Signalizace, regulace, komunikace a integrace v buňce, pletivu a organizmu

Několik poznámek.

Kvantitativně převažujícím prvkem regulace genové exprese je represe (a její odblokování).

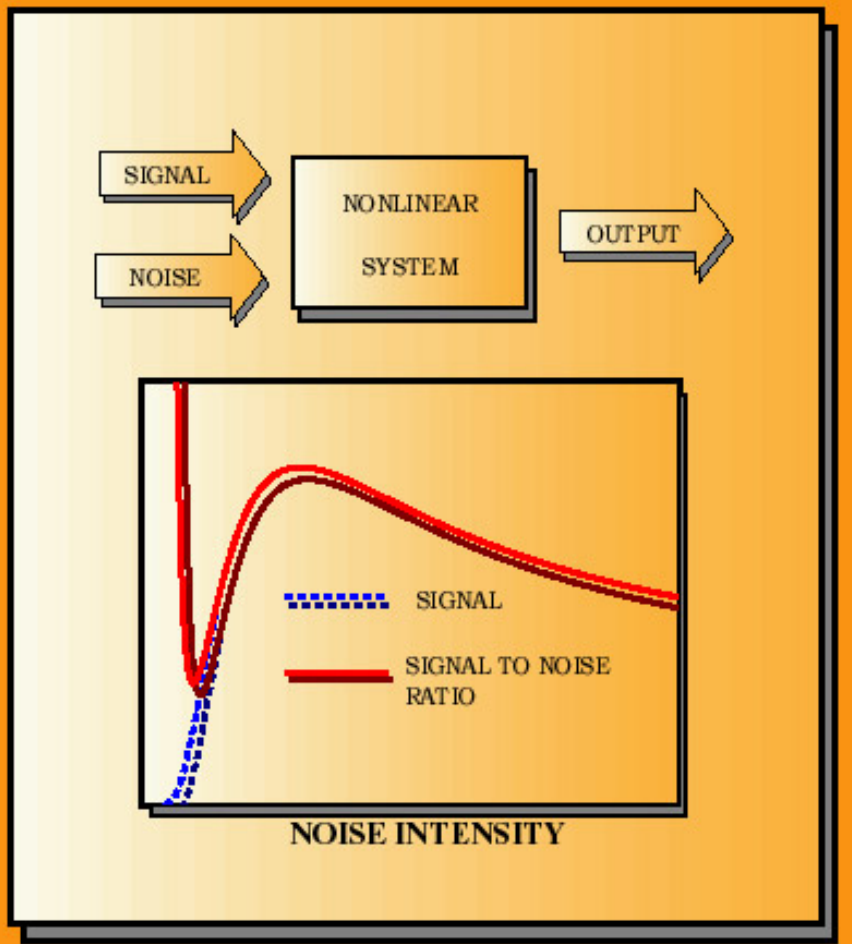
Většina bílkovin je polyfunkčních a jejich exprese a funkce je regulována na mnoha úrovních najednou.

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.



Slide 3

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.

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…)

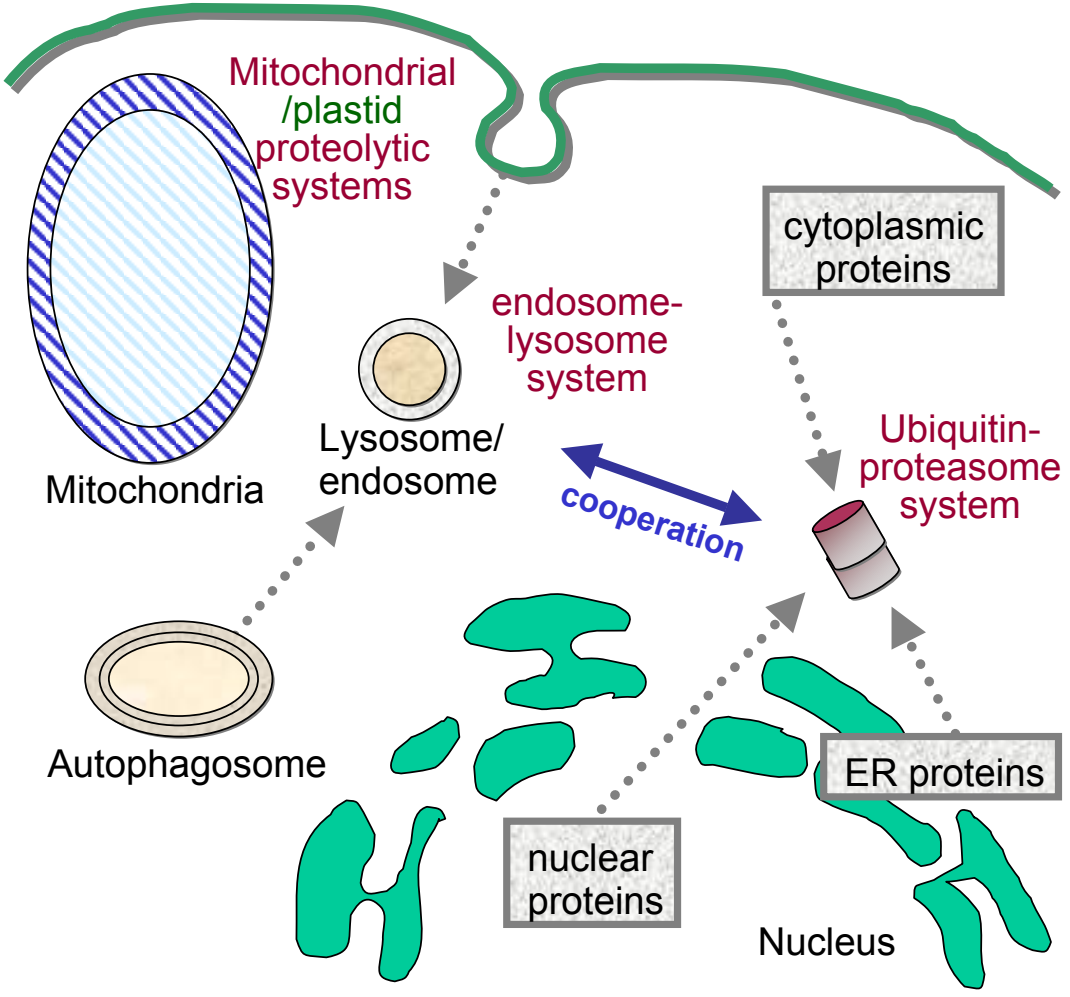
Proteolýza je ovšem také konstitutivní proces.
Až 30% translatovaných bílkovin je nefunkčních.

Degradace buněčných bílkovin

Proteolytické dráhy u eukaryot

- *1. vakuolární/lysozomální*
- *2. Na ubiquitinu-proteasomu závislá degradace*
- *3. post-proteasomálni degradace : Tricorn, TPPII?*
- *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)

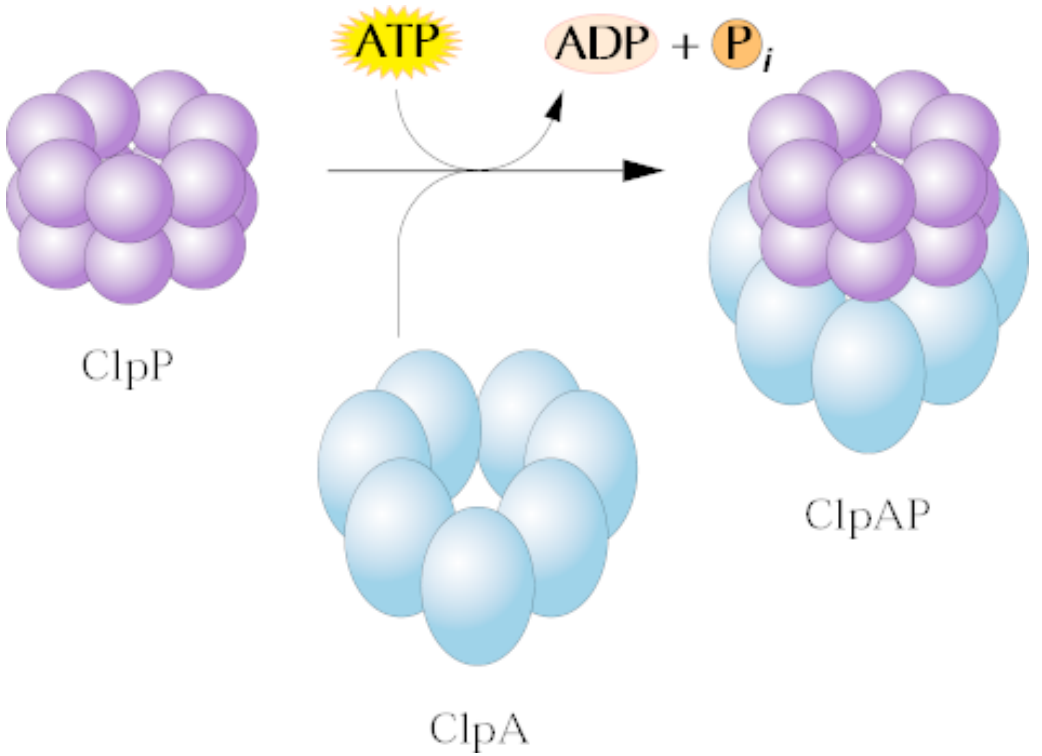
❖ combine proteolytic and chaperone activities in one system, acting as quality-control machineries

- 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

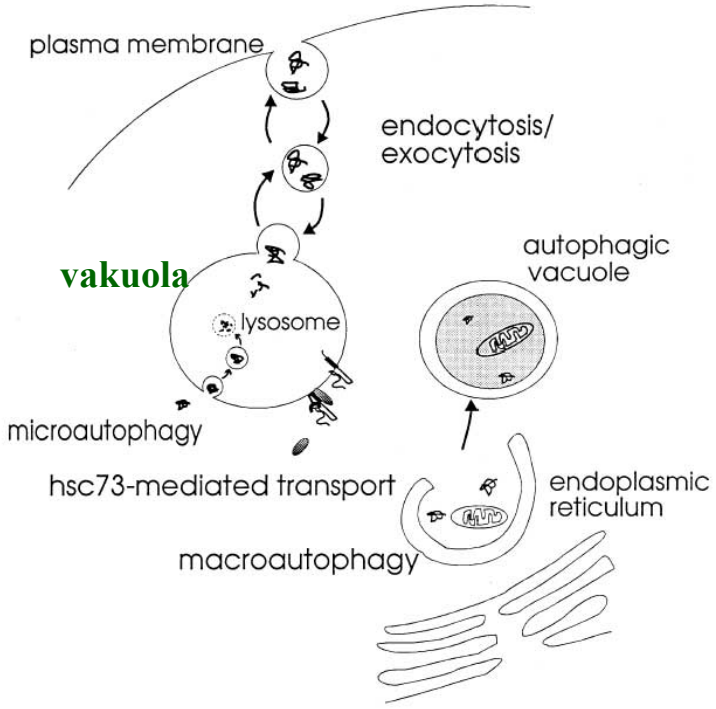


Fig. 1 Pathways of protein degradation in lysosomes. Lysosomes are able to degrade intra- and extracellular proteins following different mechanisms

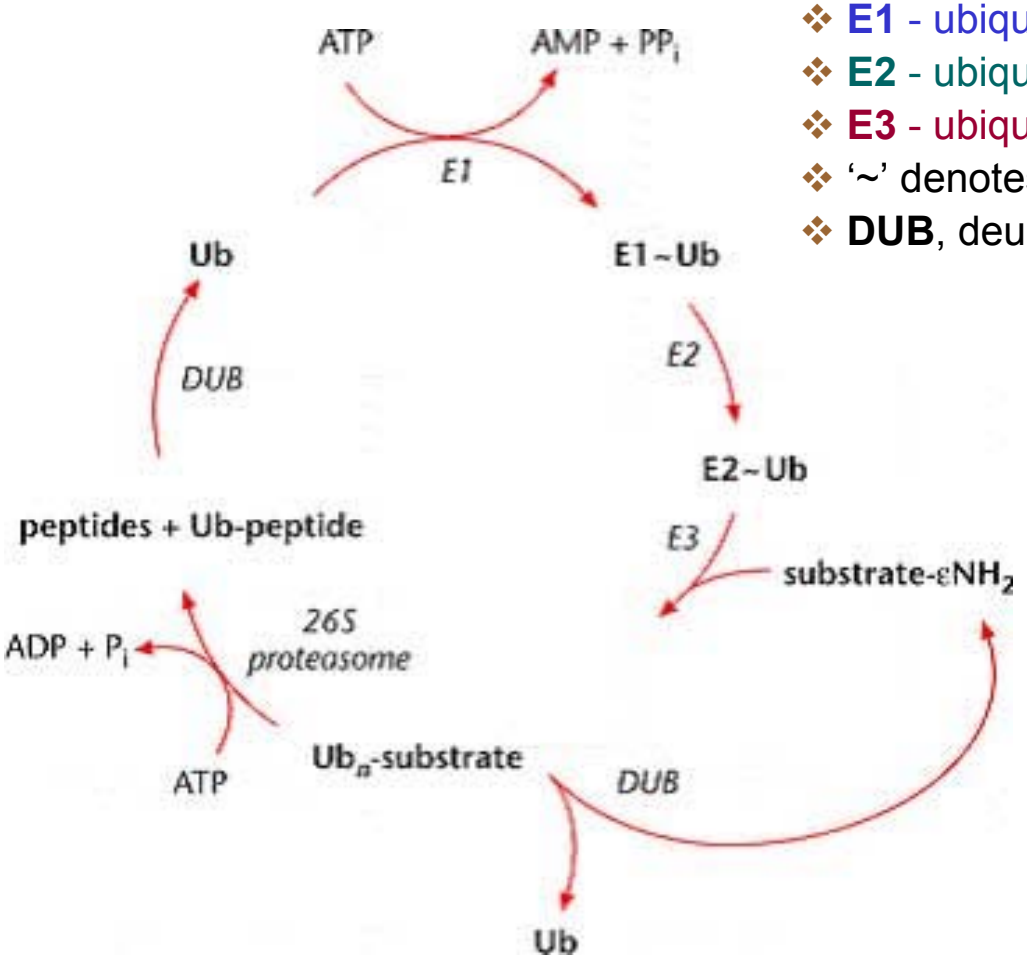
Cuervo and Dice (1998) *J. Mol. Med.* **76**, 6-12.

Bílkoviny určené k degradaci proteasomem jsou modifikovány ubiquitinem.

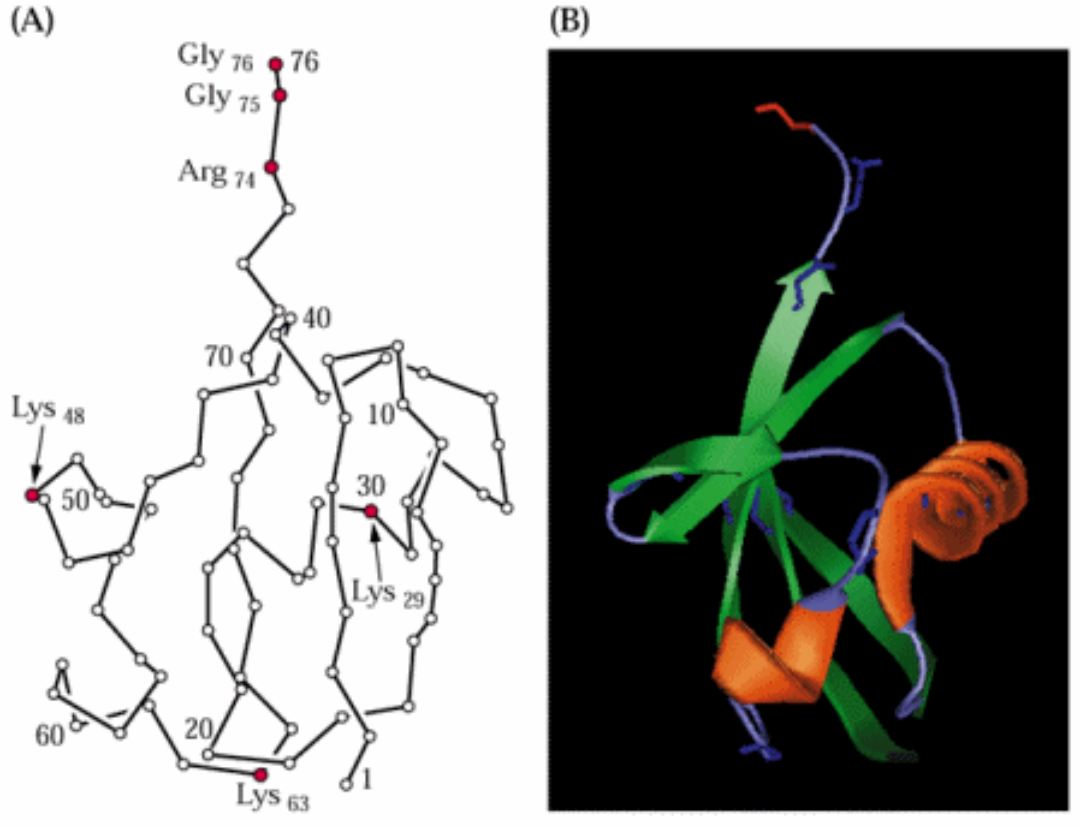
Prvním známým proteinem ubq. *in vivo* v aktivní formě byl u rostlin fytochrom.

Většina bílkovin je před ubiquitinací specificky fosforylována.

Ubiquitinová dráha



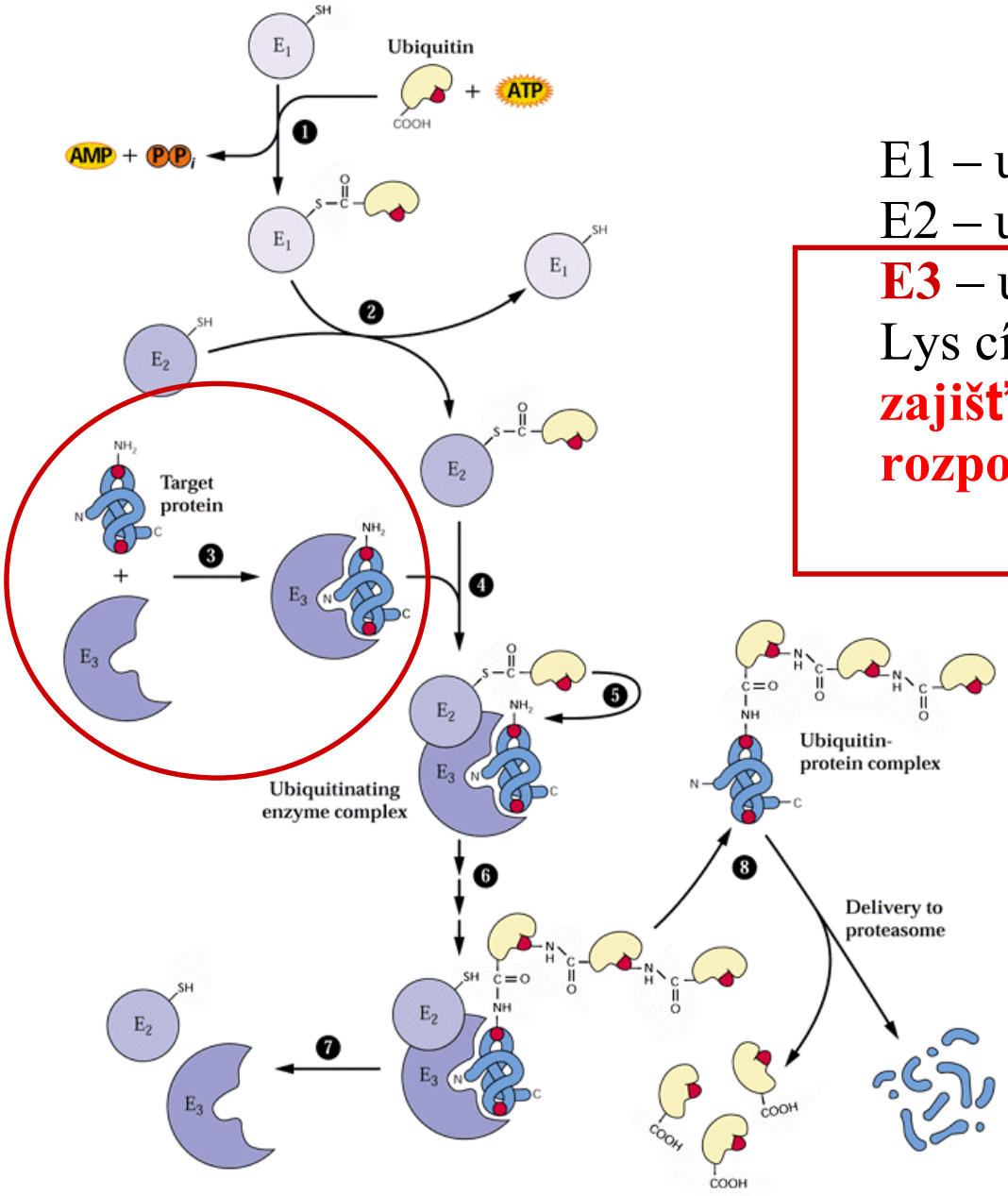
- **E1** - ubiquitin activating enzyme
- **E2** - ubiquitin conjugating enzyme
- **E3** - ubiquitin ligase
- '~' denotes high-energy thioester bond
- **DUB**, deubiquinating enzyme



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.

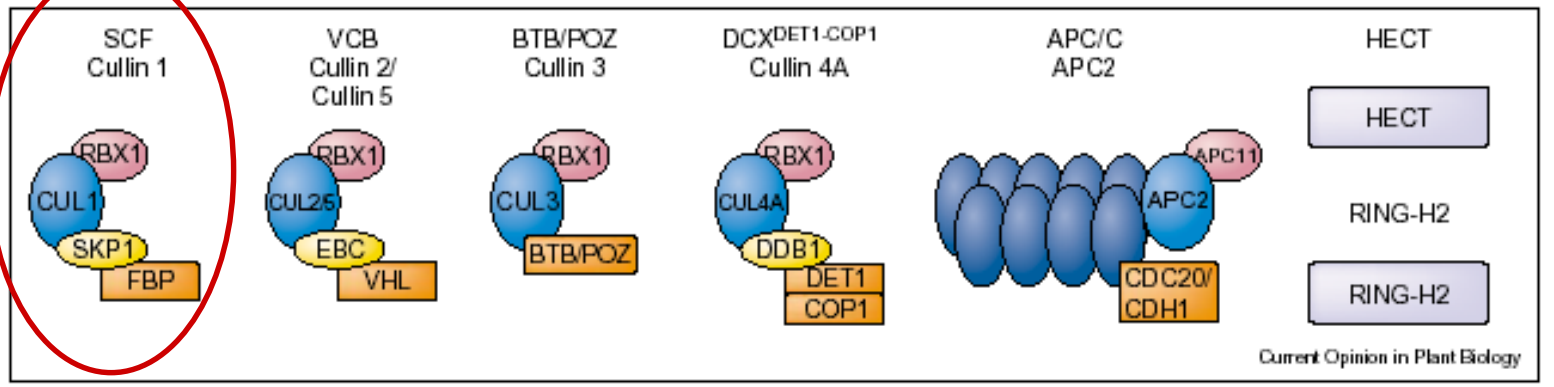
E3 ubiquitin ligázy

❖ **4** základní typy E3 ubiquitin ligáz **u rostlin**: kol.**1300 genů Arabidopsis kóduje podjednotky E3 ligáz.**

- s **HECT** domains (E6AP-related proteins) - monomerní **17x** u A.t.
- s **Ring/U-Boxem** (VHL, SCF, APC, MDM2, c-CBL, etc.) **480x** RING a 64x U-Box u A.t
- **SCF komplex 4 podjednotky - podjednotka řídící specifitu F-box 700x u A.t.**
- **APC**

ale také 37x AtE2 = potenciálě obrovské množství spec. ubq. komplexů.

Figure 2



Proteins and protein complexes that have reported E3 ubiquitin ligase activity. Many of these complexes share a common architecture. They are composed of a cullin subunit that associates with the RING-domain protein RBX1 and a receptor subunit. The E3 APC/C also contains a cullin-related subunit, APC2, and a RBX1-related RING-domain protein APC11. There is no evidence for a conservation of a VCB complex in plants.

vedle 700 F-boxů má Arabidopsis 21 SKP bílkovin.
Opět velká kombinatorika komplexů – existují ovšem **preferované** kombinace.

