Rostlinné "hormony"

- Auxins
- Gibberellins
- Cytokinins
- Abscisic acid
- Ethylene
- Brassinosteroids
- JA, SA…. nové ...př. 2008 objevený Strigolacton

TABLE 12.1 Plant Hormones: Structure and Effects

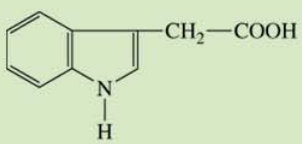
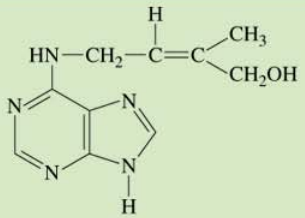
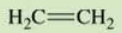
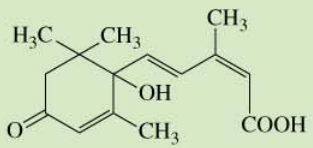
Plant Hormones	Chemical Structure	Functions
auxins	 indoleacetic acid (IAA)	Apical bud dominance (retards growth of lateral buds immediately below); mediate growth response to light direction; induce development of vascular tissue; promote activity of secondary meristems; induce formation of roots on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

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TABLE 12.1 *continued*

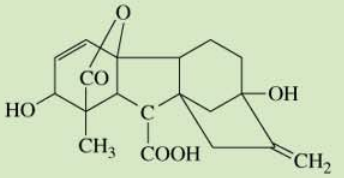
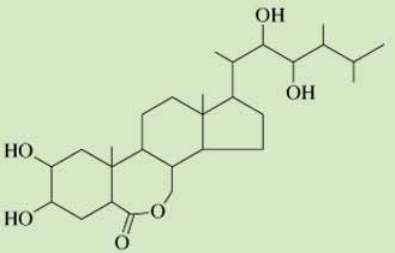
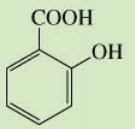
gibberellins	 gibberellic acid (GA)	Stimulate both cell division and cell enlargement during shoot elongation; promote seed germination; stimulate flowering in some plants
brassinosteroids	 brassinolide	Stimulate shoot elongation; reduce plant stress caused by heat, cold, drought, salt, and herbicide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

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

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TABLE 12.1 *continued*

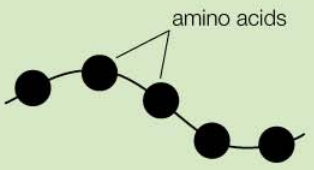
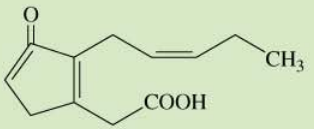
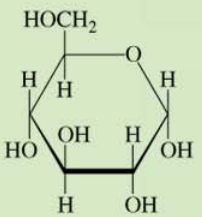
Plant Hormones	Chemical Structure	Functions
systemin	 systemin	Signals that wounding has occurred
jasmonic acid	 jasmonic acid	Helps plants resist fungal infection and other stresses; induces plant production of protective secondary compounds (alkaloids)
sugars	 glucose	Helps regulate amounts of chlorophyll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

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

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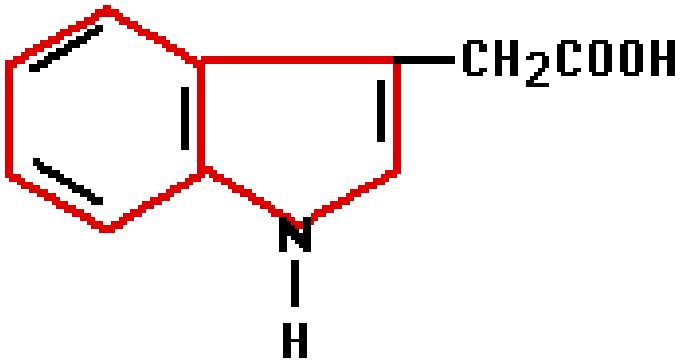
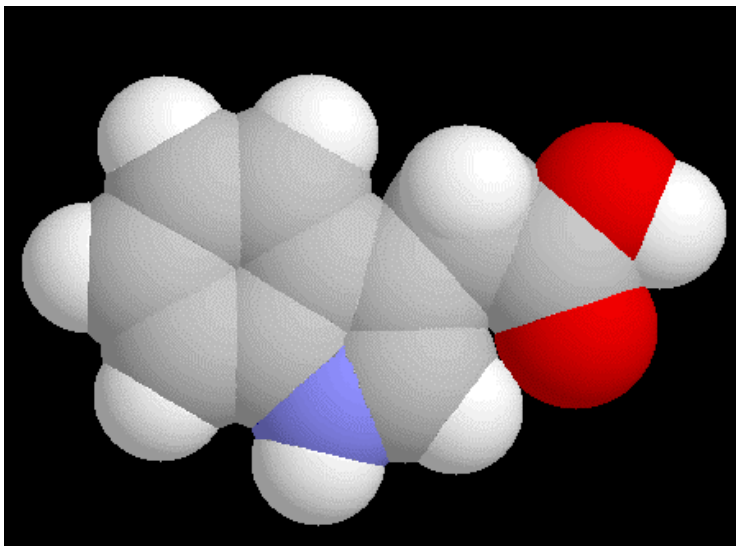
Auxin DOMINANTNÍ MORFOGEN



Indole-3-acetic acid (IAA)



ABP1

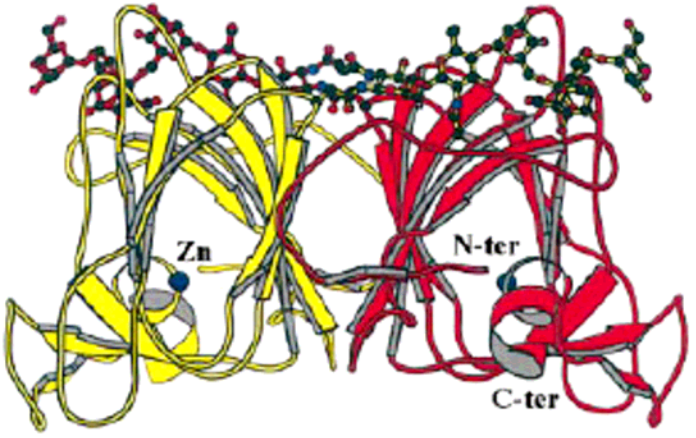


FIG. 4. Ribbon diagram showing the structure of an ABP1 dimer. The β-sheets are shown as broad arrows. ABP1 is *N*-glycosylated and some of the sugar residues are shown at the top of each monomer. Three C-terminal residues were not resolved and would extend the α-helices at the foot of the molecules.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

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

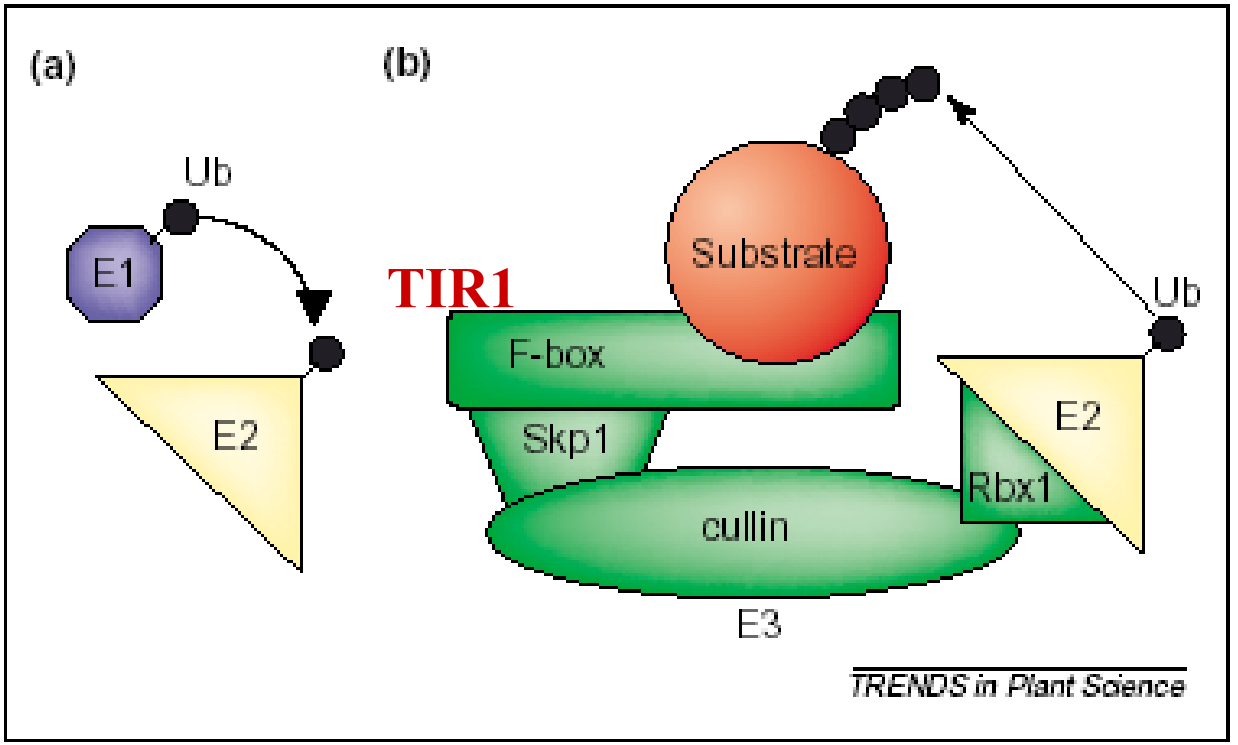


Figure 1. Key steps in the pathway of polyubiquitylation by SCF E3 ligase, which targets substrate protein and leads to degradation by the 26S proteasome. **(a)** Ubiquitin (Ub) is linked via a thioester bond to the ubiquitin-activating enzyme (E1). Ubiquitin is transferred from E1 to the cysteine of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

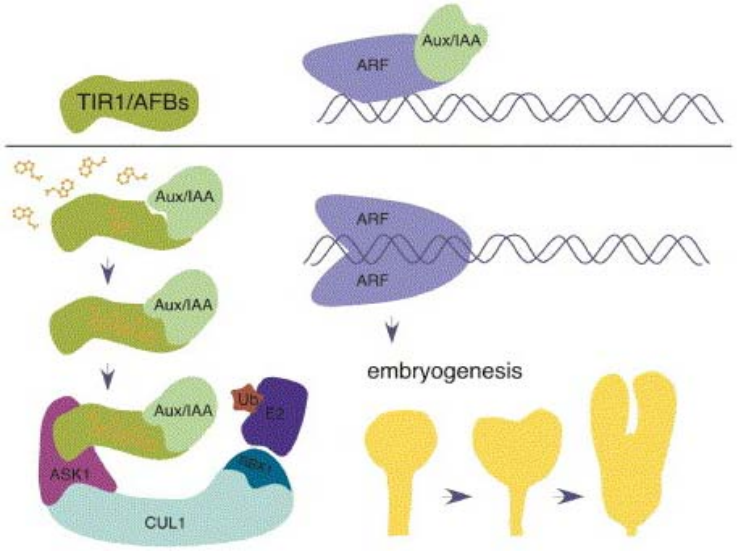


Figure 1. Auxin Signal Transduction Pathway

(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).

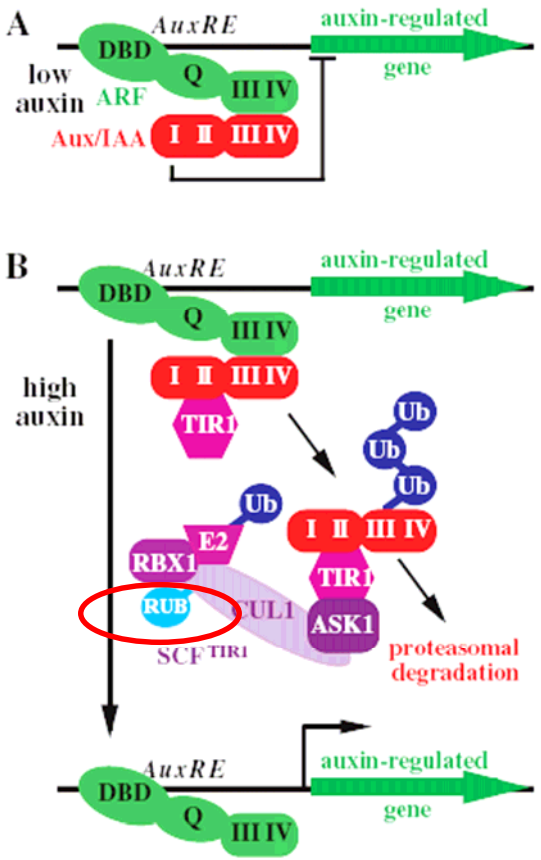
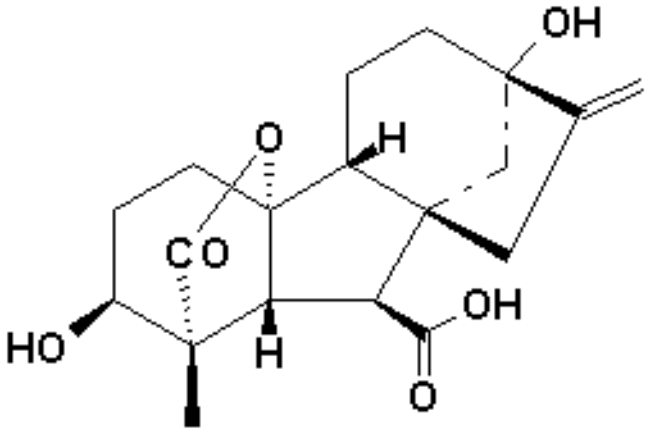


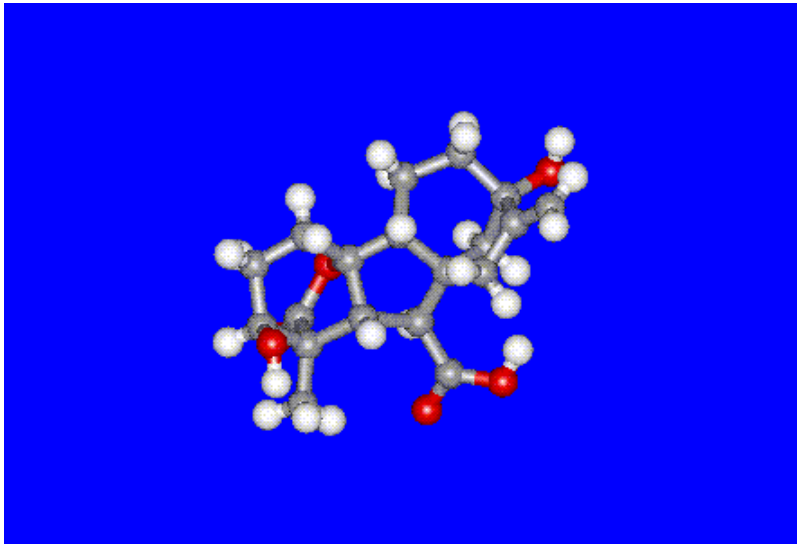
FIG. 4. The SCF^{TIR1} relieves Aux/IAA repression of activating ARFs. (A) An activating ARF protein (green) binds an *AuxRE* promoter element via an N-terminal DNA binding domain (DBD). Under low-auxin conditions, an Aux/IAA repressor (red) binds the activating ARF via heterodimerization between Aux/IAA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

Aktivita SCF/TIR1

je regulována také
signalosomem/CSN

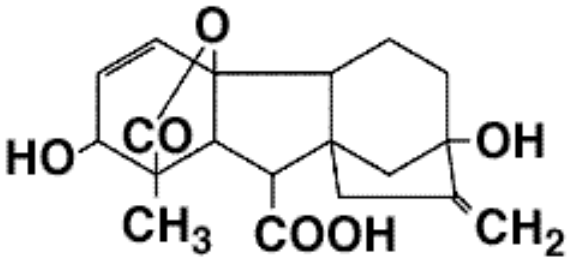
Gibberelliny -
desítky příbuzných různě aktivních molekul



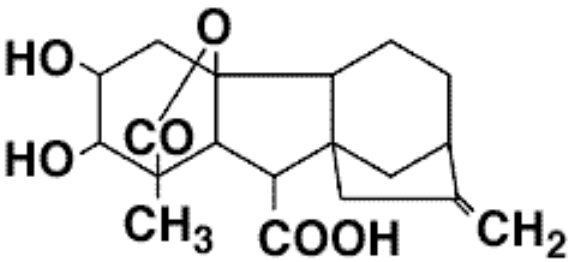


Randy Moore, Dennis Clark, Darrel Vodopich, Botany Visual Resource Library © 1998 The McGraw-Hill Companies,Inc. All rights reserved.

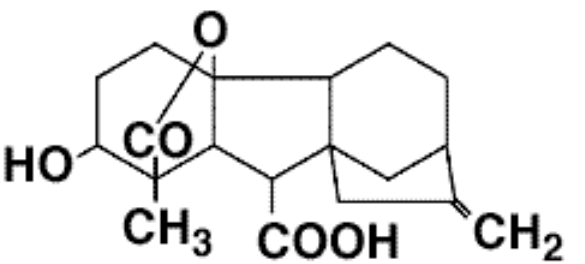
Three of the More Than Eighty Gibberellins



Gibberellic acid (GA_3)



GA_{13}



GA_4

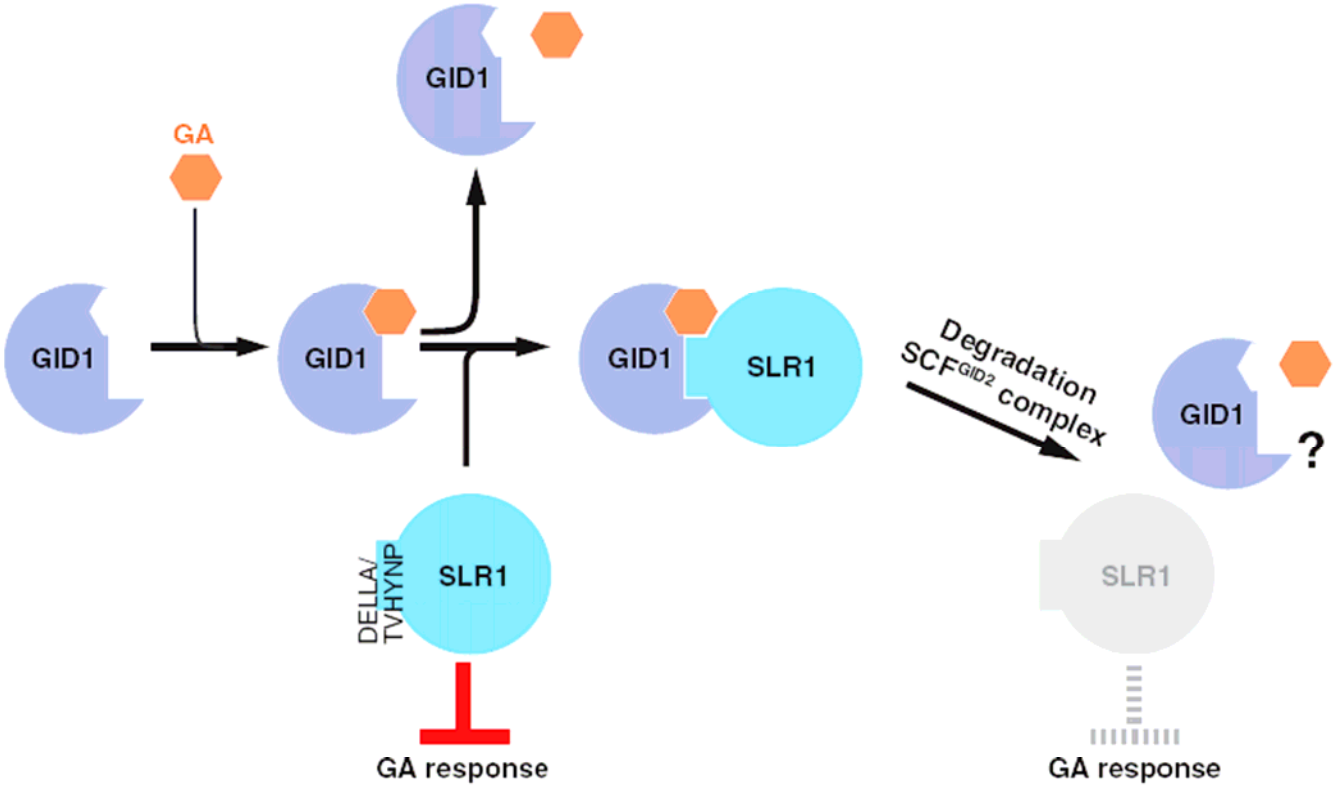


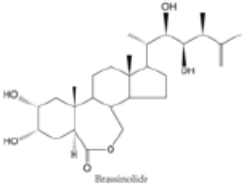
Figure 1

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.

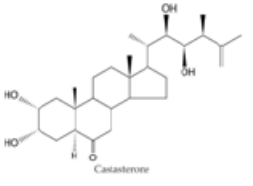
Brassinosteroidy

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

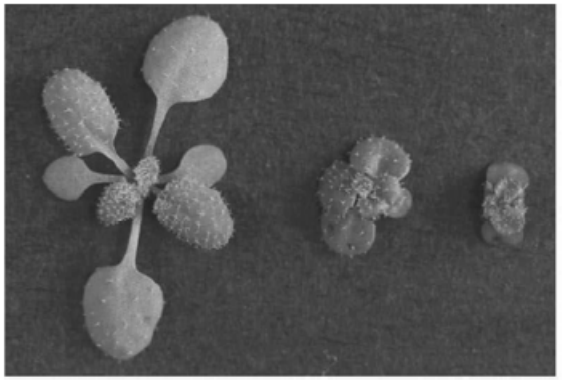


Figure 1. Comparison of *brassinosteroid-insensitive 1* (*bri1*) and wild-type *Arabidopsis thaliana* phenotypes.

The two plants shown are both light-grown and are of the same age: the *bri1* mutant on the left shows characteristic extreme dwarfism and dark-green curled leaves; *bri1* mutants also exhibit delayed senescence and flowering, and are male sterile.

