seedlings are etiolated and photomorphogenesis is suppressed. The pleiotropic *cop/det/fus* mutants have a photomorphogenic phenotype in darkness, indicating that the *COP/DET/FUS* genes are required to suppress photomorphogenesis in dark-grown seedlings.

Phytochrome function specificity during early Arabidopsis development

phyA:	**Far-red**	**De-etiolation (major)**
phyB:	**Red**	**De-etiolation (major)**
phyC:	**Red**	**Primary leaf expansion**
phyD:	**Red**	**De-etiolation (minor)**
phyE:	**Red**	**Internode suppression**

PhyB se např. účastní úniku ze stínu



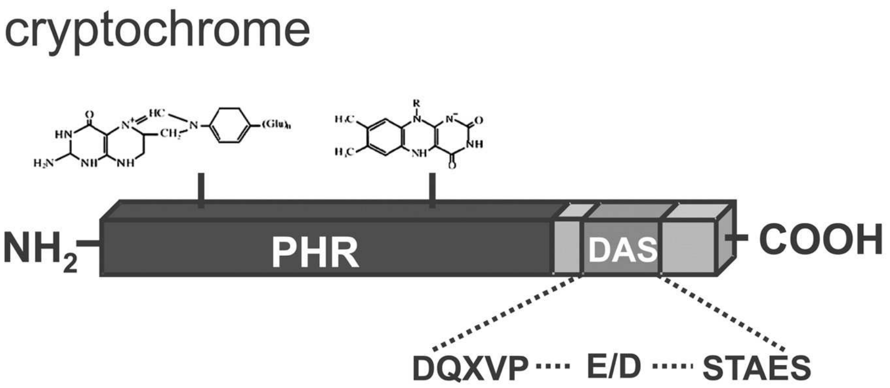
(a) **Light intensity at different wavelengths in sunlight and beneath a canopy**

(b) **The effects of red and far red light on shade escape**

Fig. 7.8 The ratio of red light to far red light regulates the shade escape response. (a) Comparing spectral fluence rates (light intensity at different wavelengths) in sunlight and canopy shade shows that the ratio of red light to far red light (R : FR) is much higher in sunlight. Therefore, R : FR is an indicator of the degree of shading by neighbouring plants. (b) If red is high relative to far red, as in sunlight, then Pr will convert to Pfr. High Pfr inhibits the transcription of the *ATHB-2* gene and hence inhibits shade escape. The line ending in a bar and accompanied by a 'minus' sign indicates negative regulation. The arrow accompanied by a 'plus' sign indicates positive regulation. ((a) from Smith, 1994 (Fig. 1) with kind permission from Kluwer Academic Publishers.)

Kryptochromy

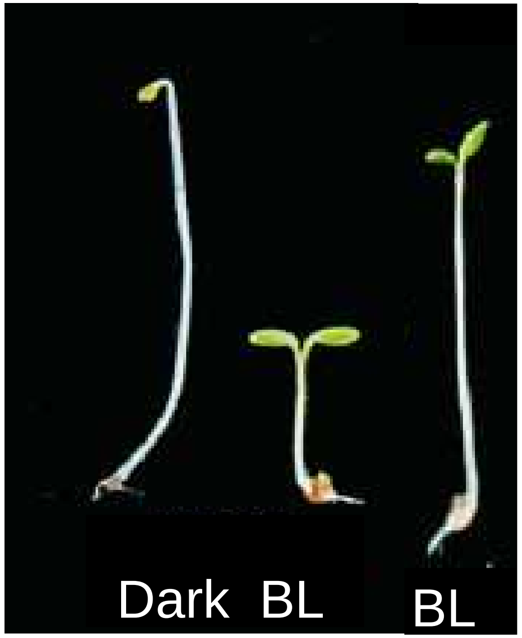
- Vyvinuly se z fotolyáz – nezávisle u rostlin a živočichů



Cryptochromes

Many plant responses were not R-FR reversible and had action spectra with peaks in the blue and near-UV. There must be a BL receptor(s).

Due to their elusive nature, Gressel (1977) described BL receptors as "cryptochromes".
hy4 mutants showed a lack of hypocotyl growth inhibition under blue light,but were normal under red and far-red.



Wild-type ***cry1***

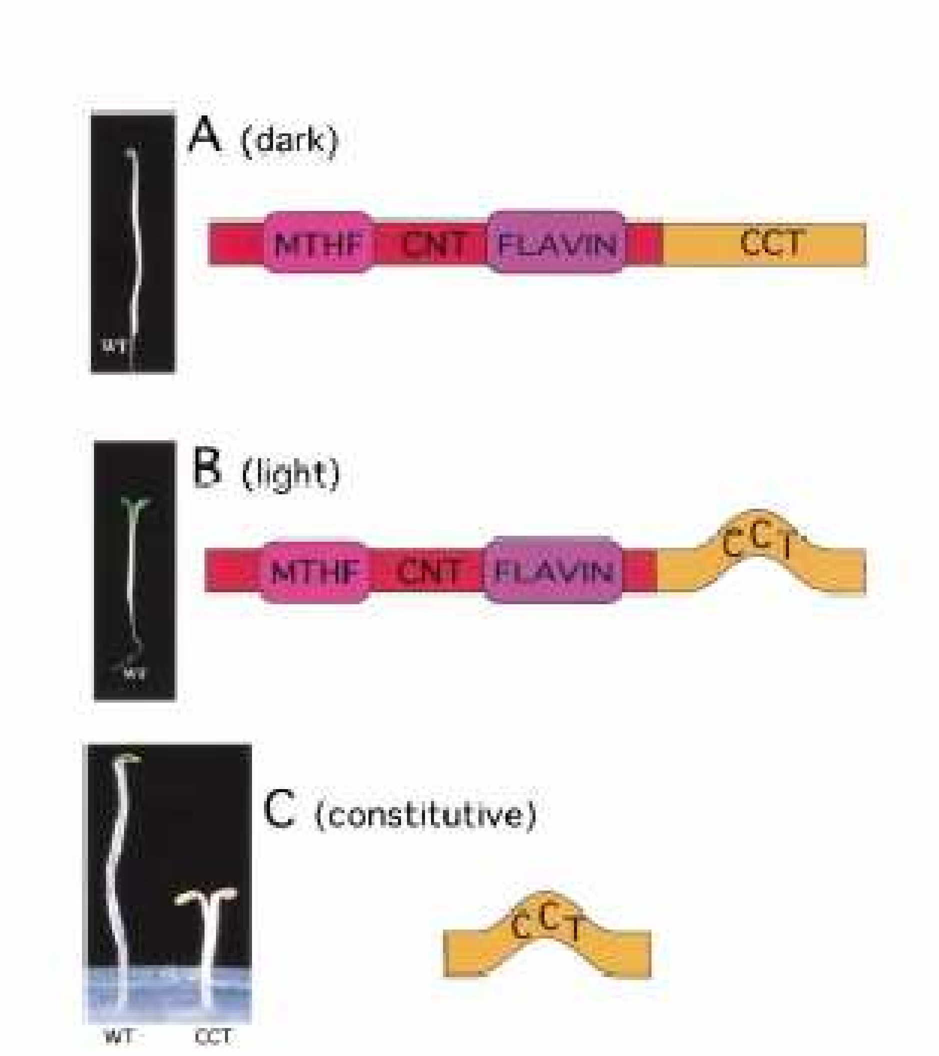
adapted from Neff and Chory, 1998

The sequence of *Hy4* was reported in 1993, and it was similar to DNA photolyase (Ahmad and Cashmore 1993, Sancar 1994) yet has no photolyase activity. Re-designated "Cryptochrome 1" (Lin et al., 1995)

Involvement in circadian rhythms; led to discovery of animal crys (Cashmore, 2003)

cry's are phosphorylated when illuminated (Shilatin et al., 2003, Bouvy et al., 2003). The timing of phosphorylation agrees well with the time course of early physiology (Folta and Spalding, 2001)