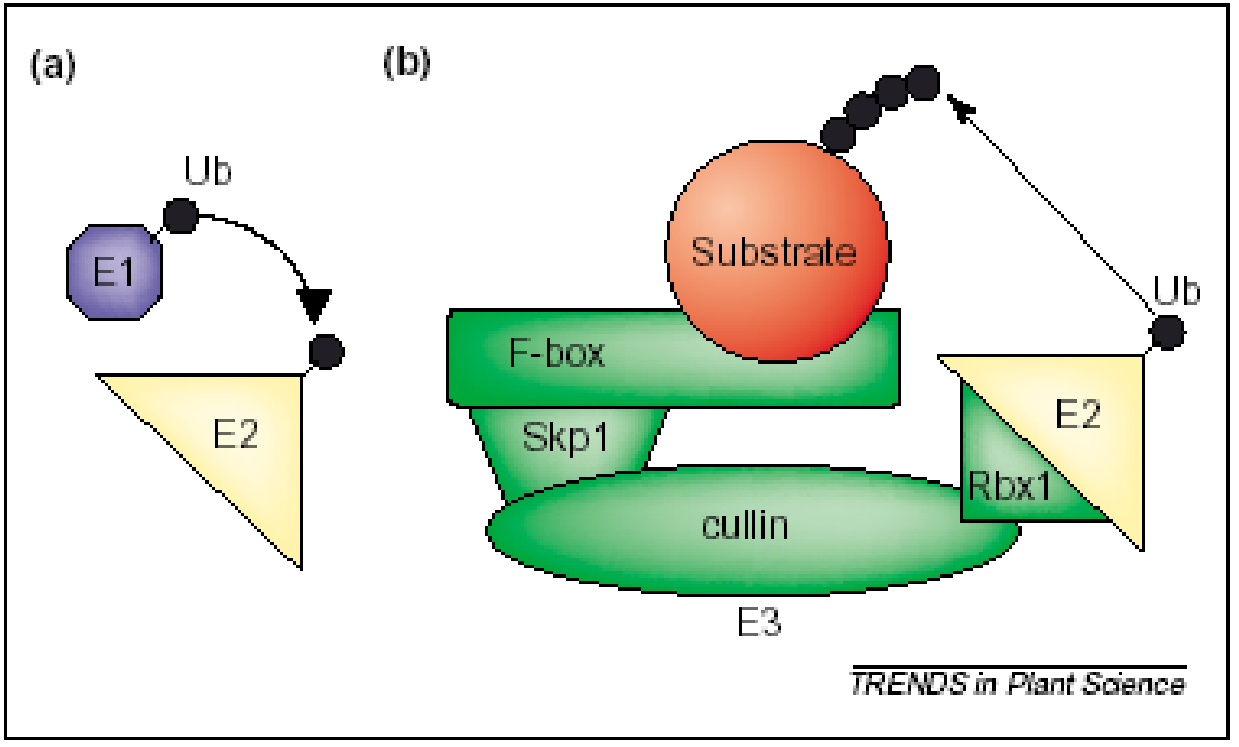
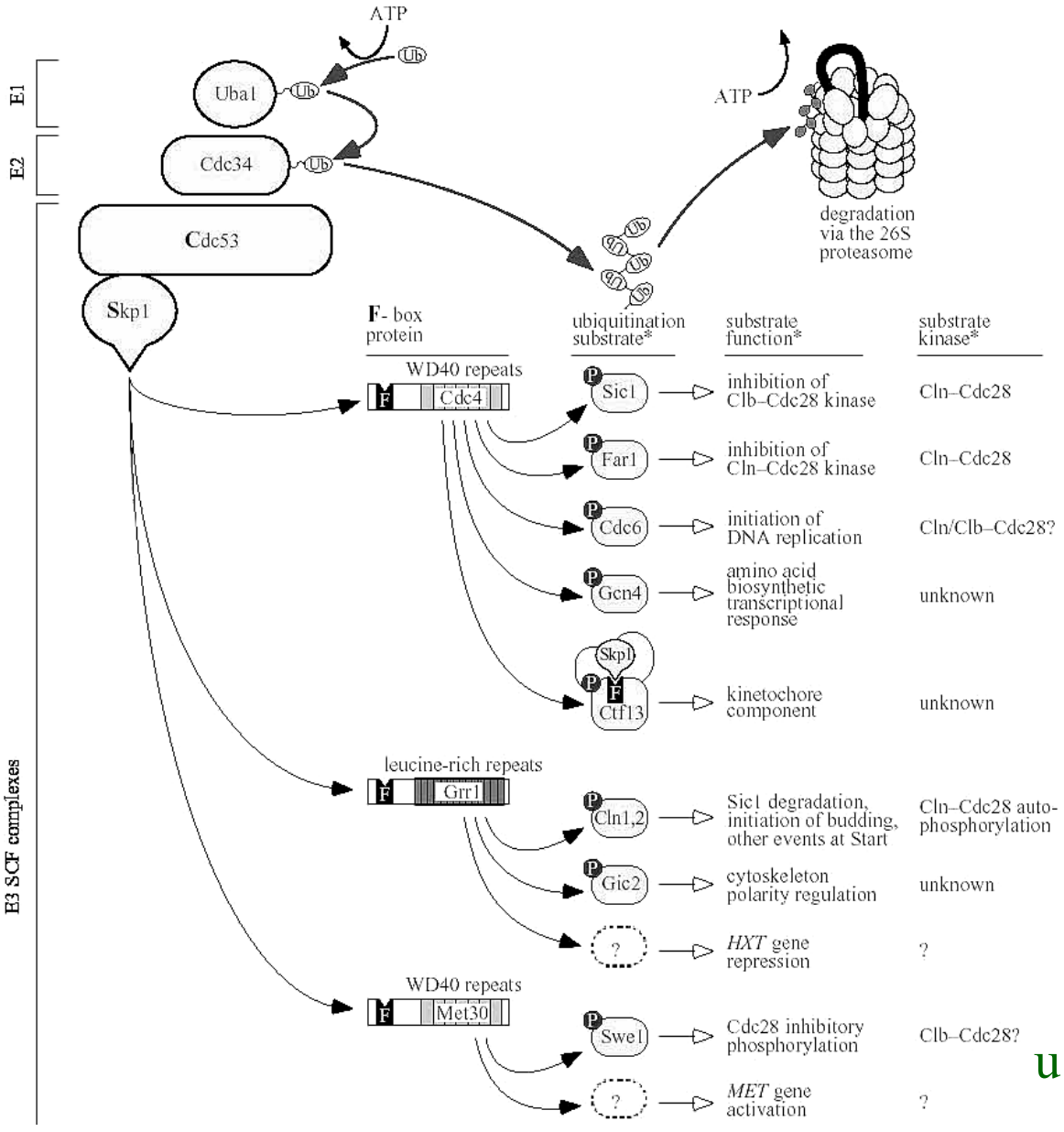


Figure 1. Key steps in the pathway of polyubiquitylation by SCF E3 ligase, which targets substrate protein and leads to degradation by the 26S proteasome. **(a)** Ubiquitin (Ub) is linked via a thioester bond to the ubiquitin-activating enzyme (E1). Ubiquitin is transferred from E1 to the cysteine of 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.

SCF-dependentní ubiquitinace u kvasinek



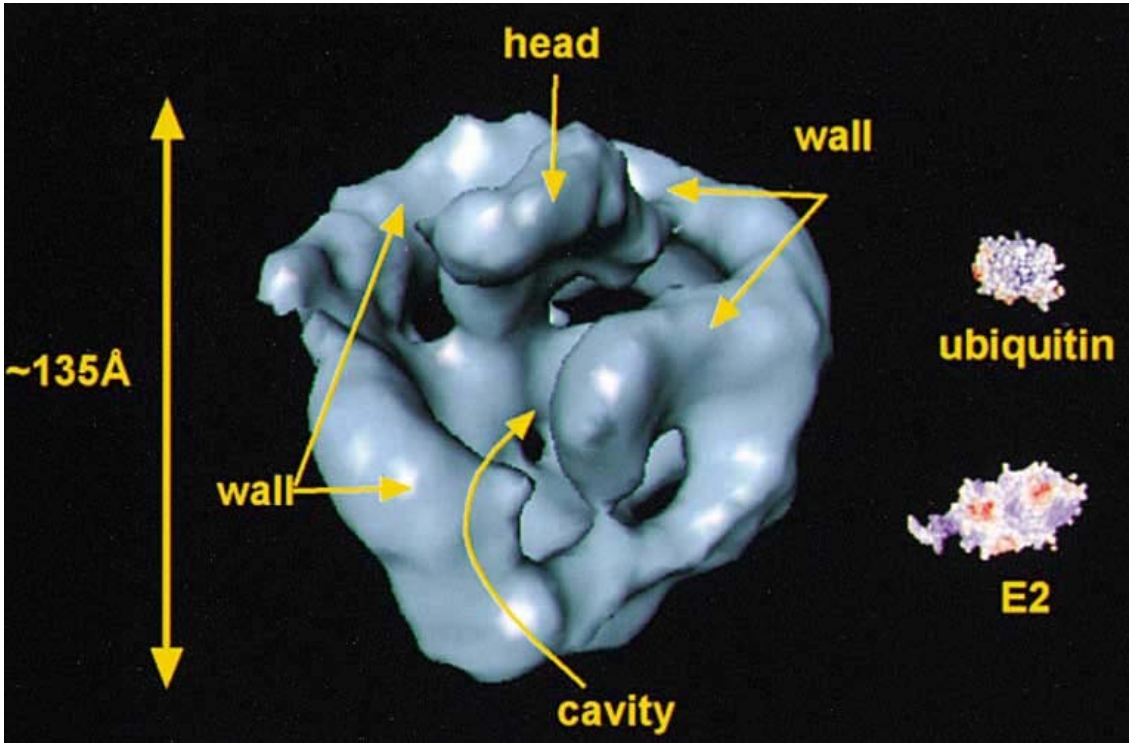
- **F-box proteins mediate substrate selectivity** in degrading various yeast proteins
- many (all?) of the substrates **need to be phosphorylated** to be recognized by the F-box protein
- **WD40 and leucine-rich repeats (LRRs) present in F-box proteins mediate protein-protein interactions**

u rostlin je to podobné

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

Anaphase promoting complex (APC)

- The anaphase-promoting complex (also termed ‘cyclosome’) is a ubiquitin-protein ligase that controls important transitions in mitosis by ubiquitinating regulatory proteins
- consists of many different proteins, including some related to SCF (*e.g.*, ring protein)
- To initiate sister chromatid separation, the APC has to ubiquitinate the anaphase inhibitor securin, whereas **exit from mitosis requires the ubiquitination of B-type cyclins**



Gieffers *et al.* (2001) *Mol. Cell* **7**, 907-913.

TABLE 1 E3s and targets of the Ub/26S proteasome pathway involved in plant growth and development

	E3 (Type)[a]	Target protein(s)	References
Cell cycle			
G1/S (Rb pathway)	SKP2 (F-Box)	E2Fc	(26)
Mitosis	APC	CYCB1, CYCA3, CDC6	(16, 51)
Hormone regulation			
Auxin	TIR1 (F-Box)	AUX/IAA family	(56, 178)
Auxin	SINAT5 (Ring HC)	NAC1	(166)
Auxin	?	EIR1	(130)
Abscisic acid	?	ABI5	(95, 132)
Brassinosteroids	?	BZR1 and BZR2	(68)
Ethylene	EBF1 and 2 (F-Box)	EIN3	(50a, 60a, 110a)
Gibberellins	SLY (F-Box)	RGA	(98)
Gibberellins	GID2 (F-Box)	SLR1	(118)
Jasmonic acid	COI1 (F-Box)	RPD3b	(30)
Responses to the abiotic environment			
Light	COP1 (Ring HC)	HY5, HYH, LAF1	(73, 106, 125)
Light	CIP8 (Ring HC)	HY5, HYH	(63)
Red/far red light	?	PhyA	(21, 22)
Red/far red light	EID1 (F-Box)	?	(35)
Red/far red light	AFR (F-box)	?	(63a)
Blue light (circadian rhythm)	FKF1, LKP2 (F-Box)	?	(102, 120a)
Blue light (circadian rhythm)	ZTL (F-box)	TOC1	(96a, 133)
Circadian rhythm	?	ZTL	(81)
Heat and cold shock	AtCHIP (U-Box)	Denatured proteins	(171)
Cold signaling	HOS1 (Ring HC)	?	(91)
Responses to the biotic environment			
NIM1 pathway	SON1 (F-Box)	?	(79)
Virus spread	?	MP	(114)
Self-incompatibility	SFB (F-Box)	?	(143)
Self-incompatibility	ARC1 (U-Box)	?	(135)
Development			
Flower development	UFO/FIM/PFO/ STP (F-Box)	?	(117, 179)

Proteasom

TABLE 1 (*Continued*)

	E3 (Type)[a]	Target protein(s)	References
Senescence/shoot branching	ORE9/MAX2 (F-Box)	?	(134, 163)
Trichome development	UPL3 (HECT)	?	(39)
Wax biosynthesis	CER3 (RING HC)	?	(62)
Metabolic pathways			
Glycolysis	?	$PyrKin_c$	(139)
Alkaloid biosynthesis	?	TDC	(1)
N-end rule pathway	PRT1 (Ring HC)	N-end rule substrates	(111)

[a]For SCF E3s, only the F-Box mutants are included.

? Unknown.

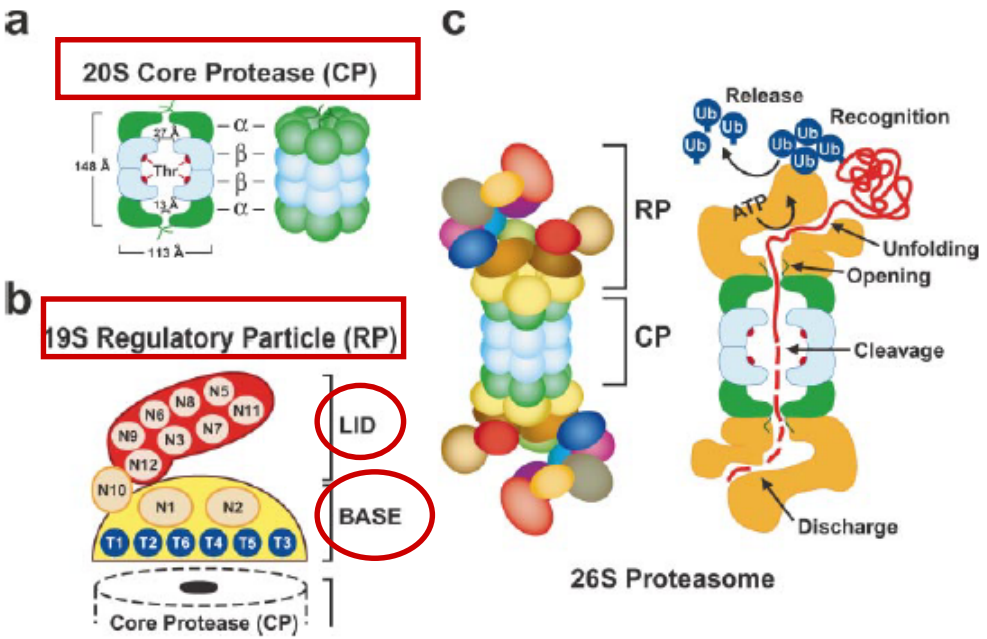
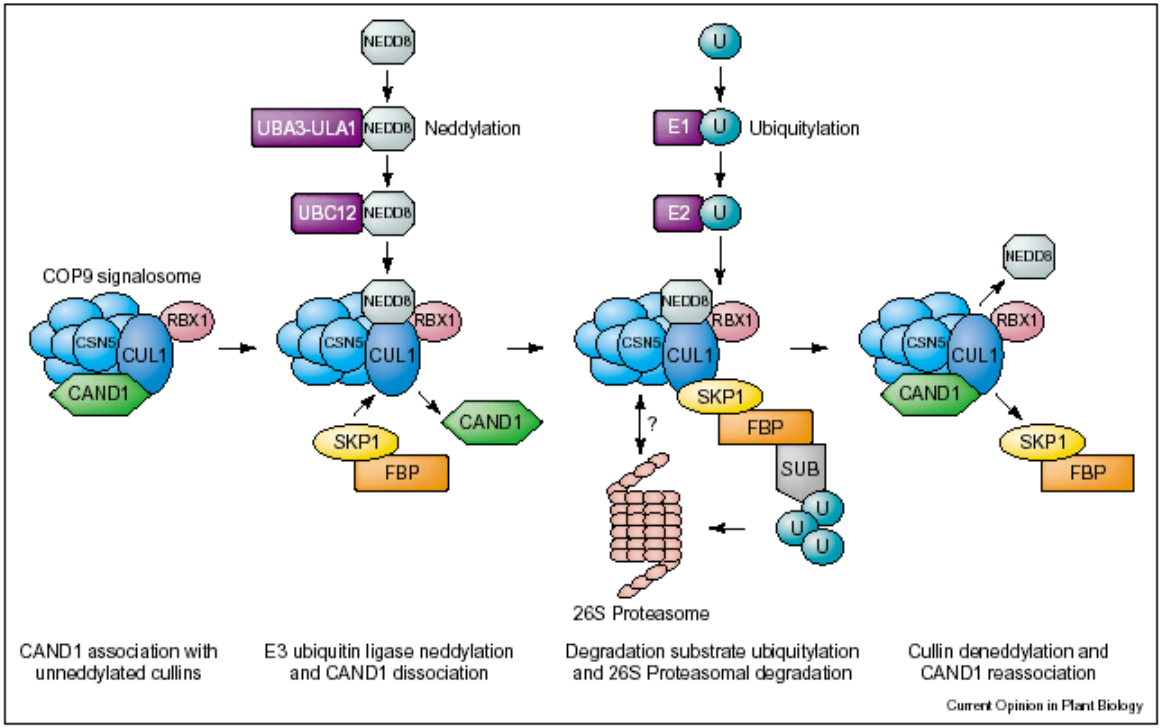


Figure 2 Organization and structure of the 26S proteasome. (*a*) Organization of the 20S core protease (CP) based on the crystal structure of the yeast particle (60). The positions of the active-site threonines are shown. (*b*) Predicted organization of the 19S regulatory particle (RP) based on its subunit 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**.



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].

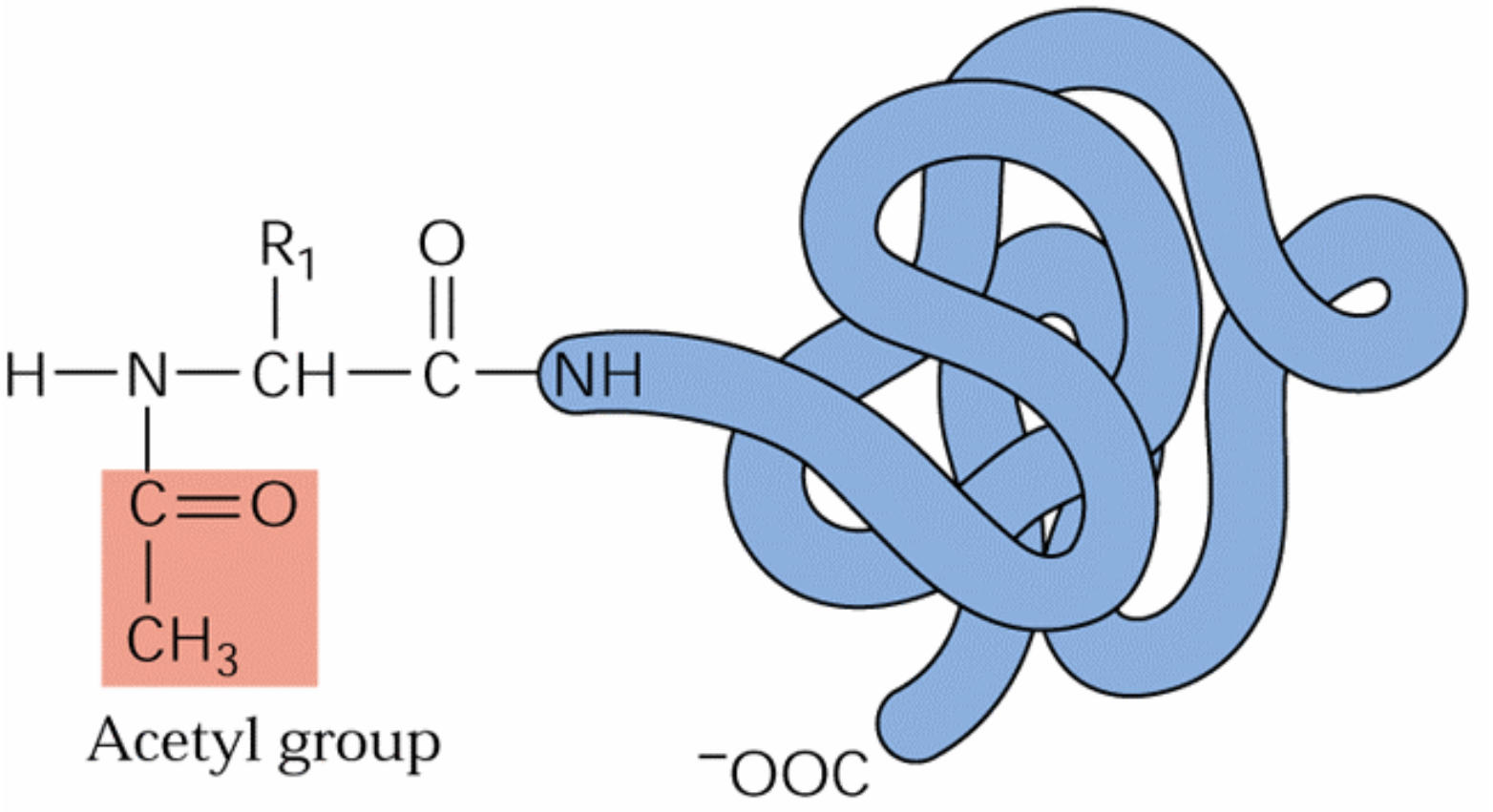
Abbreviations

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

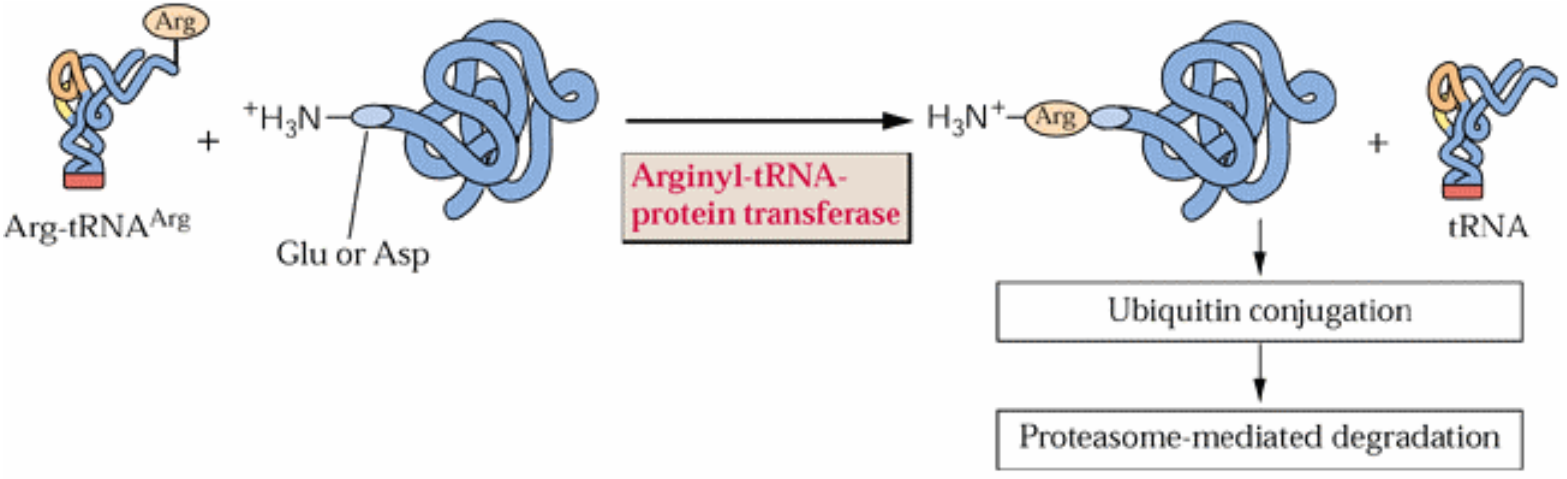
CSN kontroluje aktivitu E3 SCF ligázy prostřednictvím neddylace či deneddylace (NEDD8 či RUB1 jsou peptidy podobné ubiquitinu)
a degradaci bílkovin interakcemi s proteasomem (alternativní „víko“).

N-koncové pravidlo

Met, Thr, Ser, Gly a Val na **N´**stabilizují bílkovinu, zatímco **Lys, Arg, His** ji destabilizují.



N-koncová acetylace stabilizuje bílkovinu.



U některých prot. je N´Met odštěpen aminopeptidázou a novou počáteční AA bývá Glu nebo Asp;takové bílkoviny se stávají substrátem ubiquitinace teprve po přidání N´- Arg.