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

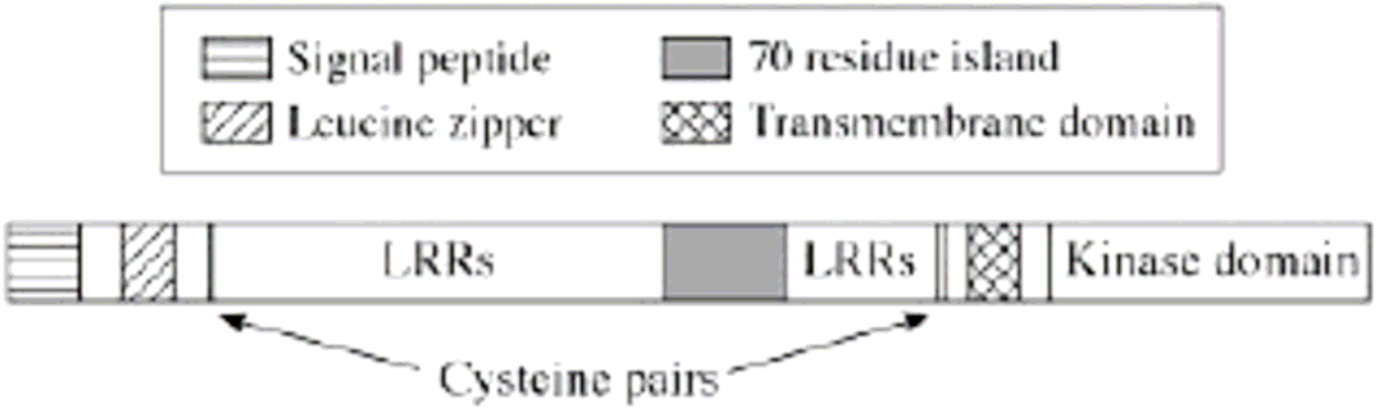
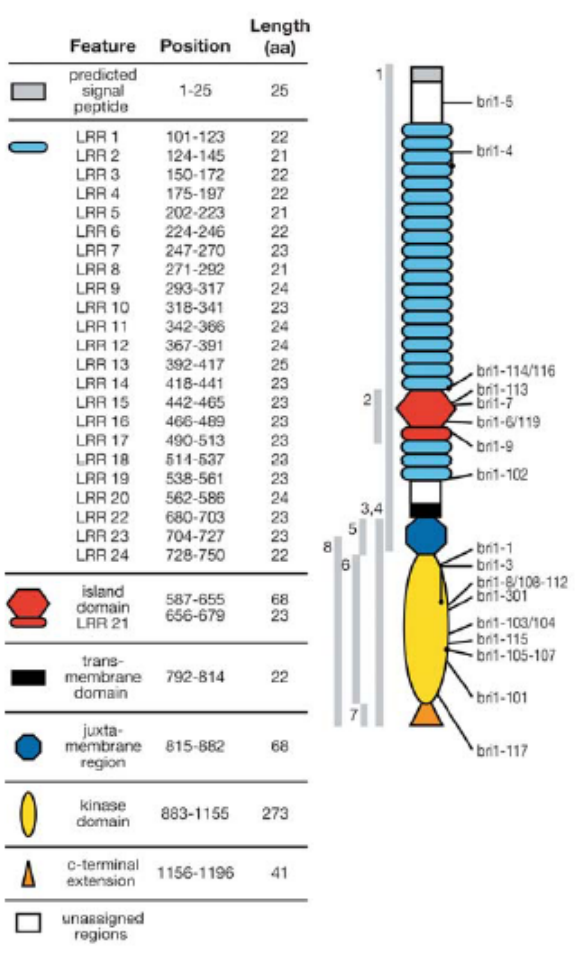


FIG. 3. Representation of BRI1.



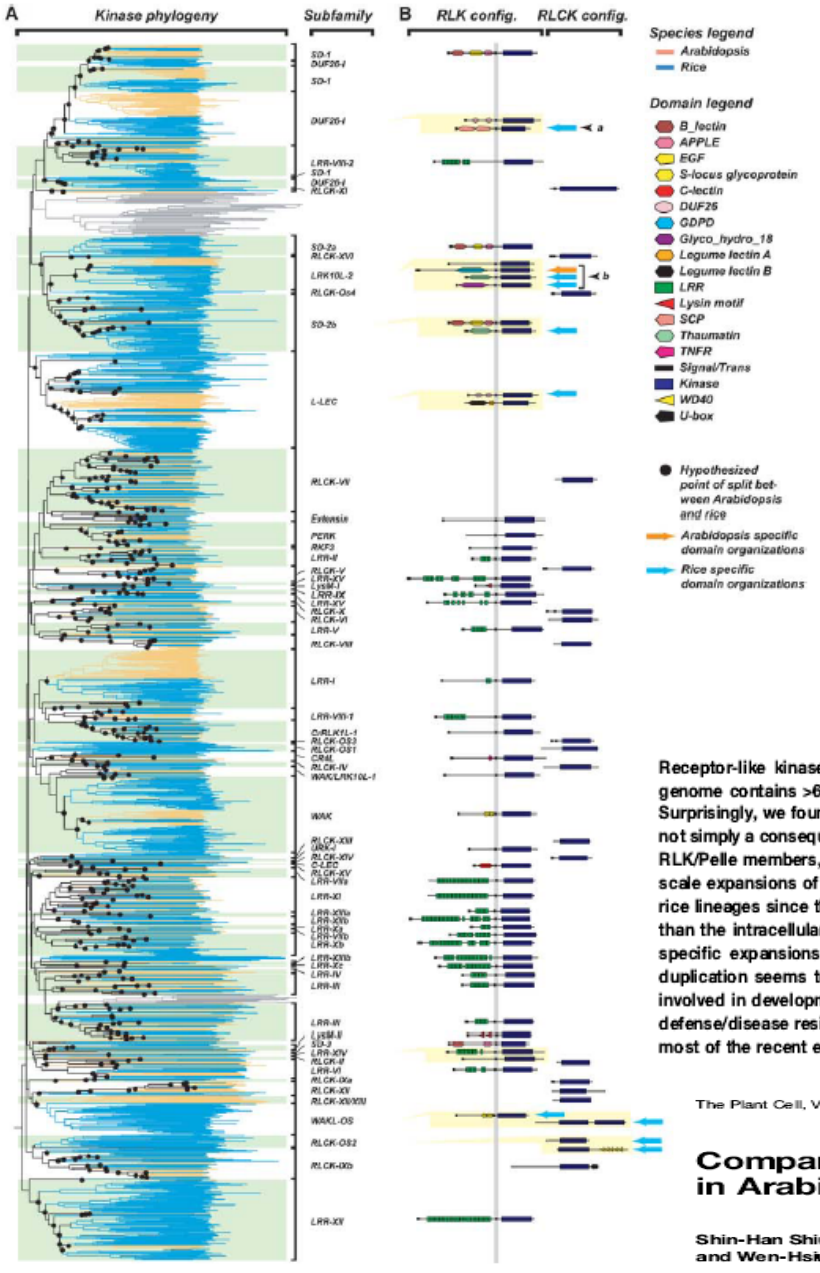
Feature	Position	Length (aa)
predicted signal peptide	1-25	25
LRR 1	101-123	22
LRR 2	124-145	21
LRR 3	150-172	22
LRR 4	175-197	22
LRR 5	202-223	21
LRR 6	224-246	22
LRR 7	247-270	23
LRR 8	271-292	21
LRR 9	293-317	24
LRR 10	318-341	23
LRR 11	342-366	24
LRR 12	367-391	24
LRR 13	392-417	25
LRR 14	418-441	23
LRR 15	442-465	23
LRR 16	466-489	23
LRR 17	490-513	23
LRR 18	514-537	23
LRR 19	538-561	23
LRR 20	562-586	24
LRR 22	680-703	23
LRR 23	704-727	23
LRR 24	728-750	22
island domain LRR 21	587-655 656-679	68 23
trans-membrane domain	792-814	22
juxta-membrane region	815-882	68
kinase domain	883-1155	273
c-terminal extension	1156-1196	41
unassigned regions		

Allele	Base pair change	Amino acid change	Allelic strength	Accession
bri1-1	GCT to ACT	Ala-909 to Thr	strong	Col-0
bri1-3	4-bp deletion	STOP 44 aa downstream	strong	Ws-2
bri1-4	10-bp deletion	STOP 13 aa downstream	strong	Ws-2
bri1-5	TGC to TAC	Cys-69 to Tyr	weak	Ws-2
bri1-6, 119	GGC to GAT	Gly-644 to Asp	weak	En-2
bri1-7	GGT to AGT	Gly-613 to Ser	weak	Ws-2
bri1-9	CTT to TTT	Ser-662 to Phe	weak	Ws-2
bri1-101	GAG to AAG	Glu-1078 to Lys	strong	Col-0
bri1-102	ACT to ATT	Thr-750 to Ile	strong	Col-0
bri1-103, 104	GCG to ACG	Ala-1031 to Thr	strong	Col-0
bri1-105-107	CAA to TAA	Gln-1059 to STOP	strong	Col-0
bri1-8/108-112	CGG to CAG	Arg-983 to Gln	inter-mediate	Ws-2
bri1-113	GGA to GAA	Gly-611 to Glu	strong	Col-0
bri1-114,116	CAA to TAA	Gln-583 to STOP	strong	Col-0
bri1-115	GGT to GAT	Gly-1048 to Asp	strong	Col-0
bri1-117,118	GAT to AAT	Asp-1139 to Asn	strong	Col-0
bri1-301	not published	Gly-989 to Ile	weak	Col-0

Figure 1

(*Continued on next page*)

BRI1 je příkladem důkladně "promutovaného" lokusu. Proto jsou dobře funkčně popsány subdomény i některé jednotlivé AA.



Arabidopsis má více než **600**

RLKs a **rýže** přes **1000**.

Multiplikace RLKs je pravděpodobně spojena mj. 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 Family in Arabidopsis and Rice

Shin-Han Shiu,[a] Wojciech M. Karlowski,[b] Runsun Pan,[a,d] Yun-Huei Tzeng,[a,c] Klaus F. X. Mayer,[b] and Wen-Hsiung Li[a,1]

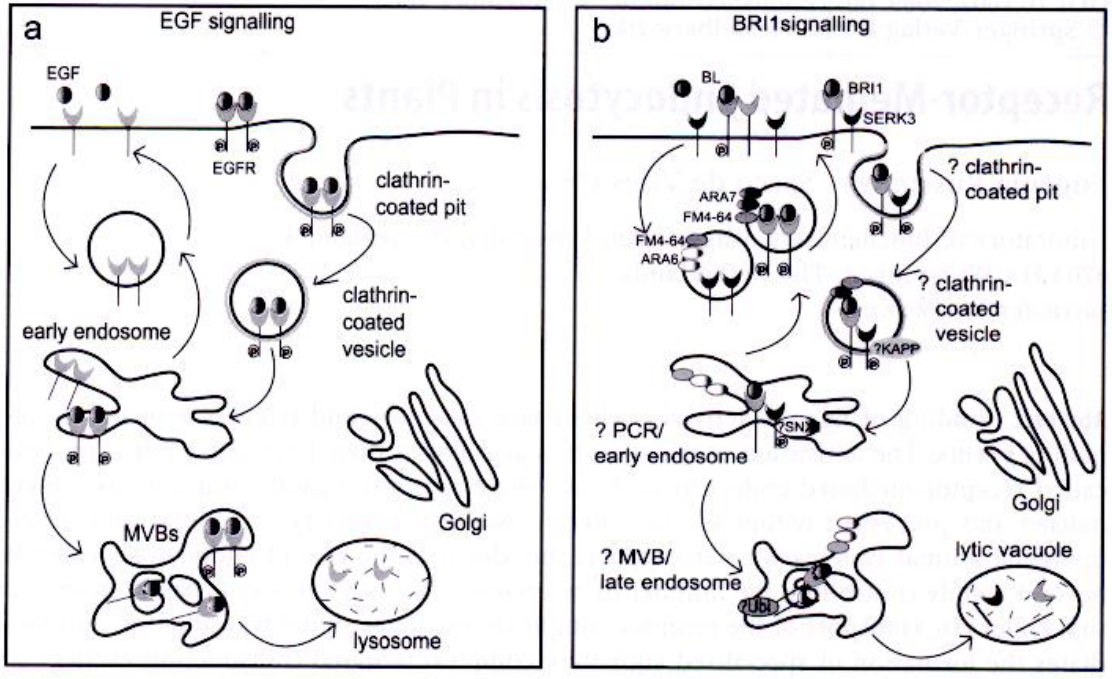
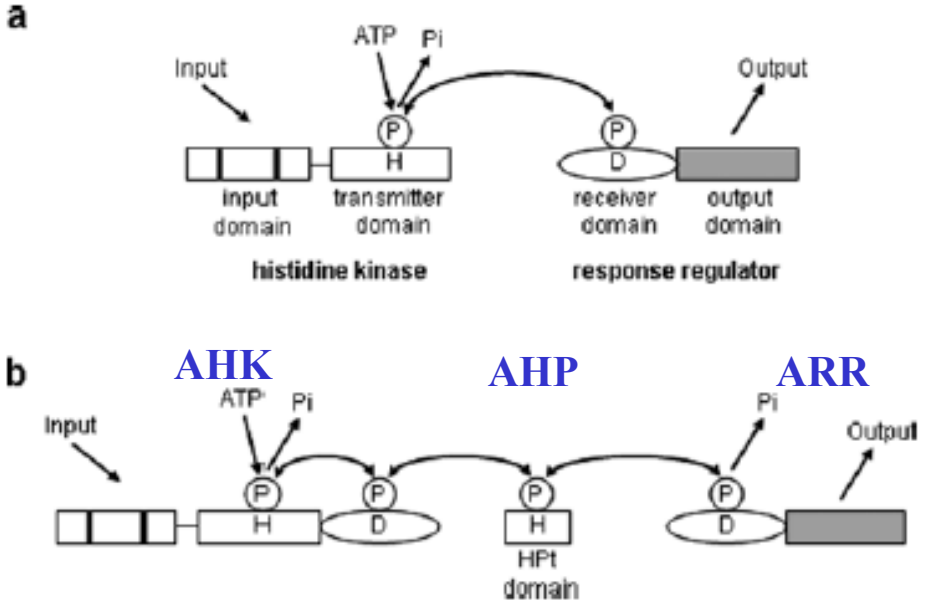


Fig. 1 A comparison of receptor-mediated endocytosis in animal and plant cells. **a** Epidermal growth factor (EGF) receptor is activated by the EGF and endocytosed mainly through clathrin-coated pits. The activated receptor accumulates in early endosomes and multivesicular bodies (MVBs). Ligand-free receptors are almost exclusively recycled to the cell surface. Ligand-bound receptors are sorted to lysosomes for degradation with an increased efficiency compared with that of the ligand-free receptors. EGF receptor remains active in early endosomes and in MVBs, indicated by the presence of phosphate groups. **b** Hypothetical model showing that brassinosteroid receptor complex including BRI1 and AtSERK3 is internalised in FM4-64 positive compartments that are colabelled with Rab5 plant homologues represented by ARA6 or ARA7. Homodimeric combinations of BRI1 and AtSERK3 are internalised and cycle back to the plasma membrane. Heterodimeric combination of BRI1 and AtSERK3 is preferentially internalised for degradation. We propose a general mechanism for degradation of the internalised receptors retained in the early endosomes and MVB compartments that involve KAPP dephosphorylation followed by ubiquitination.

Signální endosom v BR dráze.

Internalizovaný
Aktivní
Receptor
V endosomu
Je aktivní
Dokud není
Defosforylován.
SERK3=BAK1

Eukaryotické dvoukomponentní signální moduly v recepci a přenosu signálů cytokininů a ethylénu.



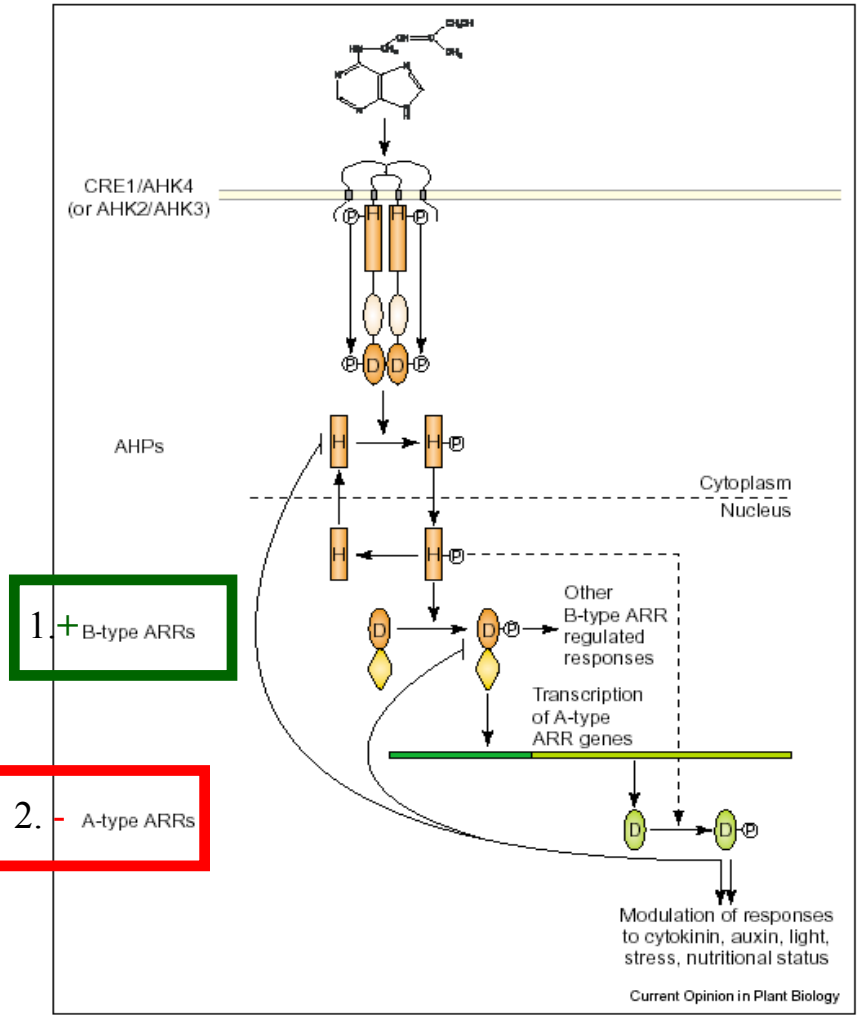
Hlavně prokaryota

Eukaryota

Fig. 1a, b Basic features of the simple (**a**) and the multistep (**b**) two-component signalling system. Signal perception by the histidine kinase induces autophosphorylation of the transmitter domain at a conserved histidine residue (*H*). The phosphoryl group (*P*) is relayed to a conserved Asp residue (*D*) that is localized either in the receiver domain of the cognate response regulator (**a**) or, in the case of hybrid histidine kinases, in an attached receiver domain (**b**). In the multistep two-component signalling system, histidine-containing phosphotransfer (*HPt*) domain proteins function as phosphor-histidine intermediates between the hybrid histidine kinase and the response regulator (**b**). *Black vertical bars* Transmembrane domain (adapted from Kakimoto 2003)

Klíčovou roli hraje "průtok" fosforylace.

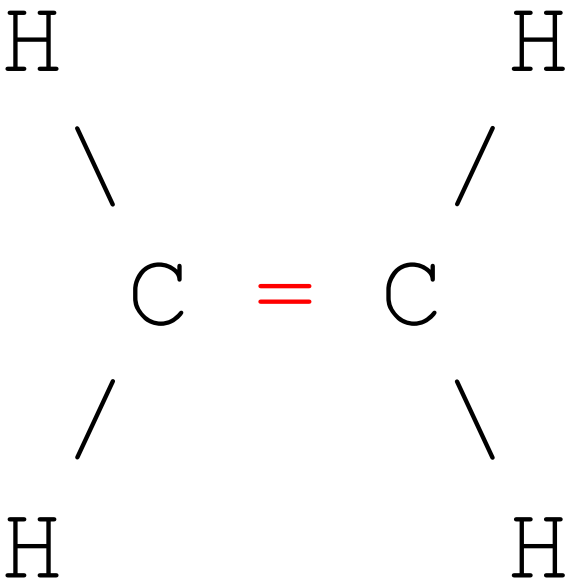
Histidin ⟶ Aspartát a příp. ještě jednou…



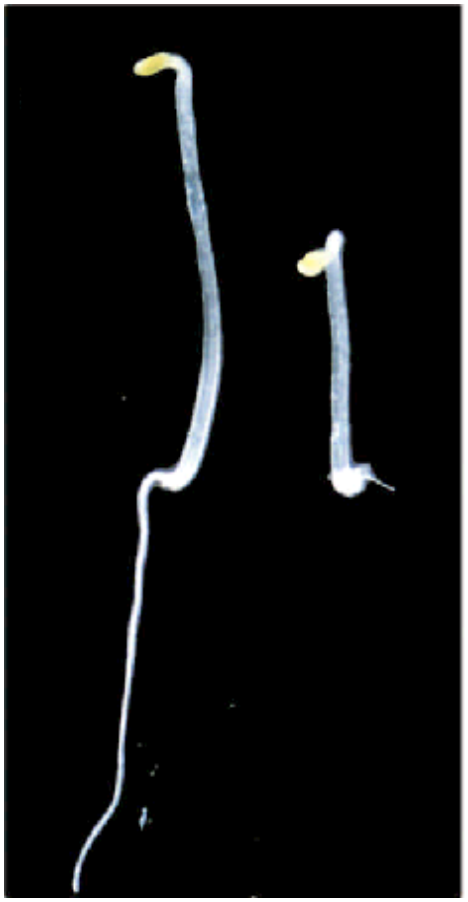
A model for cytokinin signal transduction via a His-to-Asp phosphorelay. The structure of CRE1/AHK4 is shown as an example. Ligand binding induces receptor dimerisation and autophosphorylation. Transfer of the phosphoryl group by activated receptors activates AHPs which transport the signal from the cytoplasm to type-B ARRs in the nucleus. Type-B response regulators transcribe target genes, among them type-A *ARR* genes. Type-A response regulators may downregulate the primary cytokinin signal response via a negative feedback loop, modulate downstream activities of cytokinins in a positive or negative fashion or modulate other signalling pathways through protein–protein interaction. A more complex regulation than shown in the model may exist. Abbreviations: D, aspartate residue, H, histidine residue, P, phosphoryl group.