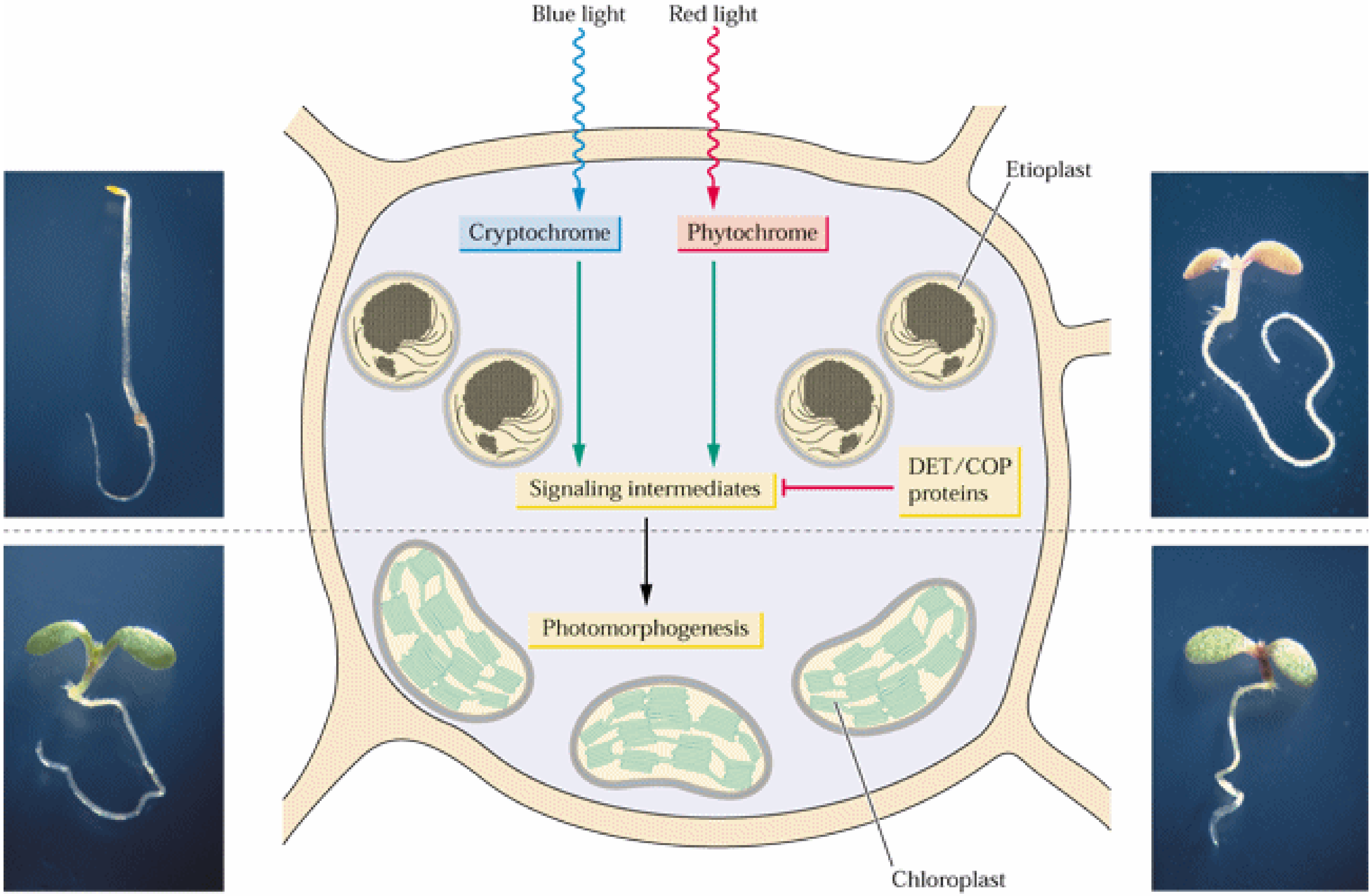
The C-Terminus CCT of cry1 Regulates Photomorphogenesis



Ectopic overexpression of the CRY1 C-terminal extension results in a constitutive-photomorphogenic phenotype.

Cashmore, 2003

Pozitivní a negativní regulace fotomorfogeneze



Wild-type plants

det/*cop* mutants

Xing Wang Deng, Yale

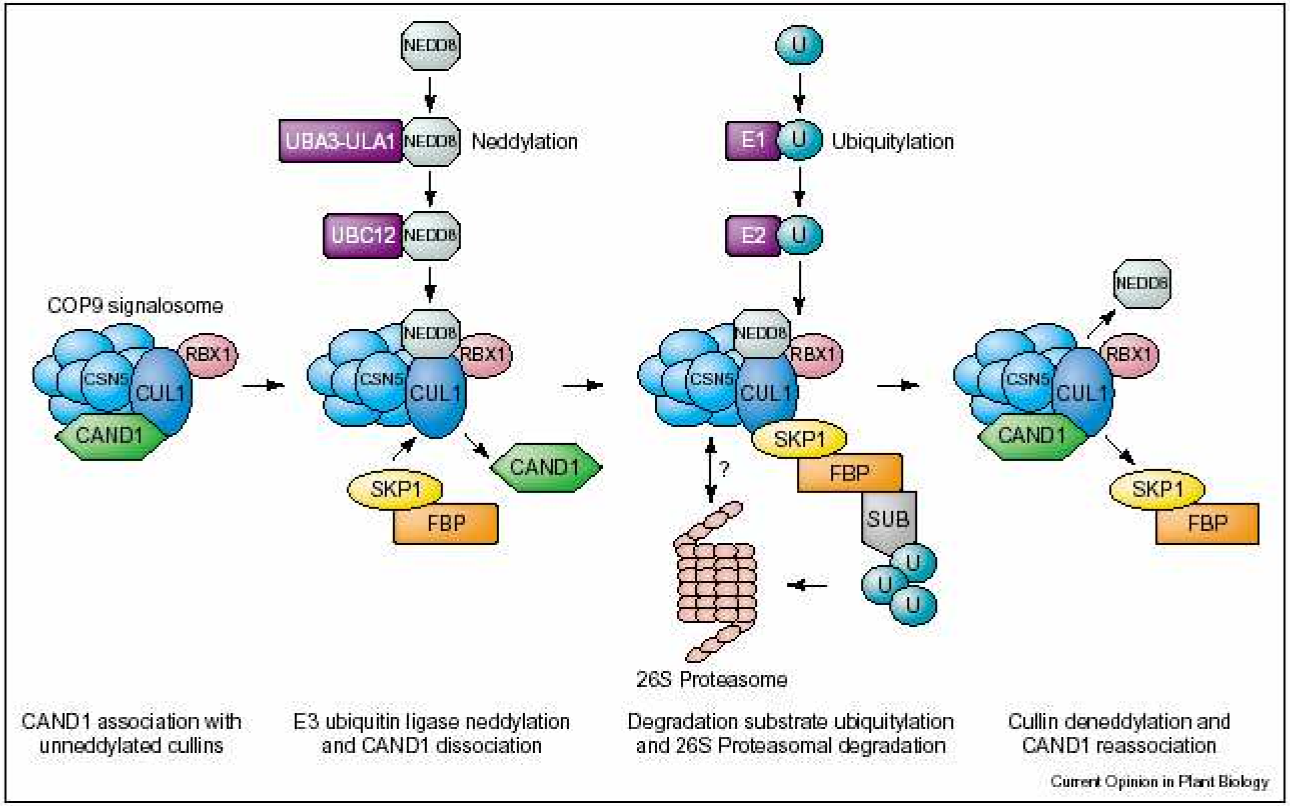
COP Mutants

First isolated by Deng et al., 1991

--Constitutive Photomorphogenic phenotype:

expanded cotyledons, short hypocotyls, light-regulated gene expression patterns in darkness

1996 Mayer et al. show that COP1 mutation affects expression of many genes– not just specific to photomorphogenesis.



Current Opinion in Plant Biology

General overview of the eukaryotic ubiquitin–proteasome system. Proteolysis substrates (SUB) are recognized by E3 ubiquitin (U) ligases (E3), exemplified here by an SCF-type E3 complex. Poly-ubiquitylation of the bound substrate also requires the activities of E1 ubiquitin-activating enzymes (E1) and E2 ubiquitin-conjugating enzymes (E2). Following poly-ubiquitylation, substrates are degraded in the 26S proteasome [1,3]. The E3 subunit cullin can be modified by NEDD8 conjugation (neddylation) [12]. At the biochemical level, ubiquitylation and neddylation are highly related processes. Cullin neddylation results in the dissociation of the cullin-interacting protein CAND1 [13,14,15•]. This process may allow the cullin–RBX1 complex to associate with specificity components of the E3, such as SKP1–F-box protein (FBP) heterodimers. The COP9 signalosome (CSN) is associated with unneddylated and neddylated cullins [16,17]. Its CSN5 subunit mediates cullin deneddylation and may therefore play a role in controlling E3 complex formation [16–18]. There is some evidence that CSN interacts with subunits of the 26S proteasome [25,74].