- **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.**

Příklady proteolytických bílk. a ovlivněných procesů u rostlin.

Summary of proteolysis components with known biological function.

E3 specificity component	Biochemical function	Pathway	Proteolysis substrate	Biochemical function	Reference(s)
PRT1	RING-domain protein		N-end rule substrates		[75]
AFP		Abscisic acid	ABI5	Transcription factor	[76]
TIR1	F-box protein	Auxin	AUX/IAAs	Transcription repressors	[77–79]
SINAT5	RING-domain protein	Auxin	NAC1	Putative transcription factor	[80]
		Auxin transport	EIR1/PIN2	Putative auxin efflux carrier	[81]
		Brassinosteroid	BZR1/BZR2		[82]
SKP2	F-box protein	Cell cycle	E2Fc	Transcription factor	[83]
APC/C	APC/C	Cell cycle	Cyclin B1, Cyclin A3	Cyclin	[84]
APC/C	APC/C	Cell cycle	CDC6		[85]
		Cell cycle	ICK1	Cell-cycle inhibitor	[86]
		Circadian rhythm	CO	Transcription factor	[87]
		Circadian rhythm	ZTL	F-box protein	[4*,88]
FKF	F-box protein	Circadian rhythm			[89]
ZTL	F-box protein	Circadian rhythm	TOC1		[4*,88,90]
AtCHIP	U-box protein	Denatured proteins			[91]
EBF1, EBF2	F-box protein	Ethylene	EIN3	Transcription factor	[50**–53**]
ETO1	BTB/POZ-domain protein	Ethylene	ACS5, ACS9	Biosynthetic enzyme	[56**]
UFO	F-box protein	Floral development			[92,93]
SLY1	F-box protein	Gibberellic acid	GAI, RGA	Putative transcription factors	[38,39,40**,41**,43]
COI1	F-box protein	Jasmonic acid			[94]
SON1	F-box protein	Pathogen response	NIM1		[95]
COP1	RING-domain protein	Photomorphogenesis	HY5, HYH, LAF1, phyA	Transcription factors, photoreceptor	[63–65,70,71]
DET1		Photomorphogenesis	HY5, HYH	Transcription factor	[63,64]
EID1	F-box protein	Photomorphogenesis			[96]
AFR1	F-box protein	Photomorphogenesis			[97]
ORE9/MAX2	F-box protein	Shoot branching/senescence			[98,99]
UPL3/KAK	HECT-domain protein	Trichome development			[100,101]
CER3	RING-domain protein	Wax biosynthesis			[102]

ABI, ABSCISIC ACID INSENSITIVE; AFP, ABI FIVE INTERACTING PROTEIN; AFR, ATTENUATED FAR-RED RESPONSE; AtCHIP, *ARABIDOPSIS THALIANA* Hsc70-INTERACTING PROTEIN; AUX/IAA, AUXIN/INDOLEACETIC ACID; BZR, BRASSINAZOLE RESISTANT; CDC, CELL DIVISION CYCLE; CER, ECERIFERUM; CO, CONSTANS; COI, CORONATINE INSENSITIVE; EID, EMPFINDLICHER IM DUNKELROTEN LICHT; EIR, ETHYLENE INSENSITIVE ROOT; FKF, FLAVIN-BINDING/KELCH-REPEAT/F-BOX; ICK, INHIBITOR OF CYCLIN-DEPENDENT KINASE; KAK, KAKTUS; MAX, MORE AXILLIARY GROWTH; NAC, NO-APICAL-MERISTEM/CUP-SHAPED COTYLEDON; NIM, NON-INDUCIBLE IMMUNITY; ORE, ORESARA; PIN, PINFORM; PRT, PROTEOLYSIS; SINAT, SEVEN-IN-ABSENTIA OF *ARABIDOPSIS THALIANA5*; SON, SUPPRESSOR OF *nim1-1*; TIR, TRANSPORT INHIBITOR RESISTANT; TOC, TIMING OF CAB EXPRESSION; UFO, UNUSUAL FLORAL ORGANS; UPL, UBIQUITIN PROTEIN LIGASE; ZTL, ZEITLUPE.

Inhibitory proteasomu

The peptide aldehydes, **MG 132**, **MG 115**, and **PSI**, inhibit the complex's chymotrypsin-like activity in a potent but reversible manner. **Lactacystin** is a natural, irreversible, nonpeptide, cell permeable inhibitor that is more selective than peptide aldehydes but less selective than peptide boronates, another class of proteasome inhibitors.

SVĚTLO

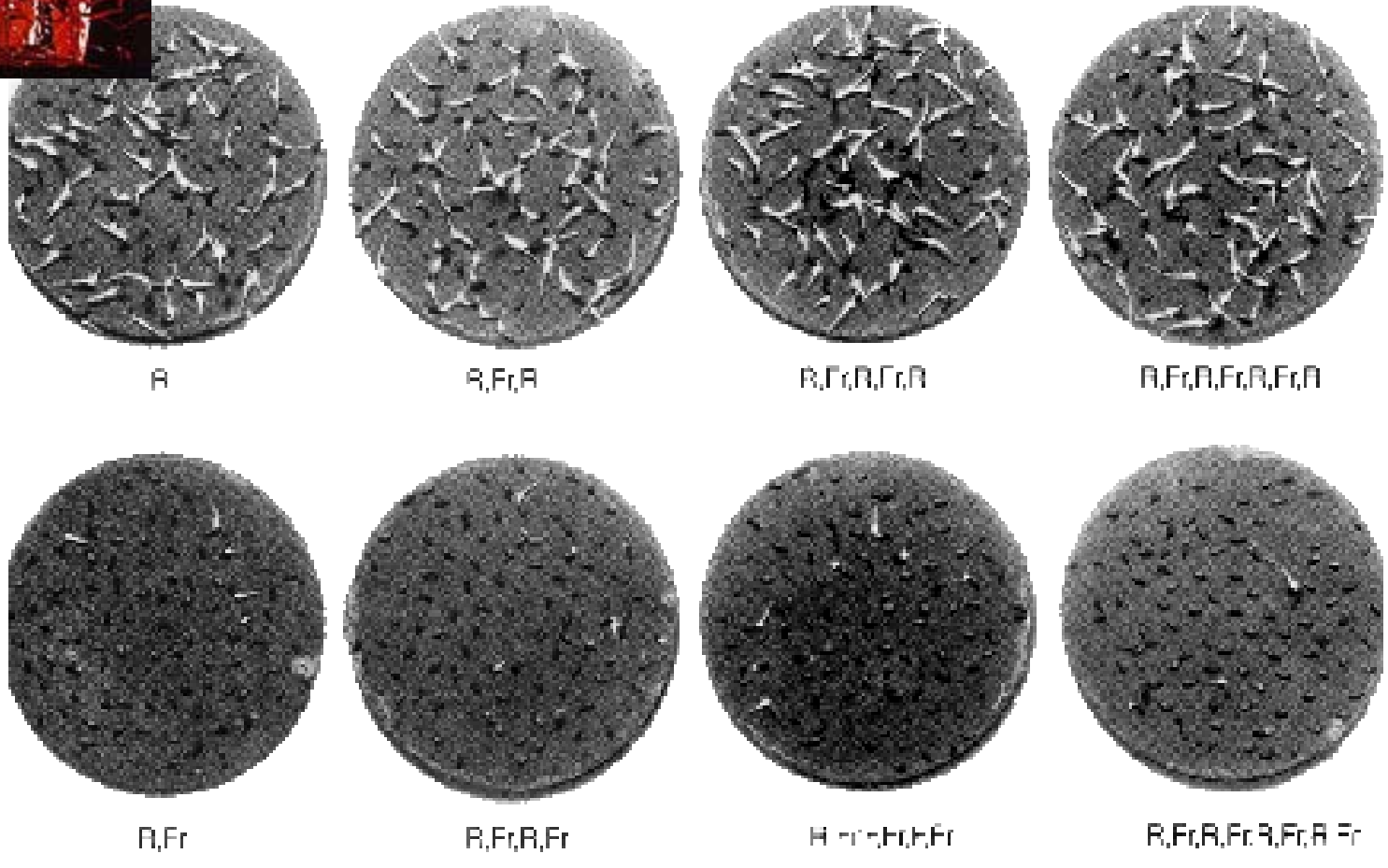
jako

SIGNÁL

- **Světlo**

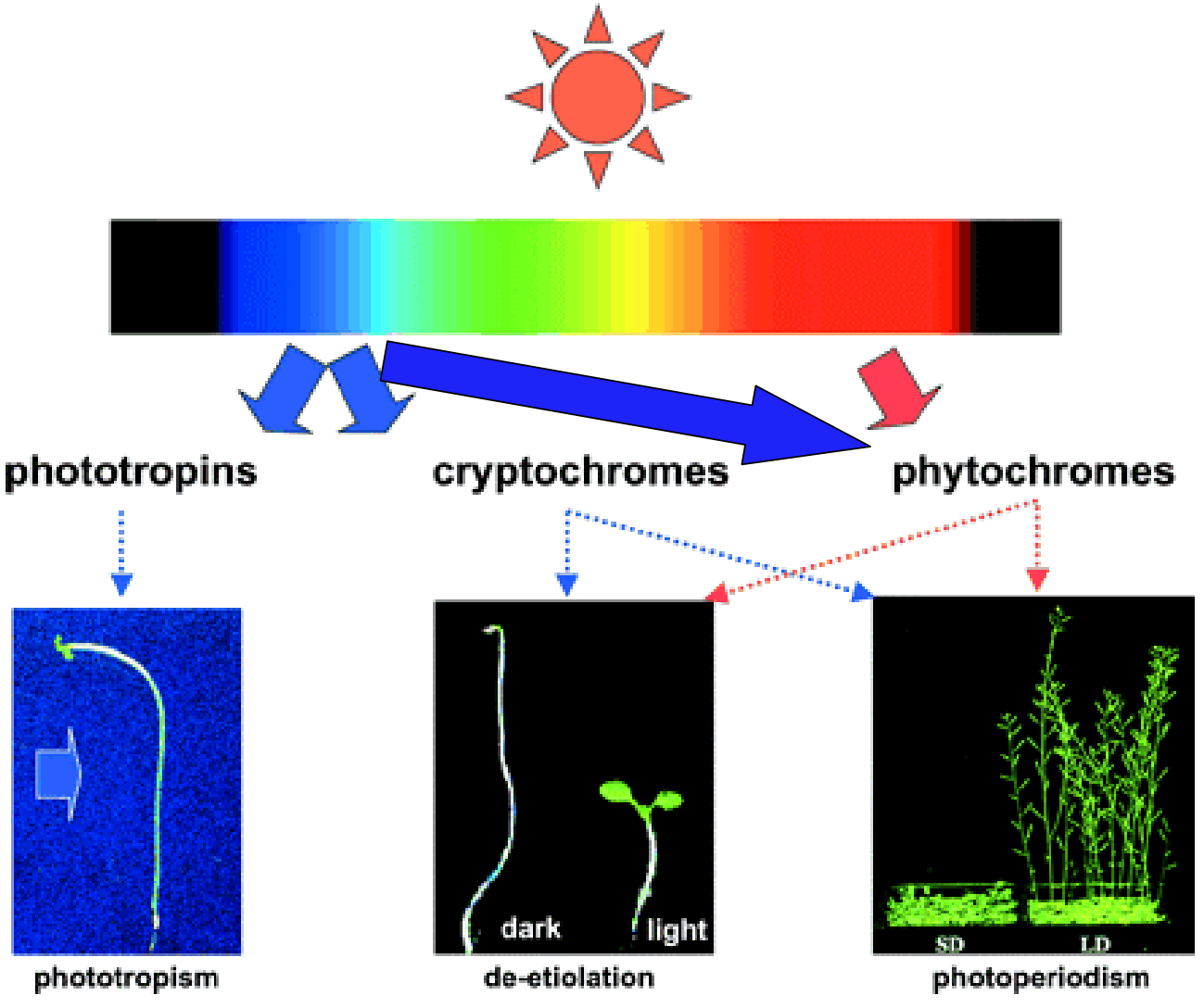
- **UV–B 280-320nm**
- **UV-A 320-380nm**
- **Modré s. 380-500nm**
- **Červené s. 620-700nm**
- **Dl. Červ. S. 700-800nm**

Borthwick's Experiment in Grand Rapids Lettuce (1952)

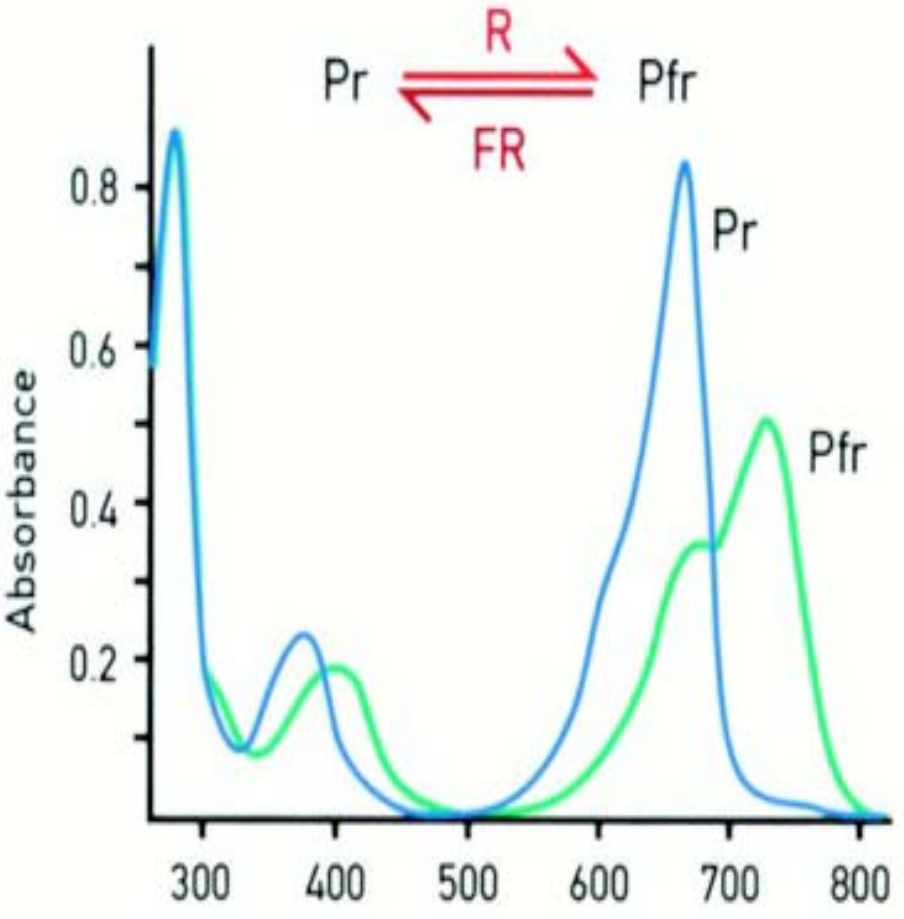


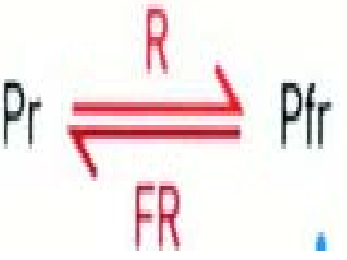
Fotoreceptory

- Fytochromy
- Kryptochromy
- Fototropiny
- Neznámý rec. UV-B


phototropins
cryptochromes
phytochromes
dark
light
SD
LD
phototropism
de-etiolation
photoperiodism

Phytochrome Exists in Two Photointraconvertible Forms





-synthesized as Pr in darkness

-converted by red light (max= 666 nm) to Pfr

-Pfr is biologically active form

-Far-red light (730 nm) converts to Pr

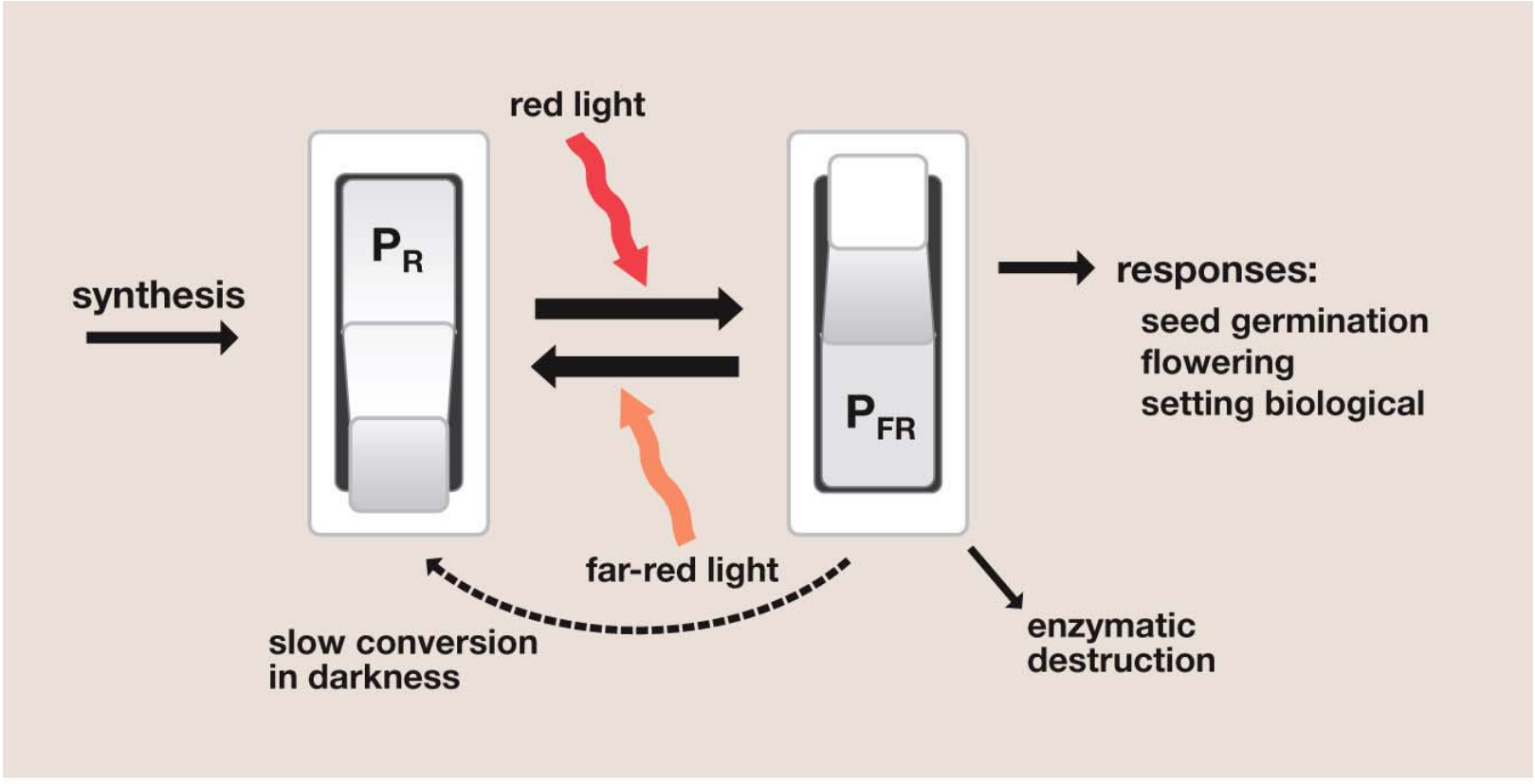


Figure 12.9 Plant Biology, 2/e

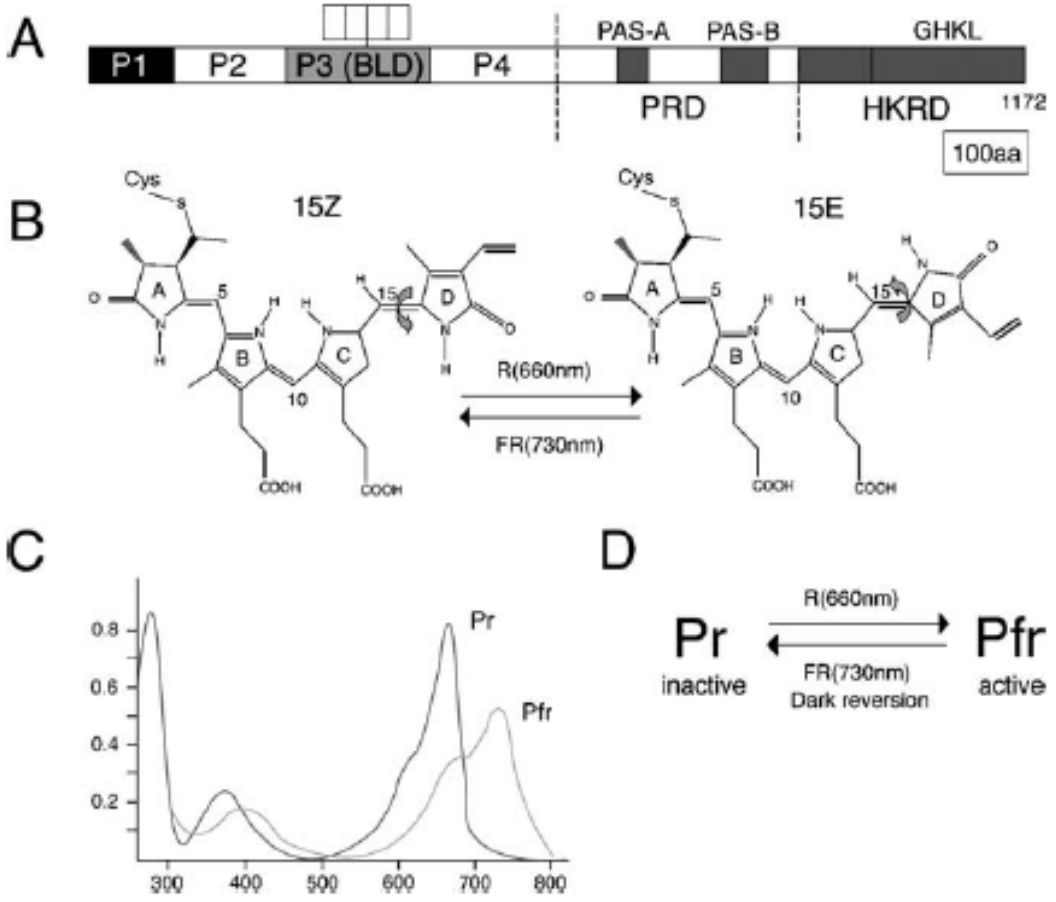
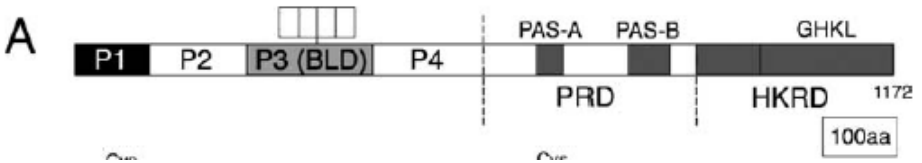
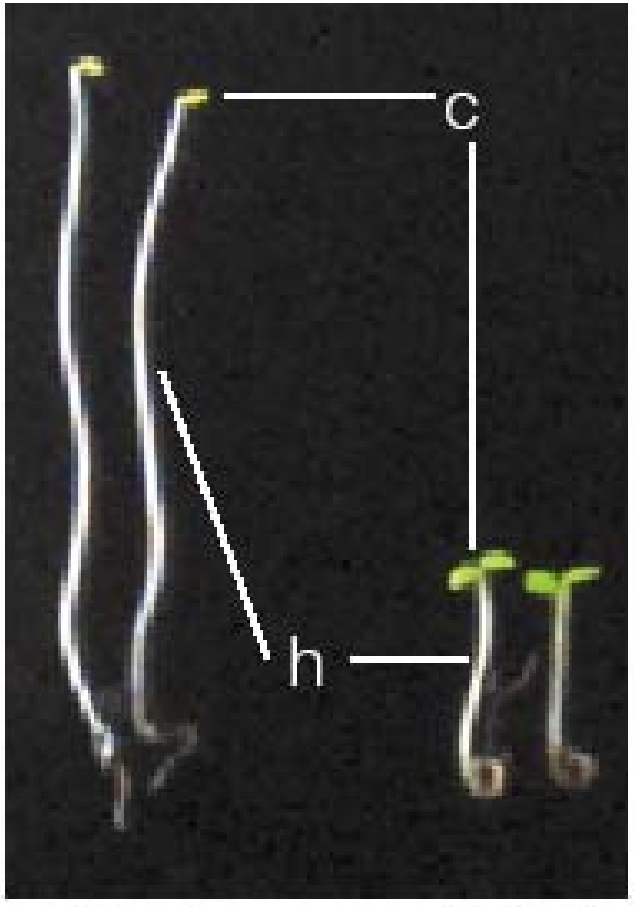


Figure 3 Domain structure and photochemical property of phytochromes. (*A*) Domain structure of phytochromes using *Arabidopsis* phyB as a model. (*B*) Two isomers of phytochromobilin, 15Z (Pr chromophore) and 15E (Pfr chromophore). (*C*) Absorption spectra of Pr and Pfr forms of phytochrome. (*D*) Photoconversion and dark reversion between Pr (inactive) and Pfr (active) form of phytochrome.

