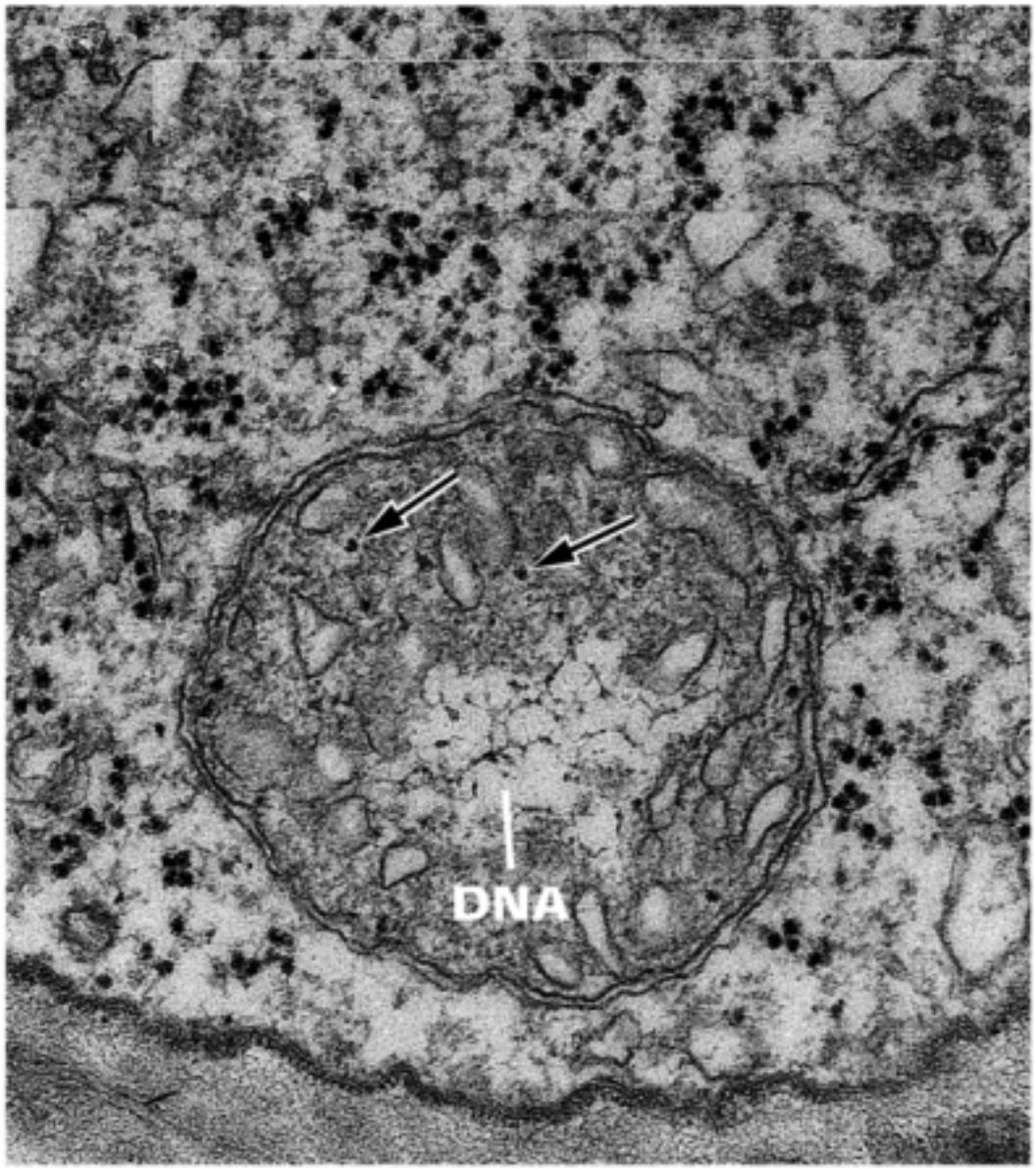
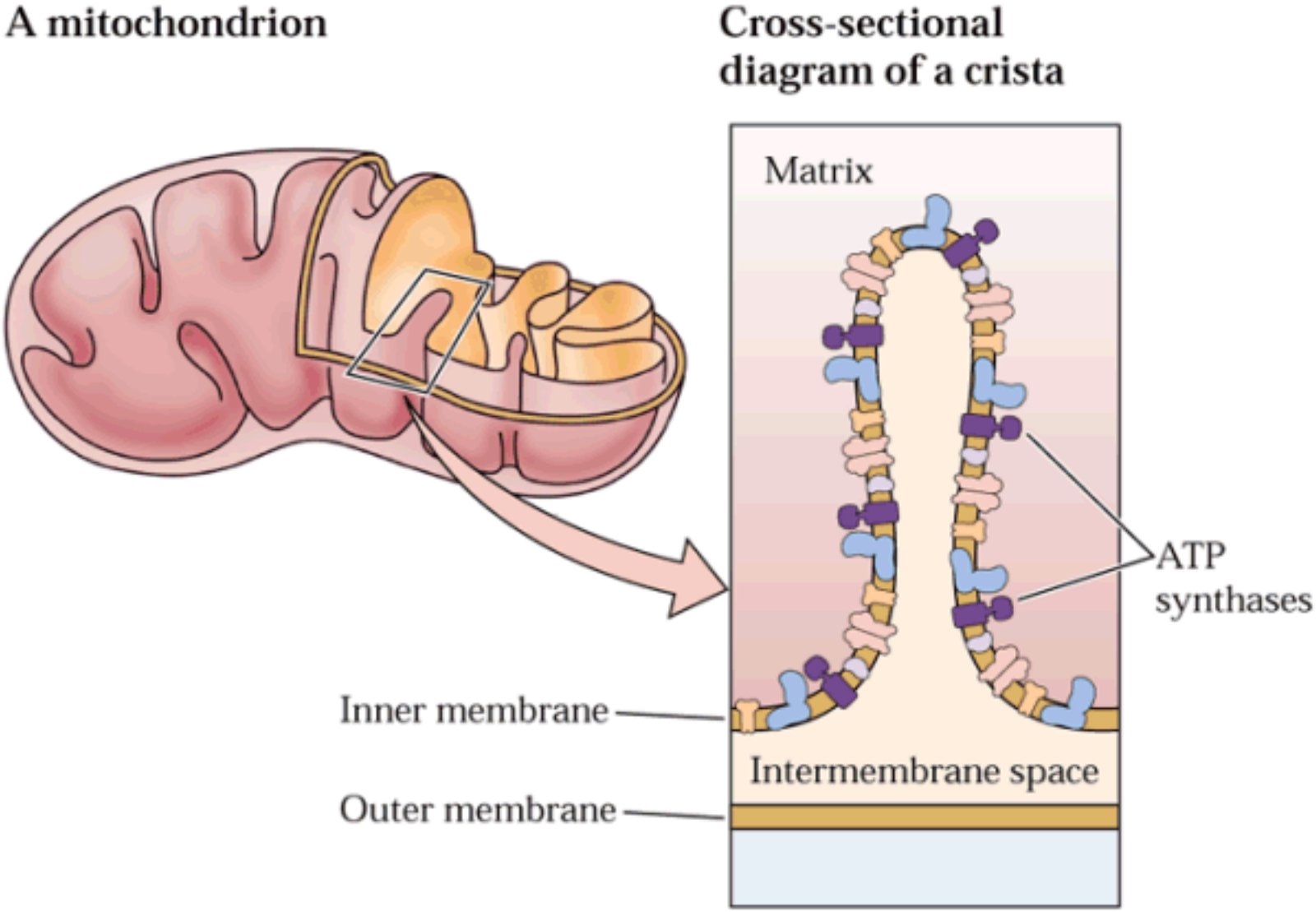
MITOCHONDRIE