Abbreviations

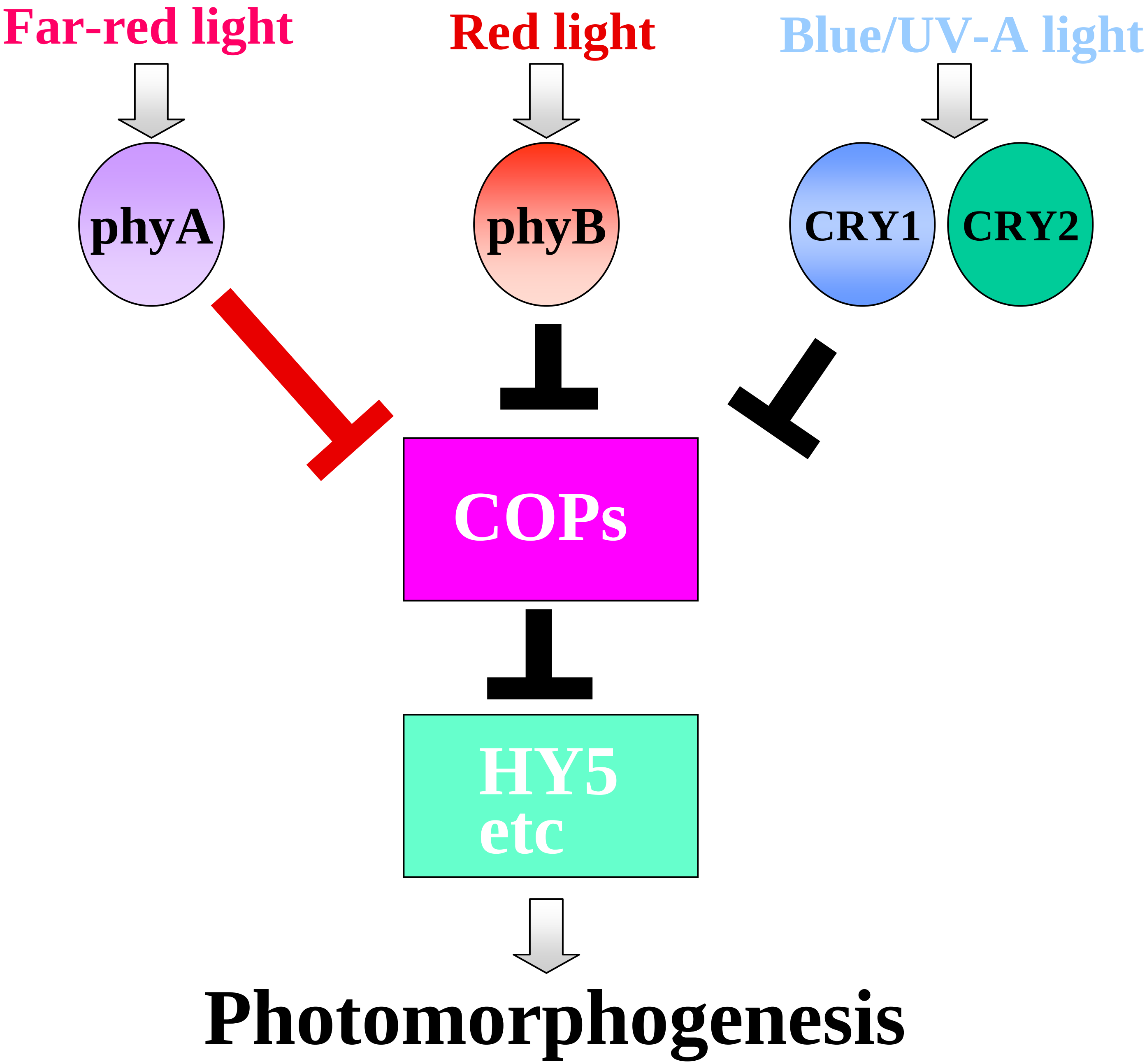
ACS	1-aminocyclopropane-1-carboxylic acid synthase
APC/C	anaphase-promoting complex/cyclosome
BTB/POZ	Bric-a-Brac Tramtrack and Broad Complex/Pox virus and Zinc finger
CAND1	CULLIN-ASSOCIATED NEDDYLATION DISSOCIATED1
COP9	CONSTITUTIVELY PHOTOMORPHOGENIC9
CSN	COP9 signalosome
DCX	DDB1/cullin 4A/X-box
DDB1	DAMAGED DNA-BINDING PROTEIN1
DET1	DEETIOLATED1
E1	ubiquitin-activating enzyme
E2	ubiquitin-conjugating enzyme
E3	ubiquitin ligase
EBF	EIN3-BINDING F-BOX
EIN3	ETHYLENE INSENSITIVE3
EIL1	ETHYLENE INSENSITIVE3-LIKE1
eto2	*ethylene overproducer2*
GA	gibberellic acid
GAI	GIBBERELLIC ACID INSENSITIVE
HY5	LONG HYPOCOTYL5
HYH	LONG HYPOCOTYL5-LIKE
LAF1	LONG AFTER FAR-RED LIGHT1
NEDD8/RUB1	NEURAL PRECURSOR CELL EXPRESSED, DEVELOPMENTALLY DOWNREGULATED 8/ RELATED TO UBIQUITIN1
phyA	phytochrome A
RBX1	RING-BOX1
RGA	REPRESSOR OF *ga1-3*
SCF	SKP1/Cullin1/F-box protein
SKP1	SUPPRESSOR OF KINETOCHORE PROTEIN1
SLY1	SLEEPY1
SPA1	SUPPRESSOR OF PHYTOCHROME A1

CSN komplex (viz. nahoře)

COP9 byla první známá podjednotka signalosomu.

HY5 is a positive regulator of photomorphogenesis and acts downstream of multiple photoreceptors

	Blue	Red	Far-red
hy1, hy2:	Normal	Long	Long
hy3/phyB:	Normal	Long	Normal
phyA:	Normal	Normal	Long
Hy4/cry1:	Long	Normal	Normal
hy5:	Long	Long	Long