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

Ethylen



Triple response of etiolated seedlings with ethylene



- short hypocotyl
- thick radial growth
- apical hook formation

Receptory etylénu jsou lokalizovány na ER.

a interakcí se tam lokalizuje také CTR1

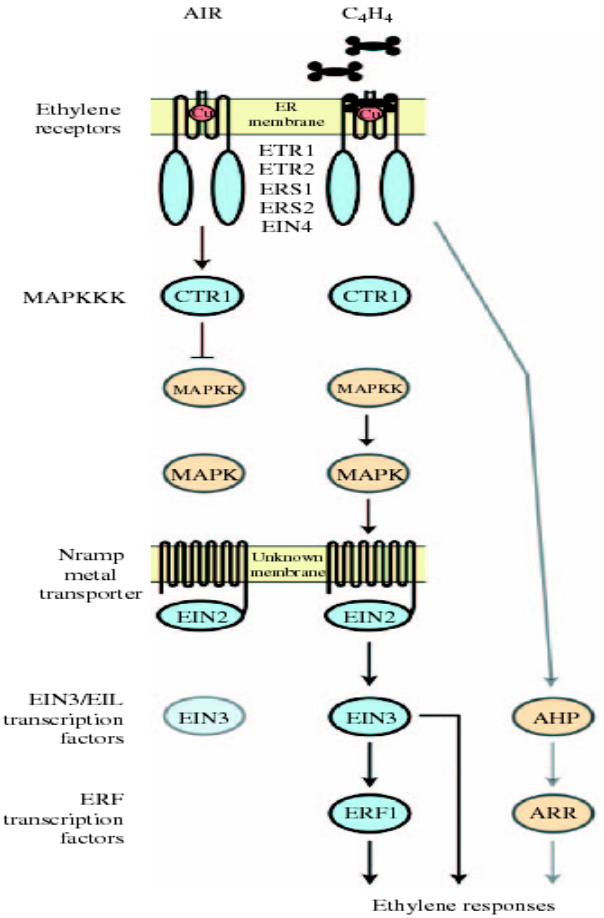
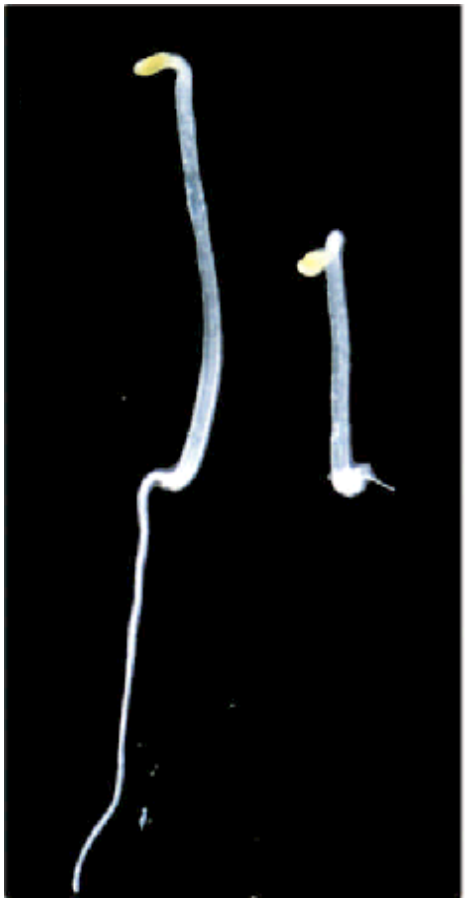
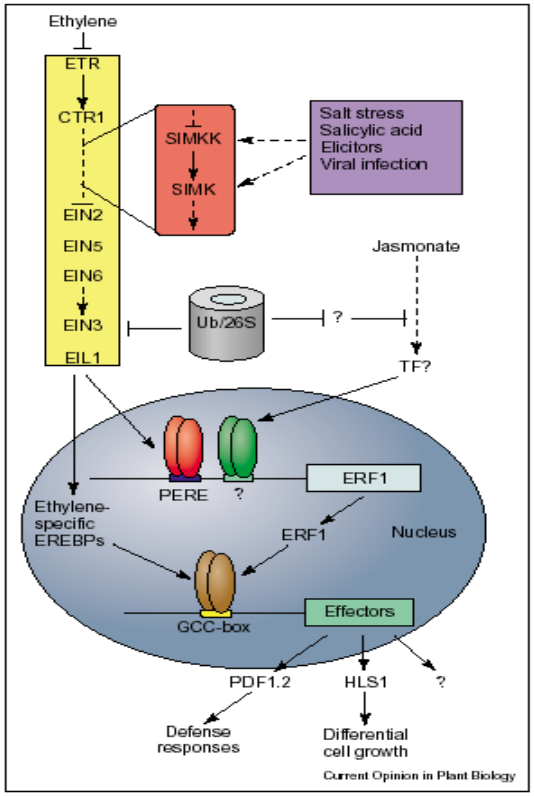


FIG. 2. Model for ethylene signal transduction that incorporates biochemical features of the pathway components. Soluble protein domains are shown as circles and predicting transmembrane structures are shown as lines. Confirmed components of the pathway are shown in blue; more recently proposed components are shown in orange. In air, ethylene receptors maintain CTR1 in an active state that serves to repress ethylene responses. In ethylene, the repression is relieved. Binding of ethylene inactivates the receptors, thereby inactivating CTR1. As a result, EIN2 is activated and a transcriptional cascade involving the EIN3/EIL and ERF transcription factors is initiated. Both families of transcription factors are involved in regulating ethylene responses. The protein level of EIN3 is lower in the absence of ethylene than in the presence of ethylene, due to degradation by the ubiquitin-proteasome pathway. The figure also incorporates components about which conflicting data has been reported, namely a MAPK module operating downstream of CTR1, and a two-component signalling pathway (AHP and ARR) functioning independently of the CTR1-mediated pathway.

Triple response of etiolated seedlings with ethylene



- short hypocotyl
- thick radial growth
- apical hook formation



A model for the ethylene response pathway in the regulation of gene expression. Ethylene gas is perceived by a family of ER-associated receptors (ETR). Ethylene binding is proposed to inhibit receptor function. CTR1 is proposed to be activated by the unoccupied receptors via physical interaction with them, and is inhibited upon binding of ethylene by the receptor. A MAPK module, consisting of SIMKK and SIMK, is proposed to act downstream of CTR1, although the biochemical consequence of this MAPK pathway is not evident. Because many biotic and abiotic stimuli activate the SIMKK/SIMK pathway, it remains to be determined whether their activation is dependent upon the functions of the ethylene receptors and CTR1. Downstream components in the ethylene pathway include several positive regulators (EIN2, EIN5, EIN6 and the transcription factors EIN3 and EIL1). The level of EIN3 protein is controlled by ethylene, possibly via the proteasome (Ub/26S). The primary ethylene signaling pathway components (indicated in yellow) are required for all known ethylene responses and, to date, none have been found to respond to signals other than ethylene. Branch points in the ethylene response pathway may lie downstream of EIN3/EIL1. Several EREBP transcription

I v signalizaci etylénu hraje roli reg. degradace bílkovin (TF EIN3).

STRIGOLACTON

- Derivát karotenoidů
- **INHIBUJE VÝVOJ POSTRANNÍCH VÝHONŮ** – SPOLU S IAA SE ÚČASTNÍ REGULACE APIKÁLNÍ DOMINANCE.

ABA

Existují pravděpodobně dva typy receptorů pro ABA - membránový a cytoplasmatický.

Membránový GCR2 není receptorem, ale α podjednotka **G**-prot. se účastní na ABA signalizaci.

Cytoplasmatický - **H podjednotka Mg-chelatázy**

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nature

ARTICLES

The Mg-chelatase H subunit is an abscisic acid receptor

Yuan-Yue Shen[1]*, Xiao-Fang Wang[1]*, Fu-Qing Wu[1]*, Shu-Yuan Du[1], Zheng Cao[1], Yi Shang[1], Xiu-Ling Wang[1], Chang-Cao Peng[1], Xiang-Chun Yu[1], Sai-Yong Zhu[1], Ren-Chun Fan[1], Yan-Hong Xu[1] & Da-Peng Zhang[1]

Abscisic acid (ABA) is a vital phytohormone that regulates mainly stomatal aperture and seed development, but ABA receptors involved in these processes have yet to be determined. We previously identified from broad bean an ABA-binding protein (ABAR) potentially involved in stomatal signalling, the gene for which encodes the H subunit of Mg-chelatase (CHLH), which is a key component in both chlorophyll biosynthesis and plastid-to-nucleus signalling. Here we show that *Arabidopsis* ABAR/CHLH specifically binds ABA, and mediates ABA signalling as a positive regulator in seed germination, post-germination growth and stomatal movement, showing that ABAR/CHLH is an ABA receptor. We show also that ABAR/CHLH is a ubiquitous protein expressed in both green and non-green tissues, indicating that it might be able to perceive the ABA signal at the whole-plant level.

TETRAPYROLOVÁ DRÁHA

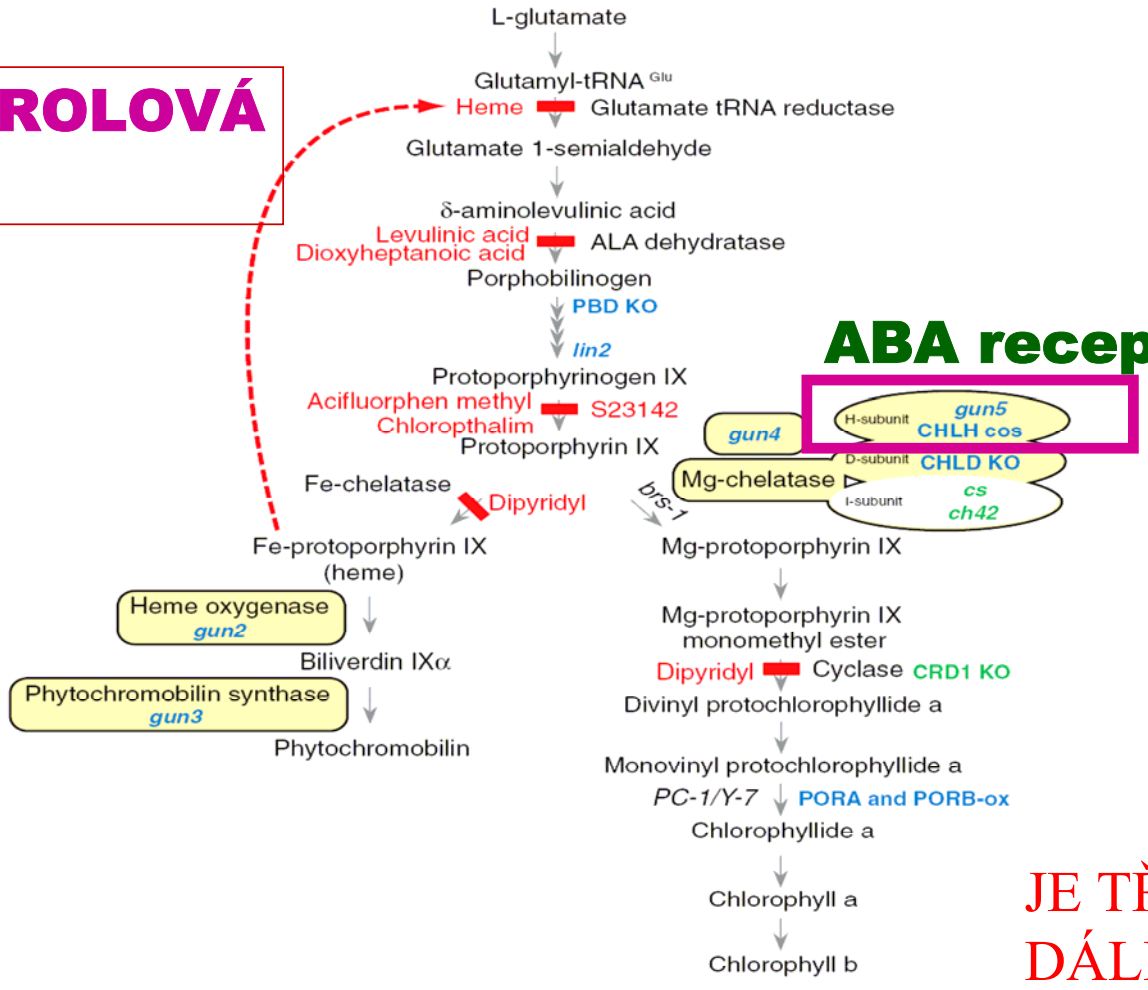


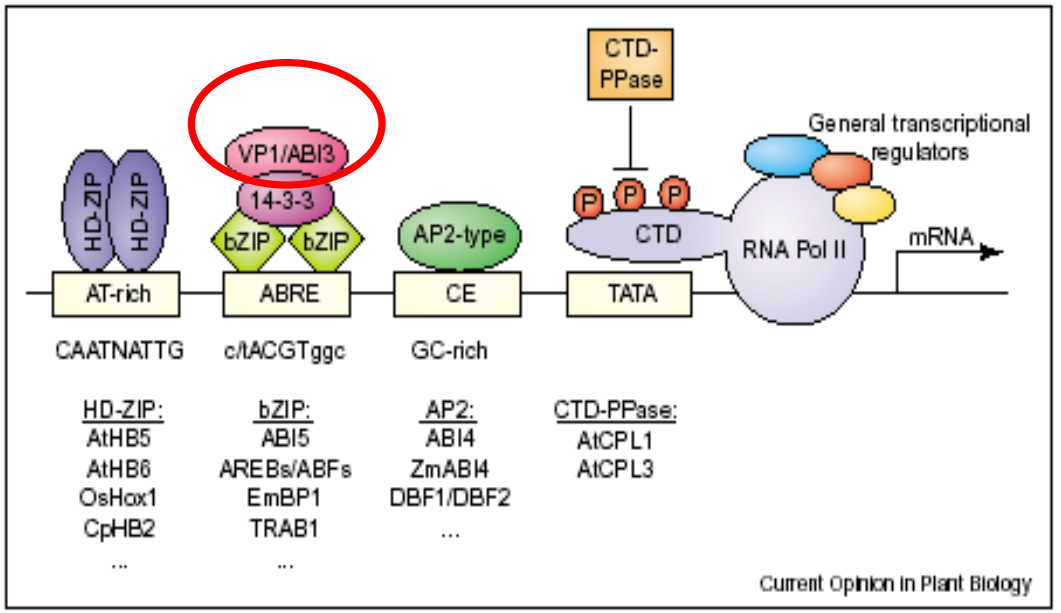
Figure 1

Tetrapyrrole biosynthetic pathway. Steps inhibited by specific inhibitors are indicated in red. Mutants with a *gun* phenotype are shown in blue, and mutants that do not show a *gun* phenotype are indicated in green. *brs-1* and *PC-1/Y-7* are *C. reinhardtii* mutants. PBD KO, T-DNA knockout of porphobilinogen deaminase; *lin2*, lesion in coproporphyrinogen oxidase; CHLD KO, T-DNA knockout of D-subunit of Mg-Chelatase; CHLH cos, cosuppression lines for H-subunit of Mg-Chelatase; CRD KO, T-DNA knockout of one subunit of the cyclase complex. *cs* and *ch42* are alleles of the I-subunit of Mg-Chelatase. PORA-ox and PORB-ox indicate overexpression of Protochlorophyllide oxidoreductase A and B, respectively.

JE TŘEBA DÁLE OVĚŘIT!?

REGULACE DORMANCE

Viviparní kukuřice **VP1**



Major *cis*-regulatory elements and transcription factors of ABA-regulated gene expression. The transcriptional complex consists of RNA-polymerase II (RNA Pol II) in association with general transcriptional regulators including the TATA-box binding protein. The transcriptional machinery is controlled by the binding of regulatory TFs to promoter-specific *cis* elements. The ABA-regulatory element (ABRE) is contacted by dimeric bZIP TFs, such as ABI5 of *Arabidopsis*, which associate with the transcriptional regulators ABI3 (maize VIVIPAROUS1 [VP1]) and 14-3-3 protein. The coupling element (CE) is targeted by AP2-type TFs, such as ABI4, which enhance ABA-regulated transcription. The HD-ZIP proteins AtHB5 and AtHB6 of *Arabidopsis* bind to AT-rich pseudopalindromic sequences. RNA Pol II is regulated by the phosphorylation of its carboxy-terminal domain (CTD). Dephosphorylation by a CTD-specific protein phosphatase (CTD-PPase), such as AtCLP3 of *Arabidopsis*, downregulates the expression of ABA-inducible genes.

INTEGRACE SIGNÁLU NA PROMOTORU

poznámka: ***ABA a cukry interagují***

Sugar Signalling

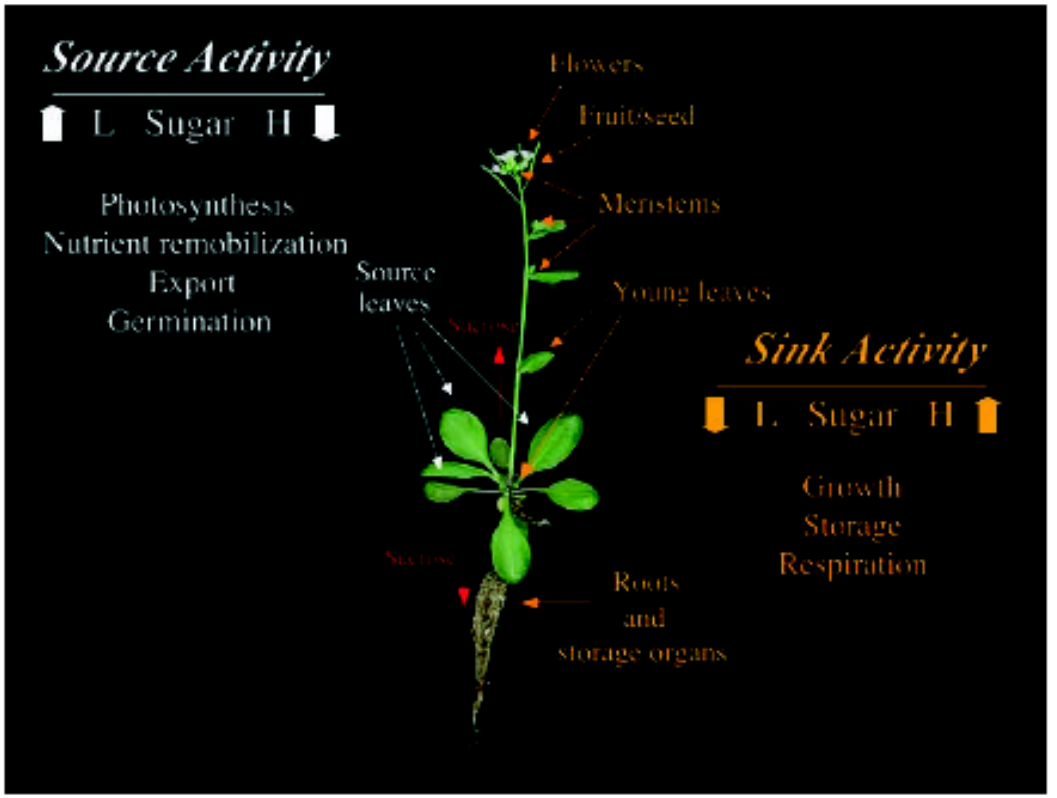


Figure 1. Differential Effects of Sugars on Plant Source and Sink Activities.

Suc is transported from photosynthesizing source leaves to sink organs such as roots, meristems, young leaves, flowers, fruit, and developing seed. Lowered (L) sugar levels can increase source activities, including photosynthesis, nutrient mobilization, and export. In contrast, higher (H) sugar levels in sink tissues stimulate growth and storage. Accumulation of higher (H) sugar levels in source tissues, however, is believed to downregulate photosynthesis, ensuring the maintenance of sugar homeostasis. The differential source-sink effects allow the adaptation of carbon metabolism to changing environmental conditions and to the availability of other nutrients.

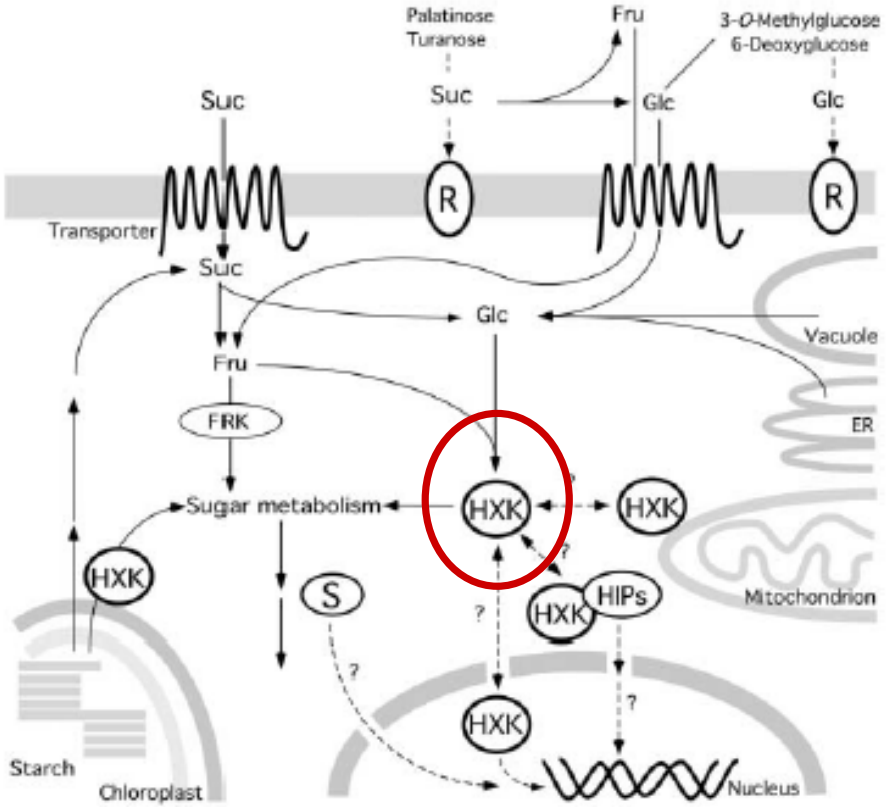


Figure 2. Possible Sugar Signals and Sensing Sites in Plant Cells.

Glc (and Fru) can be transported into the cell by hexose transporters or mobilized from cytosolic and vacuolar Suc and plastid starch. Glc then enters metabolism after HXK-catalyzed phosphorylation. The HXK sugar sensor, as a cytosolic protein or associated with mitochondria or other organelles (see text), then could activate a signaling cascade through HXK-interacting proteins (HIPs) or affect transcription directly after nuclear translocation. Possibly, different HXK (and fructokinase [FRK]) isoforms and HXK-like proteins have distinct metabolic and signaling functions. Metabolic intermediates could trigger signal transduction by activating metabolite sensors (S). Negative regulation of SnRK activity by Glc-6-phosphate, for example, suggests that SnRKs might act as sensors of metabolic activity. Finally, sugars, including Suc and hexoses and non-metabolizable sugars and sugar analogs, also could be sensed at the plasma membrane by sugar transporters or transporter-like proteins or by specific sugar receptors (R). Solid lines represent transport and enzymatic reactions involved in sugar sensing and signaling, and dashed lines represent putative interactions and translocations. ER, endoplasmic reticulum.

Hexokináza (HXK volná či vázaná) katalyzuje aktivační fosforylaci glukosy, která tak vstupuje do metabolismu.
HXK funguje jako SENZOR průtoku glukosy - sama vstupuje do jádra či prostřednictvím interagujících bílkovin (HIPs) ovlivňuje genovou expresi.