PEROXISOMY

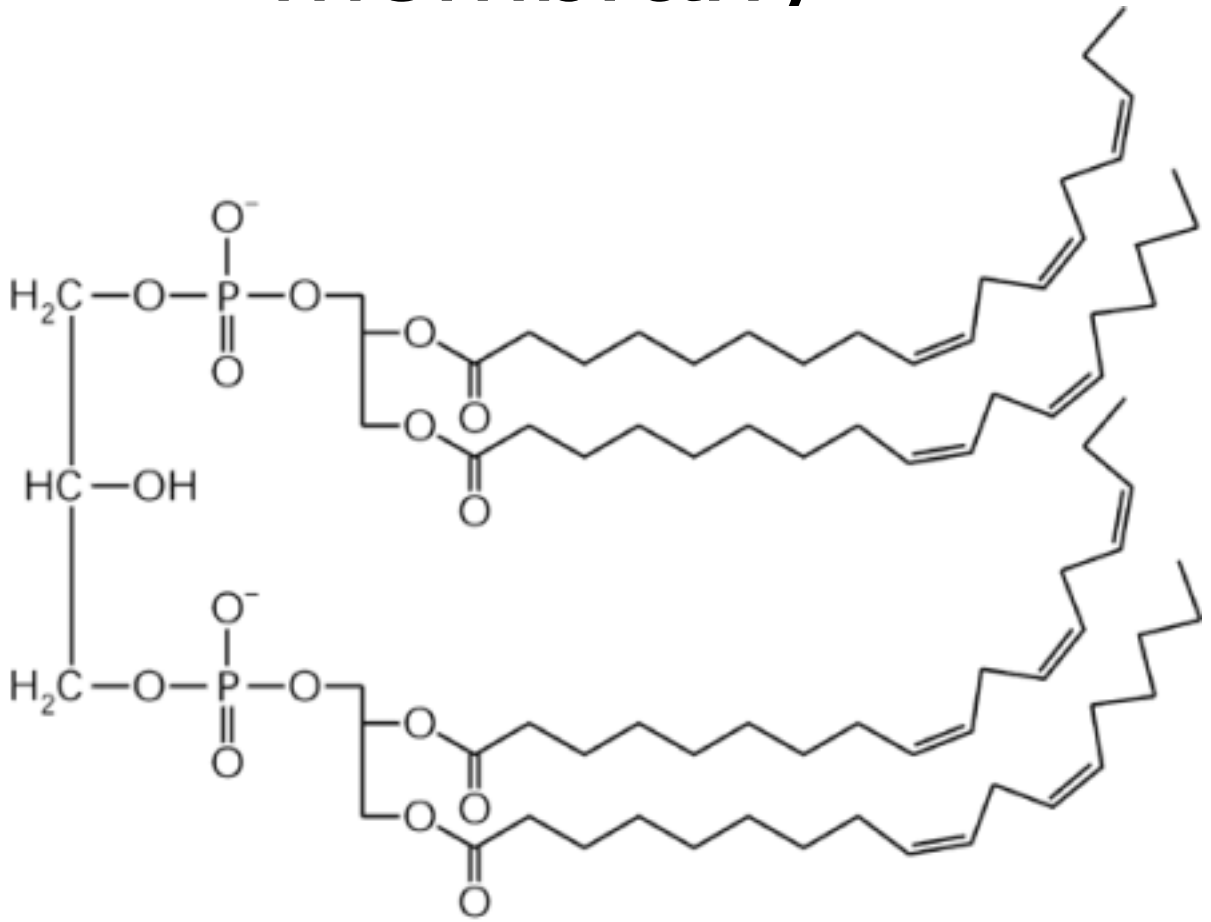
a

interakce kompartmentů


DNA


A mitochondrion
Cross-sectional diagram of a crista
Matrix
ATP synthases
Inner membrane
Intermembrane space
Outer membrane