Far-red light

Red light

Blue/UV-A light

phyA

phyB

CRY1

CRY2

COPs

HY5
etc

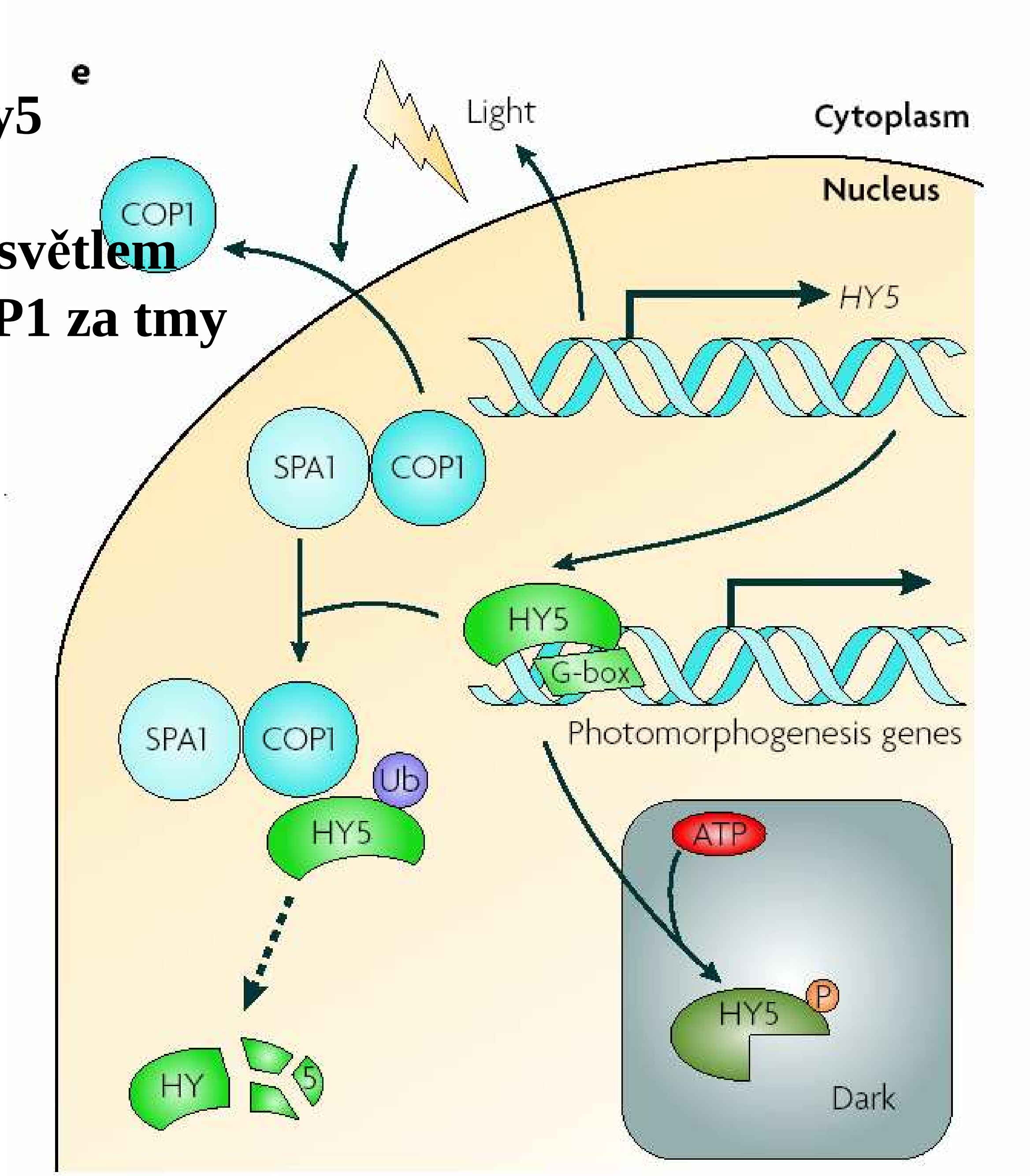
Photomorphogenesis

HY5 and COP1

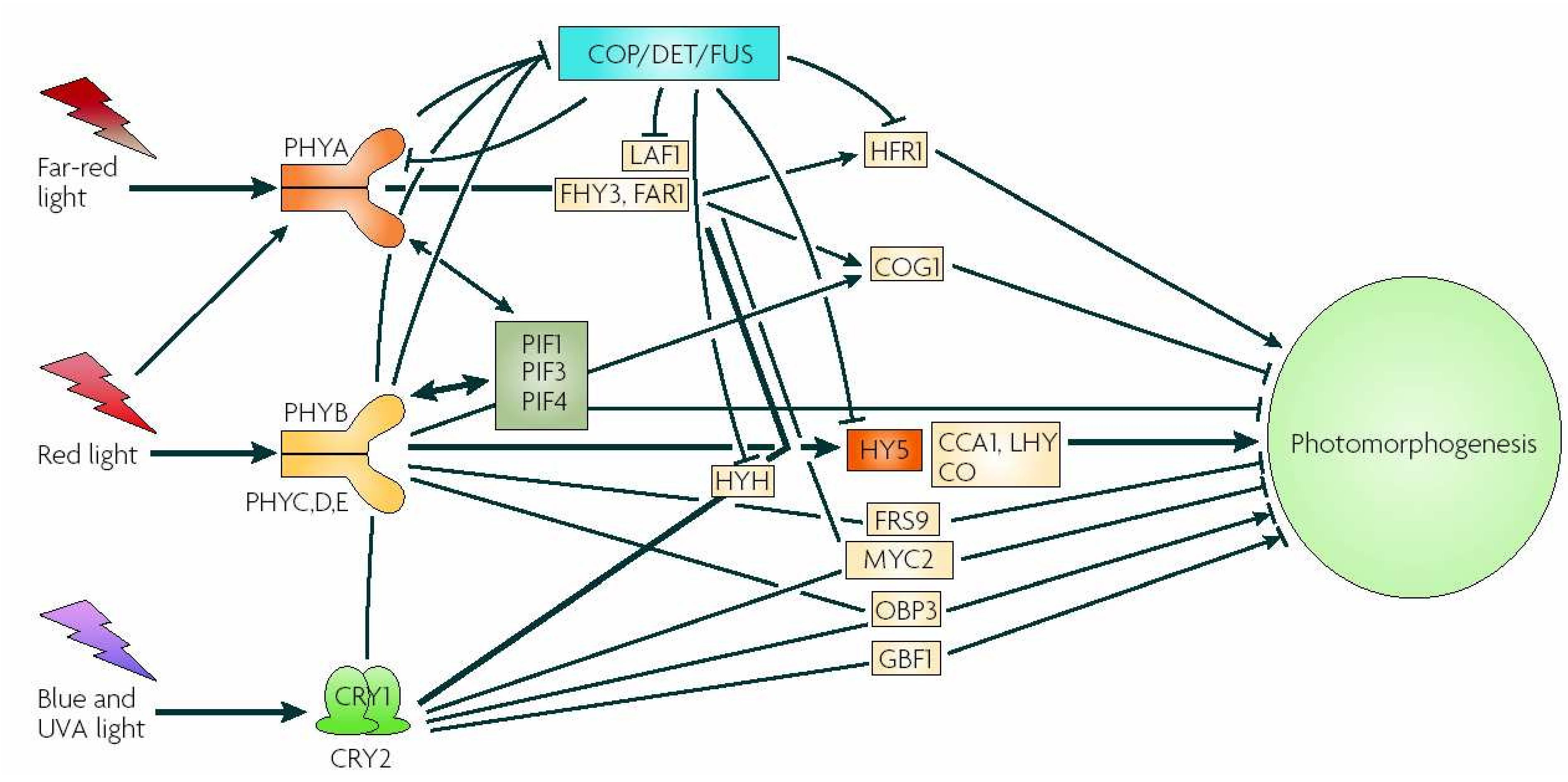
- HY5 encode a bZIP type DNA-binding transcription factor
- HY5 binds to promoters of light-regulated genes and mediates their light activation
- HY5 is a nuclear protein and directly interact with nuclear localized COP1