PHYTOCHROME STRUCTURE Phytochromes are homodimers in solution. Each monomer is a ~125-kDa polypeptide with a covalently attached linear tetrapyrrole chromophore, phytochromobilin, which is synthesized in the chloroplasts from heme (35, 104, 134, 145, 146). The phytochrome protein can be divided into two domains: an amino-terminal photosensory (signal input) domain and a carboxy-terminal domain that has been traditionally regarded as a regulatory, dimerization and signal output domain (146). The N-terminal domain comprises four subdomains: P1 (N-terminal extension, NTE), P2, P3 (bilin lyase domain, BLD), and P4 (Figure 3*A*) (130, 192). The P3 domain contains a conserved cysteine residue that forms a thioether linkage with the A ring of phytochromobilin and also autocatalyzes the bilin ligation reaction (Figure 3*B*) (94, 192). The P4 domain has been suggested to directly interact with the D ring of the chromophore to maintain its extended linear conformation in the Pr form and to stabilize the Pfr form (130). The carboxy-terminal half of phytochrome contains two subdomains: a PAS-related domain (PRD) containing two PAS domains (PAS-A and PAS-B) (11) and a histidine kinase-related domain (HKRD), which belongs to the ATPase/kinase GHKL (gyrase, Hsp90, histidine kinase, MutL) superfamily (Figure 3*A*) (42, 130, 200).



Early Developmental Effects of Phytochromes



Phytochromes regulate (100's of processes described)

-- stem elongation
-- cotyledon expansion
-- chloroplast development (greening)
-- apical hook opening
-- gene expression

Microarray analyses show that the early effects of phytochromes are to induce the expression of transcription factors that then alter the expression of genes involved in photomorphogenic development (Tepperman et al., 2001).

Fytochromy jsou syntetizovány v Pr formě

a vedle fotokonverze existuje temnostní reverze Pfr na Pr u mnoha dvouděložných (není u jednoděložných, trav)

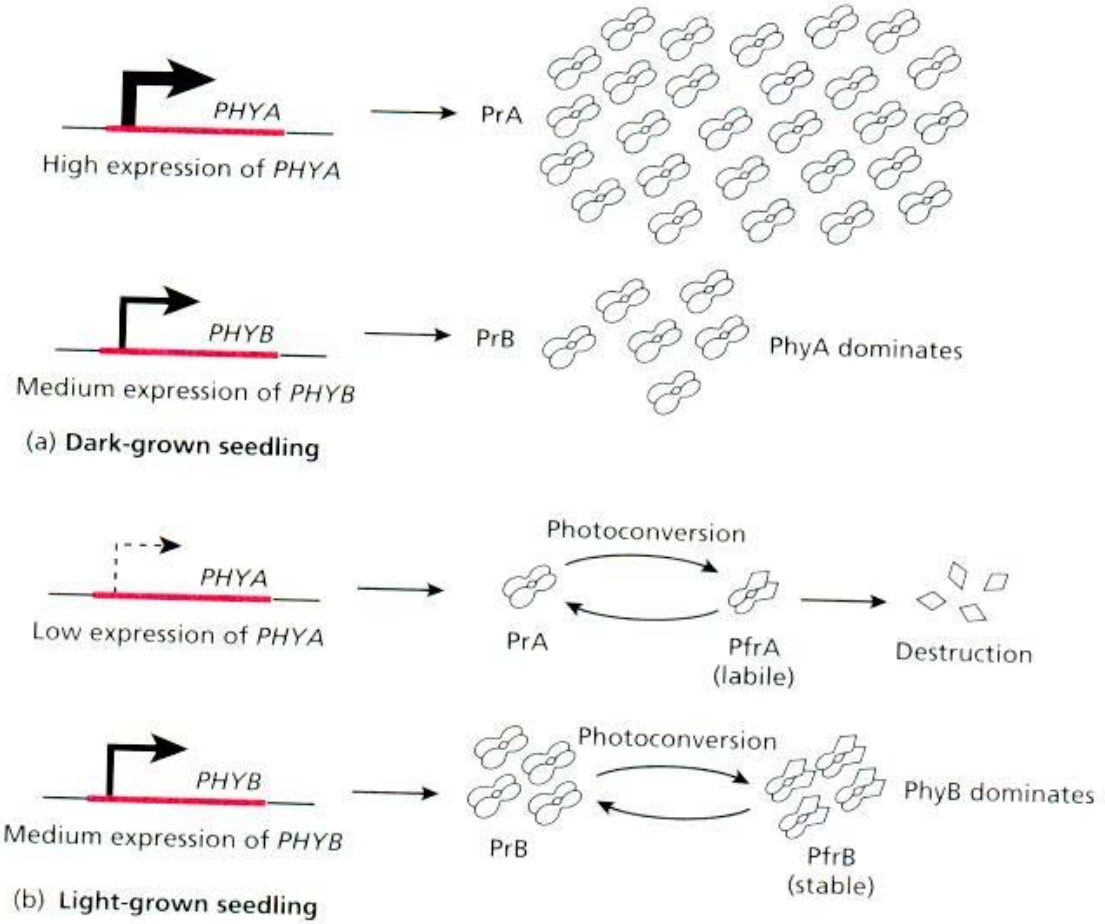


Fig. 7.2 The balance between phyA and phyB in dark- versus light-grown seedlings. (a) In dark-grown seedlings, the *PHYA* gene is highly expressed resulting in the accumulation of large amounts of phyA in the Pr form (PrA). The *PHYB* gene is expressed at a lower level. (b) The concentration of phyA 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.

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

- PhyA je nejen nestabilní na světle (PfrA-1h p.r.), ale dochází také k poklesu jeho transkripce zprostředkovaného fytochromovým systémem

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.
- PfrB váže TF PIF3 – transkr. světlem regul. genů

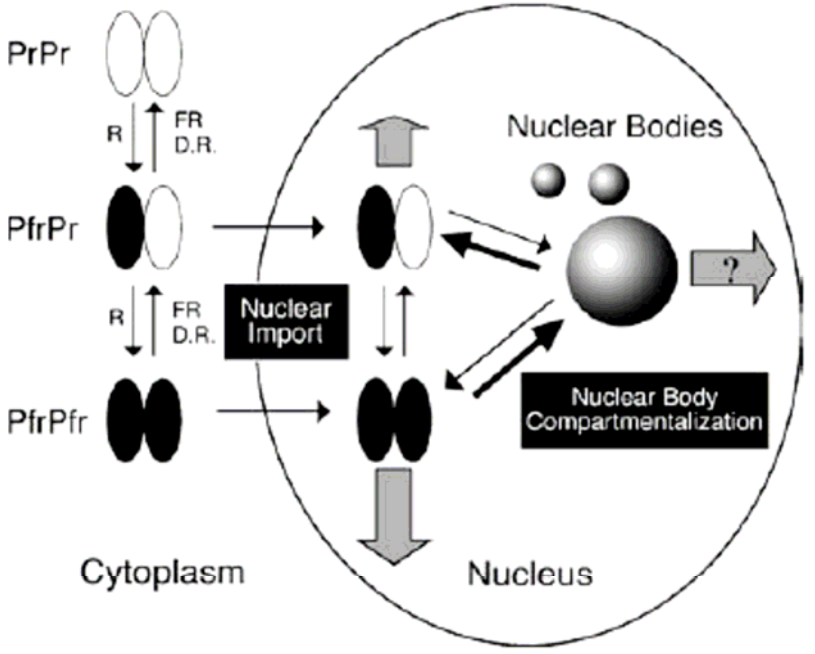
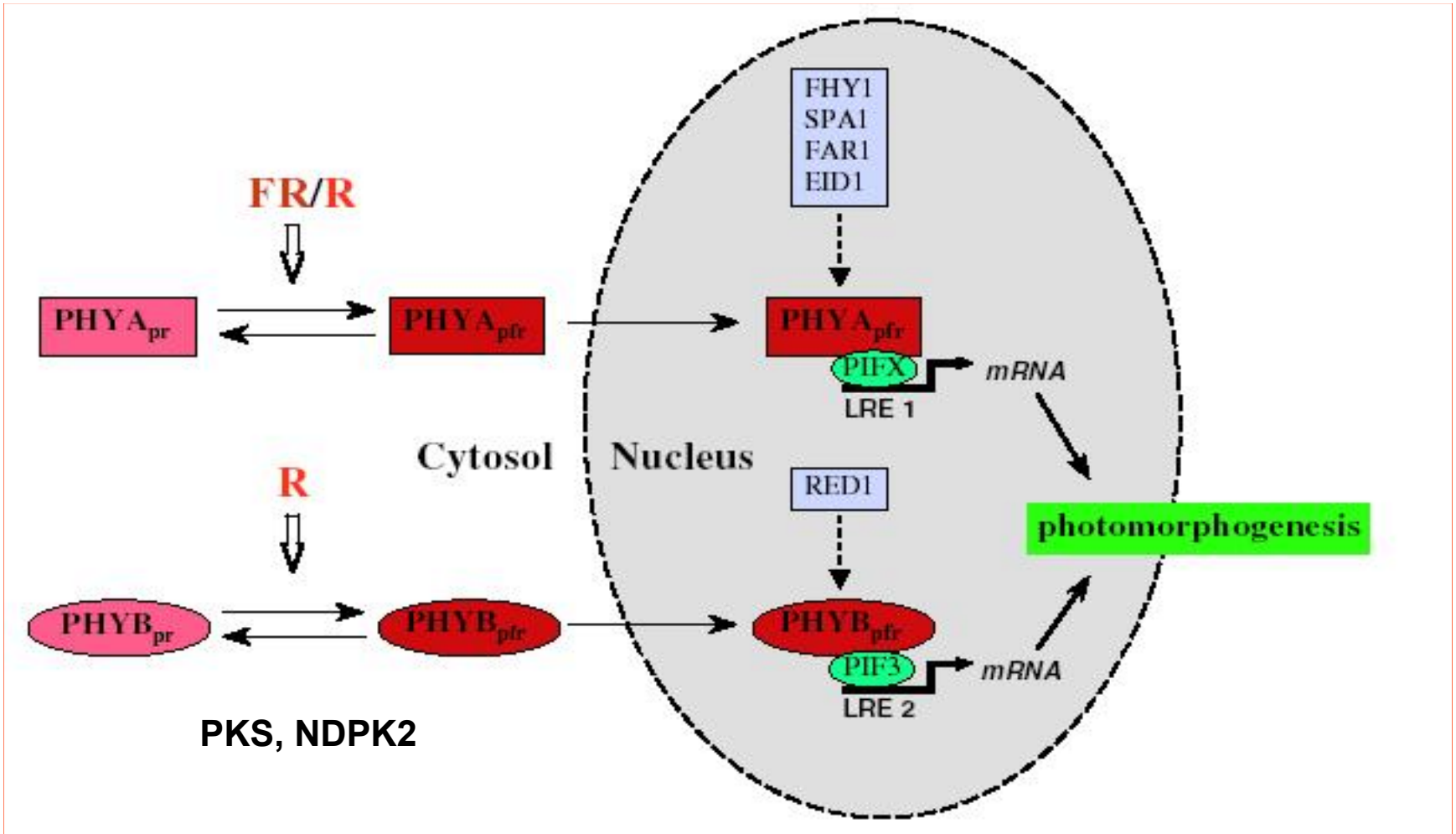


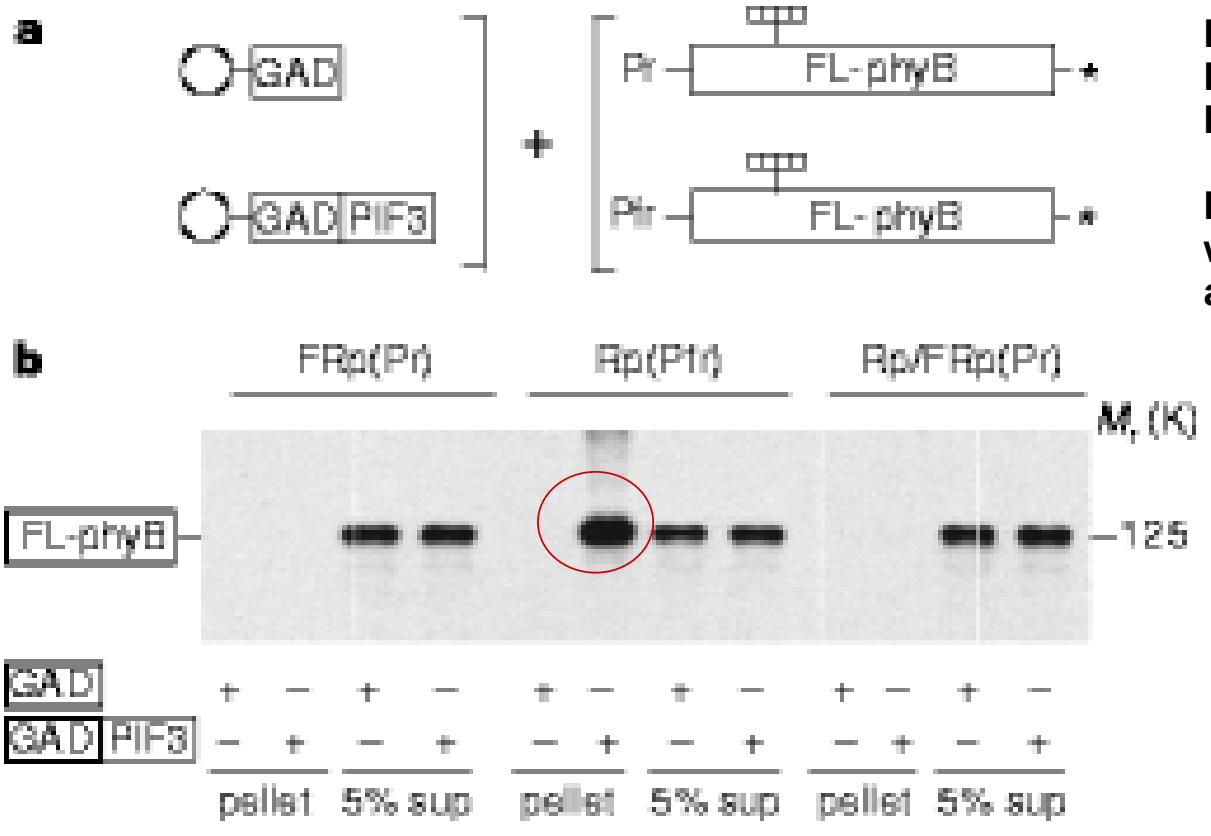
Figure 4 A schematic illustration of phytochrome localization using phyB as a model. There are two steps involved in phytochrome translocation after light activation, nuclear import and localization to nuclear bodies. Nuclear import requires at least one phytochrome molecule in the Pfr form in a phytochrome dimer. In the nucleus, PfrPfr homodimers are more likely to compartmentalize to nuclear bodies. Shaded arrows represent phyB signaling function. D.R., dark reversion.



http://bric.postech.ac.kr/~hgn/korean/lecture/plant_biology/p3.ppt

PIF3 and PHYB only interact if phyB is in Pfr state.

Interaction between PIF3 and PHYB is dependent upon state of PHYB



PIF3 bound to bead, PHYB radioactively labeled.

Extracts were treated with R or FR, spun down, and electrophoresed.

PIF3 and PHYB only interact if phyB is in Pfr state.

Ni et al., 1999

Fytochromoví mutanti

Jak je hledat?

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

THE PLANT CELL, Vol 3, Issue 12 1263-1274,

RESEARCH ARTICLES

The hy3 Long Hypocotyl Mutant of Arabidopsis Is Deficient in Phytochrome B

D. E. Somers, R. A. Sharrock, J. M. Tepperman and P. H. Quail
University of California, Berkeley/U.S. Department of Agriculture, Plant Gene Expression Center, Albany, California 94710

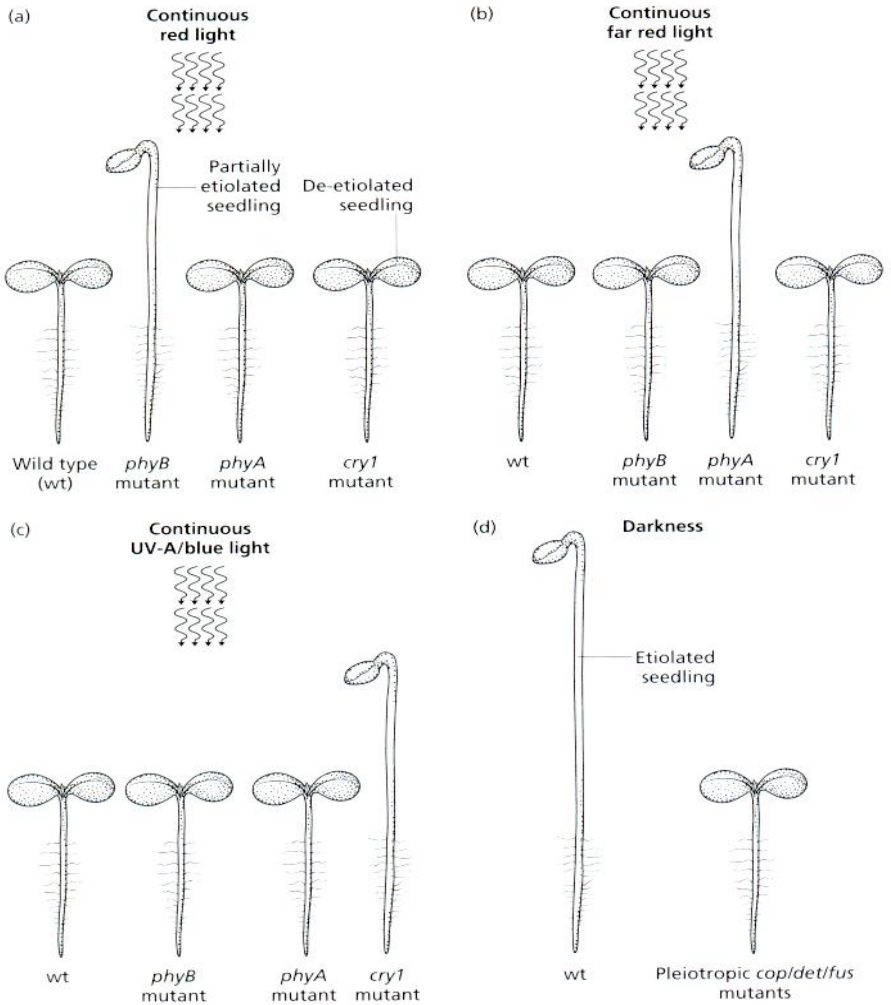
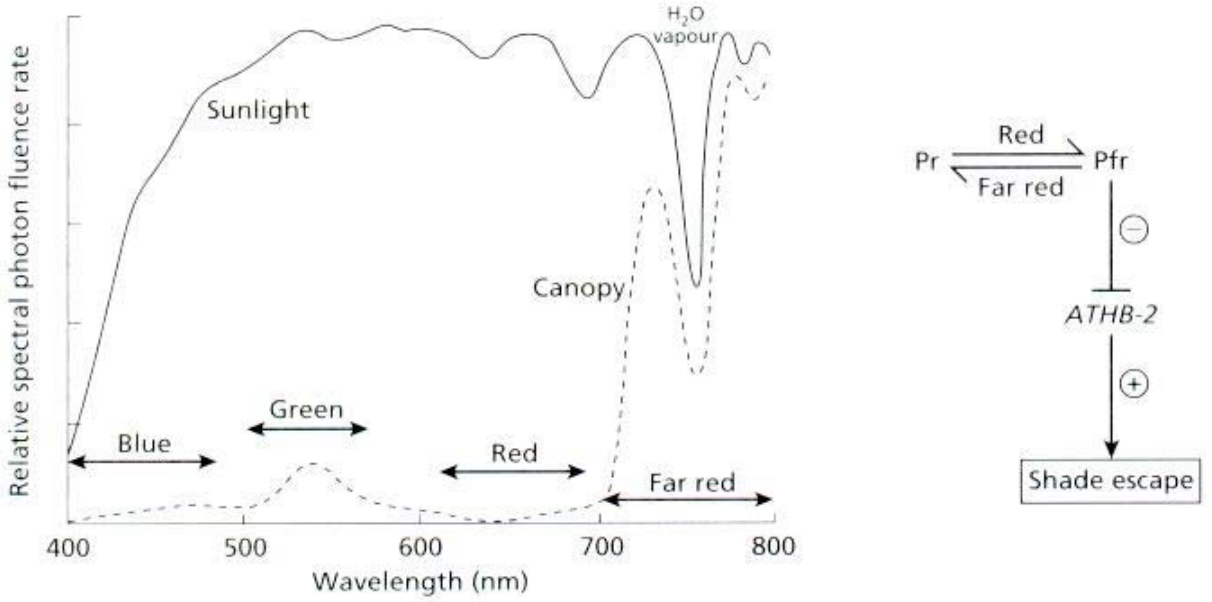


Fig. 7.6 Positive and negative regulation of photomorphogenesis in *Arabidopsis* revealed by mutants. Photomorphogenesis is promoted by continuous red light, continuous far red light and continuous UV-A/blue light in wild-type seedlings. The phenotypes of photoreceptor mutants indicate that the response 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.

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.)