Specifický lipid vnitřní mitoch. membrány



Diphosphatidylglycerol
(cardiolipin)

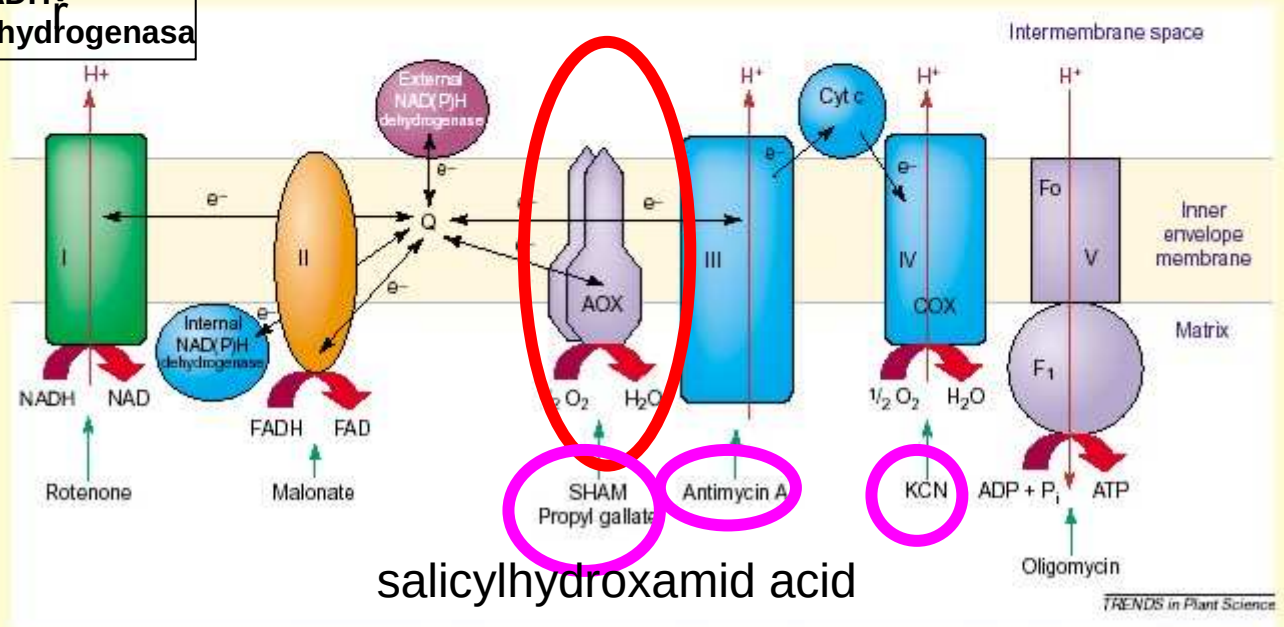
Komplexy vnitřní membrány rostlinných mitochondrií – dva systémy přenosu e-

Box 1. Cytochrome oxidase and alternative oxidase pathways of mitochondrial electron transport

The oxidative electron transport in mitochondria of higher plants uses two different pathways (Figure I) – the cyanide-sensitive cytochrome oxidase (COX) pathway and the cyanide-insensitive alternative oxidase (AOX) pathway [23,74,75]. The COX pathway accomplishes most of the ATP production in plant mitochondria, whereas energy tends to be dissipated as heat through the AOX pathway [76]. The operation of the AOX pathway is dynamic and depends on the environmental conditions [74]. AOX plays an important role in integrating the processes of carbon metabolism and mitochondrial electron transport, particularly when there is an accumulation of reduced equivalents and pyruvate (e.g. under phosphate deficiency) [23]. However, the physiological function of the AOX pathway in green tissues is not clear, although it is known to increase under stress conditions [23,74].

Experiments with inhibitors suggested that the mitochondrial pathway through COX and AOX is essential for photosynthesis [9,31]. The AOX pathway can play an important role in protecting chloroplasts against photoinhibition, by dissipating the excess redox equivalents from chloroplasts. Interestingly, the extent and engagement of AOX seems to increase when the cytosol and mitochondria are over-reduced, as happens in the light [44]. Furthermore, several pieces of evidence suggest the pronounced operation of the AOX pathway in light in green tissues: (i) increased electron flow through the AOX pathway during glycine oxidation [10,42]; (ii) synthesis of AOX protein during greening [77]; (iii) increased electron flux through the AOX pathway in light and its decrease on transition to dark [78]. Most of the experiments that suggest the importance of AOX in maintaining the redox state and optimizing photosynthesis have been conducted using salicylhydroxamic acid, whose reliability is frequently questioned [6]. However, experiments with a stable oxygen isotope revealed that ~60% of mitochondrial respiration in light occurred through AOX [78]. The concept of the participation of the AOX pathway in modulating photosynthesis or photoinhibition needs to be re-examined using other approaches, such as mutants or transgenics with altered AOX protein levels [23] and factors that modulate AOX, such as temperature [46].