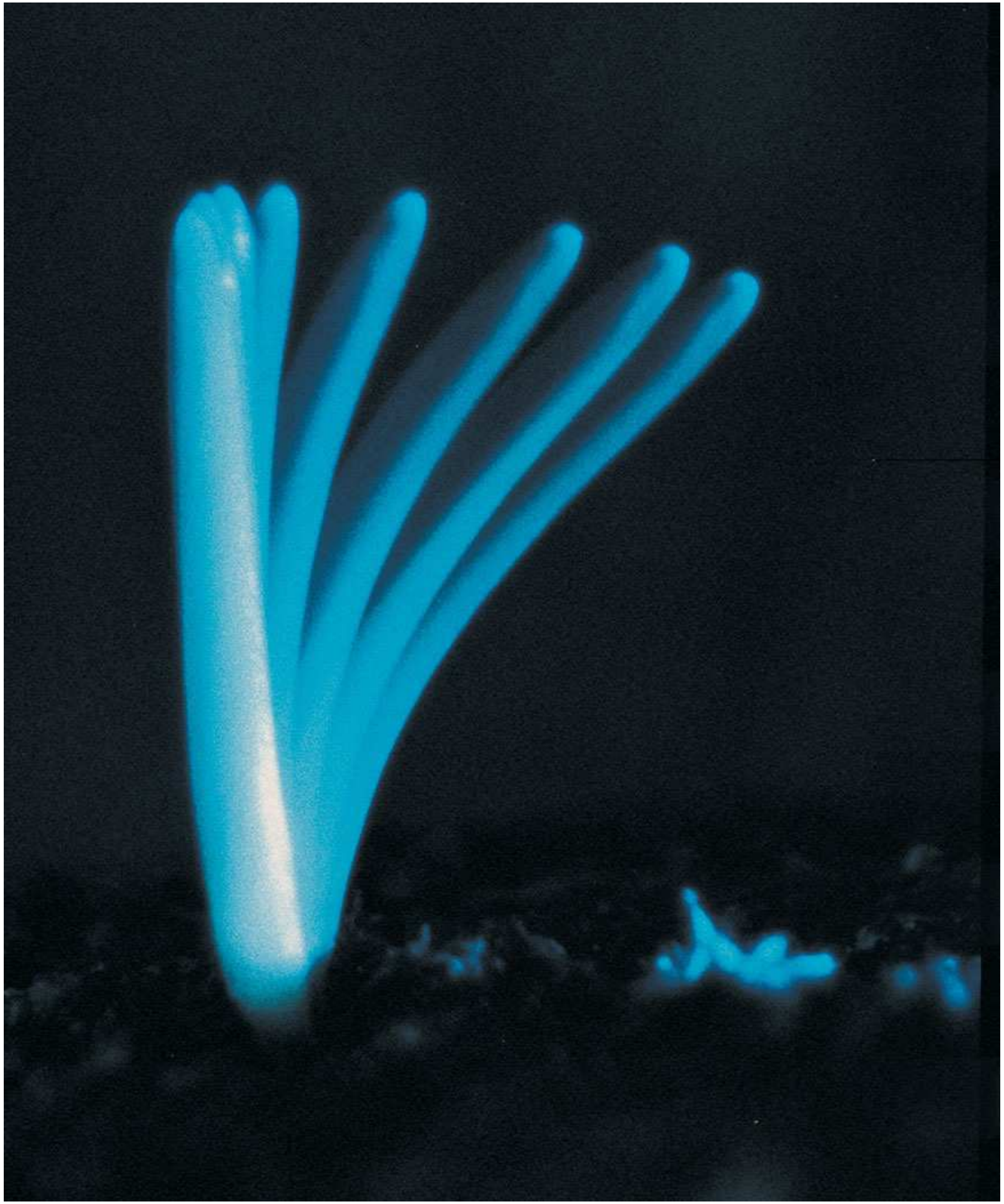
Komplexní regulace Hy5

Transkripční regulace světlem
Degradace pomocí COP1 za tmy
Fosforylace



Hy5 integruje signály z fytochromů a kryptochromů





Fototropismus

a

FOTOTROPINY

Perception of light and signal transduction in phototropism

In most species, UV-A and blue wavelengths induce the greatest phototropic

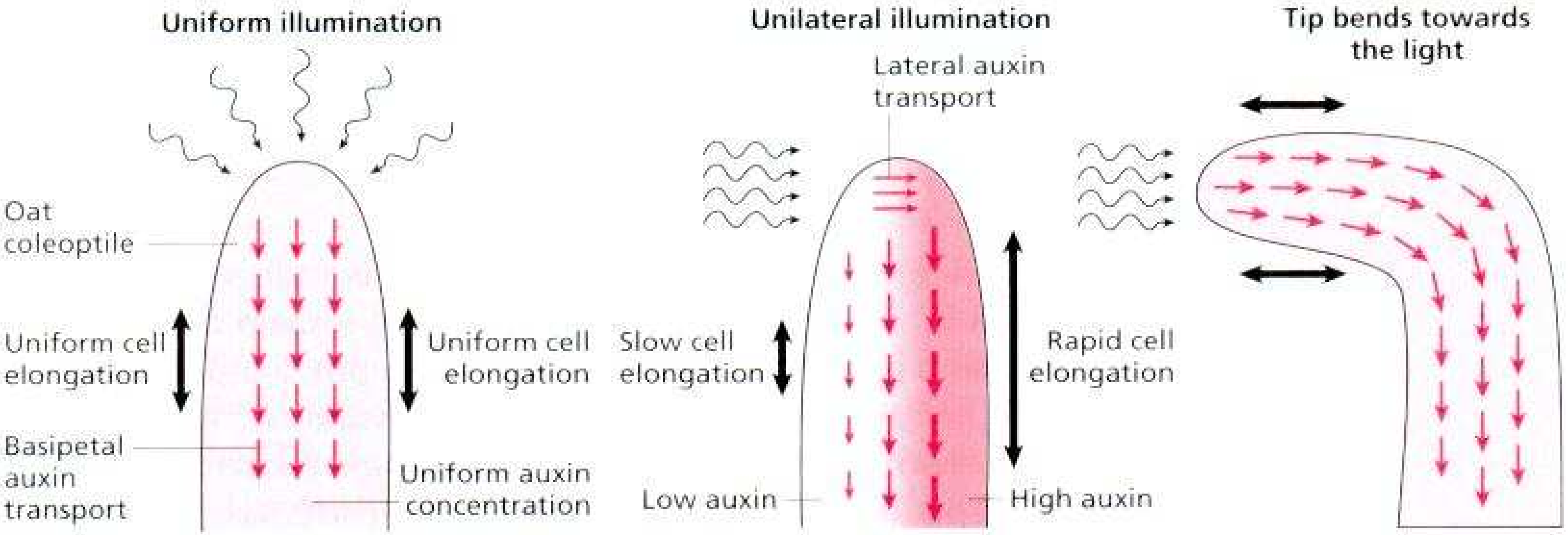
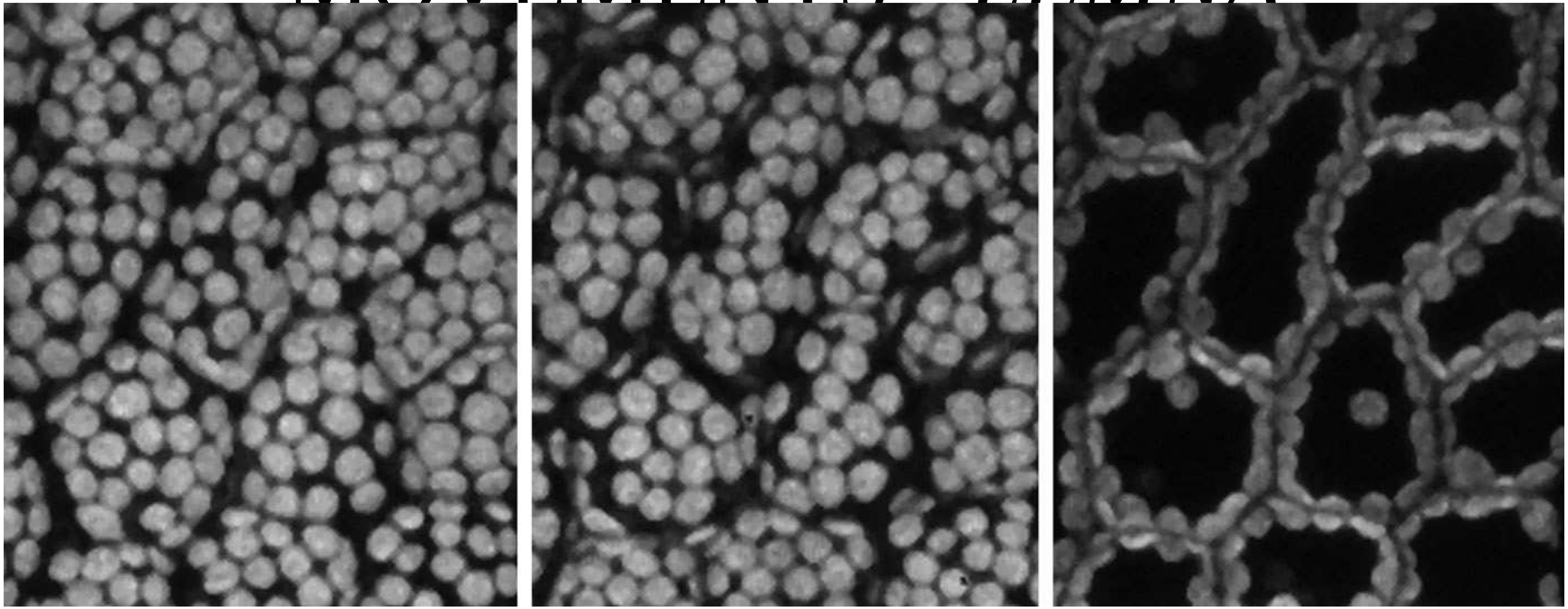


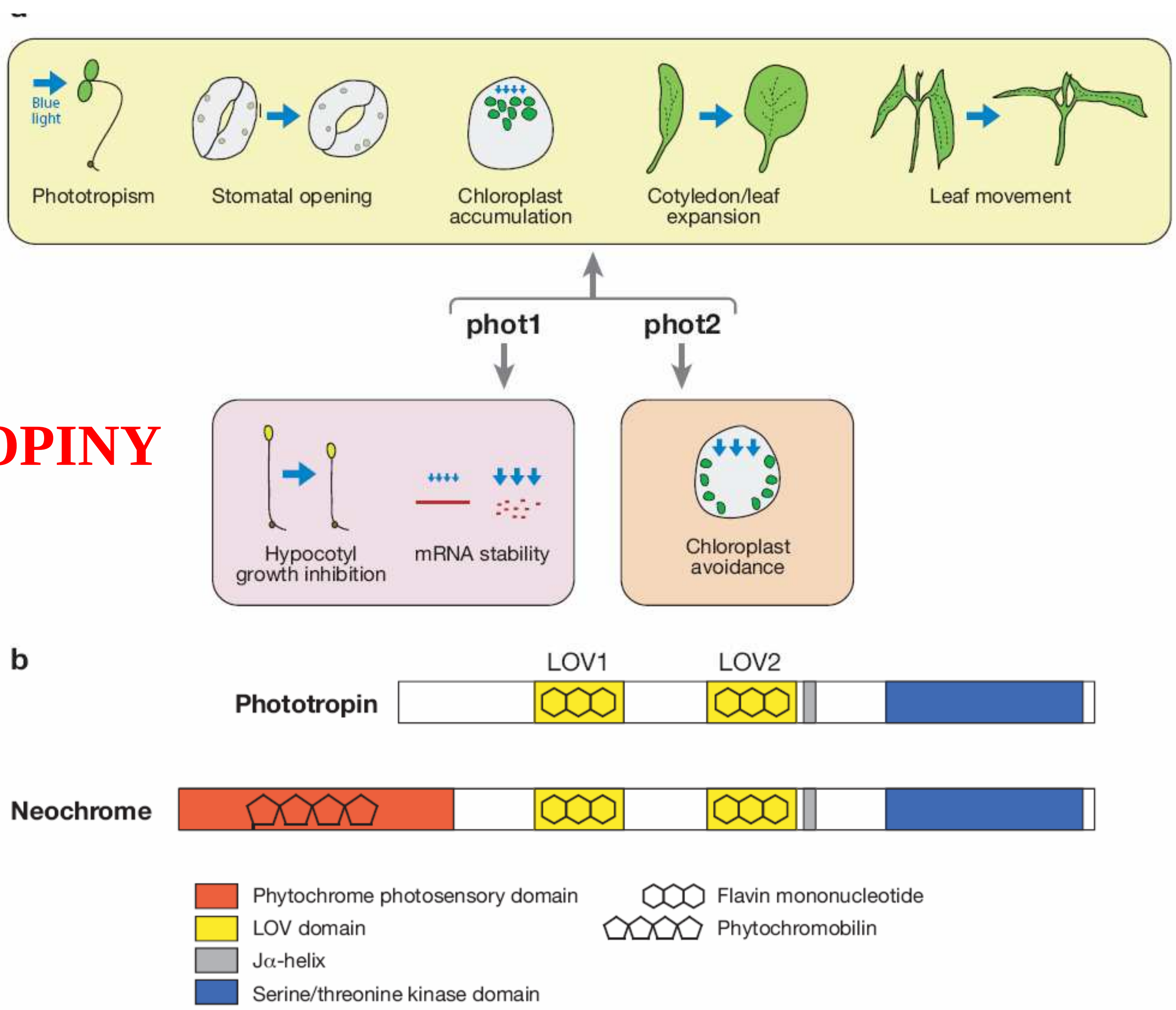
Fig. 7.9 The Cholodny–Went hypothesis of phototropism in oat coleoptiles. Unilateral illumination induces lateral auxin transport in the coleoptile tip leading to uneven auxin distribution in the coleoptile and to changes in cell expansion rates. The changes in expansion rates cause the coleoptile to bend towards the light.