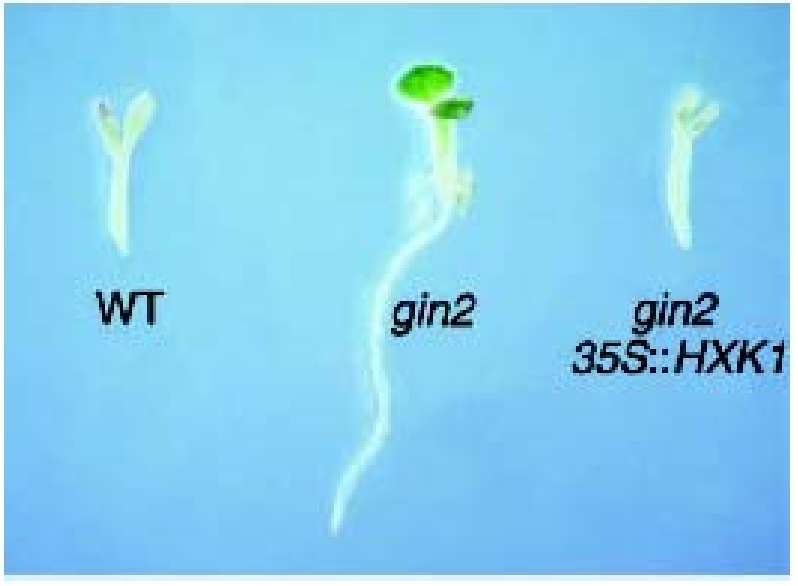


Figure 3. *gin2* Mutant Phenotype and Complementation by *35S*::*AtHXK1*.

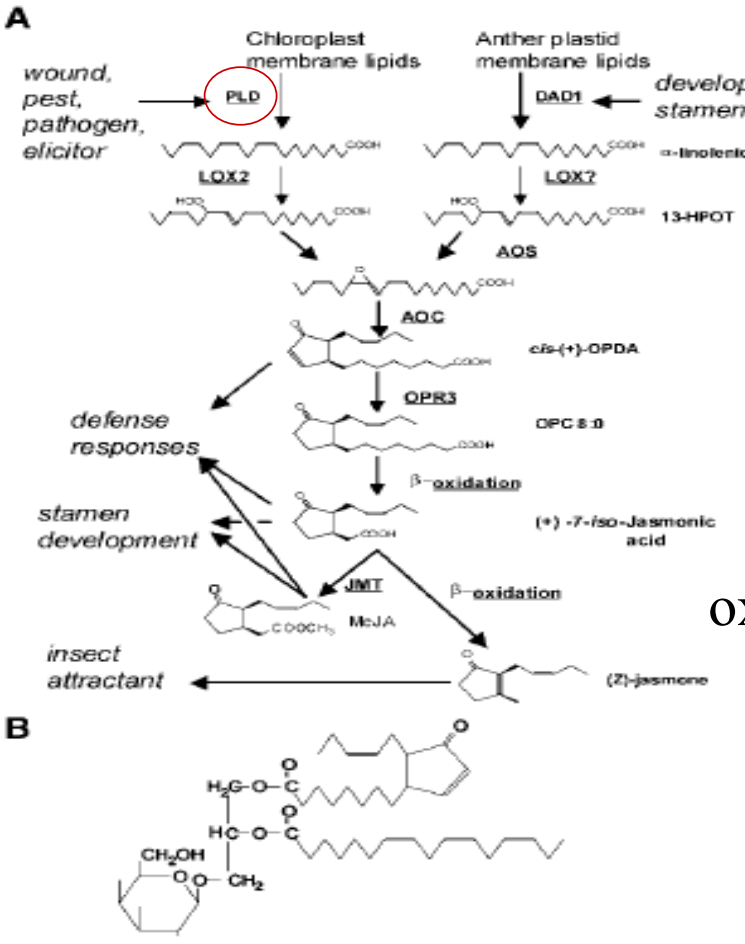
Plants were grown on 6% Glc Murashige and Skoog (1962) medium for 5 days under light. WT, wild type.

GIN2 je AtHXK1

Glukosa u WT brzdí de-etiolaci.

(met)JA
kyselina
(met)jasmonová

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



= PLA

LOX=lipoxygenáza

oxylipiny

Figure 2. Model for the Biosynthesis of JAs.

(A) Abbreviations for enzyme names are underlined; abbreviations for names of intermediates are in bold; pathway inputs and outputs are in italic.

(B) Structure of sn1-O-(12-oxophytodienoyl)-sn2-O-(hexadecatrienoyl)-monogalactosyl diglyceride, a chloroplast membrane oxylipin containing esterified OPDA.

PEPTIDY JAKO SIGNÁLY

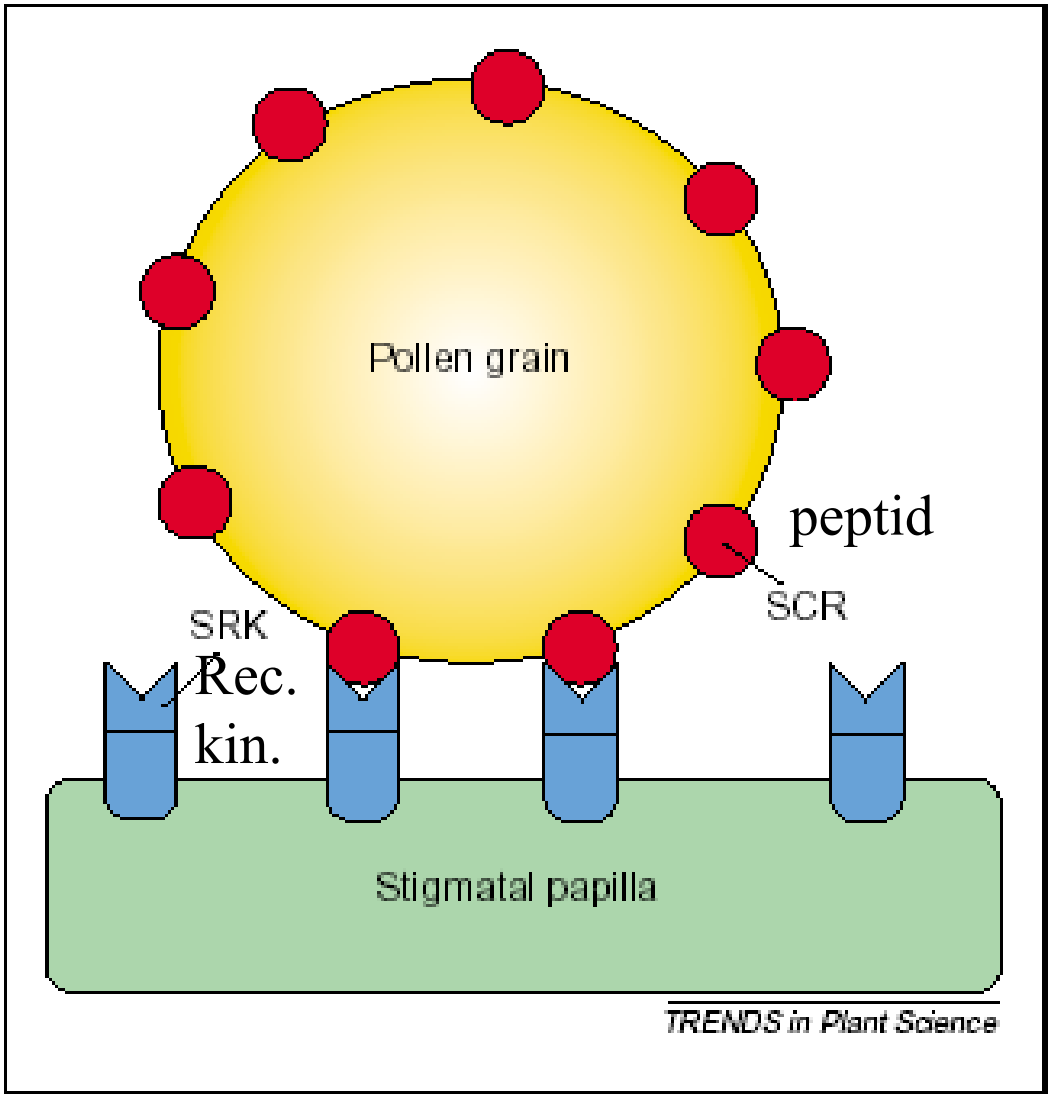
Table 1. Plant signalling peptides

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.

- **Nízkomolekulární bílkoviny hrají klíčovou roli také například ve sporofytické pylové inkompatibilitě.**

Fig. 4. The male determinant of self-incompatibility in *Brassica* is the S-locus cysteine-rich protein family (SCR) of small (<8 kDa) polypeptides. SCR is produced in microspores and tapetum, and is localized to the pollen coat. SCR might interact directly with the S-locus receptor kinase (SRK) located in the stigmatal papillae to distinguish self- from non-self-pollen.



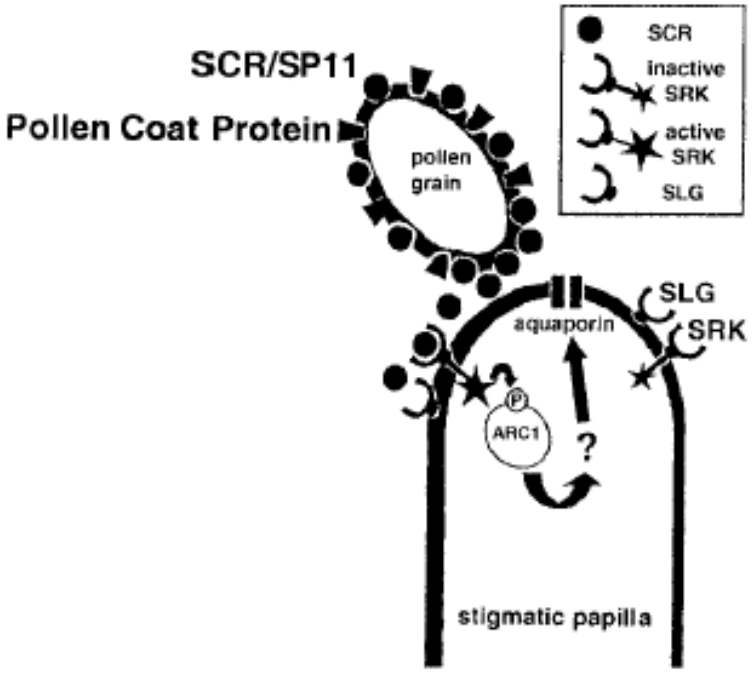


Figure 6. SI Signaling in *Brassica*.

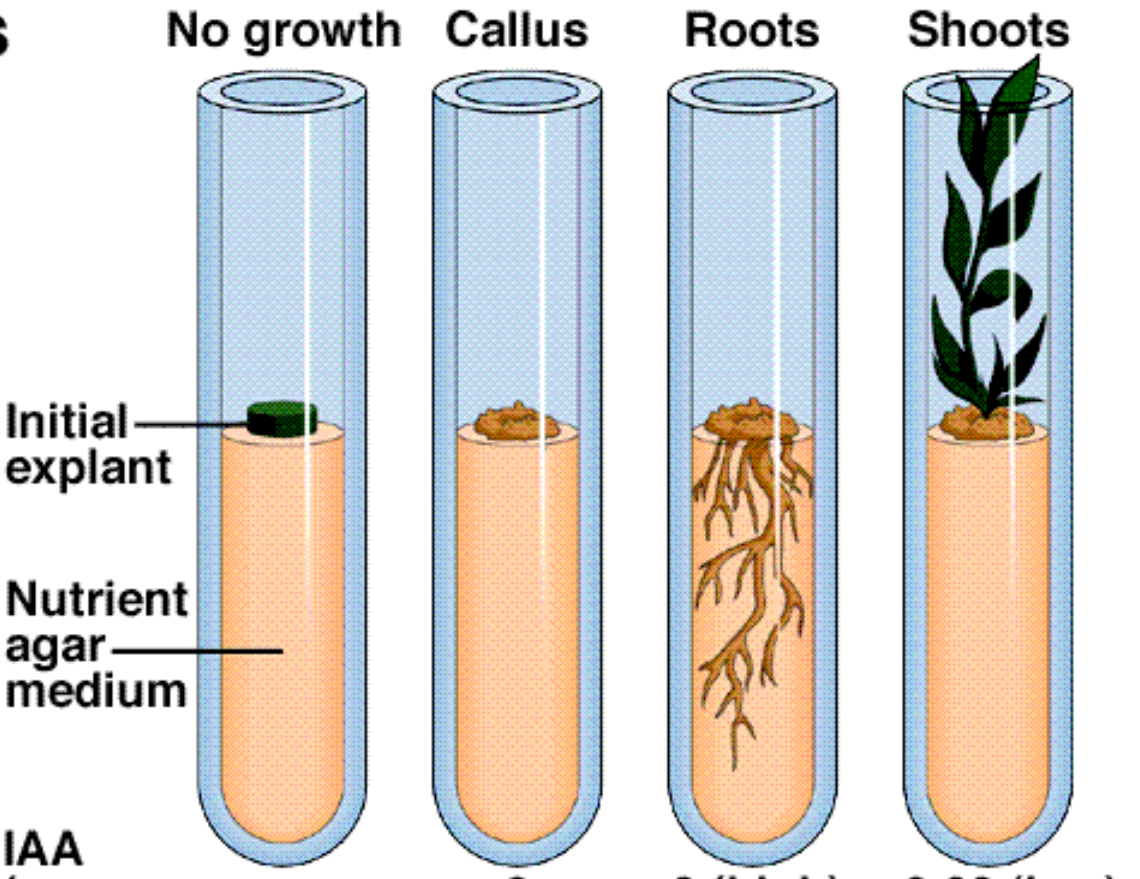
Shown is a model for the initial steps of SI signaling in *Brassica* (Franklin-Tong and Franklin, 2000). When pollen grains come in contact with the papilla surface of the stigma, the polypeptide signal, SCR, interacts with the SLG-SRK receptor kinase complex to activate a signaling cascade that leads to the incompatible SI response. The complex results in the activation of the receptor kinase and the phosphorylation of ARC1, which may lead to the activation of aquaporins to limit the access of water to the incompatible pollen. P, phosphorylation.

Integrace „hormonálních“ signálů

Randy Moore, Dennis Clark, and Darrell Vodopich, Botany Visual Resource Library © 1998 The McGraw-Hill Companies, Inc. All rights reserved.

Responses of Plant Tissue Culture to Kinetin and Auxin

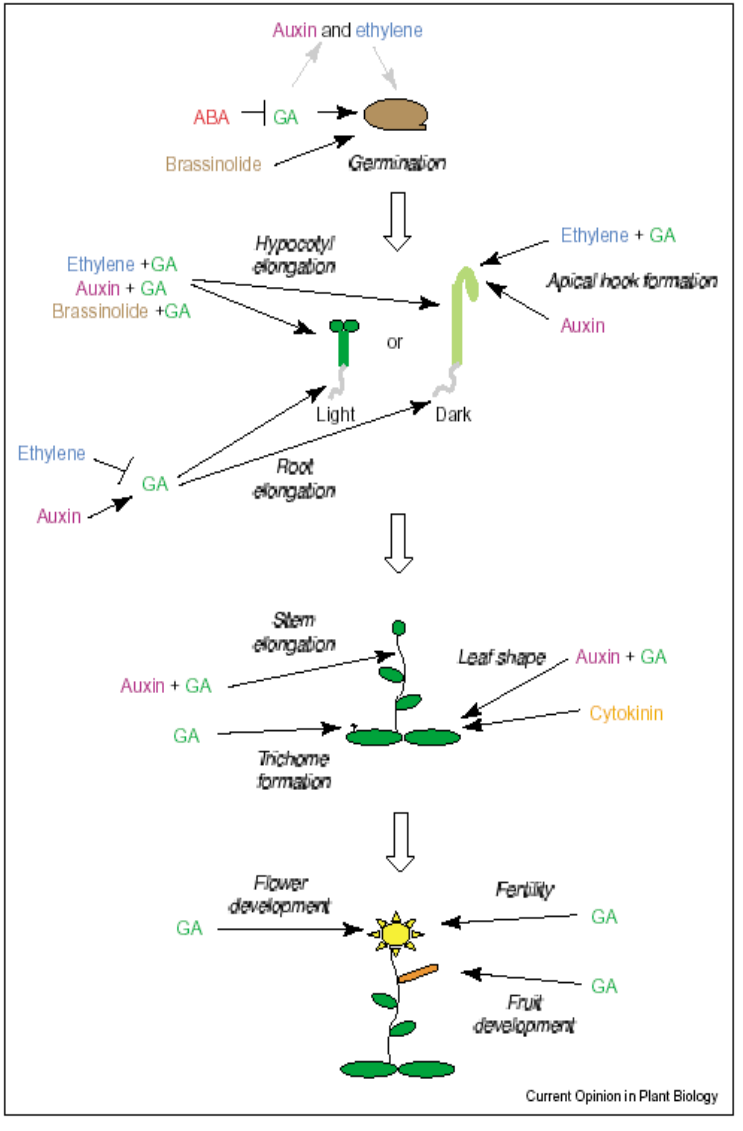
SKOOG
a
MILLER



auxin

cytokinin

Příklady: Integrace regulací GA s dalšími „fytohormony“



Interaction of GA with other hormones. Roles for hormones other than GA are indicated only when they interact with GA functions. Large arrows indicate changes in developmental stage. Italic text indicates a developmental process that is affected by the indicated hormones. T-bars denote inhibition of the indicated process, whereas black arrows denote promotion. Gray arrows denote a hypothesized role (on the basis of data from microarray analysis of transcripts in germinating seeds treated with GA). Two hormones showing synergistic interaction are denoted by a '+' between them; this does not imply an ordering of their activities. ABA, abscisic acid.

IAA spouští zvýšenou produkci GAs.

Narušení transportu IAA brzdí degradaci DELLA represorů působenou GAs.

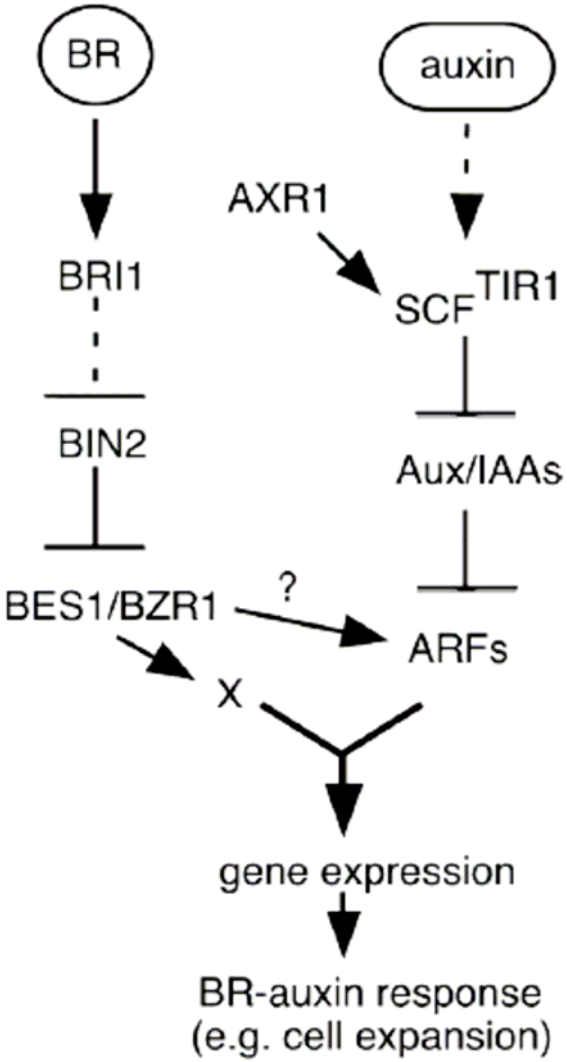


Figure 5. A Model of BR–Auxin Interaction

Auxin and BR signals are likely integrated on promoters of shared target genes. The node(s) of intersection between auxin and BR pathways must be downstream of BES1 and Aux/IAAs and upstream of gene expression. One likely mechanism is via regulation of transcriptional complexes, such as those containing the ARFs.

DOI:10.1371/journal.pbio.0020258.g005

BR a IAA

Indukují řadu
identických mRNA.

Hladiny auxinu a cytokininů korelují.

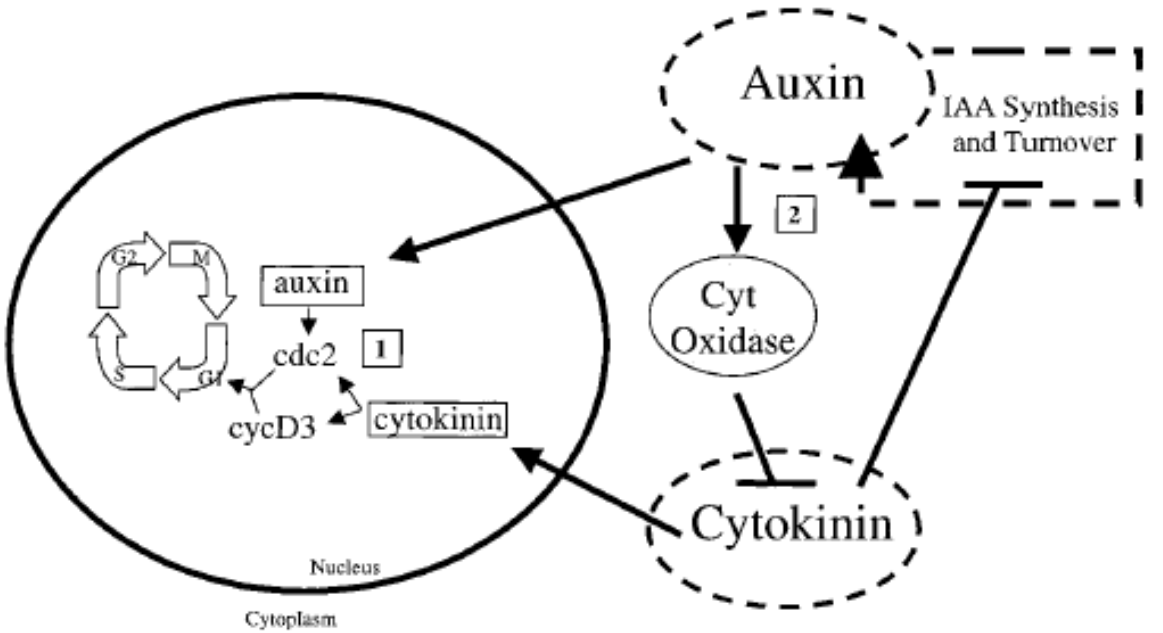


Figure 2. Auxin-cytokinin cross-talk in plant cells. Cytokinin and auxin regulate plant cell proliferation by (1) controlling the expression of the cell cycle components cdc2 and cycD3 (John *et al.*, 1993; Nogue *et al.*, 2000), whilst the size of cellular pools of auxin and cytokinin are controlled via (2) hormone-regulated enzymes such as cytokinin oxidase (Cyt oxidase; Zhang *et al.*, 1995).

Zvýšení cytokininů stimuluje syntézu IAA, ale zvýšení IAA inhibuje hladinu cytokininů.