NADH dehydrogenasa



salicylhydroxamid acid

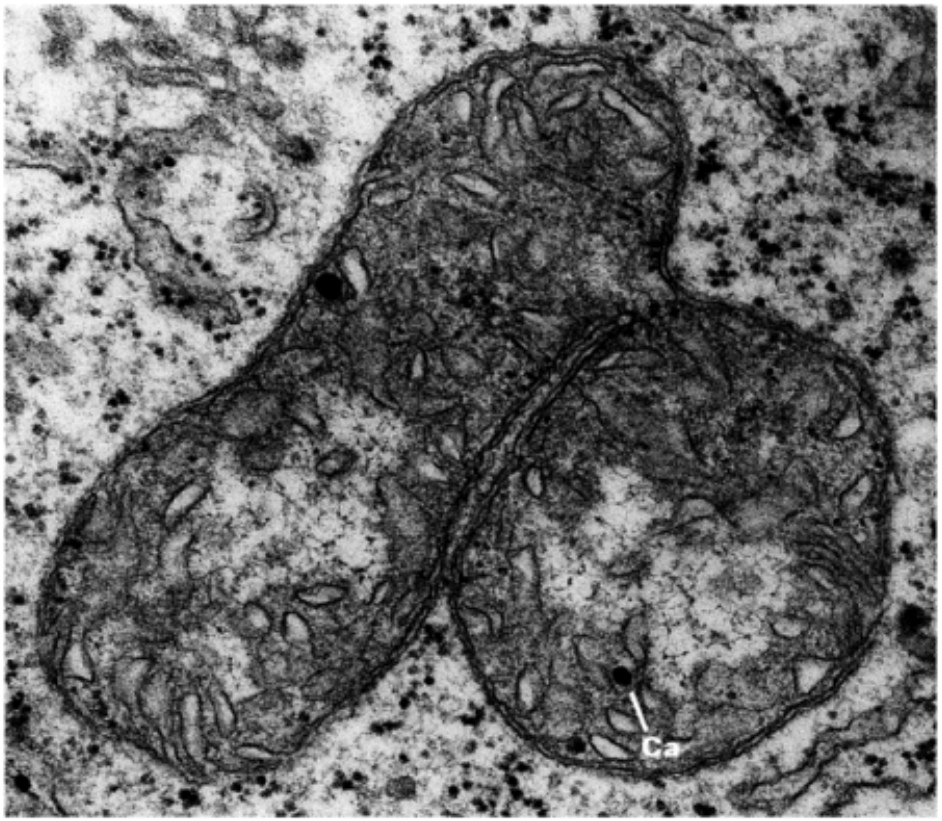
Figure I. Mitochondrial oxidative electron transport and its typical inhibitors. Abbreviations: AOX, alternative oxidase; COX, cytochrome oxidase; Cyt c, cytochrome c; SHAM, salicylhydroxamic acid.

AOS

- **ALTERNATIVNÍ OXIDÁZA**

Význam této respirační dráhy je vedle výjimečných případů produkce tepla (Arum) pravděpodobně v možnosti **reagovat na stres antioxidatčním působením**, „upouštět“ přebytečnou energii a udržet v chodu aerobní glykolýzu, pentózový a Krebsův cyklus (tj. anabolické fce mitochondrií). AOX je aktivní v případě silně reduktivní situace – např. běží-li silně fotosyntéza.

Mitochondrie se dělí „zaškrcením“ podobně jako plastidy



Vnitřní prstýnek - FtsZ1 a 2 (vzdál. prokar. příb. tubulinu GTPdep. polymerace – u krytosemenných není) a spol. Vnější prstýnek – dynamin.