opět auxin a jeho transport

CHLOROPLAST MOVEMENTS -*LEMNA*



FOTOTROPINY



Adiantum kapradina

Figure 1

Phototropin structure and function. (*a*) Diagram illustrating the range of phototropin-induced responses in higher plants. Phot1 and phot2 are activated by blue light and overlap in function to mediate several responses. These are enclosed in the yellow rectangle and include phototropism, stomatal opening, chloroplast accumulation movement, and cotyledon and leaf expansion. Phototropins have also been implicated in controlling blue-light-induced leaf movements. Chloroplast avoidance movement is only mediated by phot2. Likewise, phot1 alone plays a role in mediating the rapid inhibition of hypocotyl growth and promoting the destabilization of specific transcripts under high light intensities. (*b*) Protein structures of phototropin and neochrome photoreceptors. Domain structures of these proteins along with their respective chromophores are indicated.

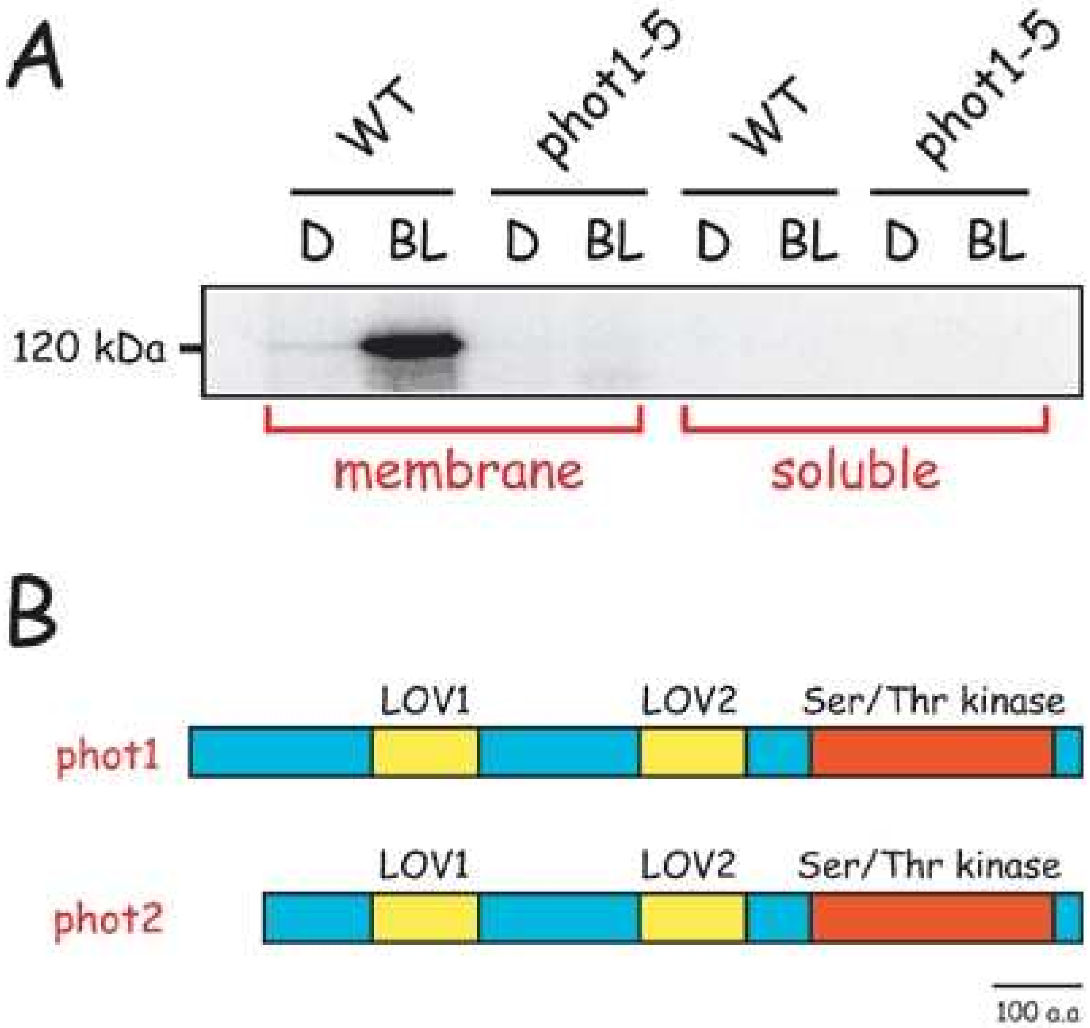


Figure 1 Kinase activity and protein structure of the phototropins. (A) Autoradiograph showing blue light-induced autophosphorylation of phot1 in protein extracts prepared from dark grown *Arabidopsis* seedlings. Protein extracts were prepared under a dim red safe light and given a mock irradiation (D for dark) or a pulse of blue light (BL) prior to the addition of radiolabelled ATP. In membrane extracts prepared from wild-type seedlings (WT), phot1 undergoes autophosphorylation in response to blue light. This response is lacking in the *phot1* null mutant (*phot1-5*). In addition, no phot1 kinase activity is detected in soluble protein extracts from wild-type seedlings, indicating that phot1 is membrane associated. (B) Protein structures of *Arabidopsis* phot1 and phot2 (996 and 915 amino acids respectively). The light sensing LOV domains are shown in yellow. The serine/threonine kinase domains are shown in red. **(Click image to enlarge.)**

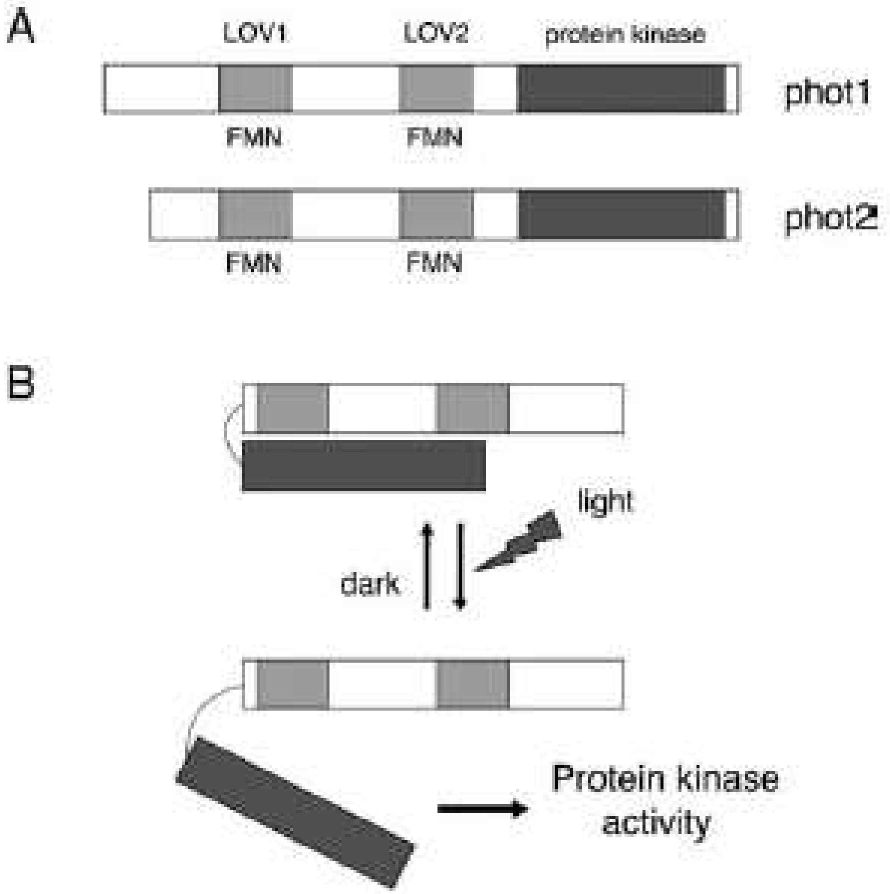


Figure 1 Structure and proposed mechanism of light activation of the phototropins. (*A*) Schematic representation of the phototropin structure. The phototropins contain two FMN binding LOV domains and a canonical Ser/Thr protein kinase domain at the C terminus. (*B*) Schematic mechanism of light activation according to the model proposed by (69).