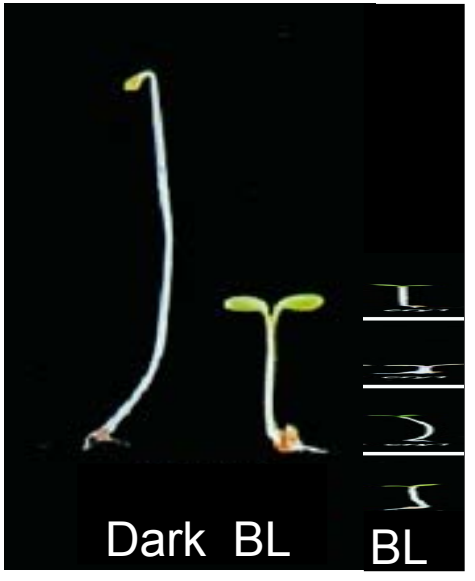
Kryptochromy

- 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***

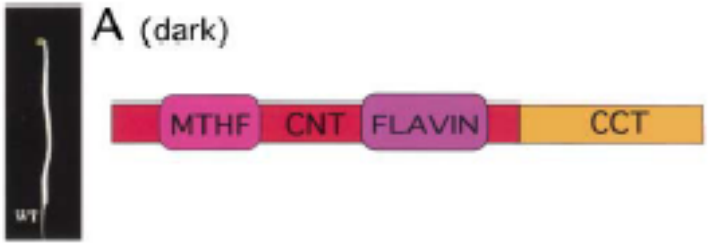
adapted from Neff and Chory, 1998

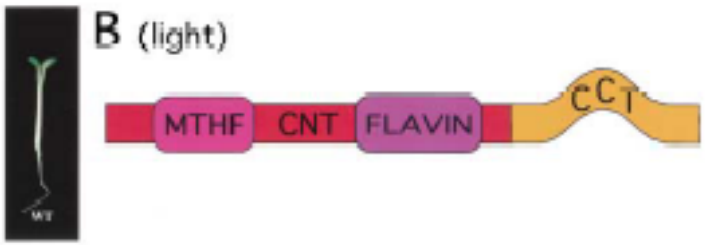
The sequence of *Hy4* was reported in 1993, and it was similar to DNA photolyase (Ahmad and Cashmore 1993, Sancar 1994) yet has no photolyase activity. Re-designated "Cryptochrome 1" (Lin et al., 1995)

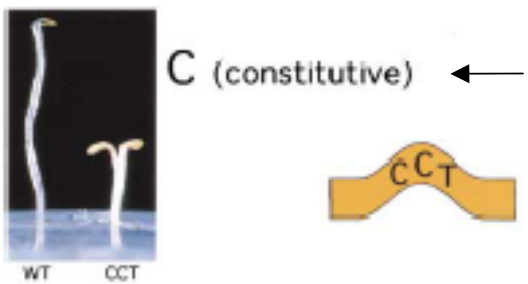
Involvement in circadian rhythms; led to discovery of animal crys (Cashmore, 2003)

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





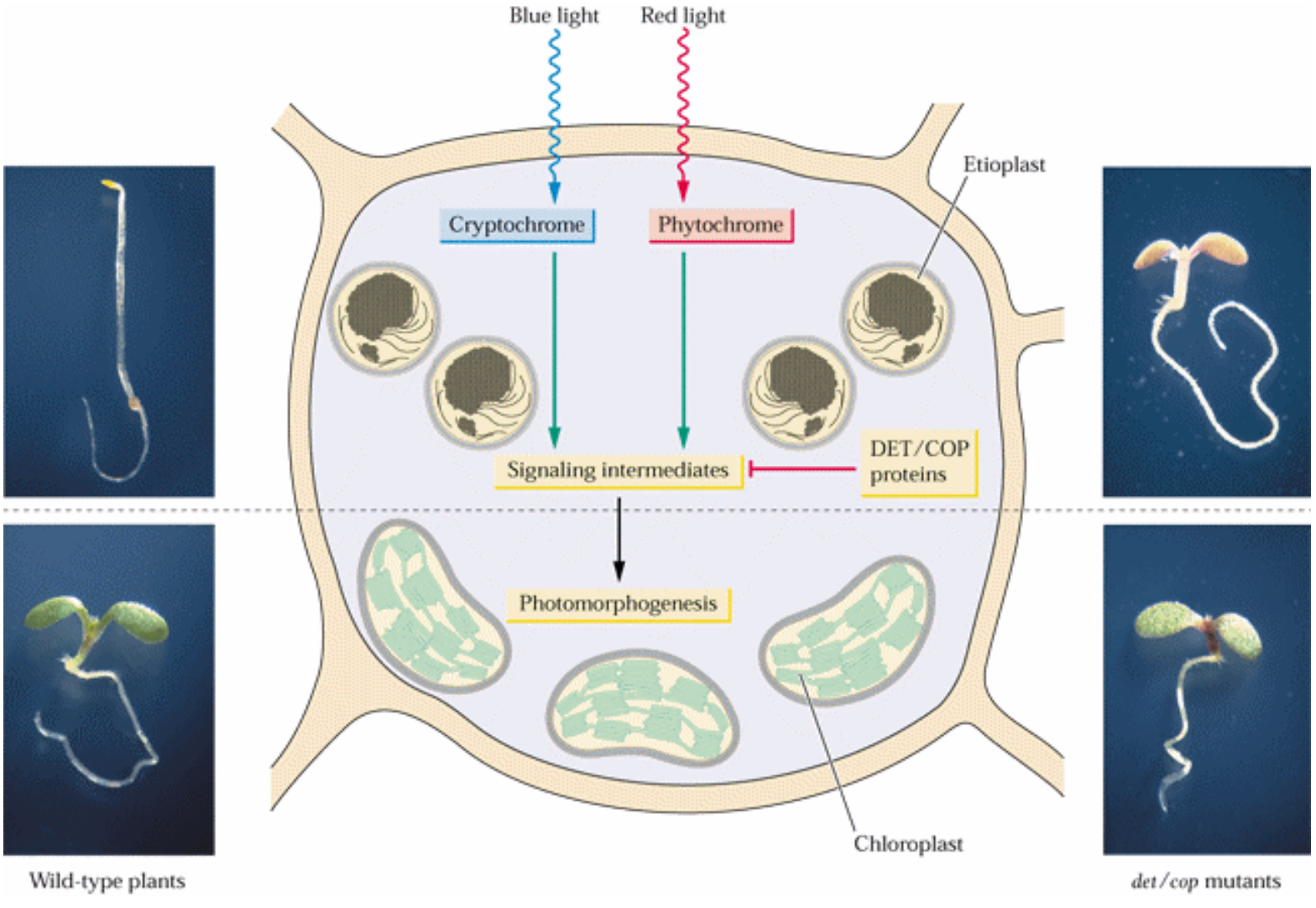


Ectopic overexpression of the CRY1 C-terminal extension results in a constitutive-photomorphogenic phenotype.

Is cry functioning through a mechanism involving COP1?

Cashmore, 2003

Pozitivní a negativní regulace fotomorfogeneze


Blue light
Red light
Cryptochrome
Phytochrome
Etioplast
Signaling intermediates
DET/COP proteins
Photomorphogenesis
Chloroplast
Wild-type plants
det/cop mutants

Xing Wang Deng, Yale

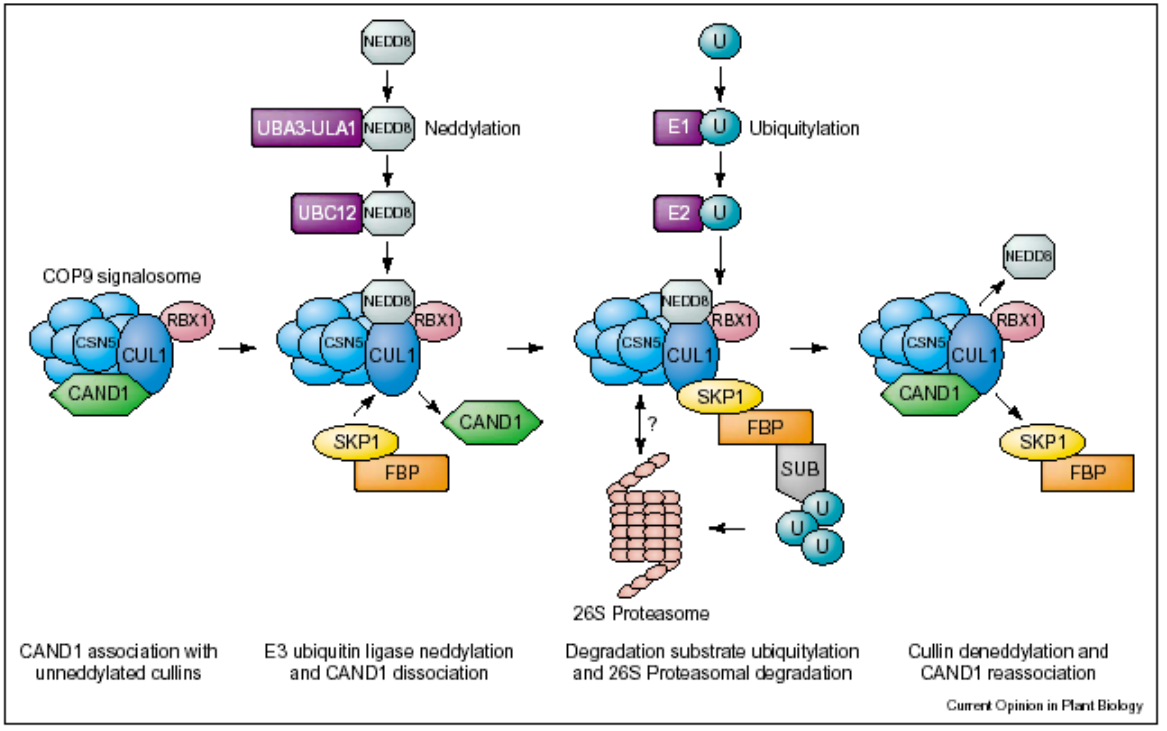
COP Mutants

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.



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].

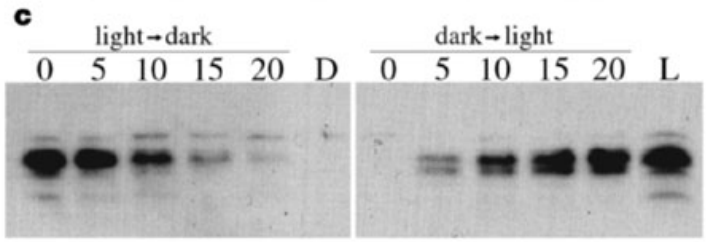
Abbreviations

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.

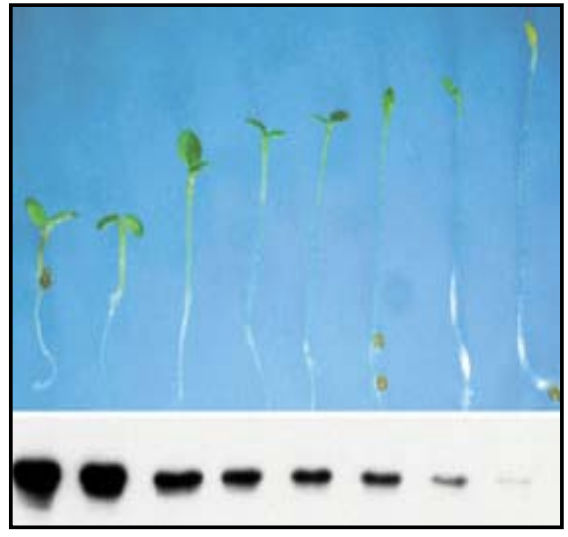
The ***hy5*** mutant – long hypocotyl under light conditions, particularly blue (Koorneef et al., 1980). Encodes a **B-zip transcription factor that is presumably a positive regulator of photomorphogenesis**.



HY5 accumulates rapidly in light and is not as detectable upon transfer of plants to darkness

Plants grown for days in light show different levels of HY5– HY5 level correlates with advanced photomorphogenic development.

fluence rate ←

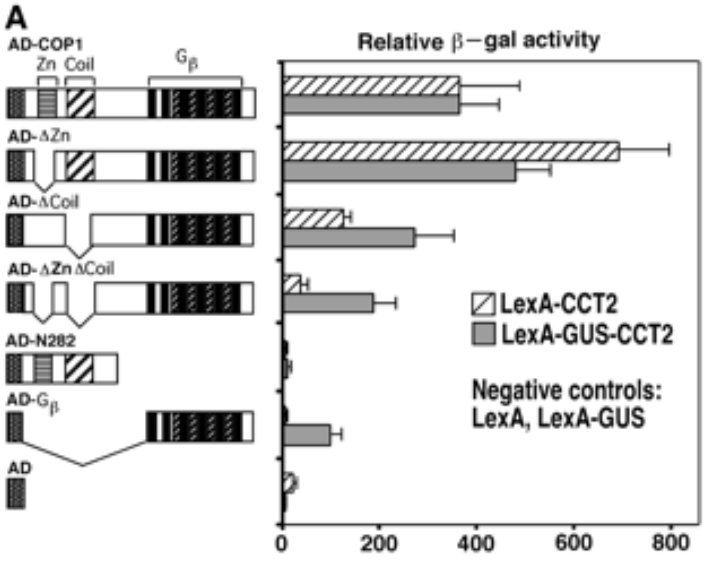


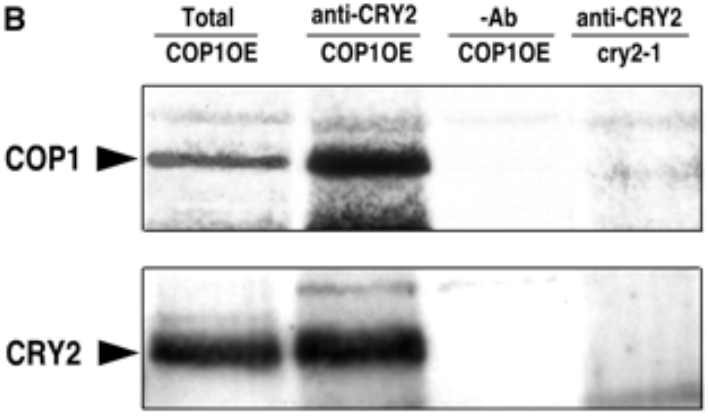
Osterlund et al., 2000

How does HY5 regulate photomorphogenesis?

Is it simply present only in light and acting as a positive regulator? Is it more complex? It seems to be acting in a manner that is antagonistic to COP1….

Does COP1 Interact with CRY?





Yeast 2-hybrid assay between COP1 truncations and CCT2 (CRY2 c-terminus)

Conclusions: The WD repeat domain is necessary and sufficient for interaction, yet binding is strongly enhanced by the coiled-coil and Zn binding domains.

CCT2-GUS interacts similarly.

Coimmunoprecipitation –

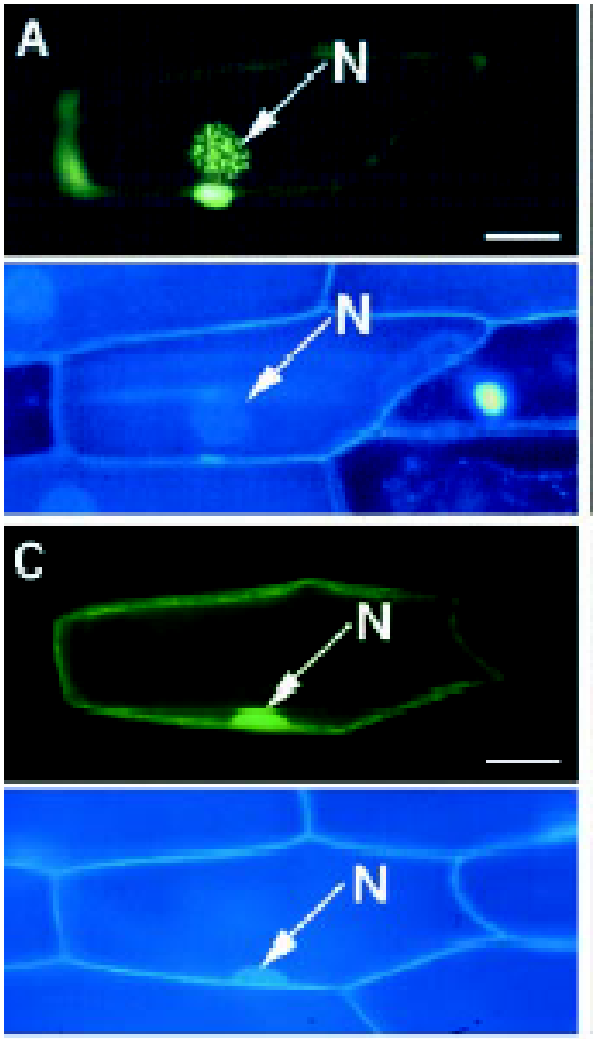
Using anti-CRY2, COP1 can be coimmunoprecipitated, demonstrating likely interaction *in vivo*.

Wang et al., 2001

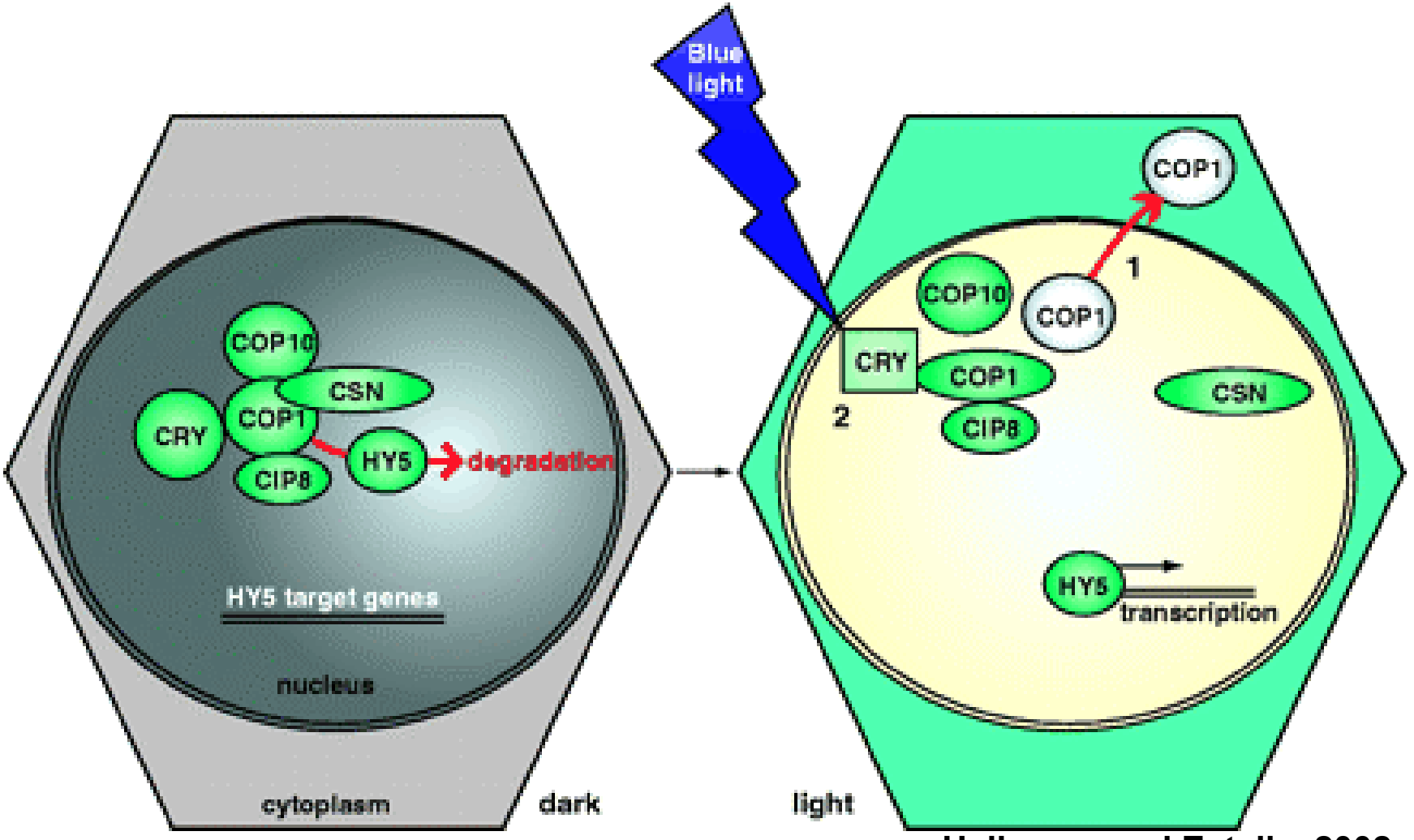
COP and CCT1 Co-Localize to the Nucleus

GFP::COP1

GFP::CCT1

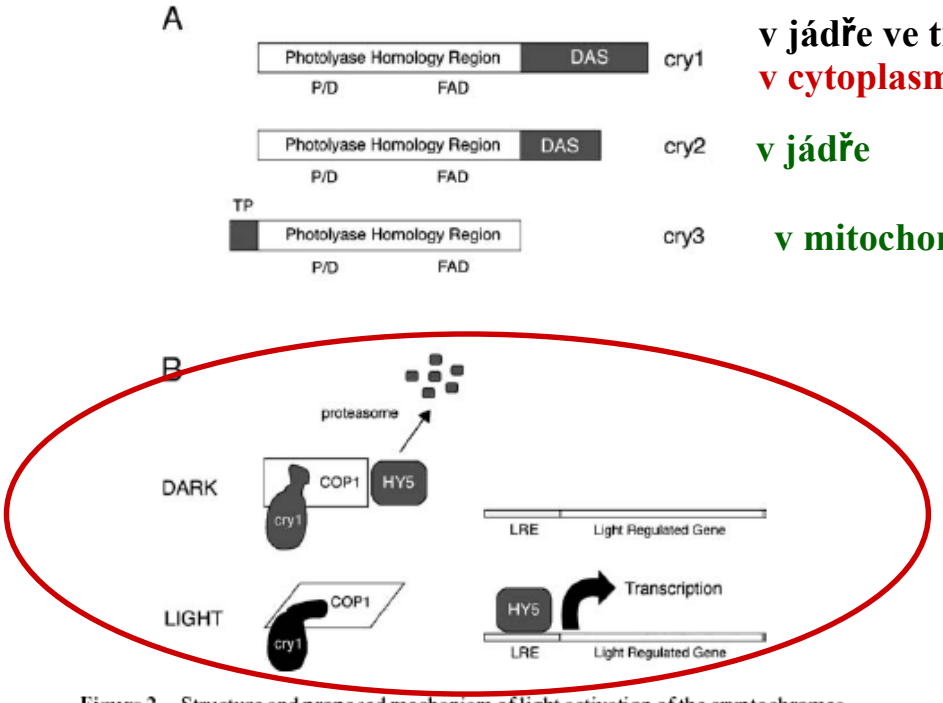


Proposed Model for Cryptochrome Function – COP1, CRY, HY5 Interaction to Regulate Degradation of HY5



Hellmann and Estelle, 2002

novější hypotéza

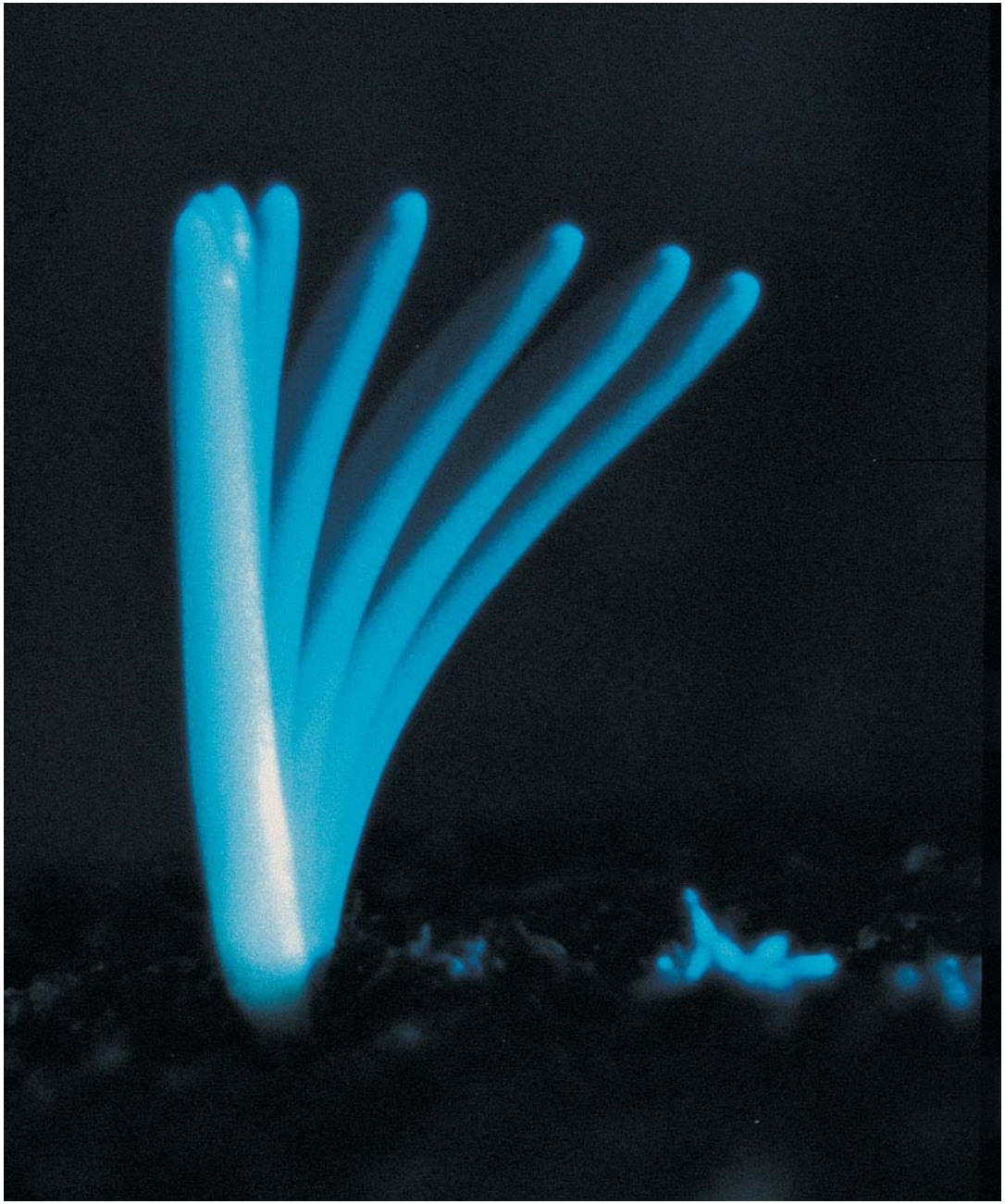


v jádře ve tmě, na světle
v cytoplasmě

v jádře

v mitochondriích i plastidech

Figure 2 Structure and proposed mechanism of light activation of the cryptochromes. (*A*) Schematic representation of the cryptochrome structure. The cryptochromes have a photolyase homology region that binds to FAD and a pterin or deazaflavin (P/T). cry1 and cry2 have short carboxy-terminal extensions with little conservation except for short stretches of homology (DAS) according to the nomenclature by Lin & Shalitin (111). cry3 has a transient peptide (TP) required for localization in the chloroplast and mitochondria. (*B*) Schematic mechanism of light activation according to the model proposed by Cashmore (23). Upon light perception the conformation of cry1 is modified, leading to a conformational change of COP1. The change of COP1 conformation releases the transcription factor HY5 that can activate light-induced genes.