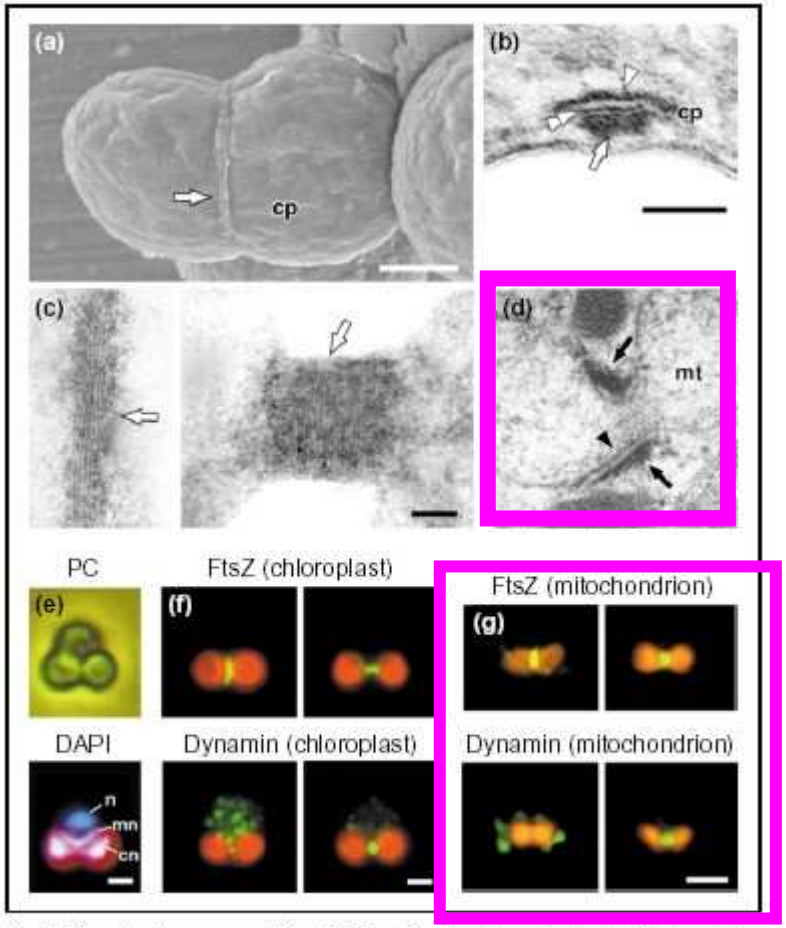


Fig. 1. Ring structures around the division site of a chloroplast and mitochondrion in the red alga *Cyanidioschyzon*. (a) A scanning electron micrograph of an isolated dividing chloroplast. (b) Magnified cross-section of the plastid-dividing (PD) ring obtained by transmission electron microscopy. The PD ring is composed of an outer ring (on the cytosolic side of the outer envelope), a middle ring (in the inter-membrane space), and an inner ring (on the stromal side of the inner envelope) [24,25]. (c) Electron micrographs of the outer PD ring obtained by treating isolated

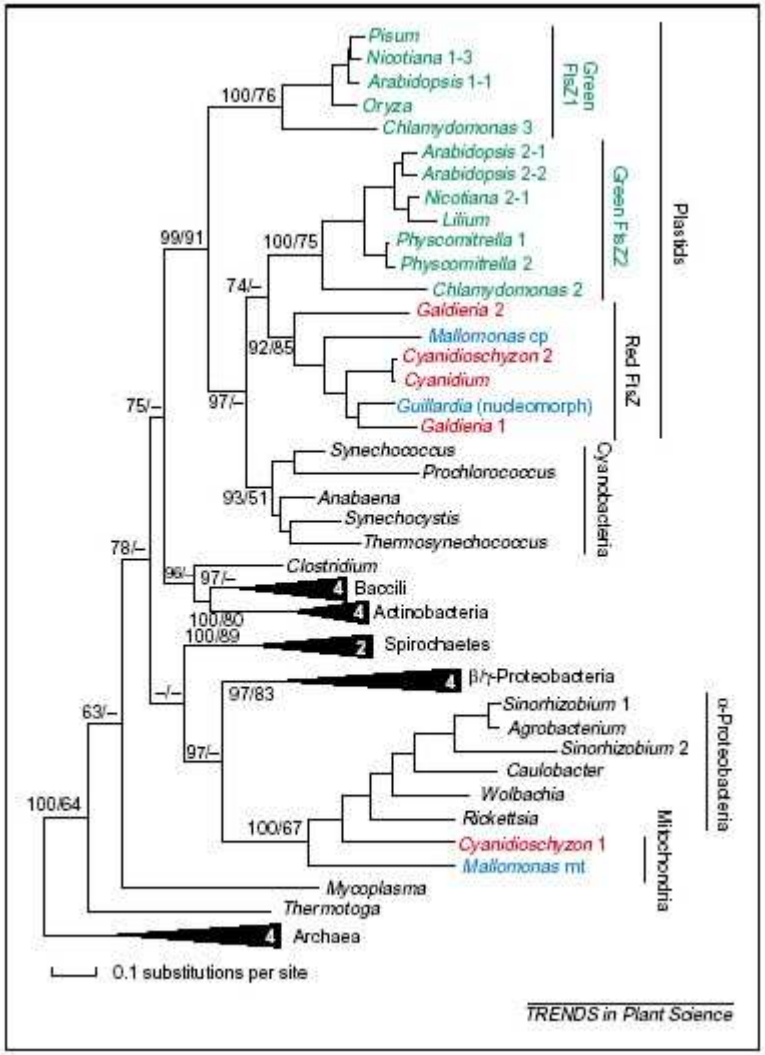


Fig. 2. Phylogeny of FtsZ, showing the origins of eukaryotic homologs. Some

Organizace mitochondriálního genomu suchozemských rostlin

208-2400 kb depending on species

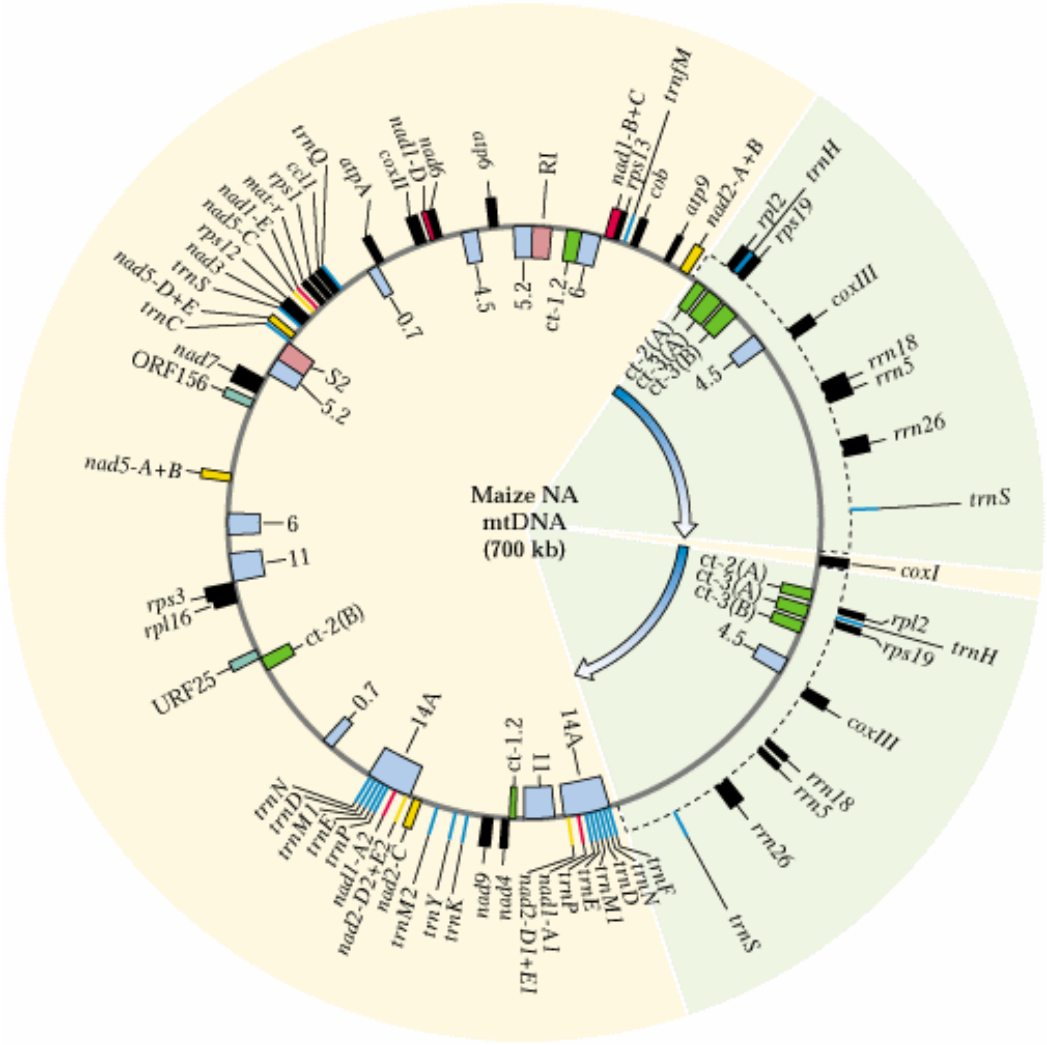
Relatively constant coding but highly variable organization among and even within a species