Mechanismus asociace s membránou je nejasný.

Po osvětlení je část Phot1 bílkoviny uvolněna do cytoplazmy.

V kortexu etiolovaného hypokotylu je Phot1 bílkovina přednostně polarizovaně kolokalizována s Pin1 na příčných stěnách.

Fotoperiodická signalizace a biologické hodiny

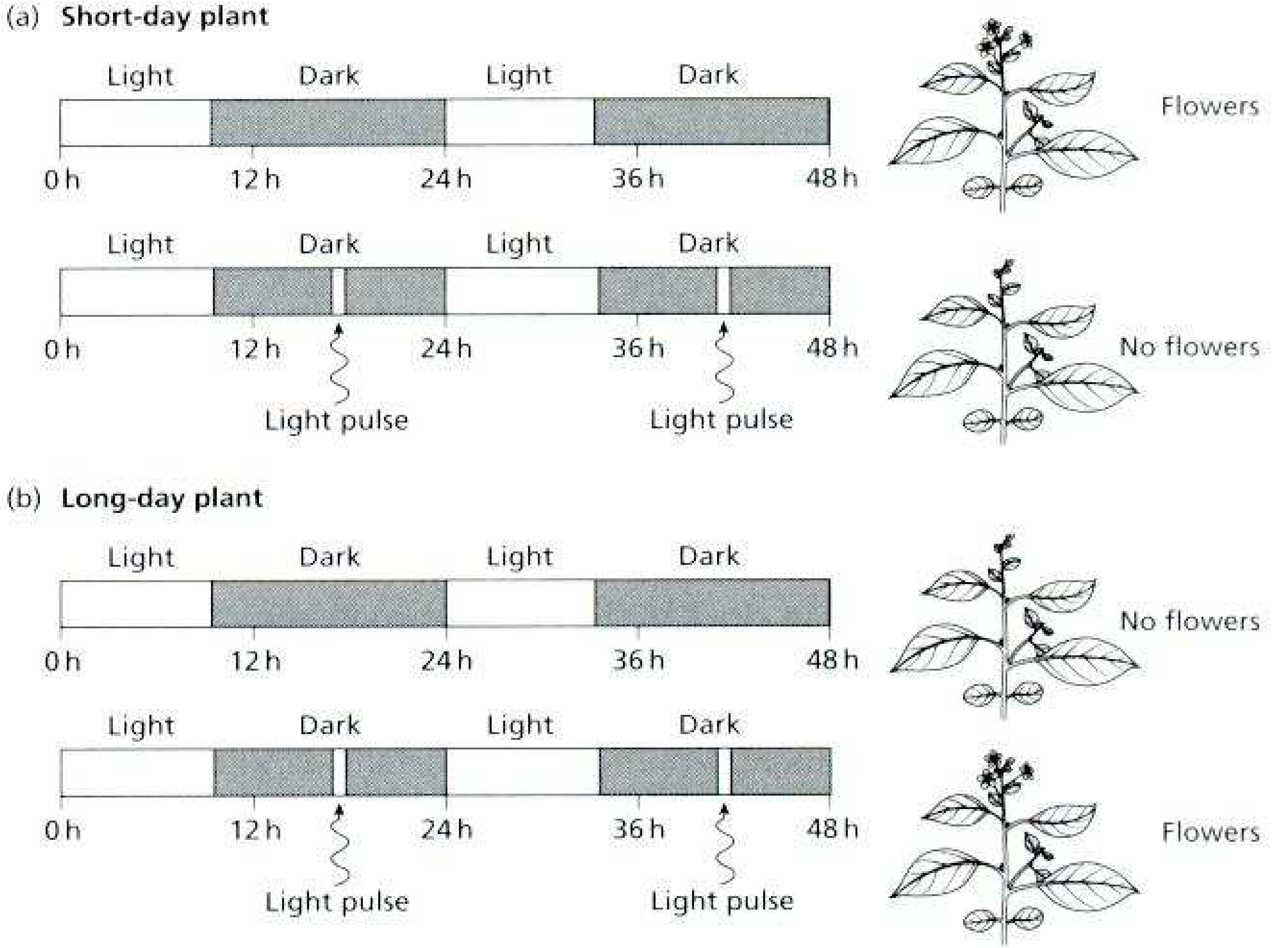


Fig. 7.10 The effects of night-time light treatments on photoperiodic flowering. Whereas day-time dark treatments have little effect on flowering, interrupting nights with brief periods of illumination can inhibit flowering in short-day plants (a) and can promote flowering in long-day plants (b).

This result is supported and extended by analysis of flowering in mutants

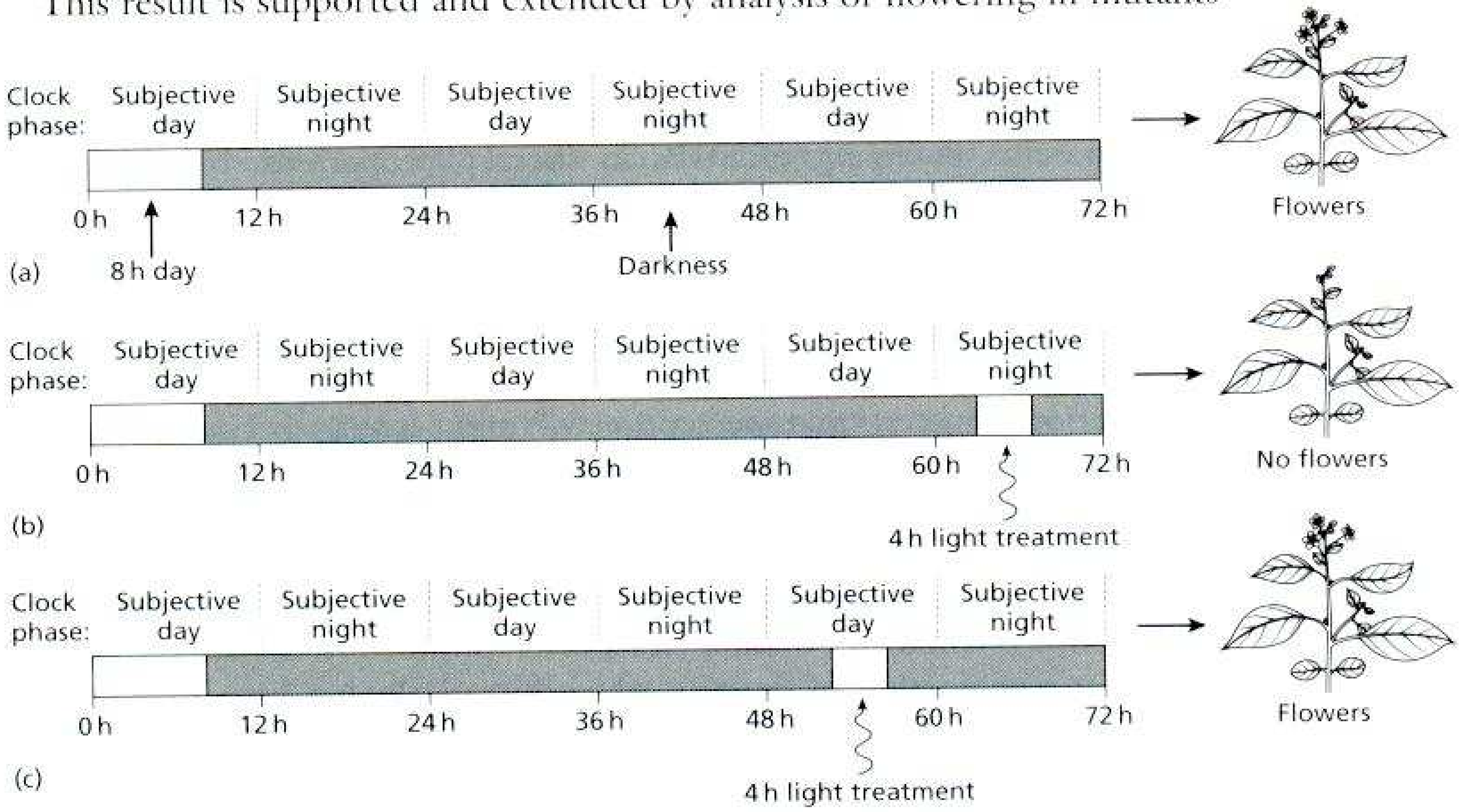
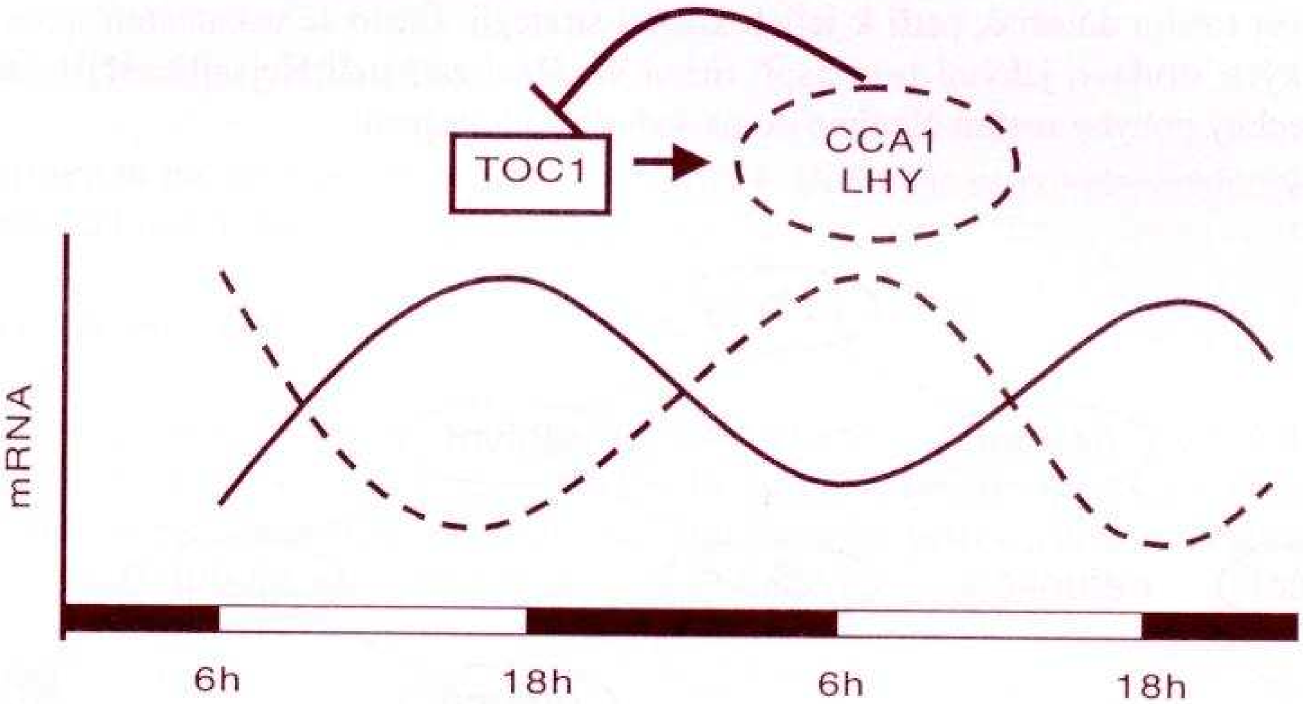