Fototropismus

a

FOTOTROPINY

Perception of light and signal transduction in phototropism

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

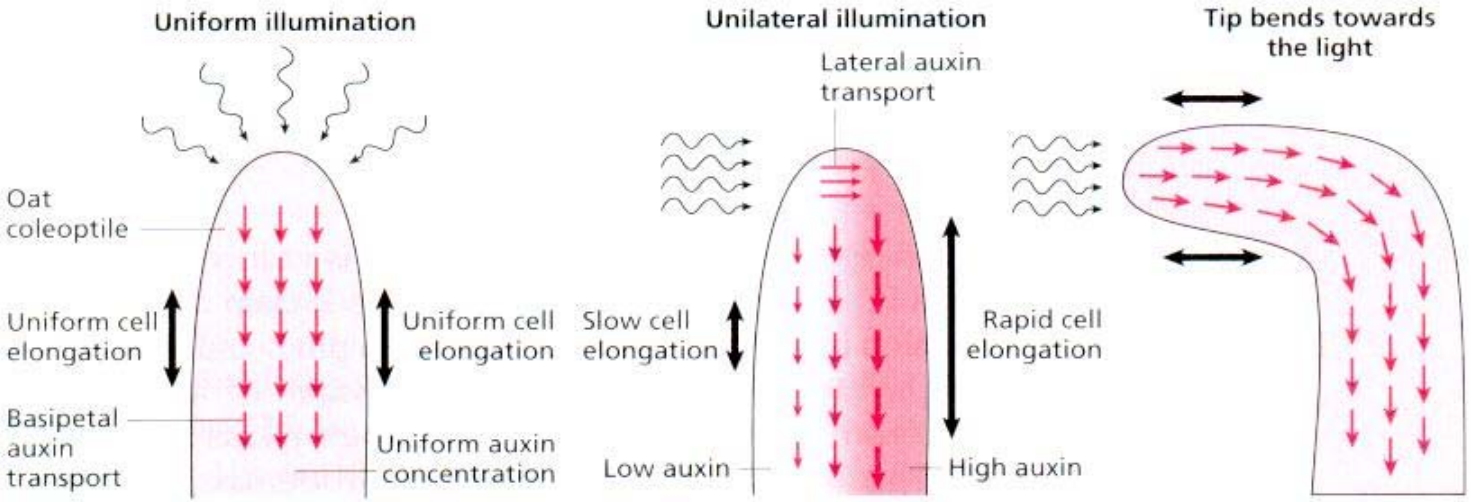
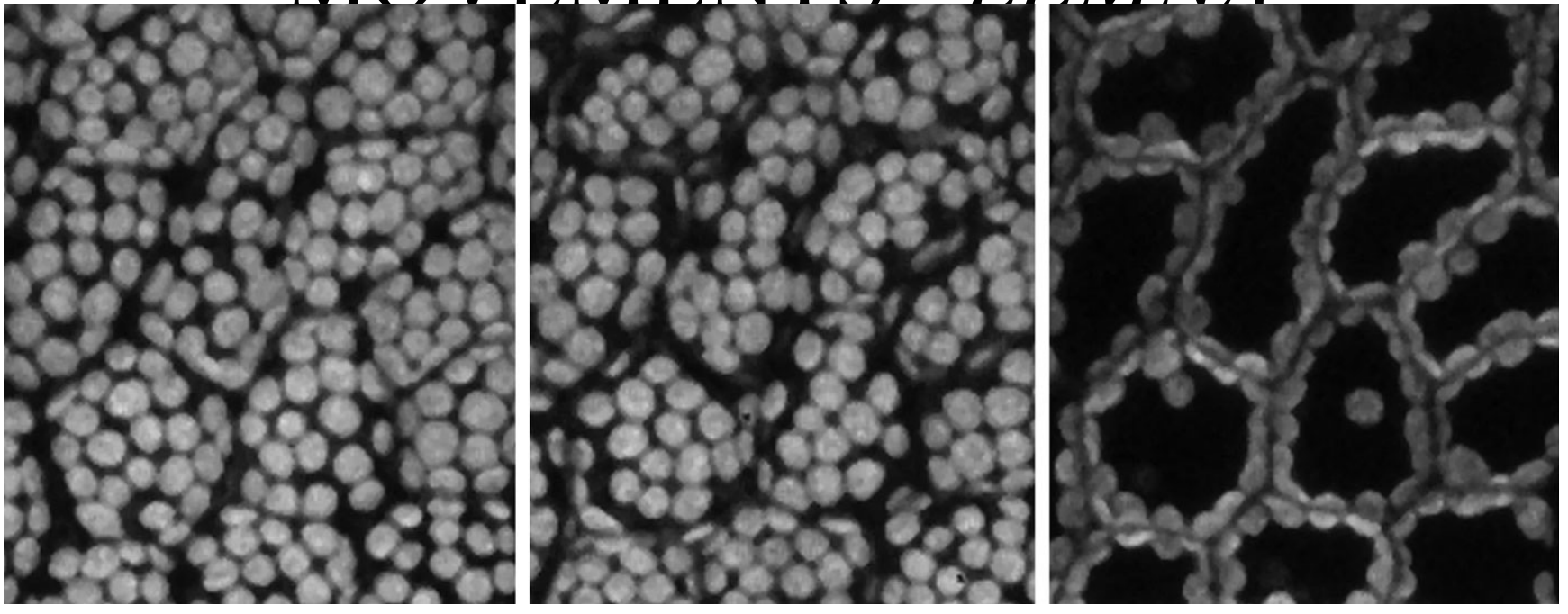


Fig. 7.9 The Cholodny–Went hypothesis of phototropism in oat coleoptiles. Unilateral illumination induces lateral auxin transport in the coleoptile tip leading to uneven auxin distribution in the coleoptile and to changes in cell expansion rates. The changes in expansion rates cause the coleoptile to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

opět auxin a jeho transport

CHLOROPLAST MOVEMENTS -*LEMNA*



FOTOTROPINY

Adiantum kapradina

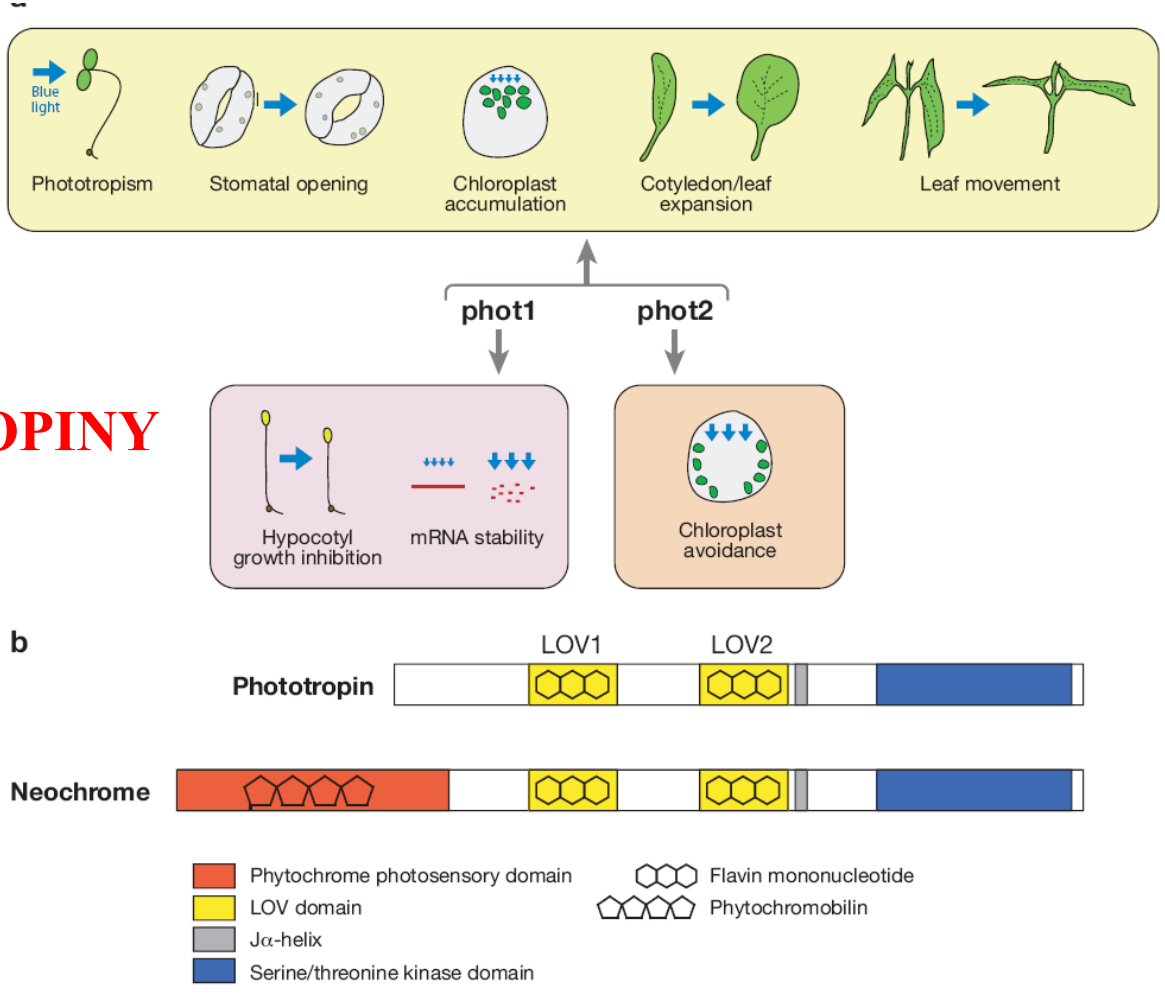


Figure 1

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.

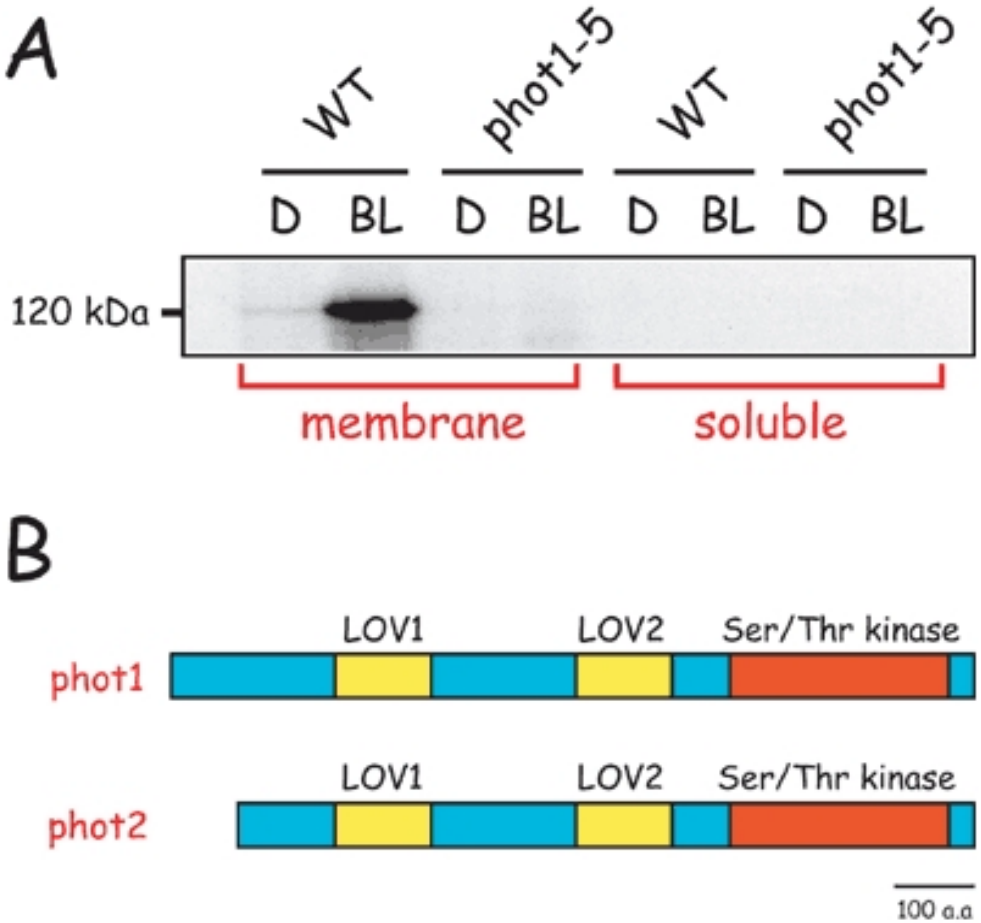


Figure 1 Kinase activity and protein structure of the phototropins. (A) Autoradiograph showing blue light-induced autophosphorylation of phot1 in protein extracts prepared from dark grown *Arabidopsis* seedlings. Protein extracts were prepared under a dim red safe light and given a mock irradiation (D 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.)**

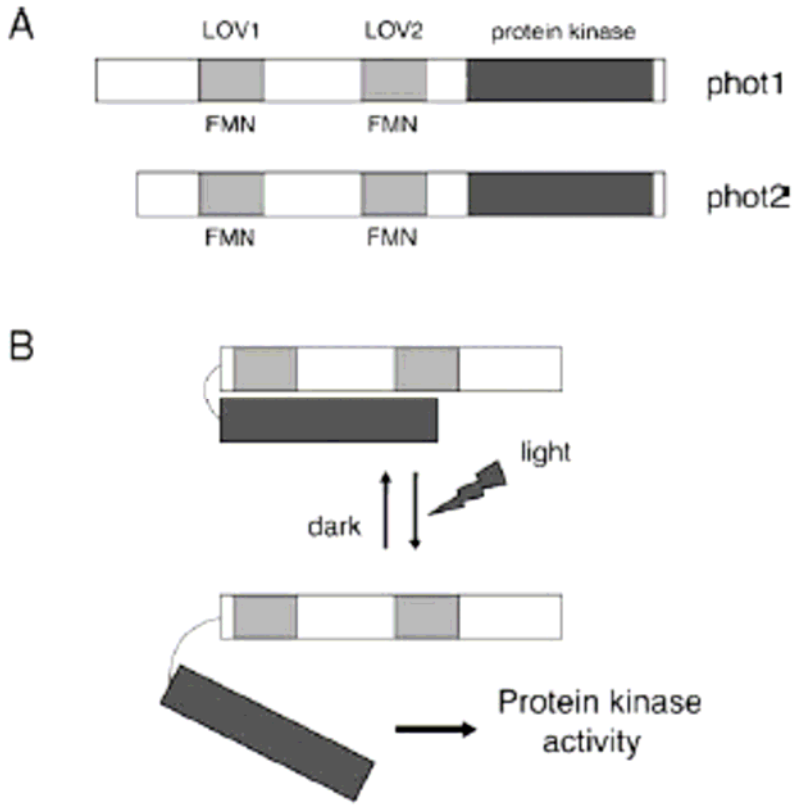


Figure 1 Structure and proposed mechanism of light activation of the phototropins. (*A*) Schematic representation of the phototropin structure. The phototropins contain two FMN binding LOV domains and a canonical Ser/Thr protein kinase domain at the C terminus. (*B*) Schematic mechanism of light activation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

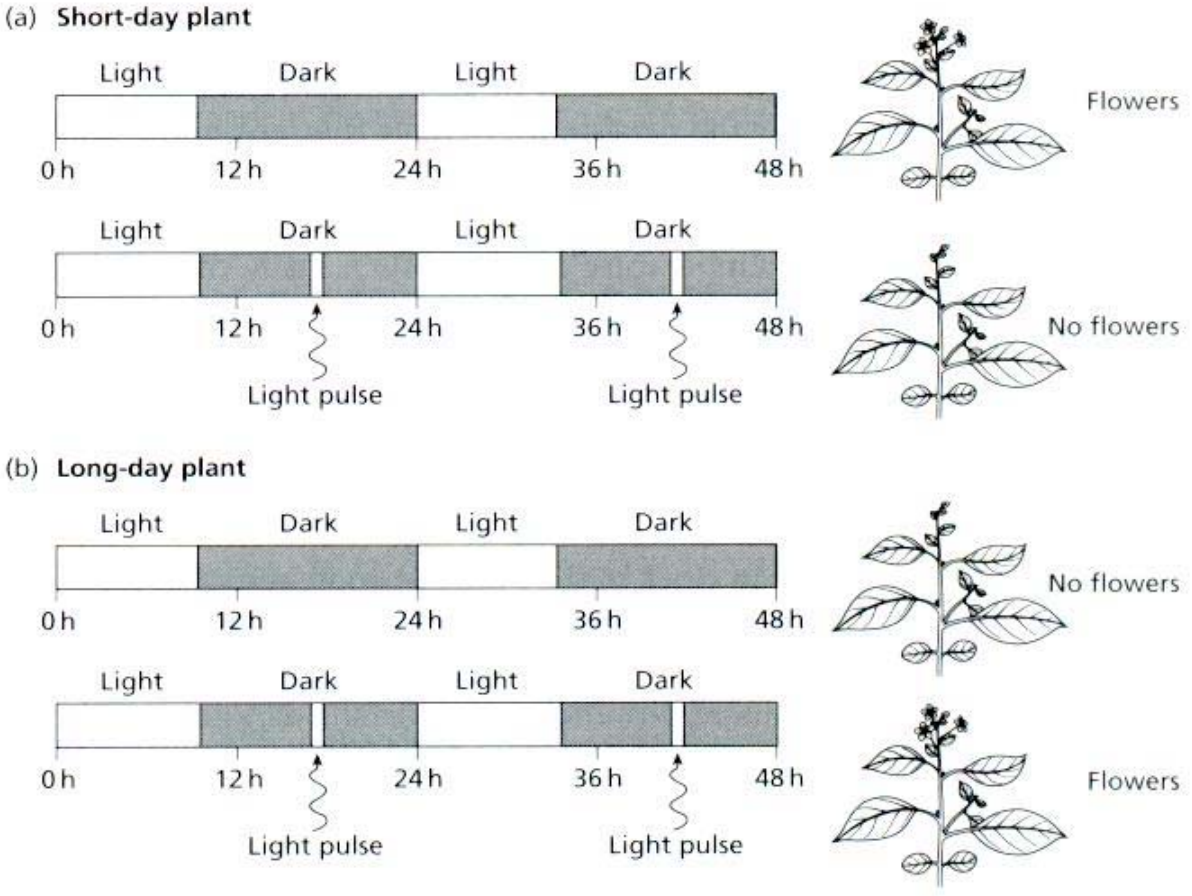


Fig. 7.10 The effects of night-time light treatments on photoperiodic flowering. Whereas day-time dark treatments have little effect on flowering, interrupting nights with brief periods of illumination can inhibit flowering in short-day plants (a) and can promote flowering in long-day plants (b).

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

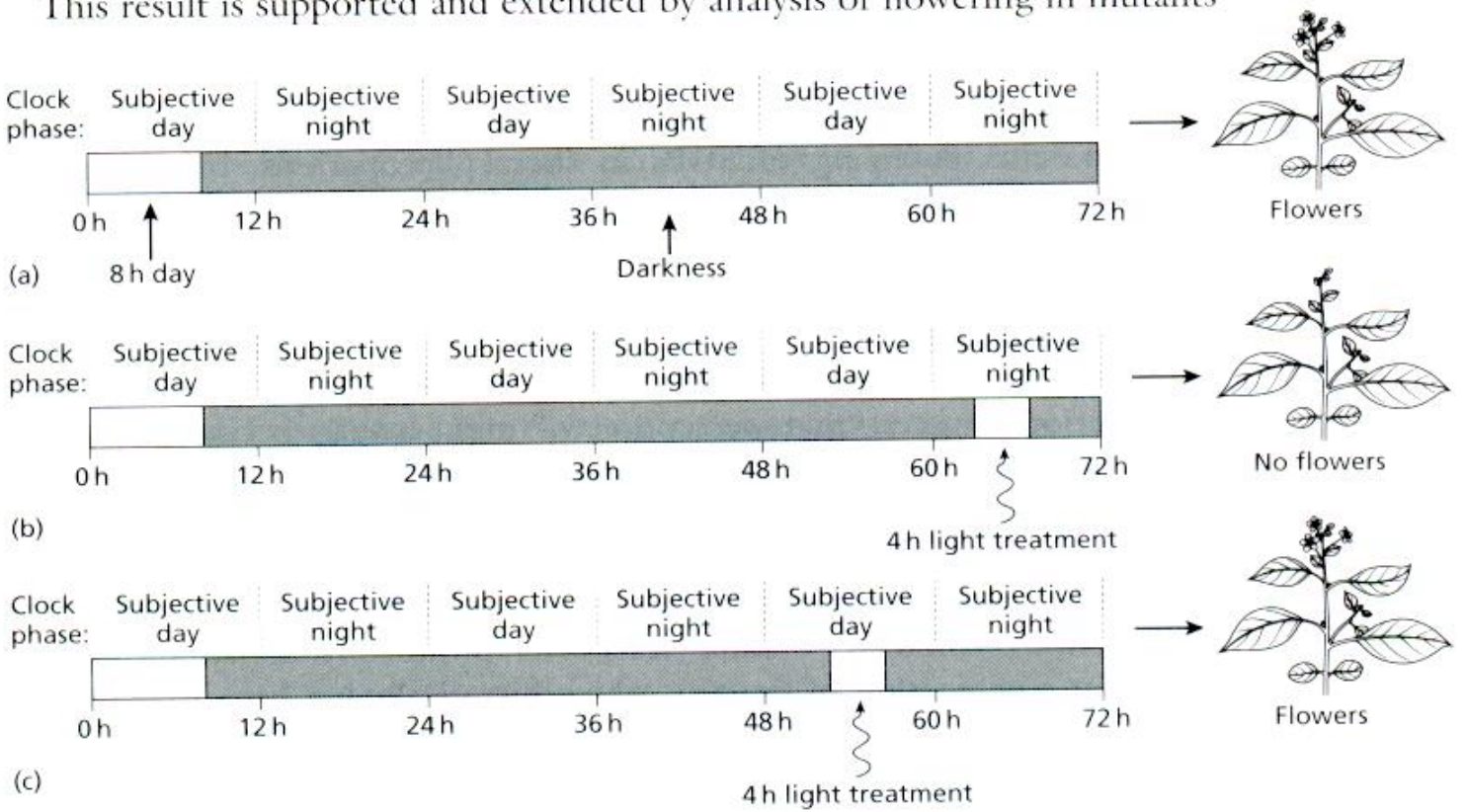
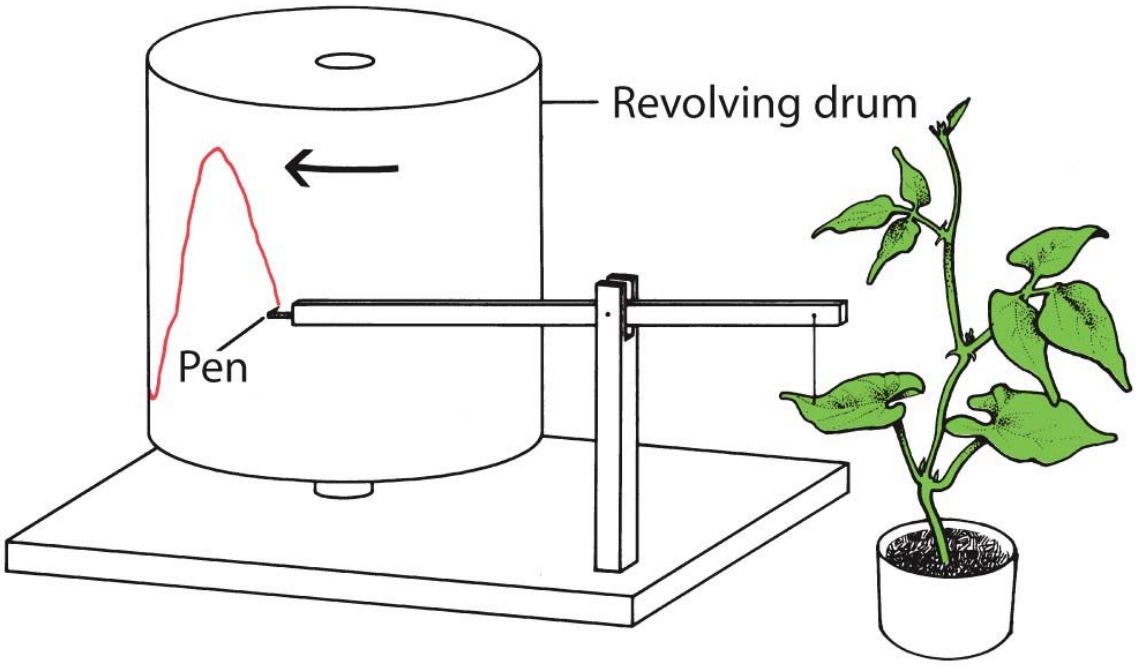
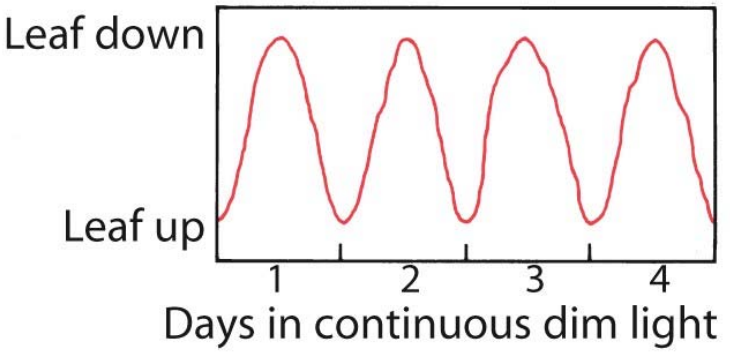


Fig. 7.11 Regulation of the photoperiodic response to light treatments by the circadian clock in soybean. Soybean is a SDP that requires several long nights to induce flowering. (a) The plant can be induced to flower if an 8-hour day is followed by 64 hours of darkness. The induction is prevented if 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.)