Entire complexity maps as a single “master circle”

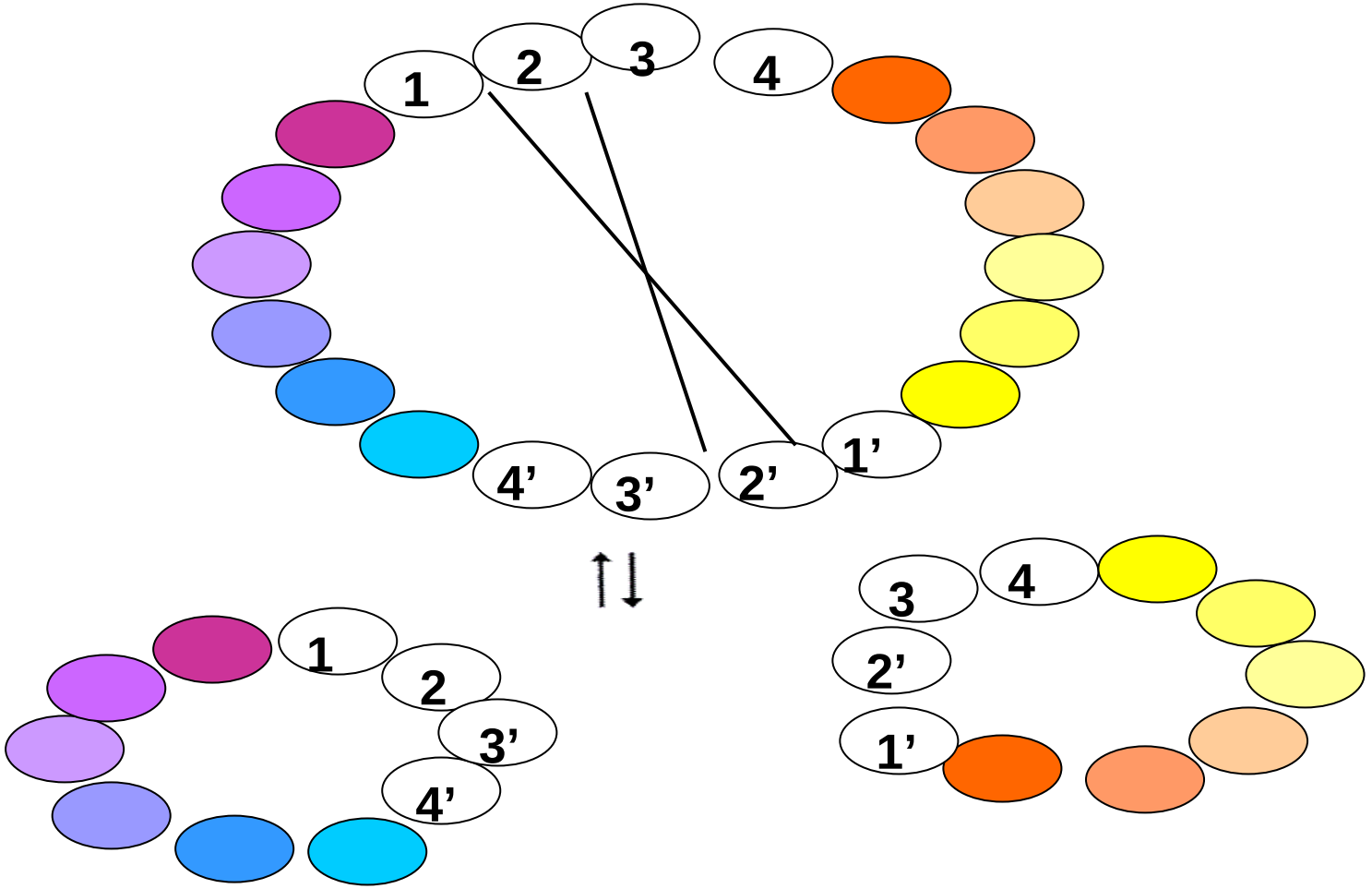
All angiosperms except *Brassica hirta* have one or more **recombination** repeats.