Fig. 7.11 Regulation of the photoperiodic response to light treatments by the circadian clock in soybean. Soybean is a SDP that requires several long nights to induce flowering. (a) The plant can be induced to flower if an 8-hour day is followed by 64 hours of darkness. The induction is prevented if a 4-hour light treatment is given during a subjective 'night' (b), but not if the light treatment is given during a subjective 'day' (c). (Data from Salisbury & Ross, 1992.)



Obr. 117. Zjednodušené schéma mechanismu negativního zpětnovazebného obvodu, který je jádrem biologických hodin (oscilátoru) rostlin. Šipka od TOC1 a LHY značí aktivaci genové exprese, zarážka v opačném směru značí inhibiční působení CCA1 a LHY na transkripci TOC1. Průběh křivek, vyjadřujících cyklické změny množství mRNA těchto transkripčních faktorů, ukazuje dvanáctihodinový posun jejich fáze.

"Biologické hodiny" tikají v každé buňce rostliny a s vnějším časovým cyklem komunikují přes fytochormový/kryptochromový systém.

Transkriptomická analýza Arabidopsis ukázala, že asi 6000 genů je exprimováno v diurnálním rytmu, z toho asi 500 přímo v závislosti na "centrálním oscilátoru/hodinách"

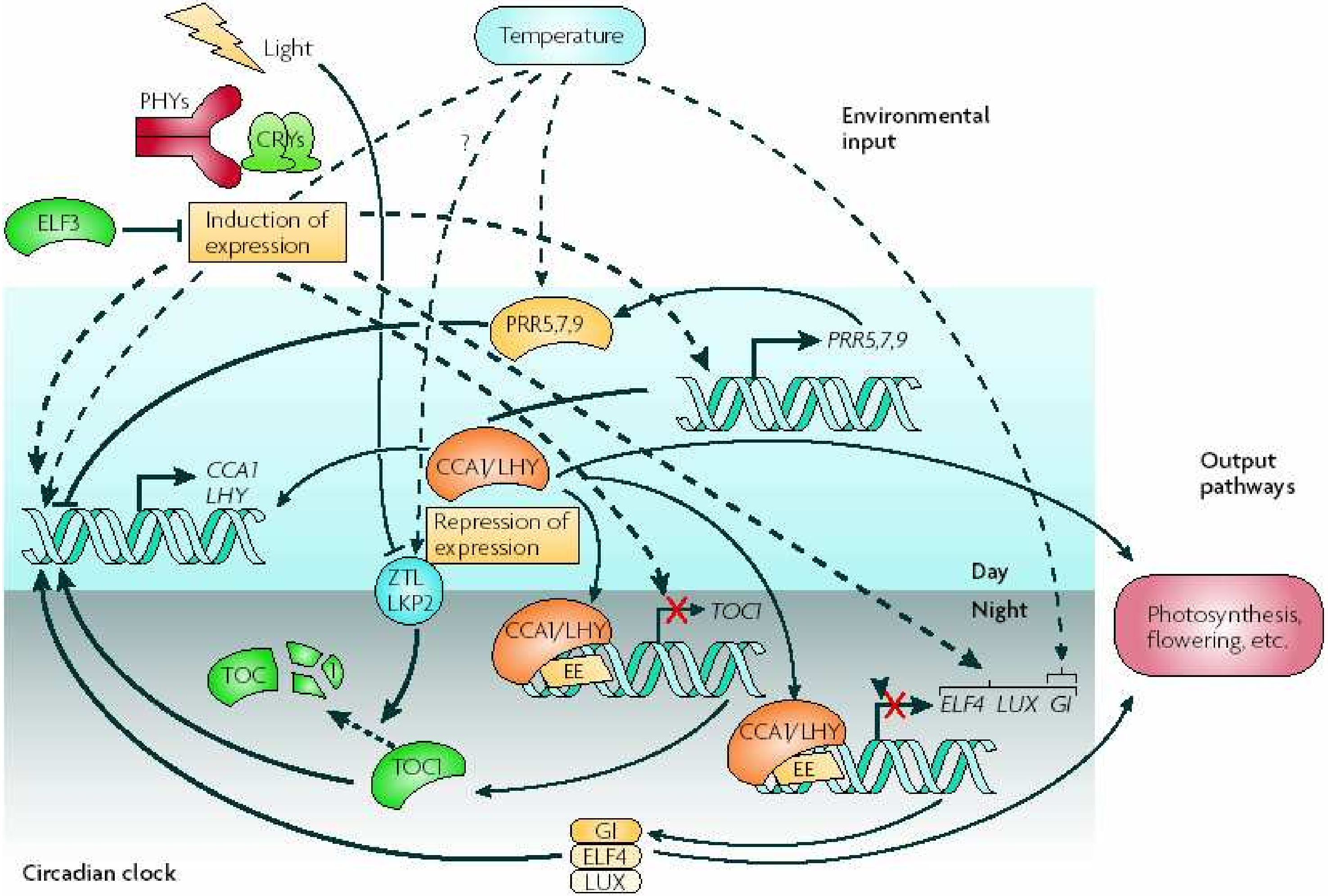


Figure 6 | **The photoperiod response in *Arabidopsis thaliana*.** The three transcription feedback loops of the circadian oscillator, which all consist of CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY), are shown in the shaded areas. In the first loop, CCA1 and LHY repress *TIMING OF CAB EXPRESSION 1* (*TOC1*) expression by binding to the evening element (EE) of its promoter, while TOC1 acts as a positive regulator for *CCA1* and *LHY* expression. Similarly, EARLY FLOWERING 4 (ELF4), GIGANTEA (GI) and LUX ARRHYTHMO (LUX) form another feedback loop with CCA1/LHY. By contrast, in the third feedback loop, CCA1 and LHY positively regulate the expression of *PRR5*, *PRR7* and *PRR9*, and these three proteins repress the expression of *CCA1* and *LHY*. The grey shaded area in the box indicates activities that peak in the subjective night, whereas the blue area indicates activities that peak during the subjective day. Light signals are perceived by phytochromes (PHYs) and cryptochromes (CRYs), and possibly by ZEITLUPE (ZTL) and LOV KELCH PROTEIN 2 (LKP2). Dashed lines indicate the extrinsic inputs that regulate gene expression.

0 2 1 3

1 2 3 0 5