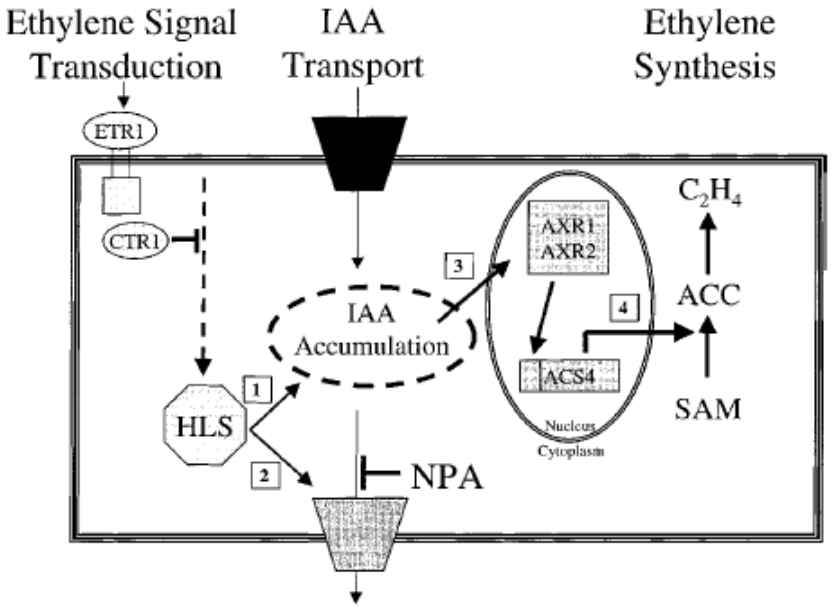
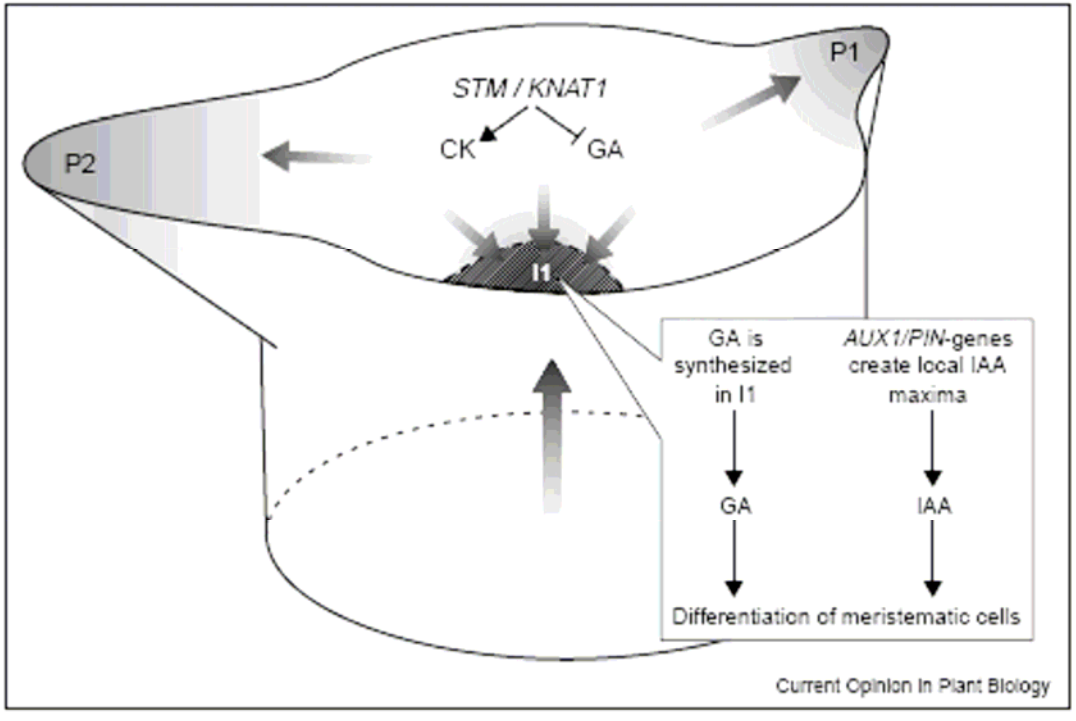


Figure 3. Auxin-ethylene cross-talk in plant cells. Ethylene up-regulates *HOOKLESS1* gene expression to facilitate apical hook formation by regulating either (1) IAA levels (Lehman *et al.*, 1996) or (2) auxin transport activity (Kieber, 1997). Auxin up-regulates ethylene biosynthesis via (3) AXR1- and AXR2-dependent expression of the (4) *ACC synthase 4* (*ACS4*) gene (Abel *et al.*, 1995).

Auxin stimuluje produkci etylenu stimulací genu pro syntézu ACC.

Etylen inhibuje polární transport auxinu.

ABA interaguje antagonisticky s GAs i IAA. Přidání ABA může snižovat hladinu volné IAA a zvyšovat podíl neaktivních konjugátů; ABA interferuje se sig. drahami GAs.



Auxin gradients in the shoot apical meristem regulate phyllotaxis. Auxin reaches the meristem via acropetal transport (upward pointing arrow). The existing leaf primordia (P1 and P2) act as auxin sinks that create a local auxin maximum (arrows) at the position of the next incipient leaf (I1). Homeobox genes (*STM*, *KNAT1*) that are expressed in the meristem inhibit the biosynthesis of GA and promote cytokinin (CK) production. They are not expressed at I1, leading to GA-production in this position (dashed line). Cells start to differentiate at I1 in response to GA and auxin, and a new leaf is formed.

Zvýšená hladina cytokinů a potlačení GAs je konstitutivním parametrem dělivého centra apikálního meristému.

Naopak pro vývoj listových primordií je podmínkou zvýšená hladina GAs.

Interaguje prakticky každý s každým a to jak na úrovni přenosu signálů/regulace genové exprese, tak

také na úrovni regulace biosyntézy - inhibice či aktivace akumulace partnerského "fytohormonu".

Plasmodesmata
(PD)
rostlina jako symplastická síť buněk (v síti apoplastu)

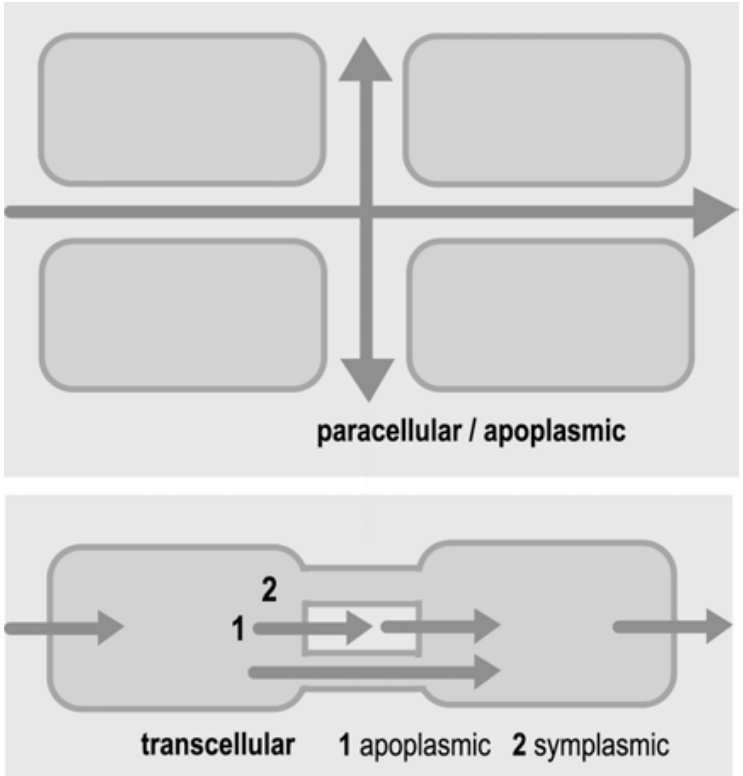


Figure 3. Schematic view of para- and transcellular solute flow. During paracellular solute flux in the aqueous cell wall space, the apoplasm, solutes neither cross membranes, nor enter the cells. Transcellular solute movement is characterized by initial solute influx into the cell. Further flux may include efflux into the apoplasm and subsequent reuptake by the neighbouring cell or symplasmic movement.

Buněčně autonomní = molekula působí jen uvnitř buňky.

Buněčně neautonomní = molekula působí mezi buňkami.

Buněčně neautonomní bílkoviny

- NCAPs – non-cell autonomous proteins

Tobacco plant expressing GFP protein
Infected with RNA virus with GFP gene
Virus infection travels through veins and PDs.

Systemické šíření RNA floemem

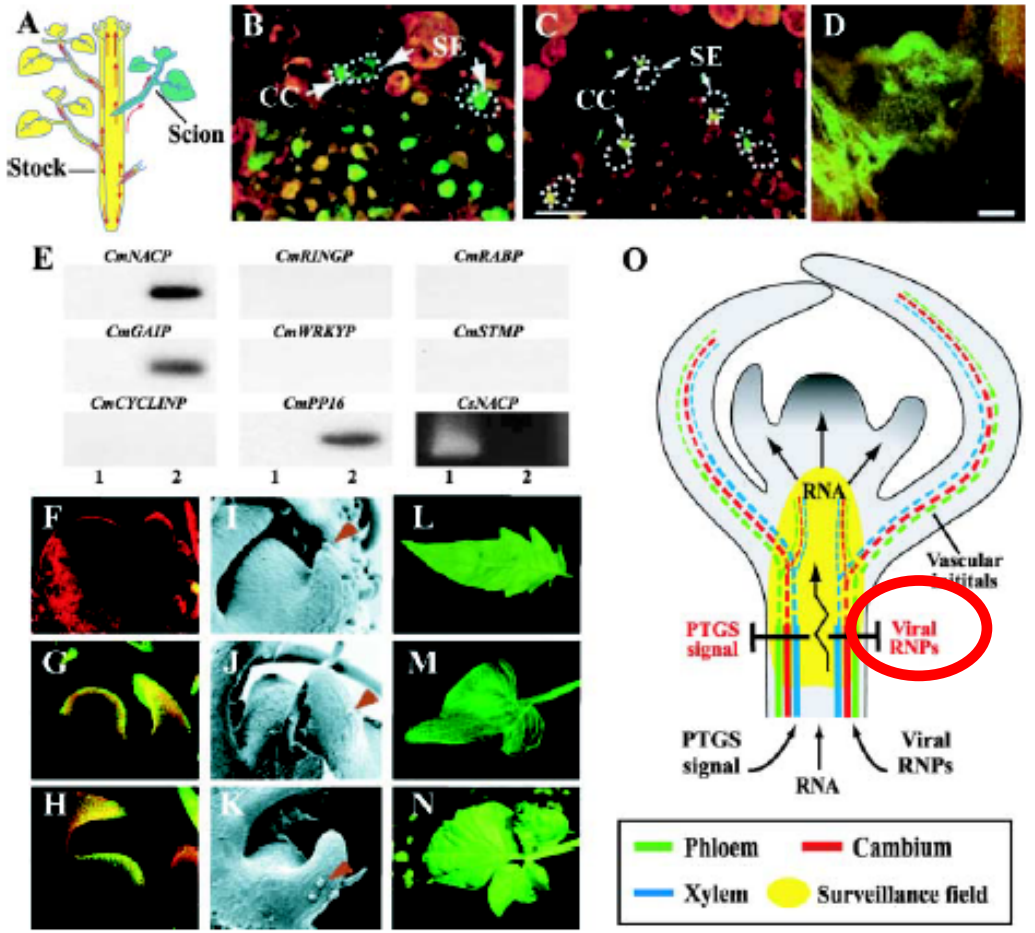
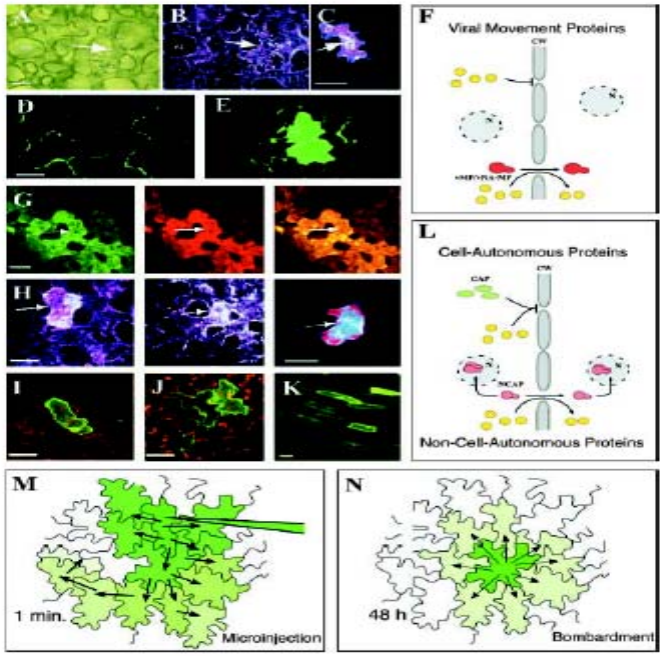


Figure 9. Developmental Regulation through Phloem-Mediated Translocation of mRNA Signals in Plants, as Demonstrated by Grafting Experiments.



injekce FITC-MP bílkoviny;C nepoh. mutant

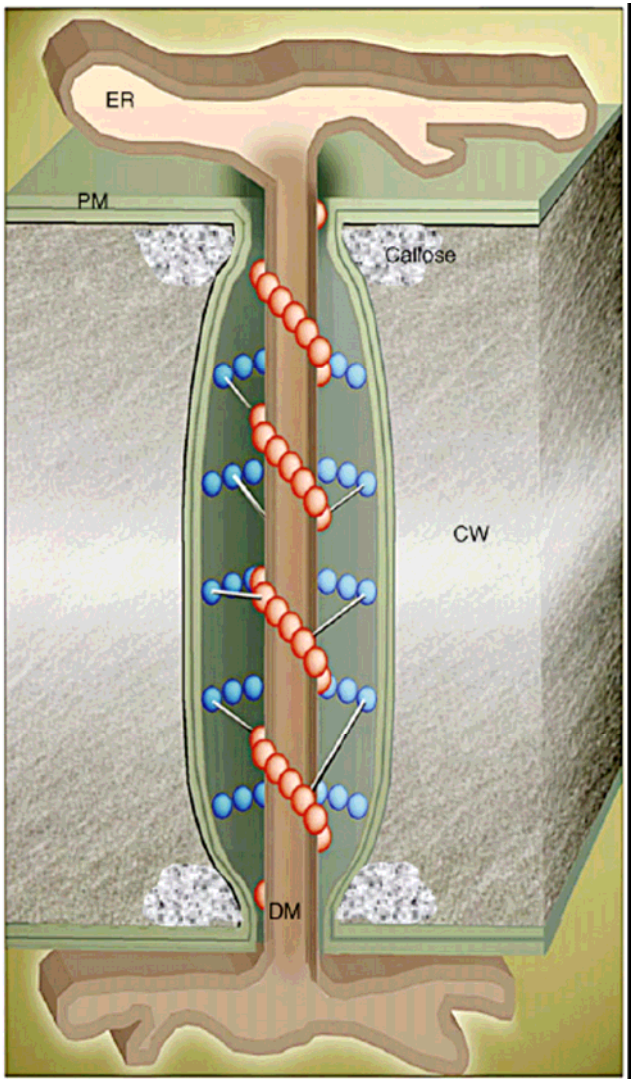
G – NCAP pomáhá pohybu dextranu.

PD=plasmodesma NCAP = non cell autonom. prot.

Figure 4. PD Potentiate Selective Cell-to-Cell Transport of Viral MPs/MP–Nucleic Acid Complexes and Endogenous Transcription Factors.

(A) and (B) Bright-field and fluorescent images, respectively, illustrating extensive cell-to-cell movement of a FITC-labeled viral MP after its injection into a *Phaseolus vulgaris* (bean) mesophyll cell. Arrows indicate injected cells. IAS, intercellular air space. (Adapted from Noueiry et al., 1994.)
(C) A mutation in this MP blocked its ability to move out of the injected cell. Arrows indicate injected cells. (Adapted from Noueiry et al., 1994.)
(D) Expression of TMV-MP-GFP in a tobacco epidermal cell, after biolistic bombardment, leads to cell-to-cell movement of this fluorescent probe. (Adapted from Crawford and Zambryski, 2001.)
(E) Control GFP bombardment experiment in which free GFP (27 kD) was produced in a tobacco epidermal cell (source leaf). Limited GFP diffusion into adjacent cells likely reflects low-frequency trafficking of endogenous NCAPs. (Adapted from Kotlizky et al., 2001.)
(F) Presence of viral MP (vMP) or MP–nucleic acid complexes (vNA-MP) (microinjected or produced in the infected cell) causes the dilation of PD microchannels, thereby permitting cell-to-cell movement of MP, MP–nucleic acid, and F-dextran/GFP probes (yellow circles). CW, cell wall; N, nucleus.
(G) Cell-to-cell trafficking of a tetramethylrhodamine isothiocyanate (TRITC)–labeled NCAP (left) permitted the simultaneous spread of an 11-kD FITC-labeled dextran (center); the yellow signal resulting from merged images (right) highlights the coupled nature of the TRITC-NCAP and FITC-dextran movement. Arrows indicate injected cell. (Adapted from Kragler et al., 1998b.)
(H) KN1 displays NCAP properties; microinjection of KN1-FITC (left) or KN1 plus 20-kD FITC-labeled dextran (center) resulted in the spread of fluorescence signal into the surrounding mesophyll cells, but movement was blocked in the case of the M6 KN1 mutant (right). Arrows indicate injected cell. (Adapted from Lucas et al., 1995.)
(I) to (K) Biolistic bombardment experiments confirm the capacity of NCAPs to traffic through PD. (Adapted from Kim et al., 2002.)
(I) Confinement to the target cell of the fluorescent signal associated with expression of GFP-YFP (52 kD) in epidermal cells of Arabidopsis.
(J) Parallel experiment to (I) demonstrating limited cell-to-cell movement of GFP-KN1 (~69 kD).
(K) Parallel experiment to (J) illustrating cell-to-cell movement of GFP-KN1 in onion root epidermal cells.
(L) Endogenously expressed or microinjected NCAPs interact with PD to induce microchannel dilation, thereby permitting their entry into the next cell as well as the co-diffusion of F-dextran/GFP probes (yellow circles). Cell-autonomous proteins (CAPs) lack this capacity to interact with PD. CW, cell wall; N, nucleus.
(M) and (N) Schematic illustrations of the patterns of NCAP cell-to-cell movement after delivery by microinjection or plasmid bombardment, respectively. In microinjection experiments, an NCAP generally spreads through some five cells within 1 min; by 10 min it will have moved out in a radial direction through some 10 cells. In bombardment experiments, NCAP-GFP expression takes 24 to 48 hr before a fluorescent signal can be detected, and then radial movement is often restricted to one or two cells.
Bars = 50 μm.

S transportem jsou spojeny regulované změny SEL (Size Exclusion Limit) – průchodnosti PD.



Basic structure of a simple primary plasmomodesma formed at cytokinesis. The figure presents one of a number of very similar published models for the structure of a simple Pd. Cell wall (CW) formation at cytokinesis traps components of the endoplasmic reticulum (ER) that becomes appressed to form the central axial desmotubule (DM) surrounded by the plasma membrane (PM) continuum between adjacent cells. The DM is sometimes also referred to the 'appressed ER'. Molecular flux is proposed to occur through the cytoplasmic sleeve between the PM and DM and to be regulated by callose deposition in the neck region of the wall. The cytoplasmic sleeve is proposed to be interrupted by components such as actin (red spheres) and other undefined proteins (blue spheres and spokes). Figure modified from [21••], under the terms of the Creative Commons Attribution License 2.5.

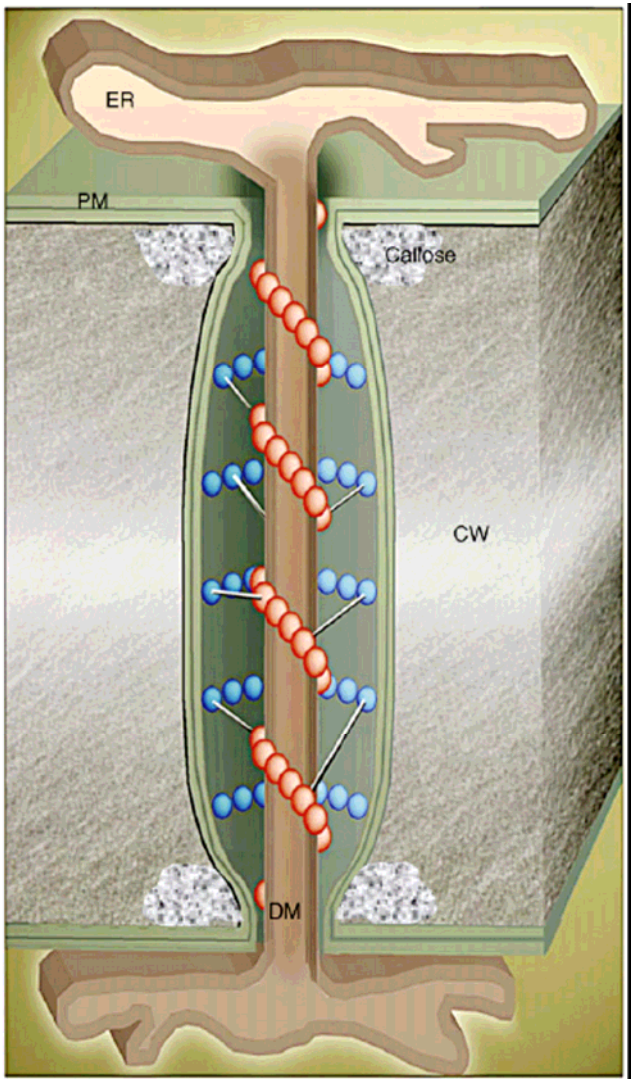
Bílkoviny plasmodesmat

- Reversibilně glycosylovaný polypeptid (RGP – napřed Chara, pak i Arabidopsis).
- HSC70 (HSP70 příb. chaperon - dtto)
- RNA helikázy (dtto) – mutant Arabidopsis *ise2* má větvená plasmodesmata.
- β-1,3-glukanáza (dtto)

β-1,3-glukanáza Arabidopsis AtBG_ppap

- Ztrátový **mutant má snížený metabolismus kalózy a s tím spojený snížený tok plasmosematy.**

- **Kalózový „limec“ pravděpodobně reguluje SEL plasmodesmat.**
- AtBG_ppap má C´- term sign. pro připojení GPI kotvy, což implikuje lokalizaci na povrchu PM.



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dále byly identifikovány

- Rodina transmembr. receptorových bílkovin(PLDLP1..8) – OX PLDLP1 snižovala plasmodesmový transport
- Rodina kalózu vážících polypeptidů (22kDa) s C´-GPI kotvou.

Buněčně neautonomní bíloviny

- (NCAPs – non-cell autonomous proteins)

- Nelze identifikovat signál-konsensus-sekvenici AA pro cílení transportu do PD. Je využívána řada různých.