Repeats not conserved among species
Direct and/or inverted orientations
Recombination generated inversions
(inverted repeats)
Recombination generated **subgenomic**
molecules (deletions) (direct repeats), some present at very low copy number (sublimons)
Leads to complex multipartite structures

Architektura rostlinné mtDNA je velmi variabilní – př.kukuřice

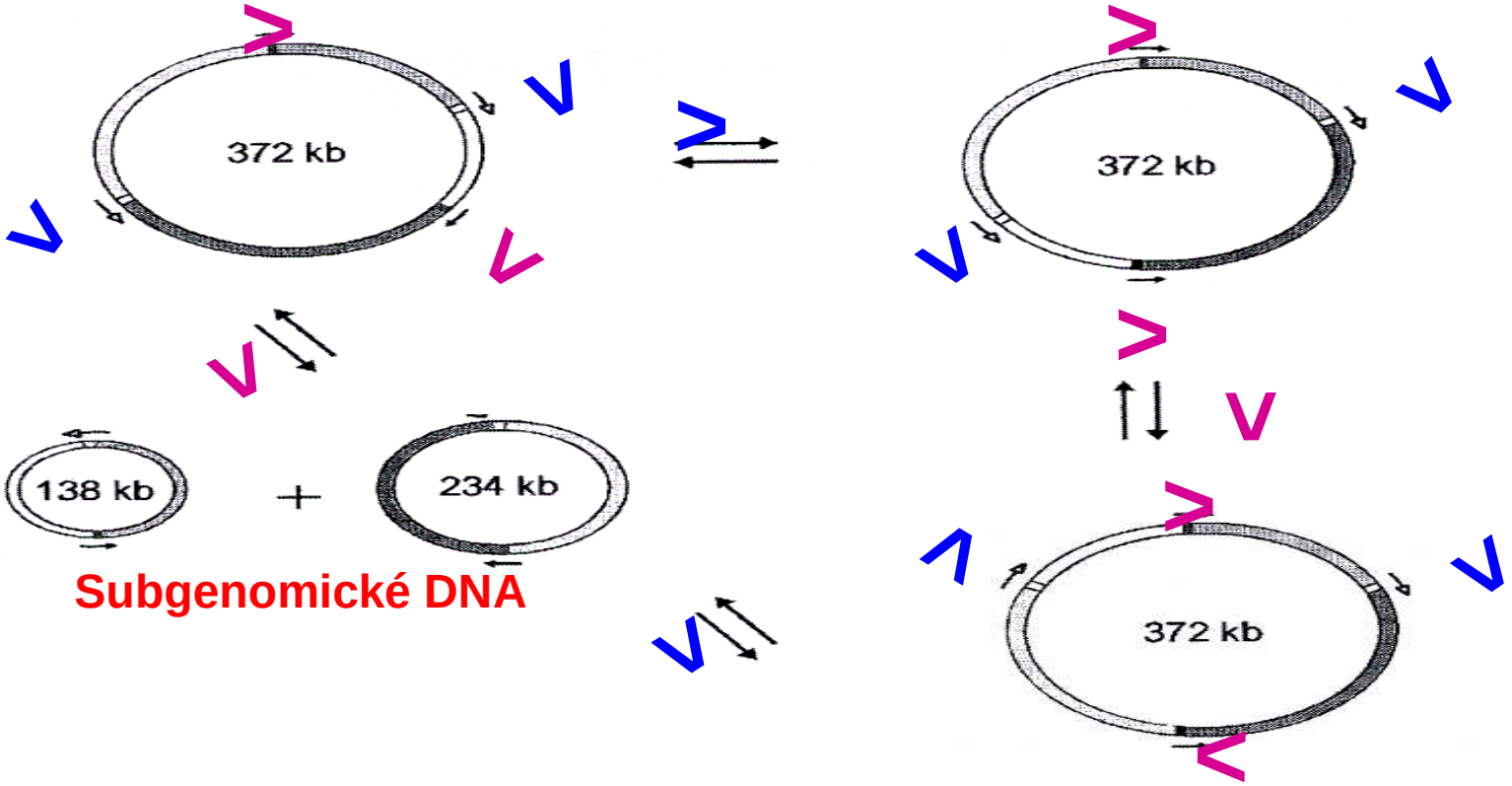


Rekombinace napříč opakováními vede k delecím a fragmentacím



Uspořádání genomu mitochondrií Arabidopsis.

(modified from Backert et al. Trends Plant Sci 2:478)



Subgenomické DNA

Fig. 1. Proposed multipartite organization model of the mitochondrial genome of *Arabidopsis*. The entire genome of 372 kb is contained in the circular master chromosome, which occurs in three different arrangements that are in balance with two subgenomic circles of 138 and 234 kb. These five circular molecules are generated by recombination across two sets of repeats. The position and direction of the repeats are indicated by open and filled arrows. *Modified from Ref. 40.*

Fyzická struktura mitochondrialní DNA rostlin

(Backert et al. Trends Plant Sci 2:478)

Conclusions

No discrete size classes

Circles including smaller than predicted

Linears including longer than predicted

Potential Mechanisms

Rosettes

- Nucleoid complexes
- Recombination complexes

Long linear molecules and sigma molecules

- Rolling circle replication
- Recombination

Branched linear molecules

- Recombination or replication of linear molecules

Small circles

- Recombination across short direct repeats

Questions

How is the genome stably inherited from cell to cell and generation to generation?