(a)


Revolving drum
Pen

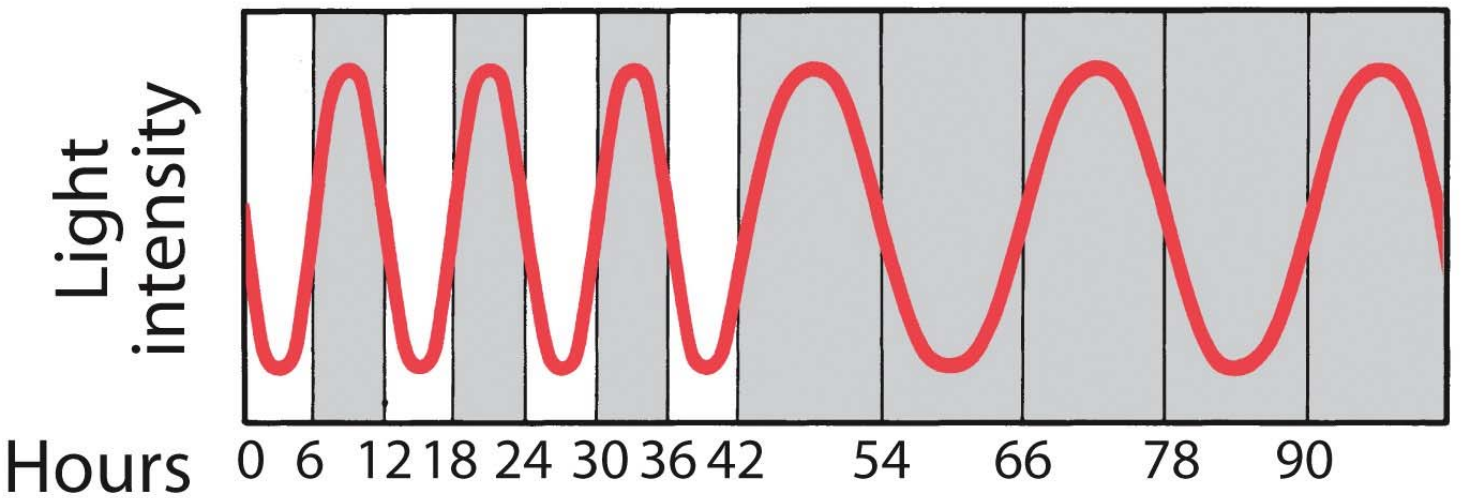
(b)

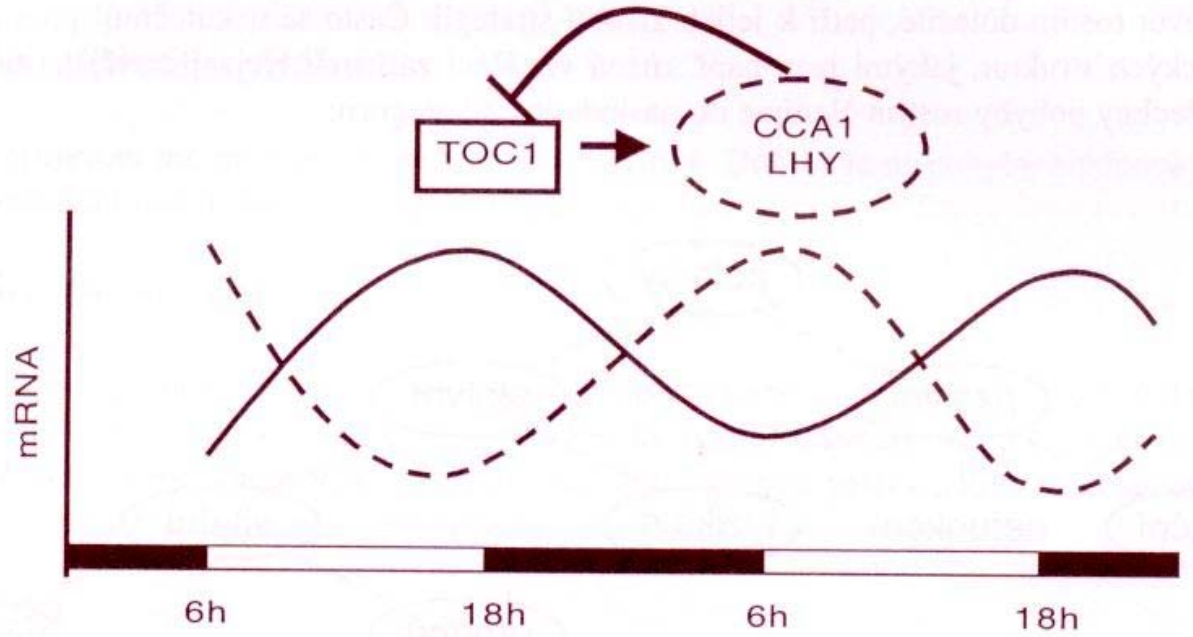

Leaf down
Leaf up
1
2
3
4
Days in continuous dim light

Biological clocks

jsou odolné vůči změnám teploty

- Two concepts associated with biological clocks
  - Free running period
  - Entrainment by light





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"

cyklus prostředí

"hodiny"

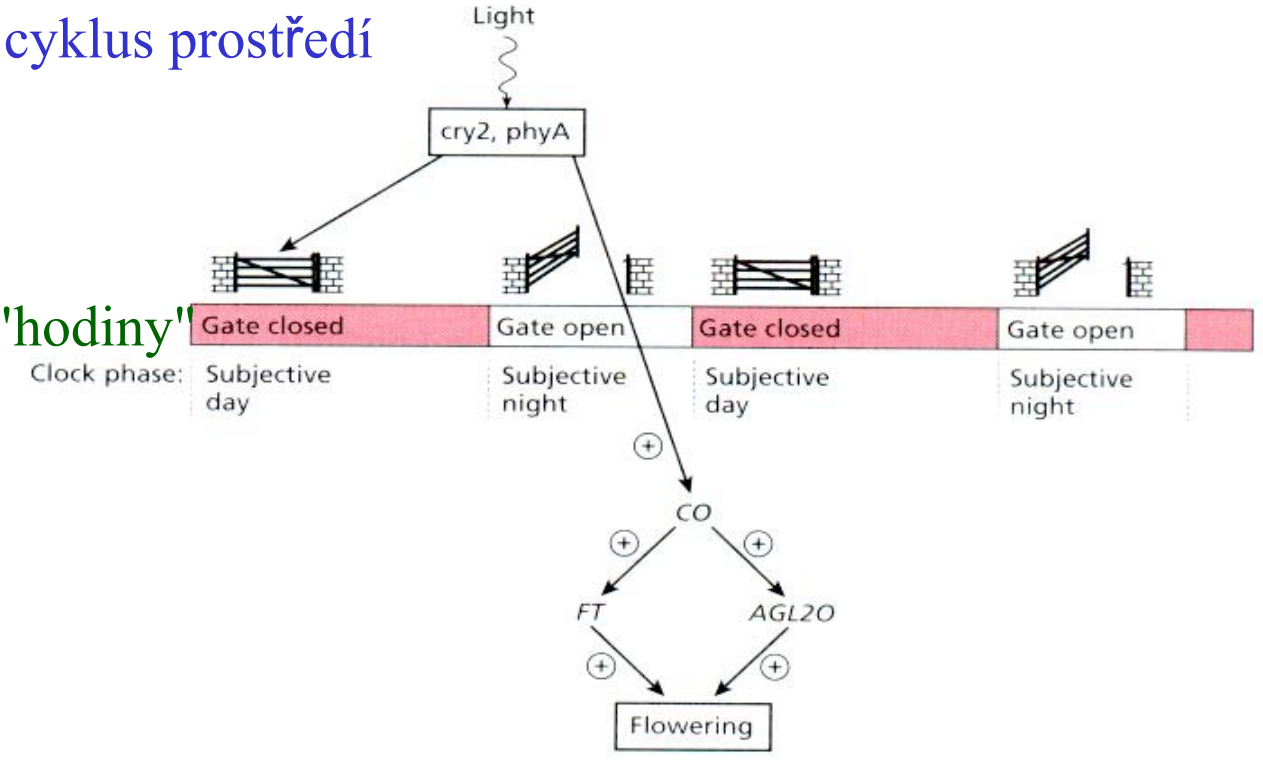
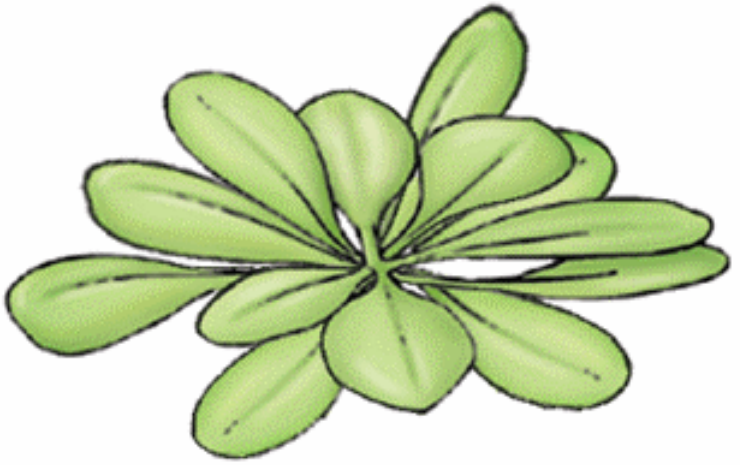


Fig. 7.12 A model for the photoperiodic regulation of flowering in *Arabidopsis*. The photoperiodic response is induced by light absorbed by cry2 and phyA. When the circadian clock is in the day phase, signal transduction between these photoreceptors and *CO* is blocked. When the clock is in the night phase, signal transduction is allowed, increasing *CO* expression and inducing flowering. Hence flowering is induced when night length is shorter than the period for which the gate is open. Arrows accompanied by a 'plus' sign indicate positive regulation.

GATING

(A)



Wild-type control
(ecotype Landsberg erecta)

(B)

35S::CO transformant

na krátkém dnu

Rostlinné "hormony"

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

TABLE 12.1 Plant Hormones: Structure and Effects

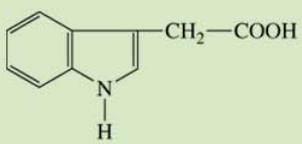
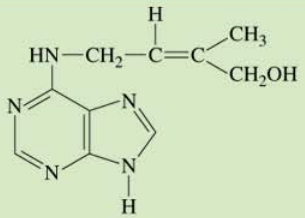
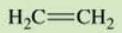
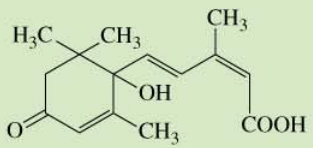
Plant Hormones	Chemical Structure	Functions
auxins	 indoleacetic acid (IAA)	Apical bud dominance (retards growth of lateral buds immediately below); mediate growth response to light direction; induce development of vascular tissue; promote activity of secondary meristems; induce formation of roots on 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	 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 (part 1) Plant Biology, 2/e

TABLE 12.1 *continued*

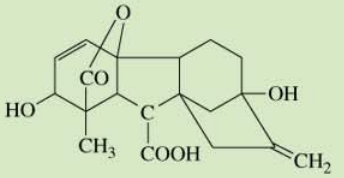
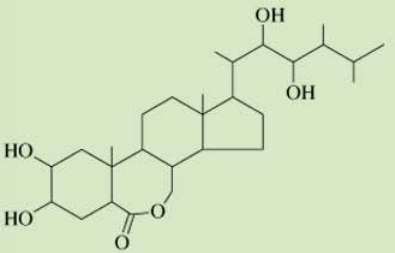
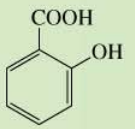
gibberellins	 gibberellic acid (GA)	Stimulate both cell division and cell enlargement during shoot elongation; promote seed germination; stimulate flowering in some plants
brassinosteroids	 brassinolide	Stimulate shoot elongation; reduce plant stress caused by heat, cold, drought, salt, and herbicide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

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

TABLE 12.1 *continued*

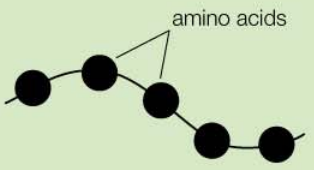
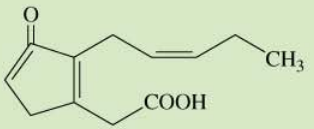
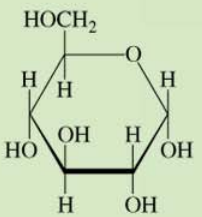
Plant Hormones	Chemical Structure	Functions
systemin	 systemin	Signals that wounding has occurred
jasmonic acid	 jasmonic acid	Helps plants resist fungal infection and other stresses; induces plant production of protective secondary compounds (alkaloids)
sugars	 glucose	Helps regulate amounts of chlorophyll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

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

Signals are perceived at the cell membrane and transduced to the nucleus, resulting in a change in gene expression

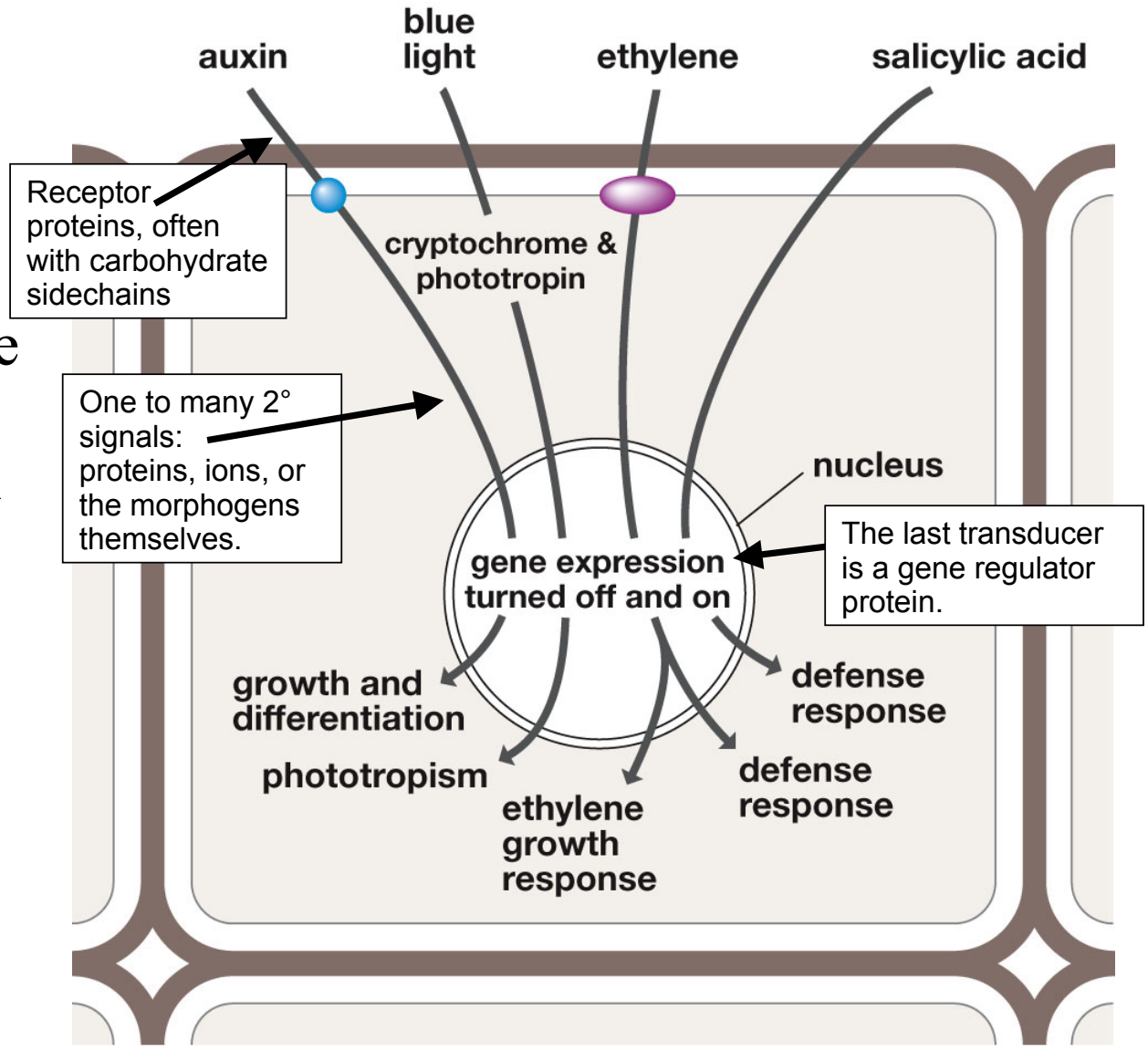
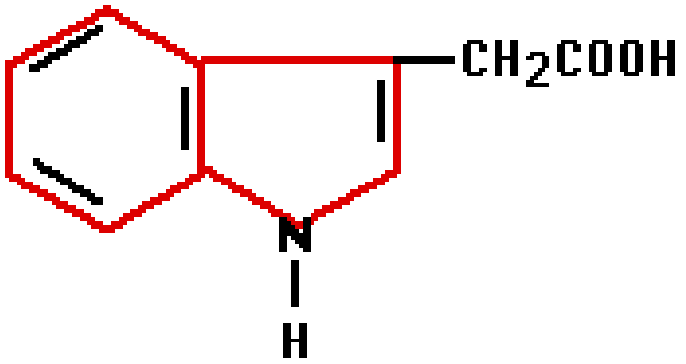


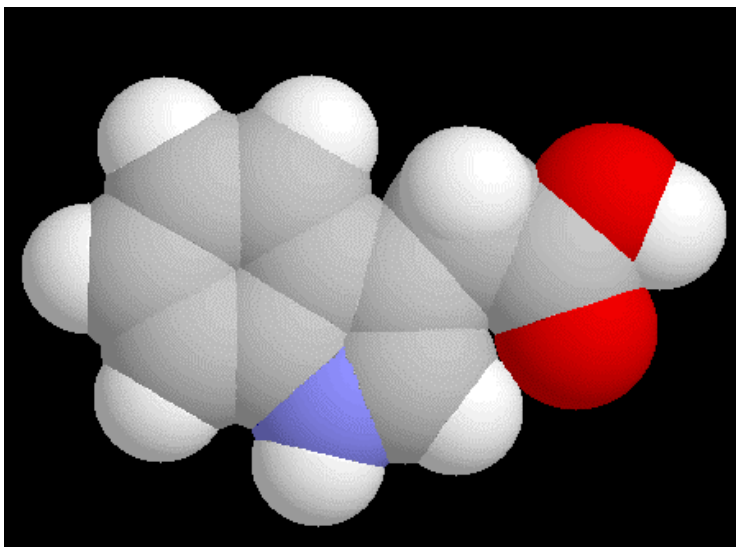
Figure 12.2 Plant Biology, 2/e

Auxiny

Auxin



Indole-3-acetic acid (IAA)



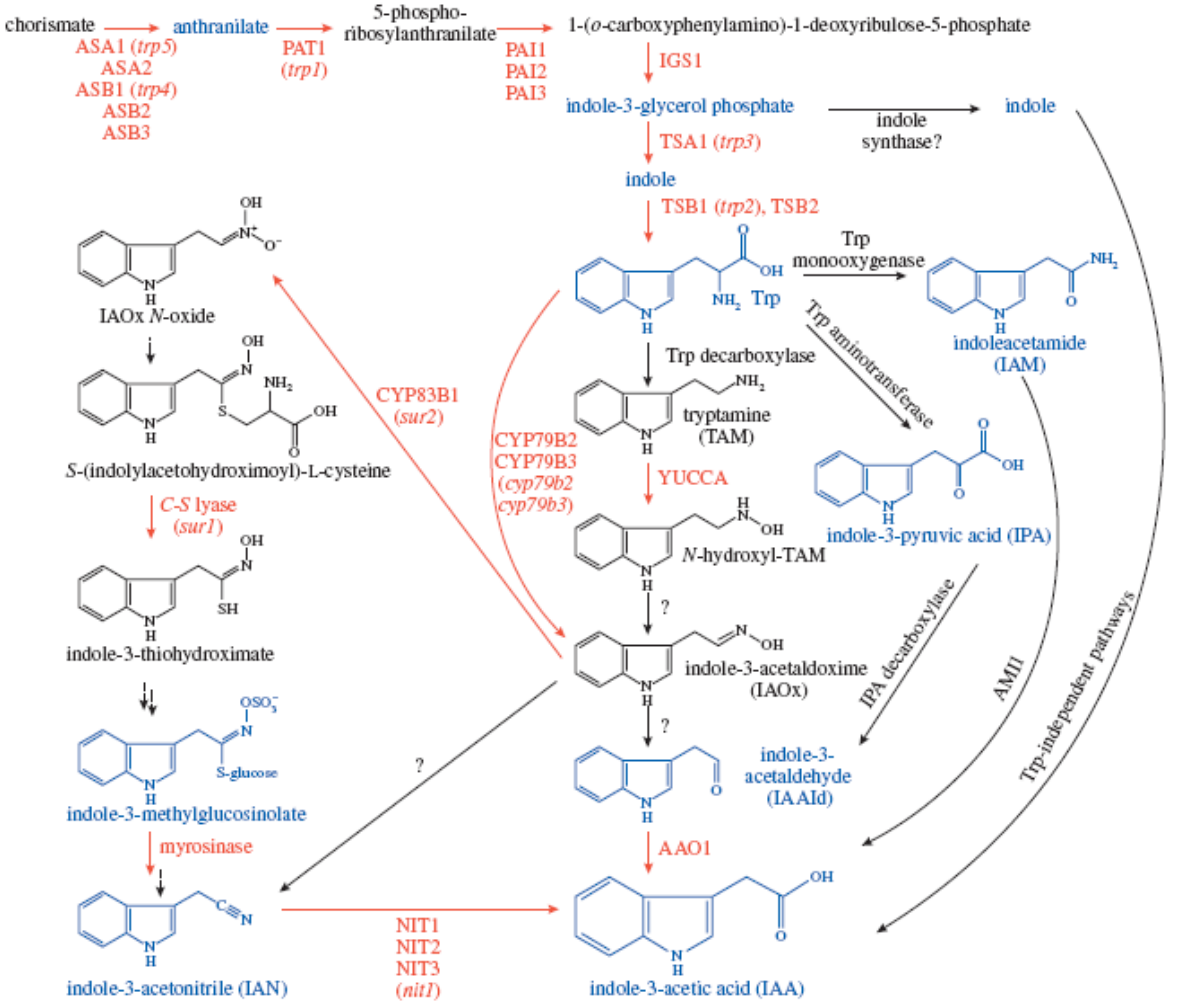


FIG. 2. Potential pathways of IAA biosynthesis in arabidopsis. *De novo* IAA biosynthetic pathways initiate from Trp or Trp precursors. Compounds quantified in arabidopsis are in blue, enzymes for which the arabidopsis genes are identified are in red, and arabidopsis mutants are in lower-case italics. 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.