Symplastická konektivita je dynamická, regulovaná!

Plasmodesmy vznikají při buněčném dělení i de novo a mohou být regulovaně uzavřena.

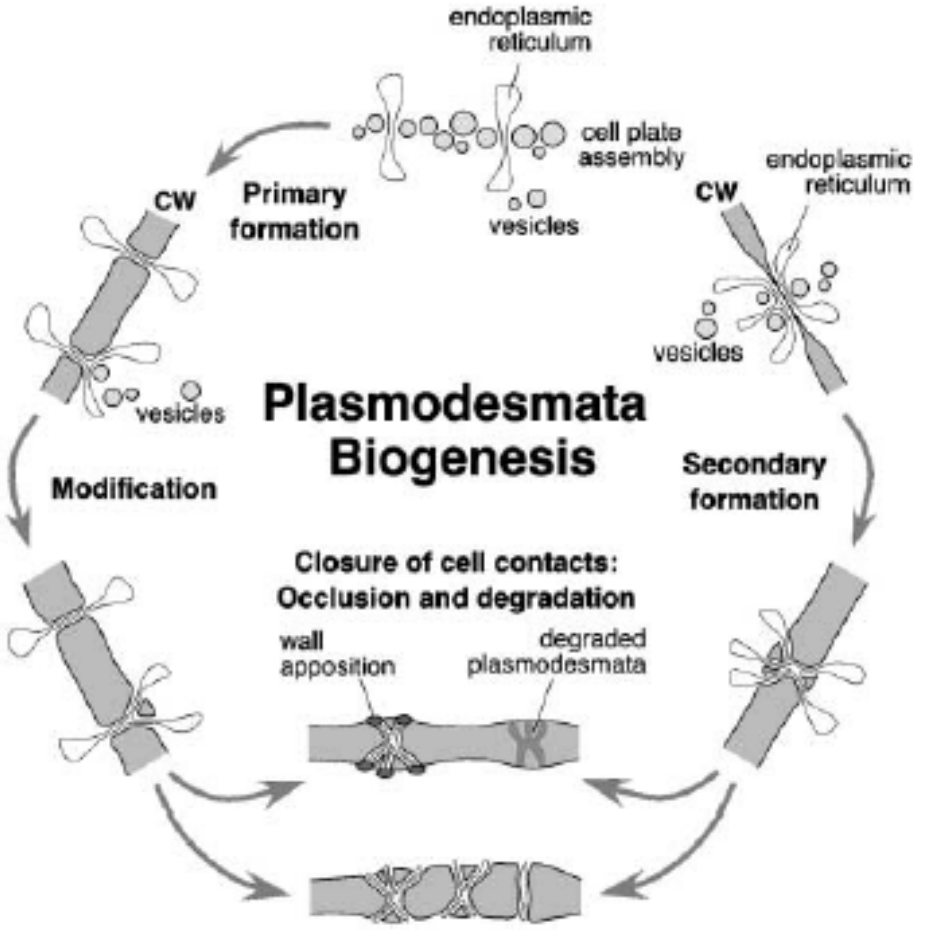
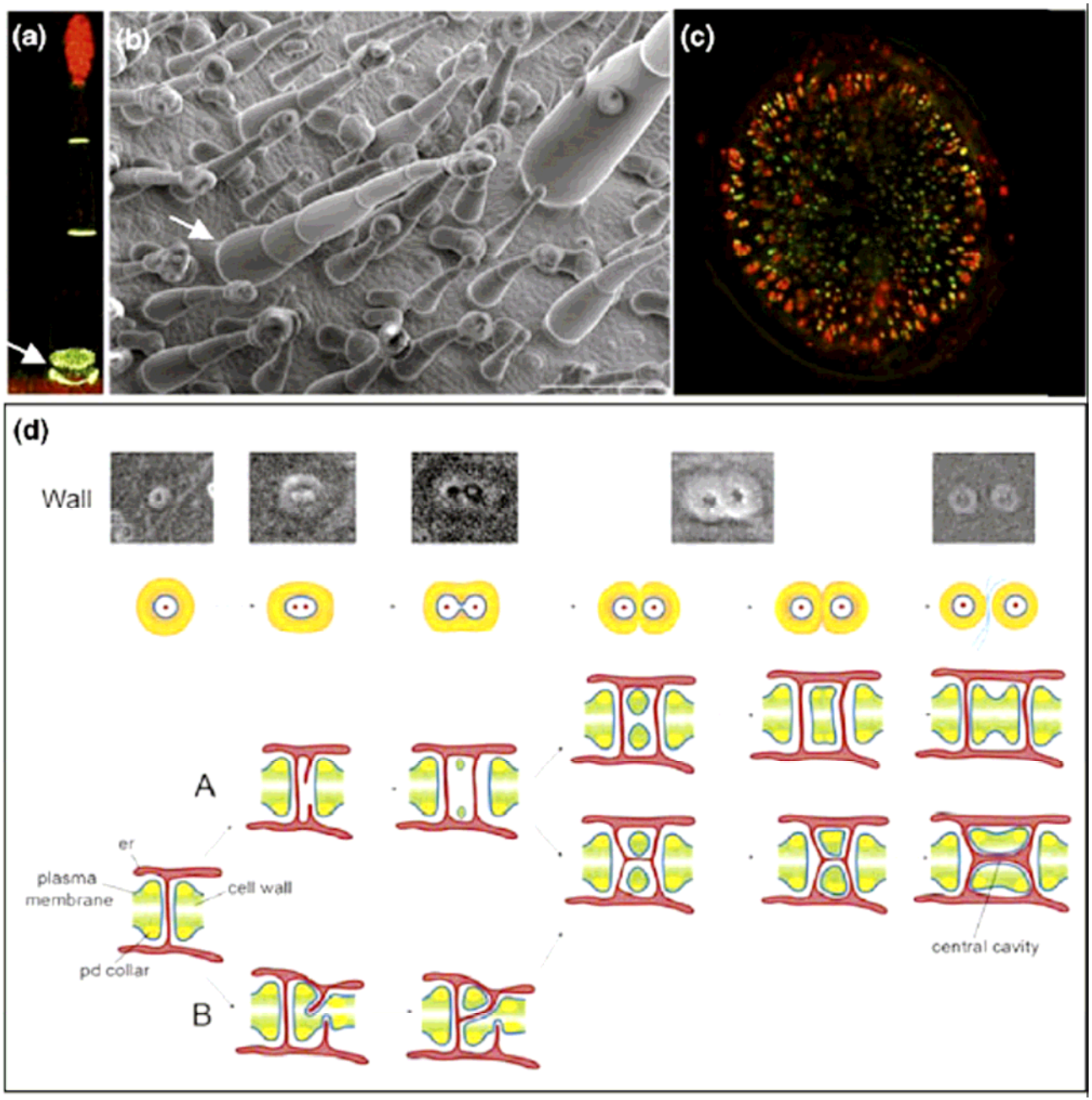


Figure 1. Formation of Primary and Secondary PD.

Formation of primary and secondary PD, in conjunction with PD occlusion and degradation, allows the plant to adjust the extent of the symplasmic/supracellular pathway interconnecting the cells of a tissue. CW, cell wall. (Adapted from Kragler et al., 1998a.)


(a)
(b)
(c)
(d)
Wall
A
B
er
plasma membrane
cell wall
pd collar
central cavity

Plasmodesmal biogenesis in the glandular trichome of *Nicotiana*. **(a and b)** *Nicotiana* leaf trichomes form as multicellular structures to support aerial glands (red). Cell–cell interfaces are populated by many Pds, revealed here using TMV MP.GFP. As the trichomes grow the basal wall interface (white arrow) expands radially resulting in a redistribution of the randomly occurring Pds. **(c)** Labelling of Pds with TMV MP.GFP (green) and antibody to calreticulin (red) reveals the radial distribution and the increase in size of Pd clusters to form pit fields at the outer edge of the interface. **(d)** Models of secondary Pd formation. Upper panels—experimental observations from scanning electron microscopy of freeze fractured trichome basal wall show single or twinned pores, sometimes separated (right) by new cell deposition. Lower panels—interpretive models describing the formation of complex structures by longitudinal fission (A) or *de novo* pore formation (B). The fission model depicts insertion of a second DM with a shared pore orifice. The DMs become separated by deposition of further wall material to create separate pores. Central cavities may or may not form between the two pores. *De novo* pores may be inserted adjacent to an existing pore from either side of the wall, merging in the middle lamellar region to form a complex structure. Figure adapted from, Figures 1, 4 and 6 in [39••] with permission.

- Časné embryo tvoří jednu symplastickou síť, v dalším vývoji se vytvářejí rozrůzněné regulační symplastické domény (např. v L1 – centrální vs. periferní zóna meristému).

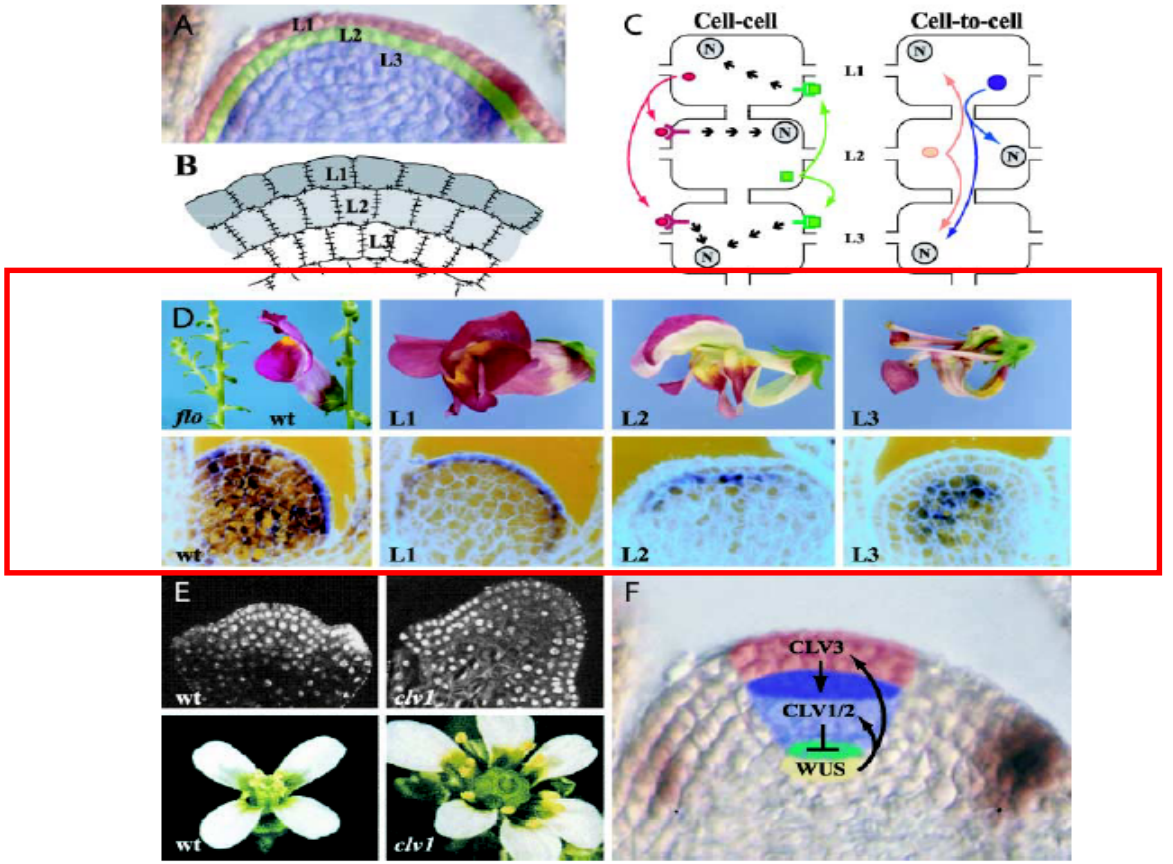


Figure 2. Non-Cell-Autonomous Signaling Molecules Mediate Control over Developmental Processes in the SAM.

(A) The dicot SAM is typically organized into three clonally distinct cell layers. Cell division in the L1 (pink) and L2 (green) occurs almost exclusively in the anticlinal plane, whereas cells of the L3 (purple) can divide in all planes. (Adapted from Bowman and Eshed, 2000.)

(B) Distribution of primary (–) and secondary (×) PD that interconnect the cells of the SAM. (Adapted from Lucas, 1995.)

(C) The possible intercellular pathways taken by non-cell-autonomous signaling molecules. Cell–cell signaling (left) takes place in the apoplasm and involves receptor–ligand-mediated interactions. Secreted ligands (red circle, green square) diffuse through the apoplasm and bind to plasma membrane–bound receptors, thereby activating downstream signaling cascades within the target cell. Cell-to-cell signaling (right) involves PD-mediated trafficking of information macromolecules. N, nucleus.

(D) Cell-to-cell signaling as exemplified by the effects on floral development by *FLO* expression in the SAM of Antirrhinum. Lack of *FLO* expression causes the production of secondary IM instead of flowers. The unstable *flo-613* mutation was caused by insertion of a transposable element, *Tam3*, into the second intron of *FLO*. Spontaneous *Tam3* excision can generate periclinal chimeras exhibiting revertant *FLO* sectors in the *flo-613* background (Carpenter and Coen, 1990). The degree to which normal floral development can be restored is here shown to depend on the layer of the SAM in which *FLO* is expressed. (Adapted from Carpenter and Coen, 1990, 1995; Hantke et al., 1995.)

(E) Phenotypes of wild-type (wt) and *clv1* mutant Arabidopsis plants. Loss of *CLV1* expression results in an enlarged SAM (at top) as well as increased production of floral organs (at bottom). (Adapted from Clark et al., 1993.)

(F) Cell–cell signaling in the SAM by the CLV regulatory pathway. (Adapted from Bowman and Eshed, 2000.)

Floemem se šíří květní signál – bílkovina FT

moves to the apex to trigger flowering. FT protein movement is the more likely hypothesis, since microarray results have shown that *FT* RNA is not detected at the shoot apex (Schmid et al., 2003). The possibility of FT protein movement is further supported by the molecular size of the FT protein (23 kD), which is below the size exclusion limit of plasmodesmata (Imlau et al., 1999). The FT protein may thus freely move to the shoot apex. However, this FT protein movement should be precisely coordinated to induce flowering since the size exclusion limit can change during organ development (Imlau et al., 1999). Nev-

Rostlinný organismus jako nadbuněčná integrovaná symplastická síť.

RNA viruses can block expression of a transgene if a copy of the transgene has been added

Tobacco plant expressing GFP protein
Infected with RNA virus with GFP gene
Virus infection travels through veins
GFP expression inhibited starting at veins.

Gene silencing and RNA viruses share potential to produce ds RNA

Fire and Mello proved that ds RNA inhibits expression of endogenous genes homologous to that dsRNA (Nobelova cena 2006).
Ve skutečnosti první důkaz byl podán u rostlin (př. v laboratoři Davida Baulcomba JIC, Norwich)

a RNA interference:

A type of gene regulation
Involving small RNA molecules
and induced by double stranded RNA

Positive Thinking & Stress

Stress Responses & Gene Expression

- **plants must adapt to stresses because of their sedentary lifestyle**

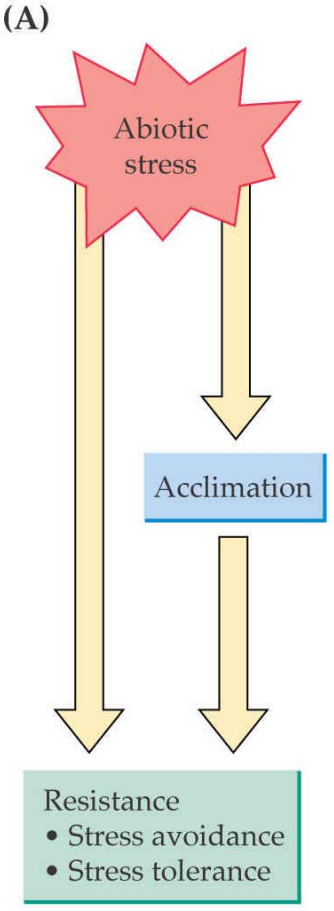


Fig. 22.2, Buchanan et al.

Regulace iontové homeostasy během reakce na zasolení.

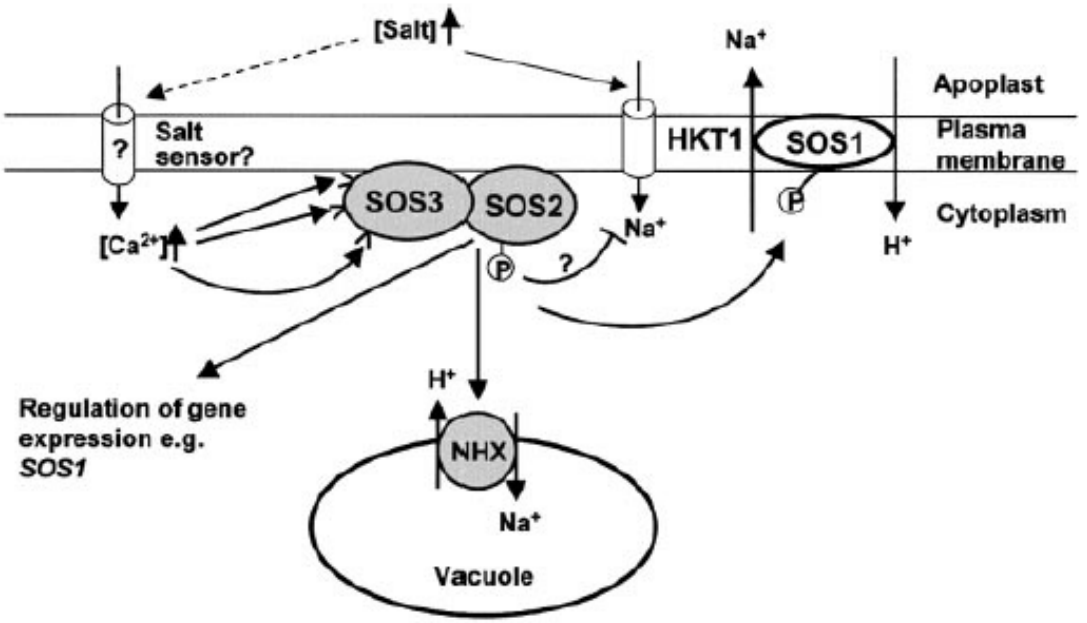


Fig. 1. Regulation of ion homeostasis by the SOS pathway during salt stress. Salt stress-induced Ca^{2+} signals are perceived by SOS3 which activates the SOS2 kinase. The SOS3–SOS2 kinase complex regulates cellular Na^+ levels by stimulating Na^+ transport out of the cytoplasm (e.g. by increasing the expression and activity of *SOS1*) and conceivably by restricting Na^+ entry into the cytosol (e.g. inhibiting HKT1 activity). An additional target of the SOS2 kinase, NHX (vacuolar Na^+/H^+ exchanger), also contributes to Na^+ ion homeostasis by transporting Na^+ from the cytoplasm into the vacuole.

Reakce na osmotický stress a ABA.

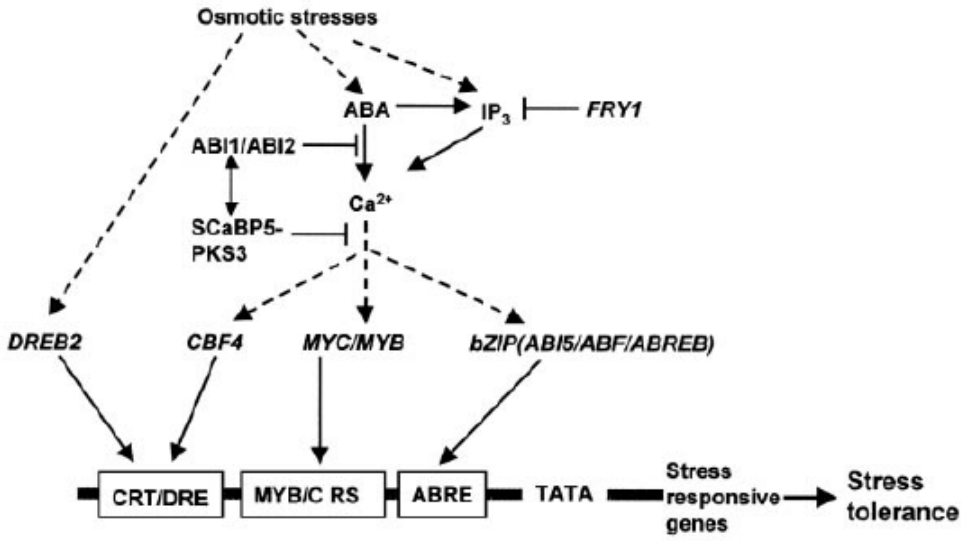


Fig. 2. Transcriptional regulation of stress-responsive genes in response to osmotic stress. Osmotic stress-induced DREB2 transcription factors induce ABA-independent transcription of stress responsive genes. ABA-dependent pathways regulate stress-responsive gene expression through CBF4, MYC/MYB and bZIP-type transcription factors, which bind to the CRT/DRE, MYB/C Recognition Sequences (MYB/C RS) and ABA Responsive Elements (ABRE) promoter elements, respectively. ABA-dependent abiotic stress signalling is mediated in part through IP_3 and Ca^{2+}. FRY1 negatively regulates IP_3 levels. ABA-induced Ca^{2+} signalling is negatively regulated by the ABI1/2 protein phosphatase 2Cs and the SCaBP5–PKS3 complex.

Transkripční regulace v odpovědi na chlad

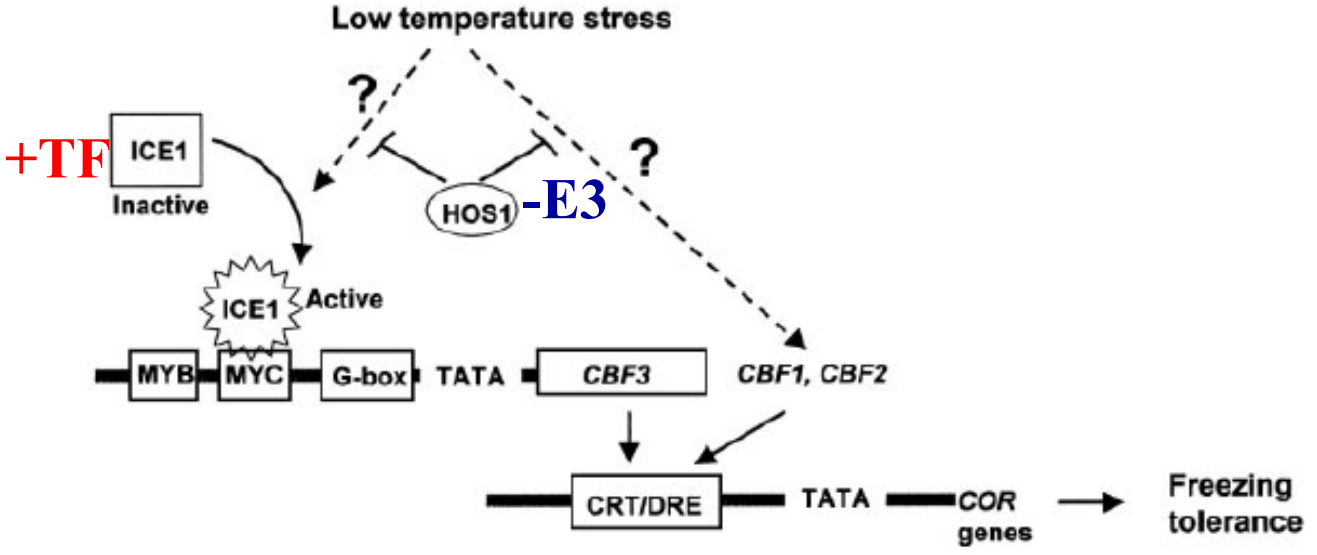


Fig. 3. Regulation of a cold-stress-responsive transcriptome and freezing tolerance. ICE1 is a constitutively expressed myc-like bHLH transcription factor, which is inactive under non-stress conditions. Low temperature stress presumably activates ICE1, which binds to *myc cis*-elements of the *CBF3* promoter and induces *CBF3* expression. CBFs binds to the CRT/DRE *cis*-elements on cold-stress-responsive (*COR*) genes inducing their expression and leading to acquired freezing tolerance. HOS1, a putative E3 ubiquitin-conjugating enzyme, appears to target a positive regulator of CBFs for degradation.

Heat Shock Proteins (hsp)

- ~100, ~90, ~70, and ~60 kDa
- Low molecular weight (LMW) hsp: ~27, ~20-22, ~15-18 kDa
- all induced within 30 min.
- **more LMW hsp in plants**
- 2-Dimensional gel electrophoresis and molecular cloning indicates most hsps are families of related proteins, particularly hsp70 and the LMW hsps

HSP70, a chaperonin

- Homologues found in cytoplasm, ER lumen, mitochondria, and chloroplasts
- function in protein targeting and assembly in normal (non-stressed) cells, hydrolyze ATP
- Constitutive & heat-induced (cytoplasmic) forms
  - the heat-induced form first appears in the nucleolus, then goes to cytoplasm (may protect pre-ribosomes from heat stress?)
- Also, some hsp70s are light-induced; chloroplast hsp70 helps protect PSII from light/heat damage in *Chlamy*

LMW HSPs

- **highly heat-induced**
- 4 nuclear gene families:
  1. Class I cytoplasmic
  2. Class II cytoplasmic
  3. Chloroplast localized
  4. Endomembrane localized (ER)
- found in organelles only in plants
- function mostly unknown
- **aggregate *in vivo* into "heat shock granules"**

HSP regulation

- most work on LMW hsp in plants
- **induction is mainly transcriptional but also translational control (hsp mRNAs preferentially translated)**
- genes induced coordinately, but not equally in all tissues
- light can also induce some LMW hsps

Cis-acting transcriptional regulatory elements

- HSE (heat shock elements) in the 5' regions:
  - ~10-15 bp **partial palindromes**
  - multiple copies required
  - also found in other HS genes (e.g., hsp 70)
  - similar to HSEs in animals

Heat-shock transcription factor (HSF)

- studied mostly in animals and yeast
- Binds to HSEs
- Contains leucine zipper motifs
- Binds DNA as a trimer
- Activity is induced by heat, and phosphorylation
- **Activity Repressed by HSP70**

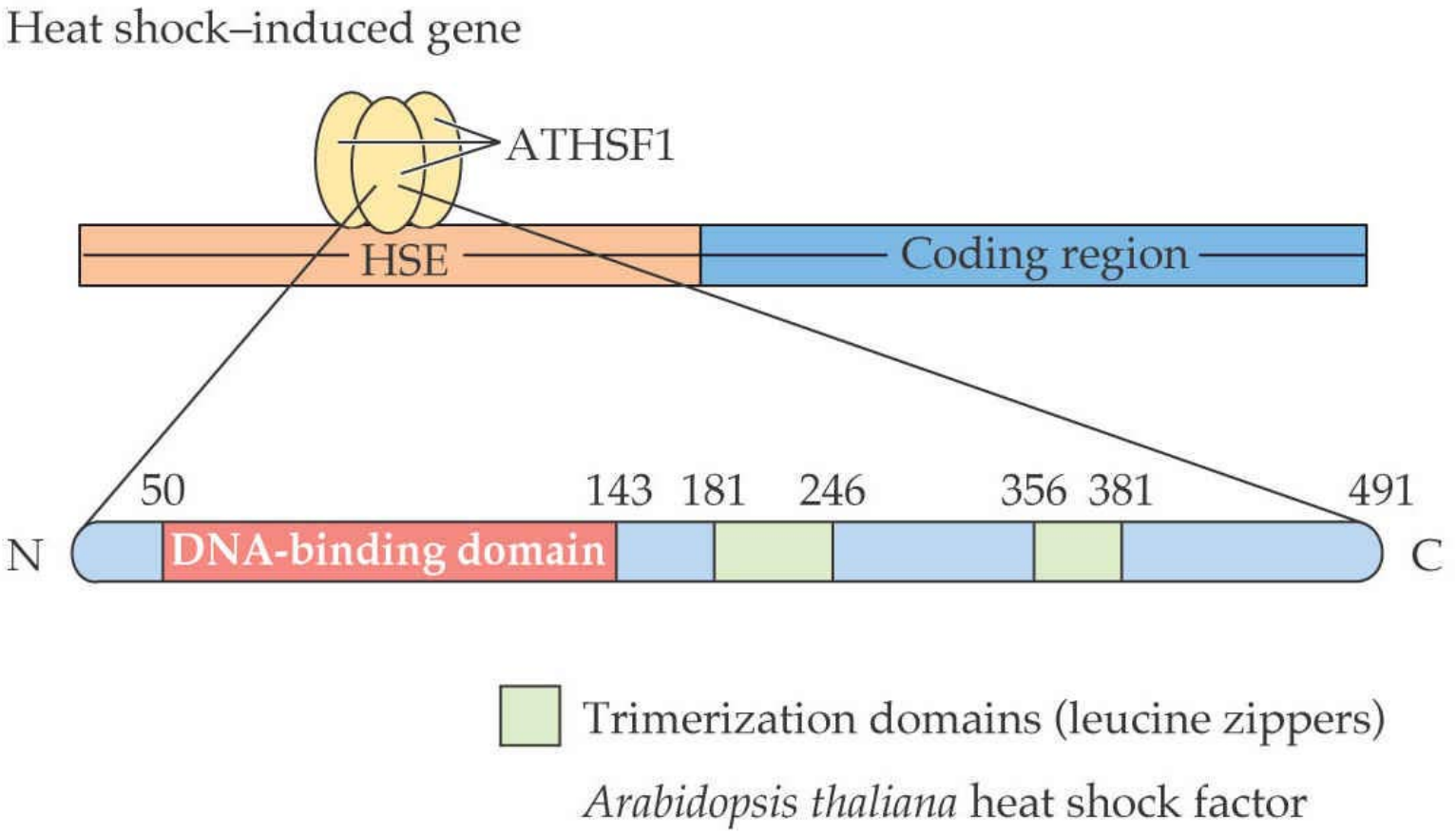


Fig. 22.43, Buchanan et al.

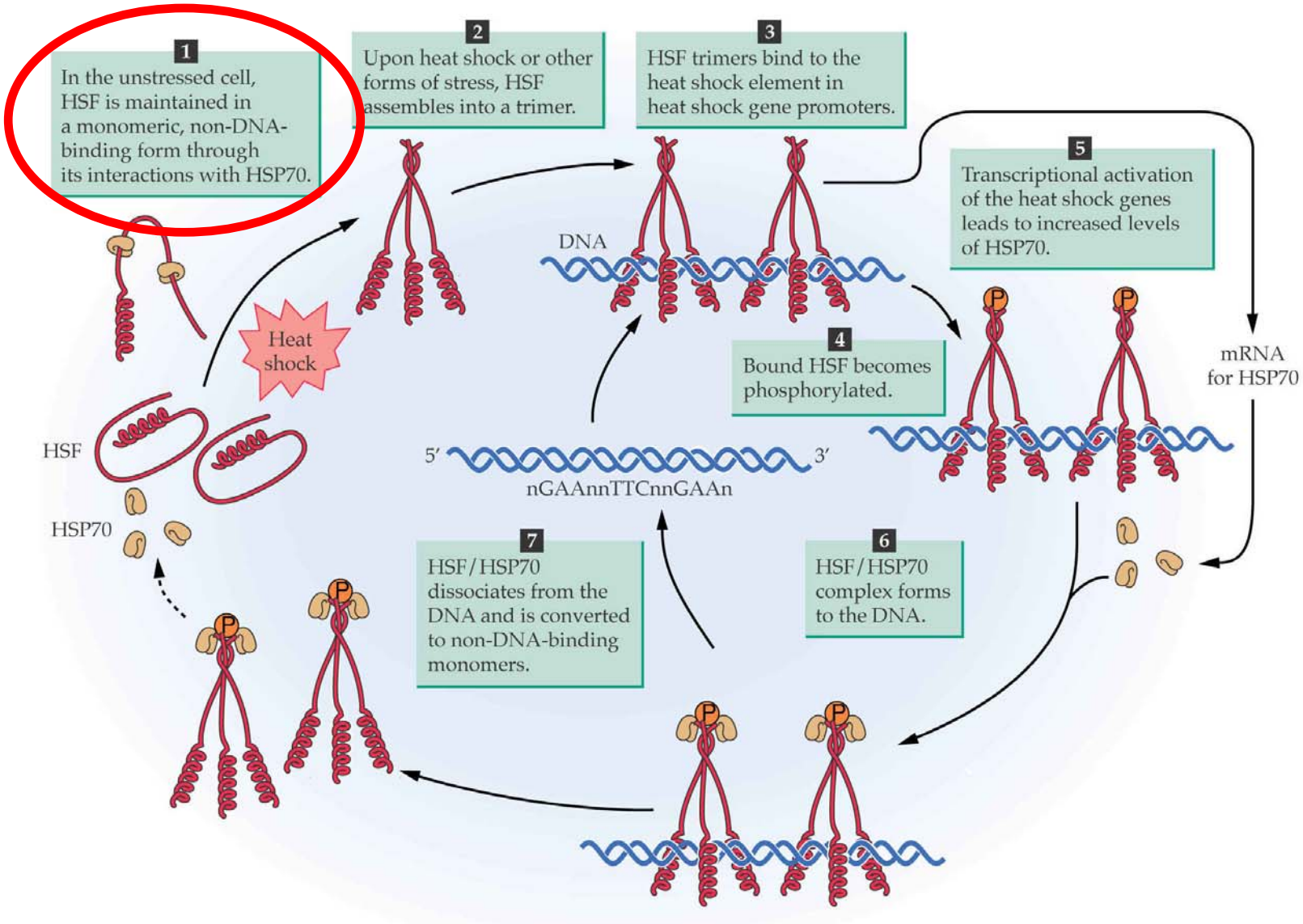
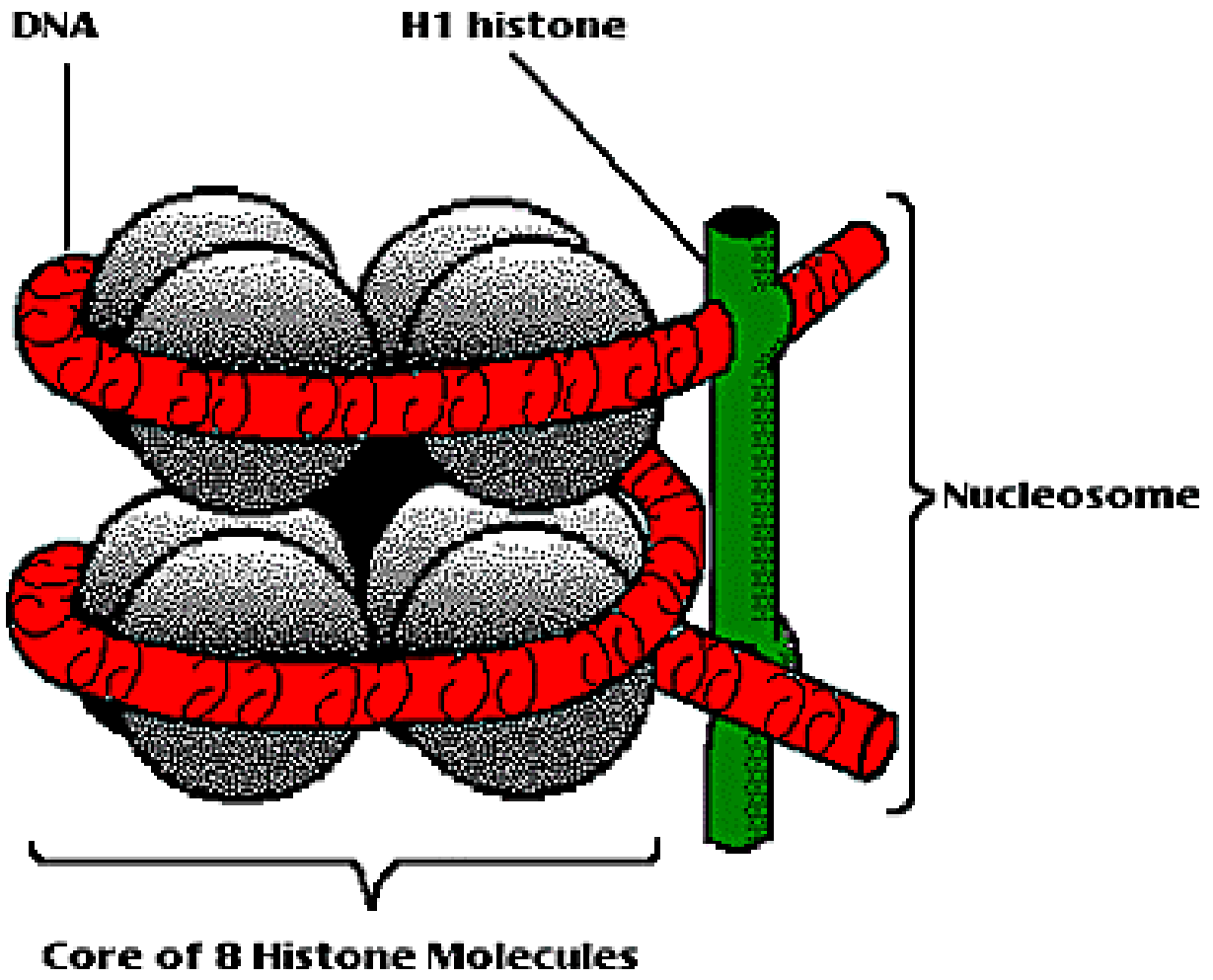


Fig. 22.44, Buchanan et al.