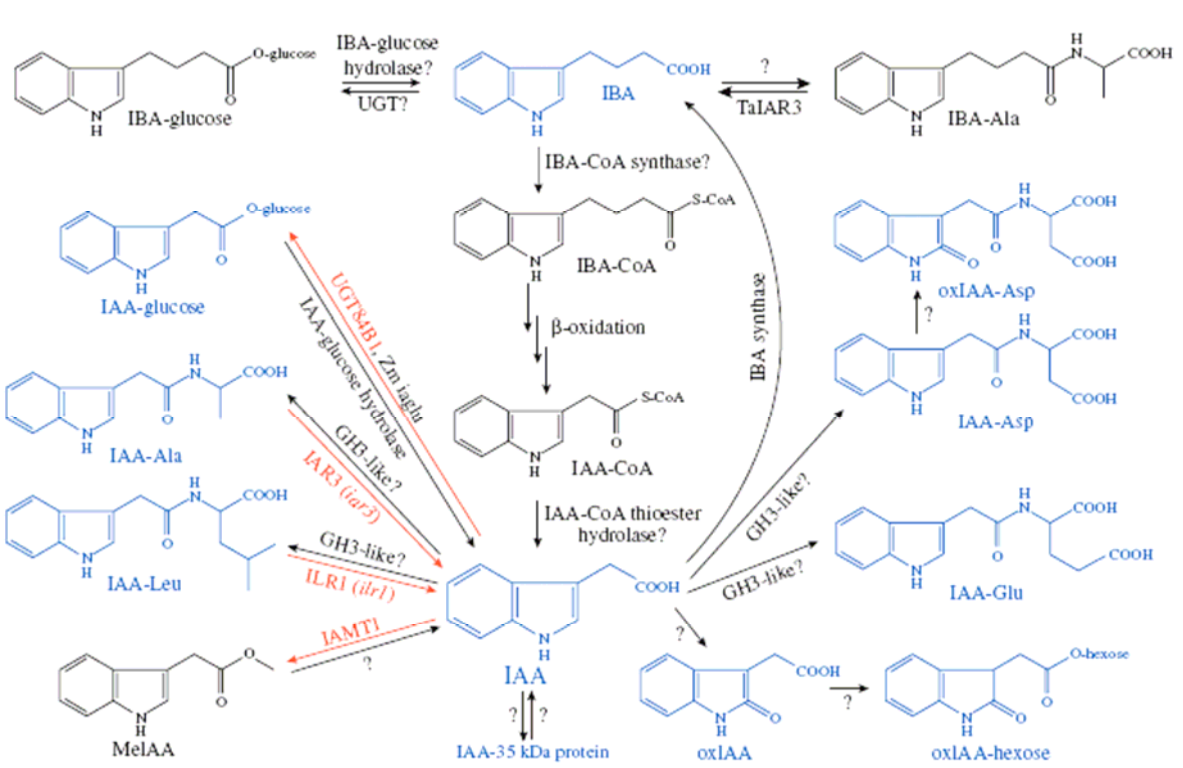
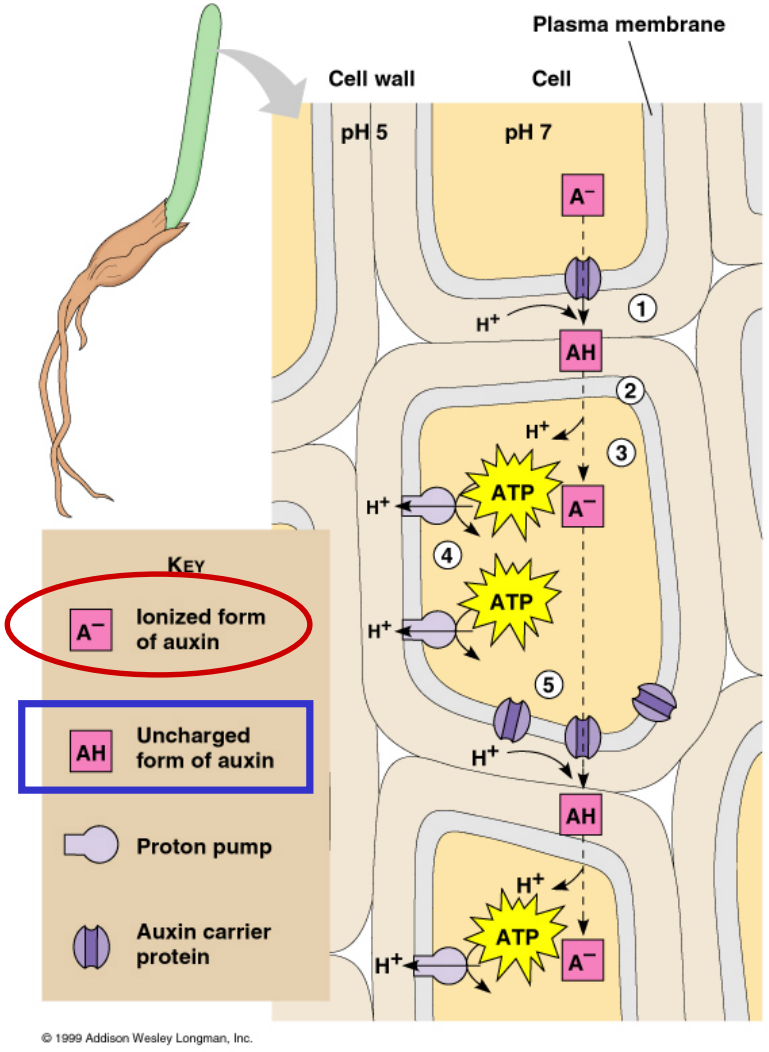


FIG. 3. Potential pathways of IAA metabolism. Compounds quantified in arabidopsis are in blue, enzymes for which the arabidopsis genes are cloned are in red, and arabidopsis mutants are in lower-case italics. Suggested conversions for which plant genes are not identified are indicated with question marks. 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

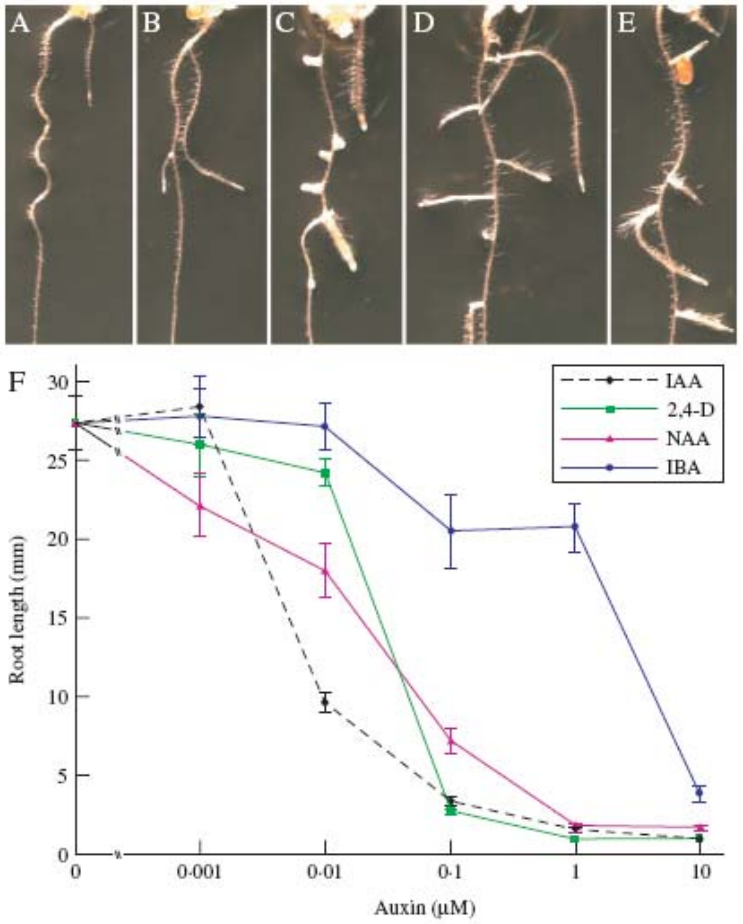


FIG. 1. Auxins promote lateral root formation and inhibit root elongation. *Arabidopsis thaliana* Col-0 ecotype plants were grown on unsupplemented medium (Haughn and Somerville, 1986) for 6 d, then transferred to unsupplemented medium (A) or medium supplemented with 10 nM IAA (B), 100 nM 2,4-D (C), 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???

ABP1

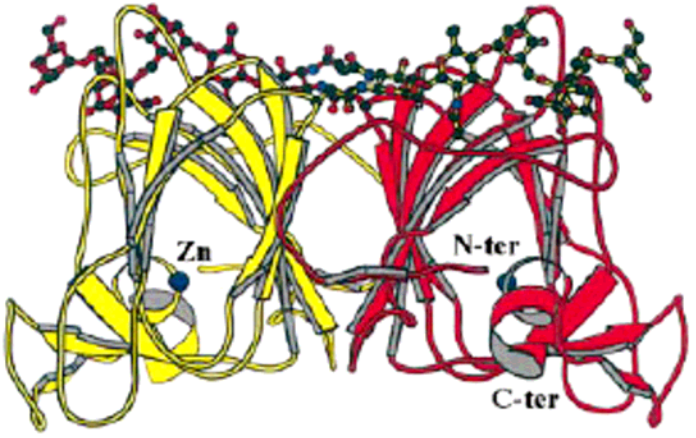


FIG. 4. Ribbon diagram showing the structure of an ABP1 dimer. The β-sheets are shown as broad arrows. ABP1 is *N*-glycosylated and some of the sugar residues are shown at the top of each monomer. Three C-terminal residues were not resolved and would extend the α-helices at the foot of the molecules. 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.

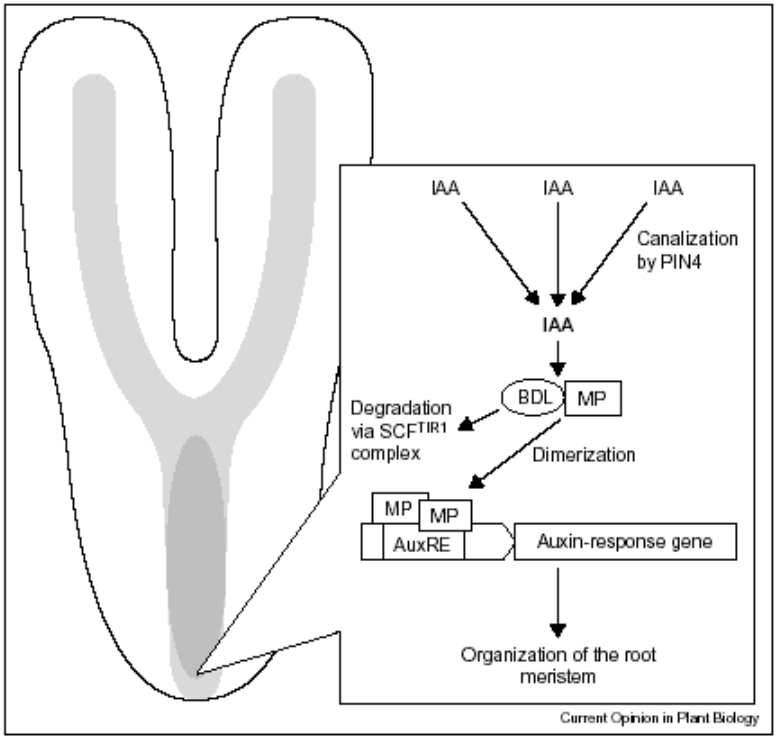
Mutanti *monopteros(mp)* a *bodenlos(bdl)* nemají kořen.

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.

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

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



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….

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

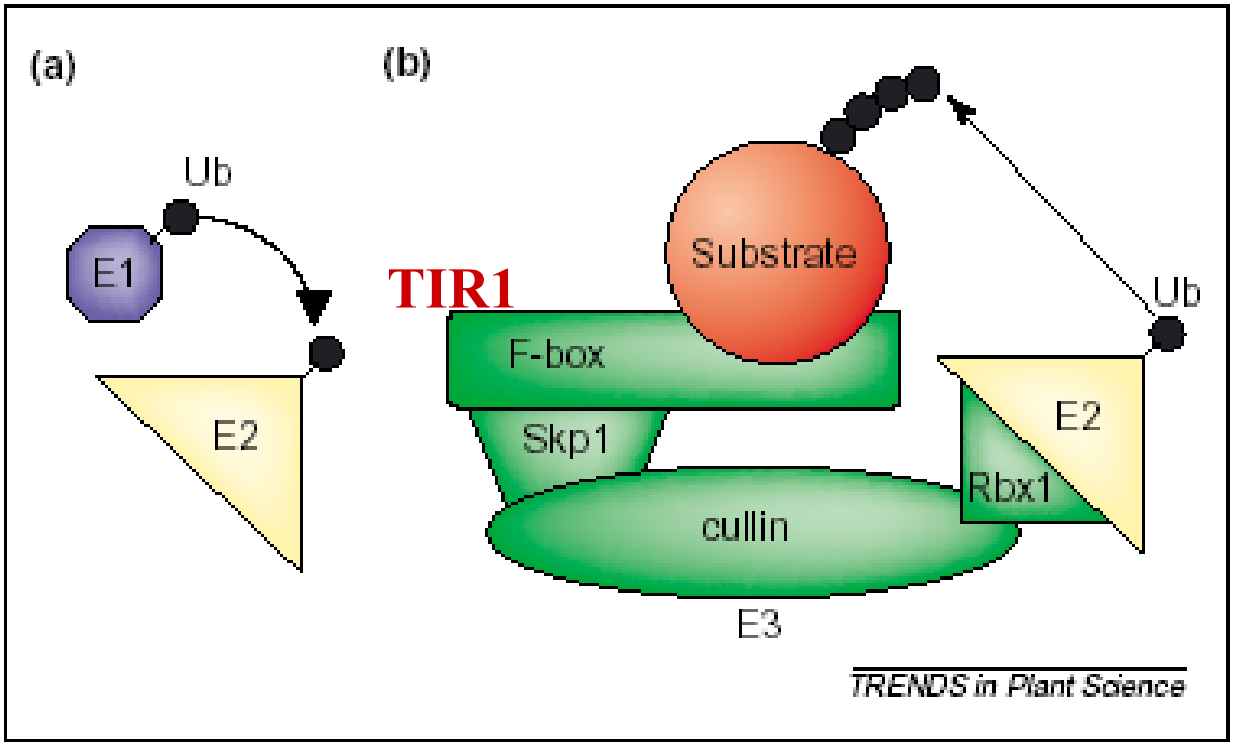


Figure 1. Key steps in the pathway of polyubiquitylation by SCF E3 ligase, which targets substrate protein and leads to degradation by the 26S proteasome. **(a)** Ubiquitin (Ub) is linked via a thioester bond to the ubiquitin-activating enzyme (E1). Ubiquitin is transferred from E1 to the cysteine of 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.

Receptorem auxinu je **TIR1 a jeho homology: F-box** podjednotka SCF E3 ligázy.

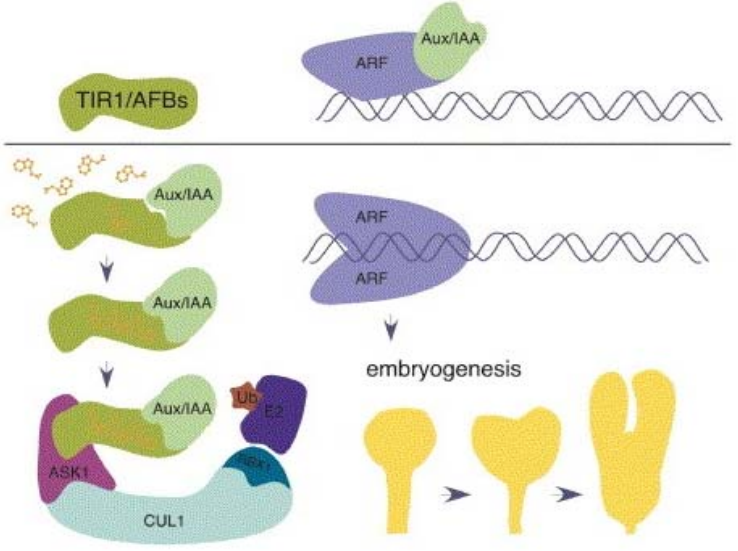


Figure 1. Auxin Signal Transduction Pathway

(Top panel) In plant cells exposed to little or no auxin, Aux/IAA transcriptional repressor proteins remain bound to the ARF (auxin response factor) transcription factor, and target genes of auxin remain switched off. (Bottom panel) When auxin (orange) binds to the TIR1 auxin receptor, TIR1 (or its family members, the AFBs) strongly interacts with Aux/IAA proteins. TIR1/AFBs are leucine-rich repeat F box proteins, which are part of an SCF-type E3 ubiquitin ligase, containing ASK1 (Arabidopsis SKP1-Like1), CUL1 (Cullin1), and RBX1 (RING-box protein1). Once assembled, this protein complex recruits an E2 ubiquitin-conjugating enzyme, and their combined action adds ubiquitin molecules (Ub) to the Aux/IAA proteins, which are subsequently degraded. When Aux/IAA proteins bind to auxin-modified TIR1/AFBs, the ARF transcription factor is no longer repressed, resulting in the expression of target genes required for *Arabidopsis* embryogenesis (yellow silhouettes).

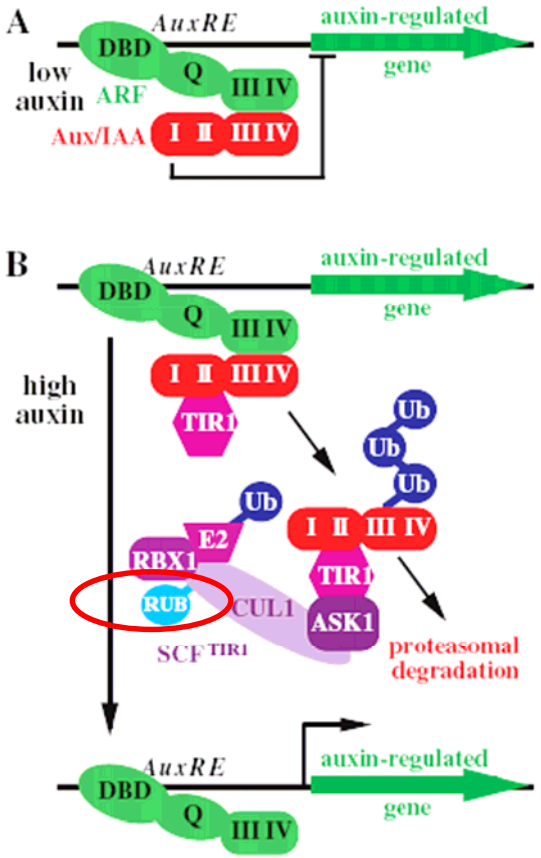
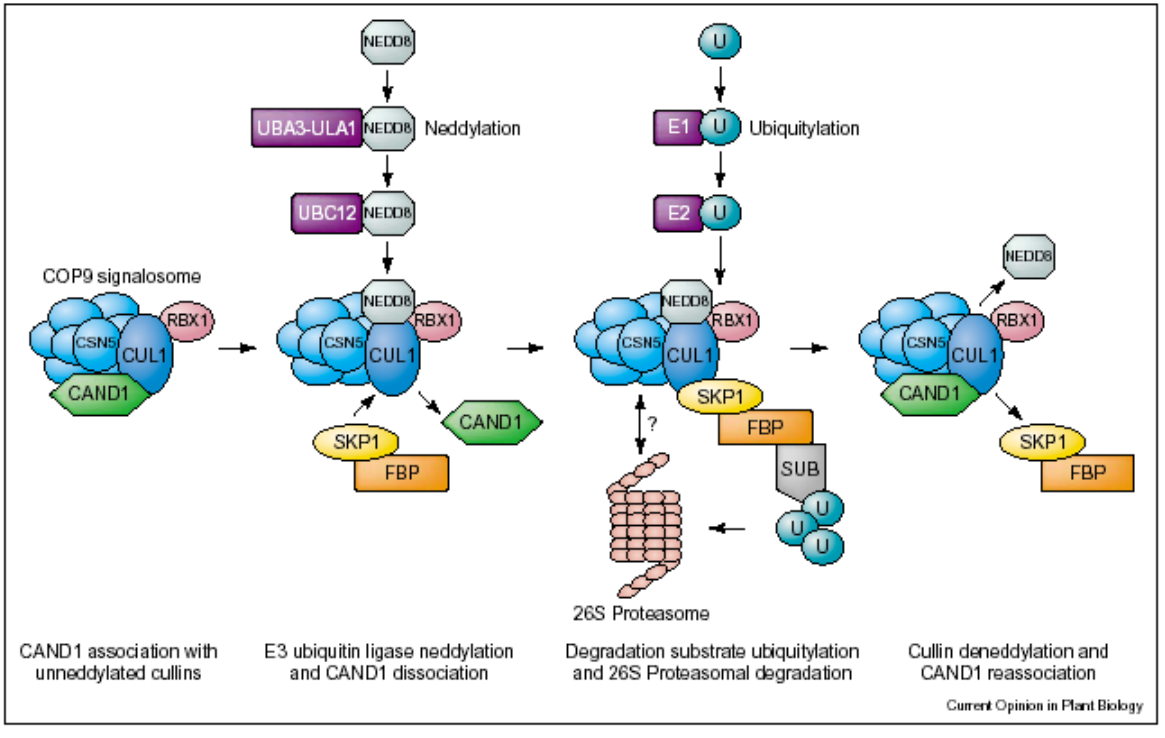


FIG. 4. The SCF^{TIR1} relieves Aux/IAA repression of activating ARFs. (A) An activating ARF protein (green) binds an *AuxRE* promoter element via an N-terminal DNA binding domain (DBD). Under low-auxin conditions, an Aux/IAA repressor (red) binds the activating ARF via heterodimerization between Aux/IAA 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.



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

TABLE 4. *Auxin-related SCF components and SCF-regulatory genes from arabidopsis*

Gene	Function	Loss-of-function phenotype*	Assay
TIR1	Auxin F-box	Auxin resistant[1]	Root elongation inhibition
		NPA, CPD resistant[1]	Root elongation inhibition
		Reduced lateral root number[1]	
		Hypocotyl elongation defect[1]	Growth at elevated temperature
		Enhances *axr1-12* dwarfism[2]	
		Enhances *cand1* dwarfism[2]	
CUL1/AXR6	SCF scaffold	Auxin resistant[3]	Root elongation inhibition
		Embryo lethal (null)[4]	
ASK	CUL1/F-box adapter	Auxin resistant (*ask1*)[5]	Root elongation inhibition
		Reduced lateral root number (*ask1*)[5]	
		Dwarf (*ask1*)[5]	
		Floral abnormalities (*ask1* and *ask11*)[6]	
		Embryo lethal (*ask1 ask2*)[6]	
RBX1	CUL1/E2 adapter	Auxin resistant[7]	Root elongation inhibition
		Reduced lateral root number[7]	
		Dwarfism[7]	
		MeJA resistant[7]	Root elongation inhibition
		Delayed cold-induced gene expression[7]	Northern blot
RUB1 RUB2	Ubiquitin-like modifier	Auxin resistant[8]	Root elongation inhibition
		Reduced lateral root number[8]	
		Dwarfism[8]	
		Ethylene overproduction[8]	Hypocotyl elongation in darkness, GC
		Embryo lethal (null)[8]	
AXR1	RUB activating enzyme component	Auxin resistant[9]	Root elongation inhibition
		Reduced gravitropism[10]	Root reorientation
		Dwarfism (severe alleles)[9]	
		MeJA resistant[11]	Root elongation inhibition
		ACC resistant[12,13]	Hypocotyl elongation inhibition in darkness
		Enhances *cop10-4* deetiolation[7]	Growth in darkness
		Floral abnormalities[14]	
		Delayed cold-induced gene expression[7]	Northern blot
ECR1	RUB activating enzyme component	Reduced auxin-induced gene expression[14]	Northern blot of wild-type plants transformed with a mutant version of ECR1
		Dwarfism[14]	
		Floral abnormalities[14]	
RCE1	RUB E2 enzyme	Auxin resistance[10]	Root elongation inhibition
		Reduced lateral root proliferation[10]	Lateral root induction by auxin
		Reduced gravitropism[10]	Root reorientation
		Dwarfism[10]	
		MeJA resistance[10]	Root elongation inhibition
		Ethylene overproduction[15]	Gas chromatography
		Reduced hypocotyl elongation in darkness[15]	
CSN5	COP9 signalosome component	Auxin resistance[7]	Root elongation inhibition
		Reduced lateral root number[7]	
		Dwarfism[7]	
		MeJA resistance[7]	Root elongation inhibition
		Delayed cold-induced gene expression[7]	Northern blot
CAND1/ETA2	SCF regulator	Auxin resistance[2, 13, 17]	Root elongation inhibition
		Reduced lateral root number[2]	
		Dwarfism[2]	
		MeJA resistance[17]	Root elongation inhibition
		ACC resistance[2]	Hypocotyl elongation inhibition in darkness
		Reduced apical hook[13]	Growth in darkness
		Floral abnormalities[17]	
		ABA resistance[13]	Root elongation inhibition
		Enhanced red light response[13]	Hypocotyl elongation inhibition
		Late flowering[17]	
SGT1b	SCF regulator	Auxin resistance[16]	Root elongation inhibition
		Reduced lateral root number[16]	
		MeJA resistance[16]	Root elongation inhibition

*Red, auxin-related phenotypes; blue, ethylene-related phenotypes; purple, jasmonate-related phenotypes; green, floral development phenotypes.
[1]Ruegger *et al.* (1998); [2]Chuang *et al.* (2004); [3]Hellmann *et al.* (2003); [4]Shen *et al.* (2002); [5]Gray *et al.* (1999); [6]Zhao *et al.* (2003*a*); [7]Schwechheimer *et al.* (2002); [8]Bostick *et al.* (2004); [9]Lincoln *et al.* (1990); [10]Dharmasiri *et al.* (2003*b*); [11]Tiryaki and Staswick (2002); [12]Xu *et al.* (2002); [13]Cheng *et al.* (2004); [14]del Pozo *et al.* (2002); [15]Larsen and Cancel (2004); [16]Gray *et al.* (2003); [17]Feng *et al.* (2004).