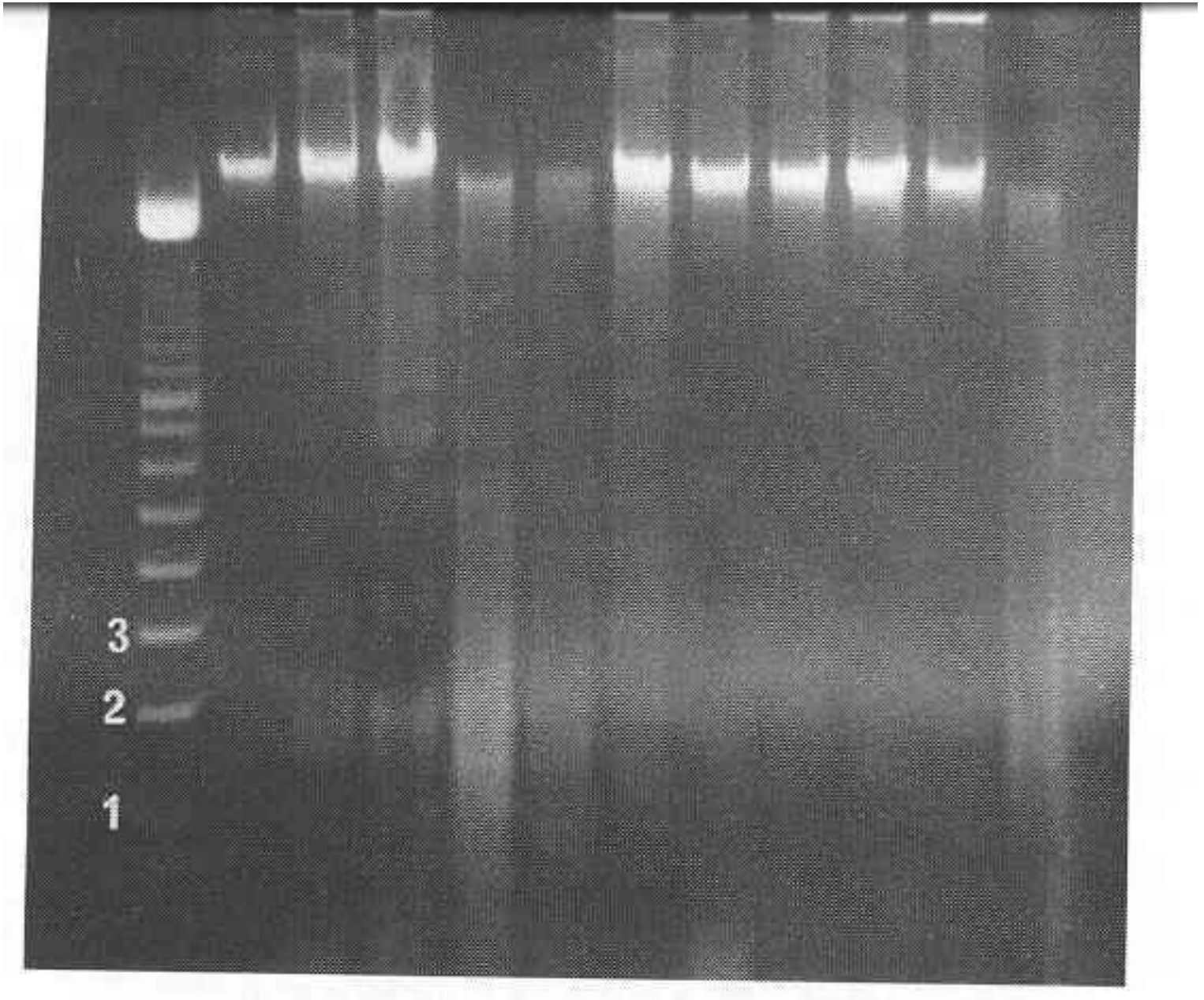
PCD rostlinné buňky

a vakuola

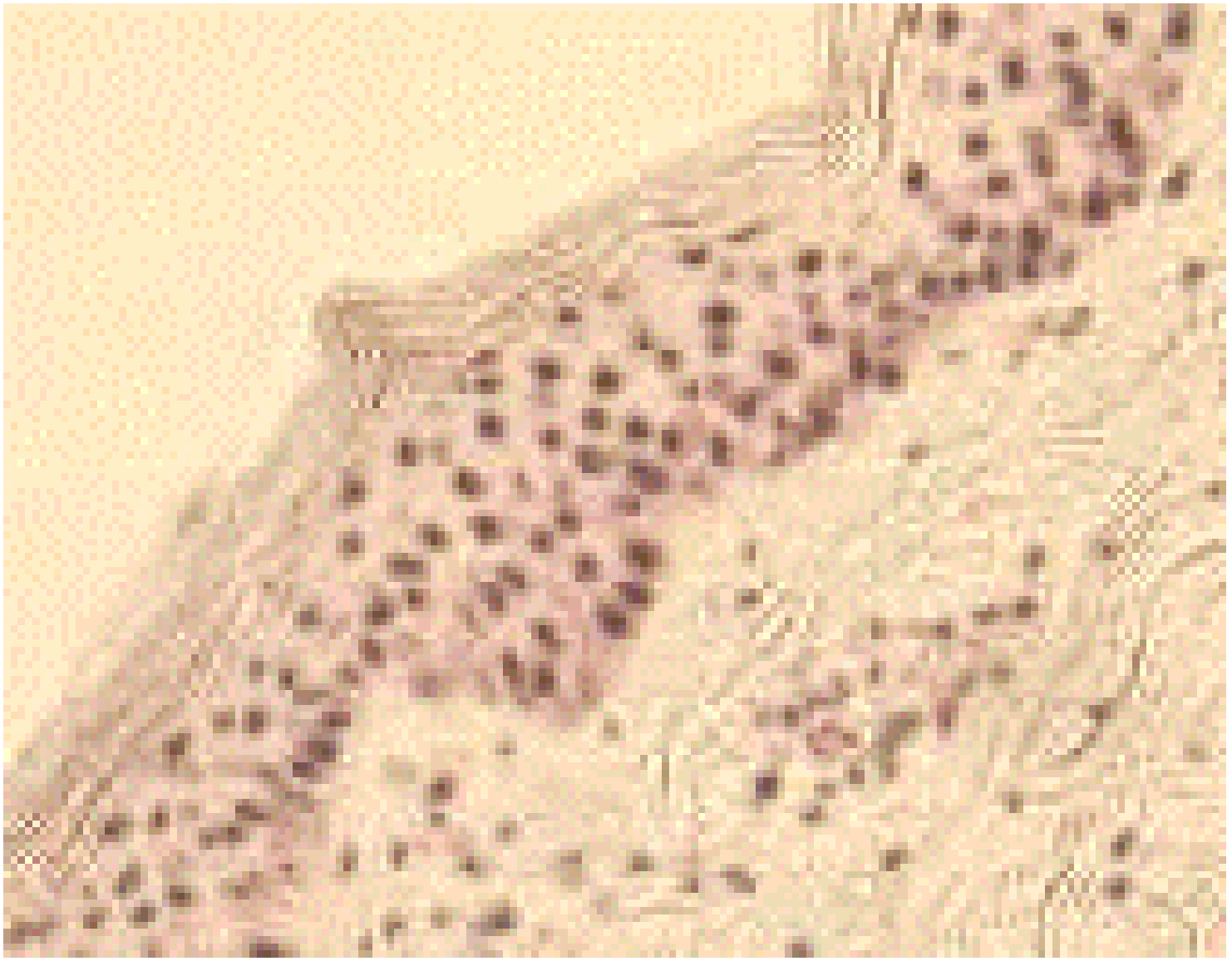


Nucleosome

http://www.accessexcellence.com/AB/GG/nucleosome.gif


3
2
1

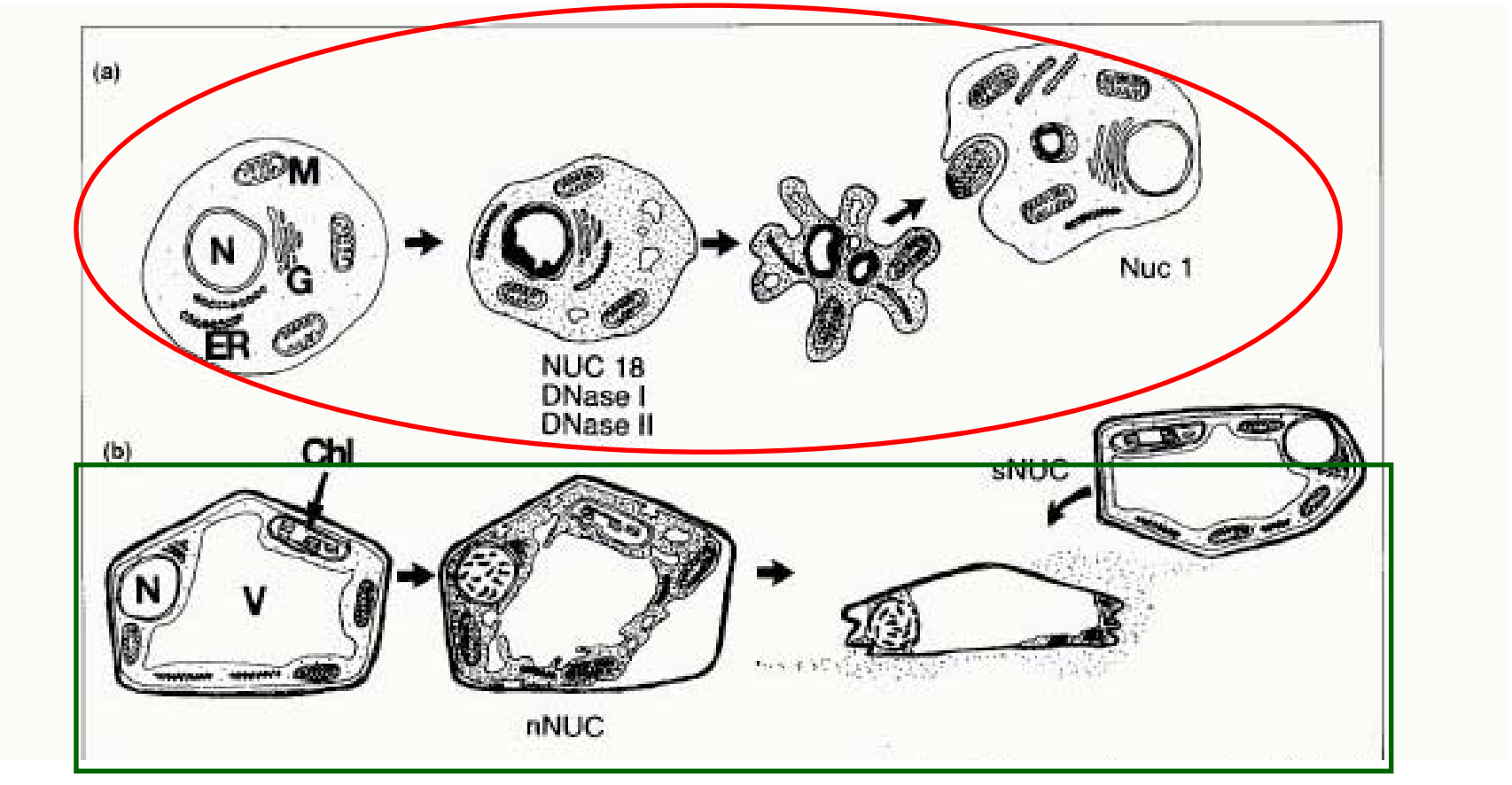
- **TUNEL assay- (Terminal deoxynucleotidyl Transferase Biotin-dUTP Nick End Labeling)**
- **uses terminal deoxynucleotidyl transferase (TdT) to transfer biotin-dUTP to these strand breaks of cleaved DNA. The biotin-labeled cleavage sites are then detected by reaction with HRP conjugated streptavidin and visualized by DAB showing brown color.**



Důležitou roli hrají mitochondrie

- **Leakage of cytochrome C from mitochondria into cytoplasm precedes death**

a výtok cytochromu C z nich.



Živočišná vs. rostlinná buňka = autofágie.

Iniciace PCD je regulována preteázami - kaspázami

- **Regulated by caspases- cysteine proteases**
- **Very specific proteases known- usually no more than 1 or 2 breaks substrate**
- **Orchestrates cell death- eg. Caspase-3 an executioner caspase that starts a cascade**

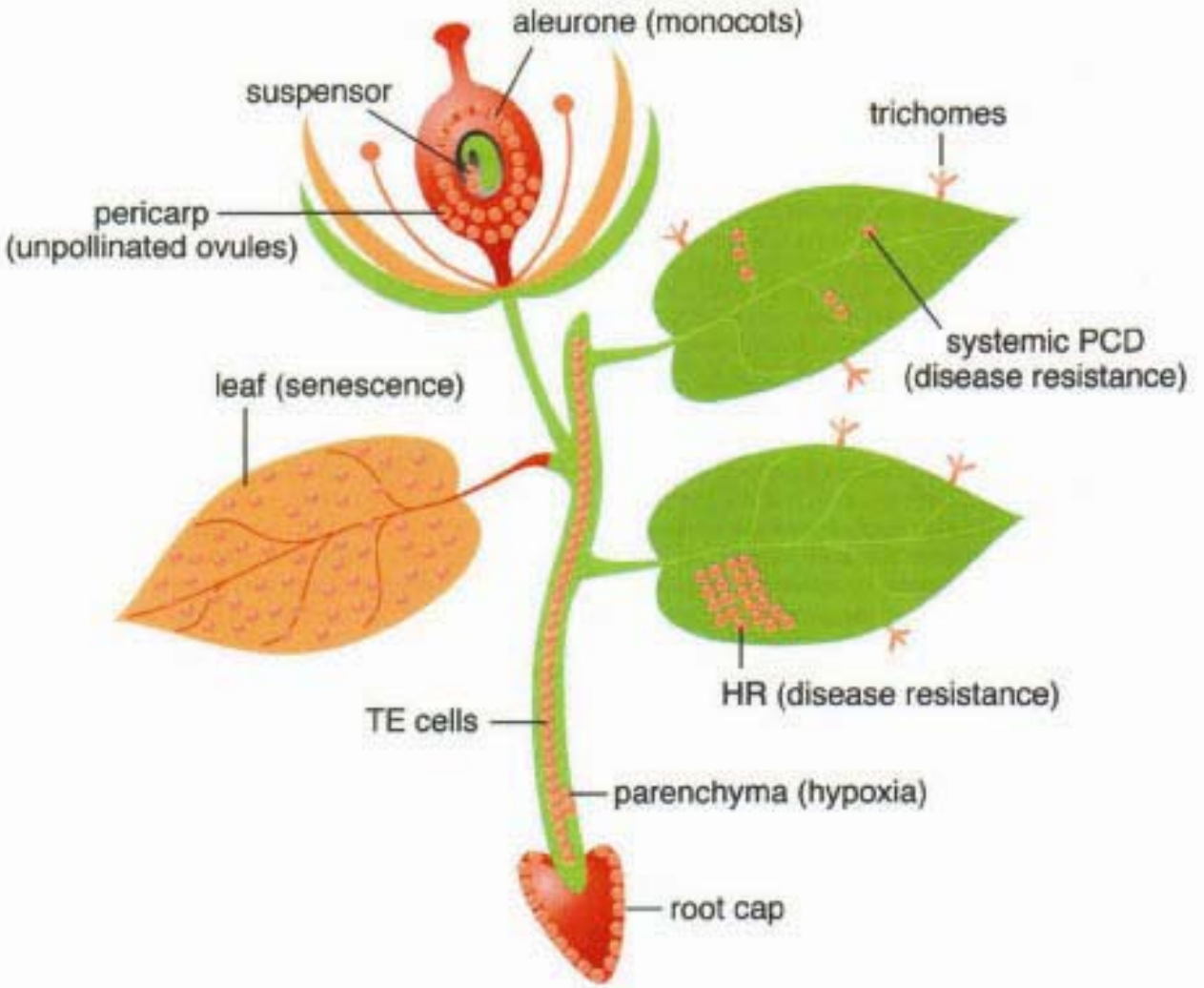


Figure 1. Sites of PCD in a Vascular Plant.

The orange spheres represent internal dead cells, and the branched structures on the leaves represent trichomes.

Hypersensitivní Reakce hypersens. resp. (HR)

- **A hallmark of resistance- non-host, host specific**
- **the rapid death of plant cells in association with the restriction of pathogen growth**
- **Effective against biotrophic fungi, bacteria, and viruses**

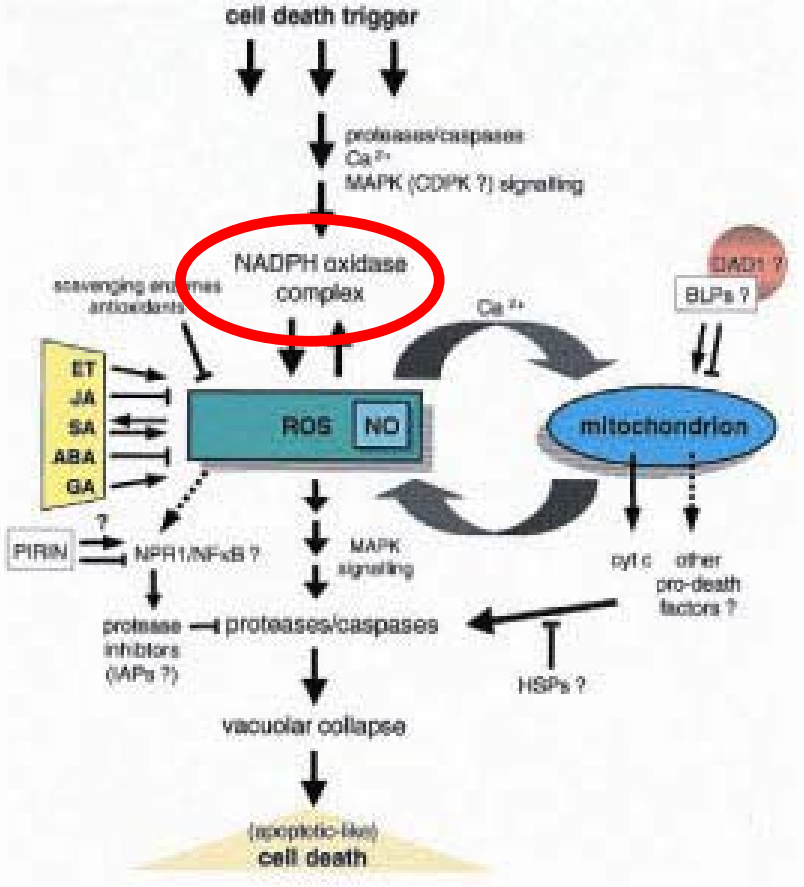
…je nespecifická a širokospektrá

JE HR FORMOU PCD ?

- **Do plant cells show PCD or apoptotic characteristics during infection?**

  • Ryerson and Heath 1998- showed TUNEL and DNA laddering in resistant reaction with soybean rust.

A N O



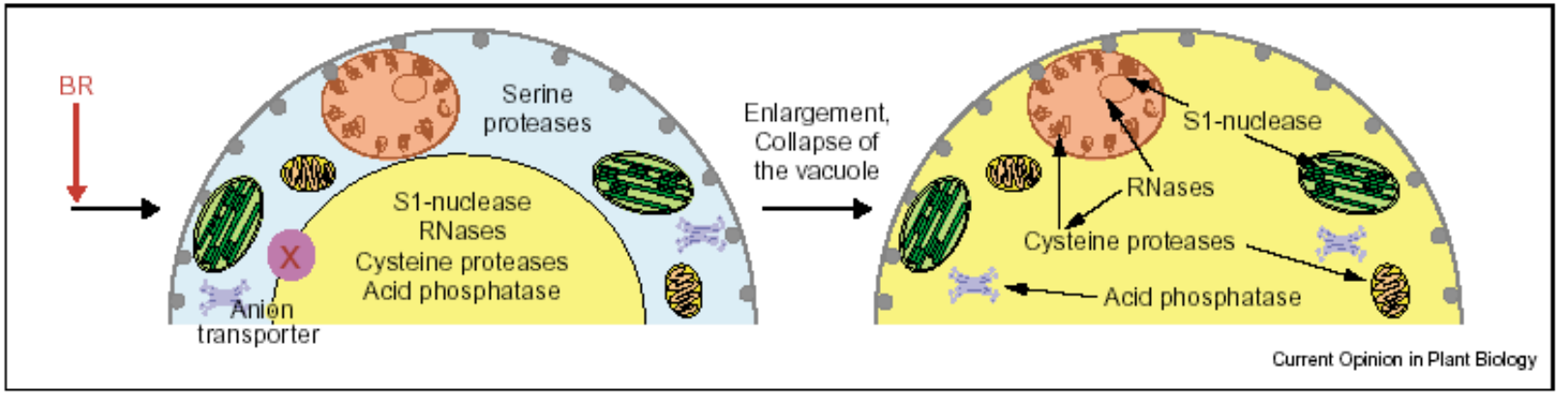
RBOH
respir. burst. ox.

Figure 2. Model of plant programmed cell death. For explanation, see text.

Rop GTPázy všude – i v PCD

Fig. 2. ROP-mediated signaling pathways leading to diverse cellular responses.

VPE - vakuolární kaspázy (Casp.LikeProt.) u rostlin



TE PCD-specific hydrolytic enzymes. Brassinosteroids (BRs) induce PCD, as well as the formation of secondary walls. PCD-specific hydrolytic enzymes, such as an S1-nuclease, RNases and cysteine proteases, are synthesized and accumulate in the vacuole. The transport of organic anions into the vacuole is inhibited, in association with the enlargement of the vacuole. The enlarged vacuole bursts, then shrinks and fragments. The collapse of the vacuole causes hydrolytic enzymes to invade the cytoplasm and to attack various organelles, resulting in the degradation of cell contents and part of the cell walls. Finally, perforation of the wall causes TEs to lose all of their cell contents and to form mature hollow tubes that are reinforced by secondary walls. It takes 20 minutes to lose nuclear DNA after the collapse of the vacuole. Note that hydrolytic enzymes that are located in the cytoplasm are also expressed in association with TE PCD.

Vacuolar **P**rocessing **E**nzyme (VPE) je vakuolární cysteinová proteáza, která štěpí substráty specifické pro kaspázy.

Rostliny s potlačenou expresí VPE mají částečně potlačen také kolaps vakuoly při HR.
Ale PCD je celkem normální u kombinovaných mutantů bez VPE proteáz…

TRANSFORMACE
A GENOVÉ MANIPULACE U ROSTLIN

Hlavní nástroj genetické transformace rostlin je derivátem biotické interakce mezi rostlinami a Agrobakteriemi.

Genetic engineering of plants with *Agrobacterium tumefaciens*

- ***A. tumefaciens:*** used extensively for genetic engineering of plants.
- **Contains T-DNA** (bacterial plasmid)
- Genes colud be integrated into the plant chromosomes when the T-DNA is transferred.

http://courses.washington.edu/z490/gmo/natural.html

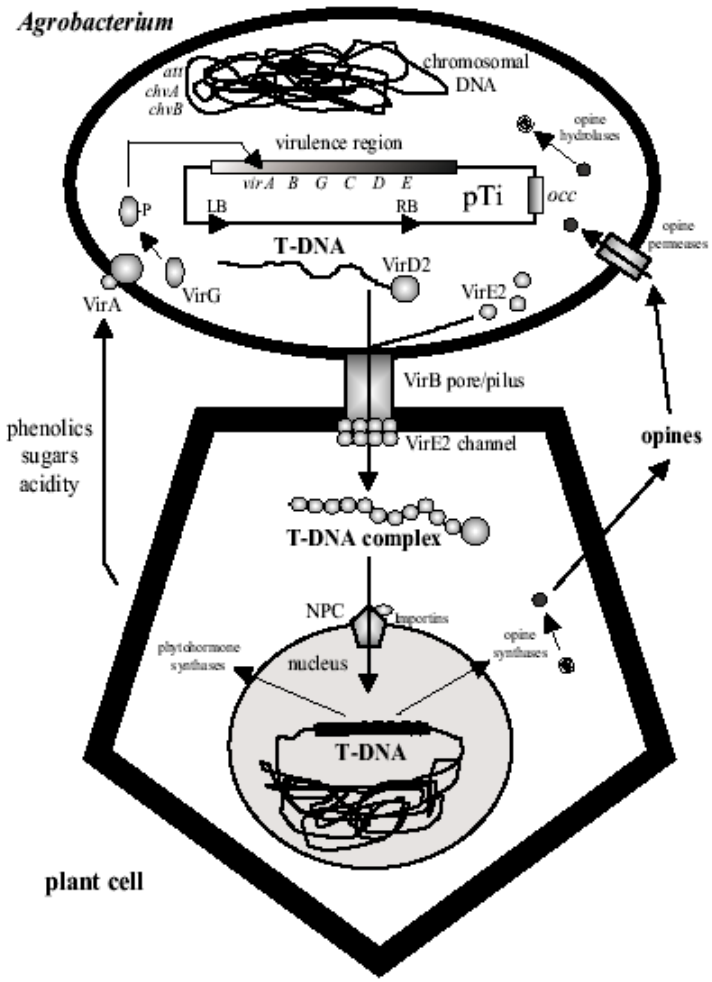


Figure 1. Simpified representation of T-DNA journey from *Agrobacterium* to the plant cell.

LB and RB, left and right border repeats, respectively; NPC, nuclear pore complex. See text for the details.

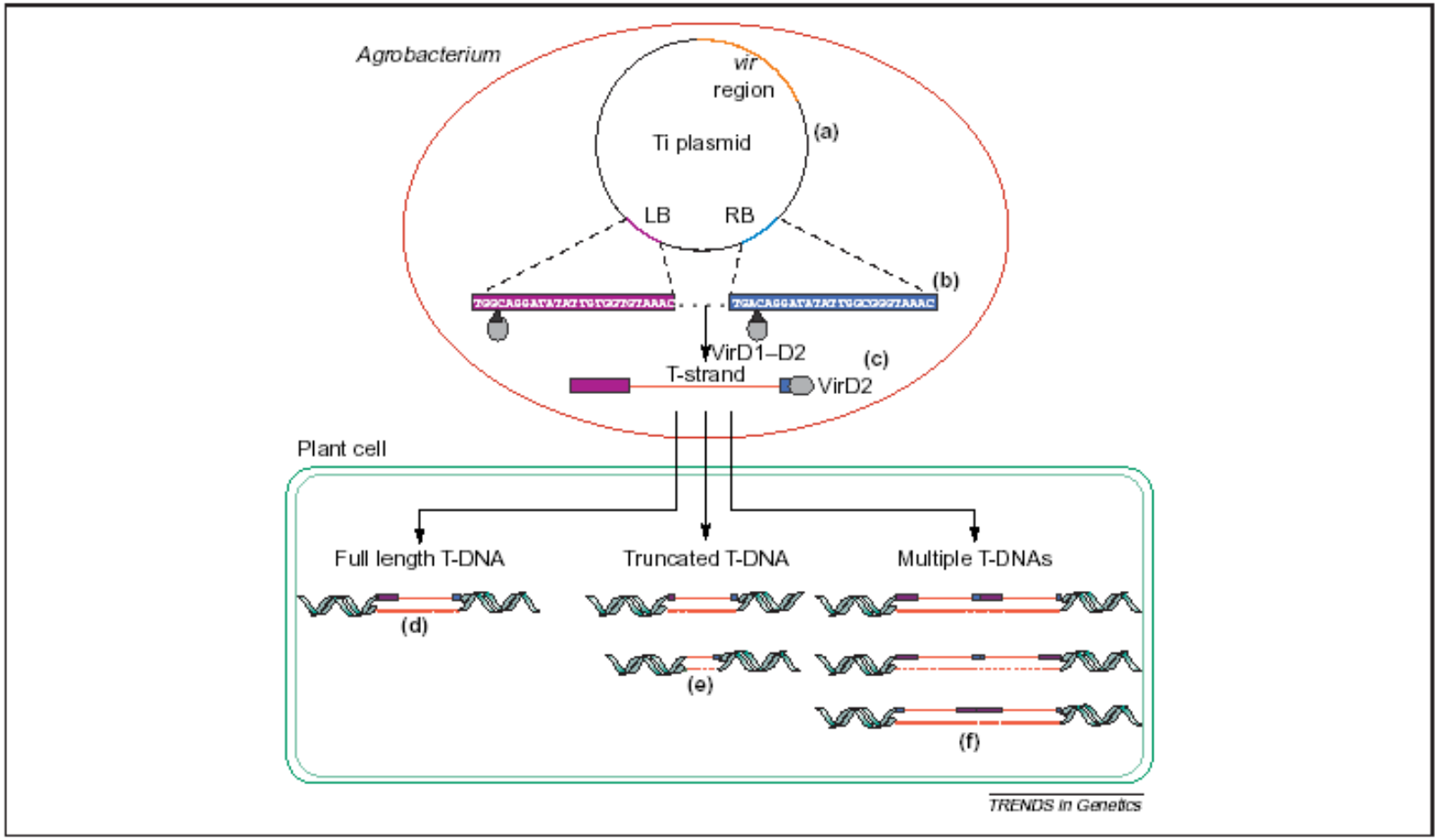


Figure 1. Schematic structure of the *Agrobacterium* Ti-plasmid and mobile and integrated T-DNA molecules. **(a)** The Ti plasmid contains the T-DNA region, which is defined by its right border (RB, in blue) and left border (LB, in purple), *in trans* to the virulence (*vir*) region (orange). **(b)** The T-DNA borders are 25-bp repeats and serve as targets for the VirD2–VirD1 endonuclease complex, which nicks between the third and the fourth nucleotides of each of the border sequences [15,71,72]. **(c)** The mobile copy of the T-DNA is released as single-stranded (ss) DNA molecule (T-strand) with a single VirD2 molecule attached to its 5′-end. The residual sequences of the T-DNA borders, which no longer have biological function, are often used as reference points for T-DNA orientation and integrity in the plant cell. T-DNA typically integrates into the host genome **(d)** as a single full-length or **(e)** truncated molecule in addition to **(f)** multiple molecules ligated to each other in various orientations.

BINÁRNÍ VEKTORY

- Vir oblast je na zvláštním vektoru/plasmidu I..
- T-DNA s R a L hranicí zbavena původní genové výbavy a nesoucí námi zvolený ektopický gen je na dalším vektoru/plasmidu II., jehož hostitelem je nejen *Agrobacterium*, ale také *E.coli.*

U Arabidopsis je možná vysoce účinná transformace ponořením mladých květenství do suspenze Agrobacteria. Dochází posléze k transformaci zárodečných vaků a vzniku heterozygotních transformantů po opylení. Účinnost je kol. 2%.

POZNÁMKY A OTÁZKY K ROSTLINNÝM BIOTECHNOLOGIÍM