Fyzická struktura mitochondrialní DNA rostlin
(modified from Backert et al. Trends Plant Sci 2:478)

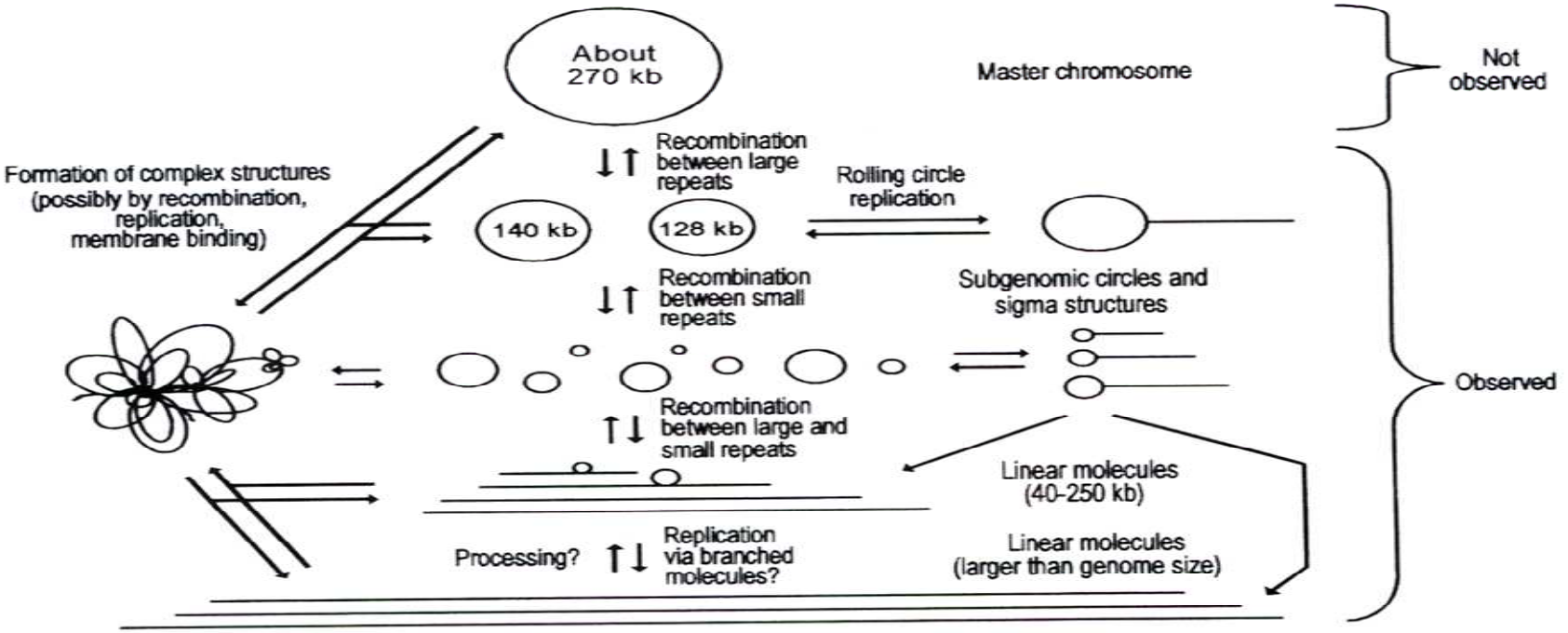
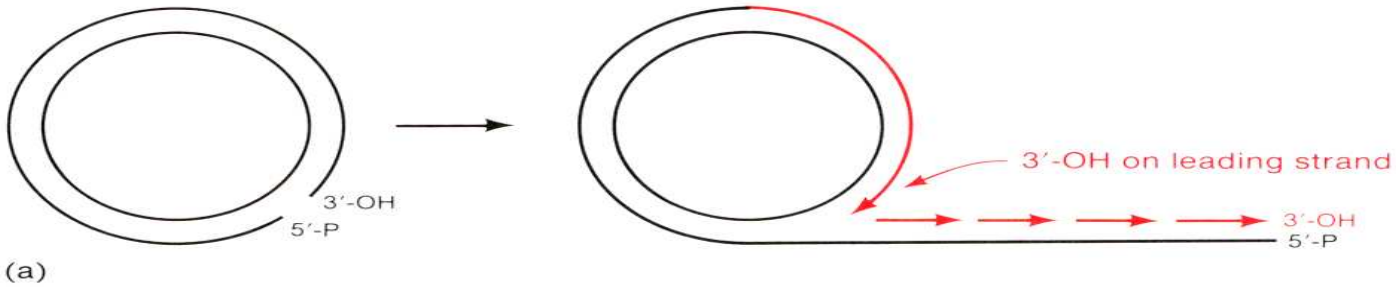


Fig. 2. Hypothetical model of the structural organization and replication of the mitochondrial DNA in *Chenopodium album*. Circular molecules (including circles with tails, which comprise 13–26% of the molecules, about one third of which are represented by plasmid *mp1*), linear molecules (56.5–81.5%) and more complex molecules (4.2–17.5%) are expected to exist in dynamic equilibrium as observed in electron microscopic studies[12]. The organization of DNA may be more simple or even more complicated in the mitochondria of other species. The enormous diversity in the size of molecules (including molecules larger than the putative master chromosome, such as oligomers and concatemers), and in their shape, may be generated by various mechanisms: inter- and intramolecular recombination events between large and small repeats; the formation of higher ordered (nucleoid-like rosette) structures; and specific types of replication.

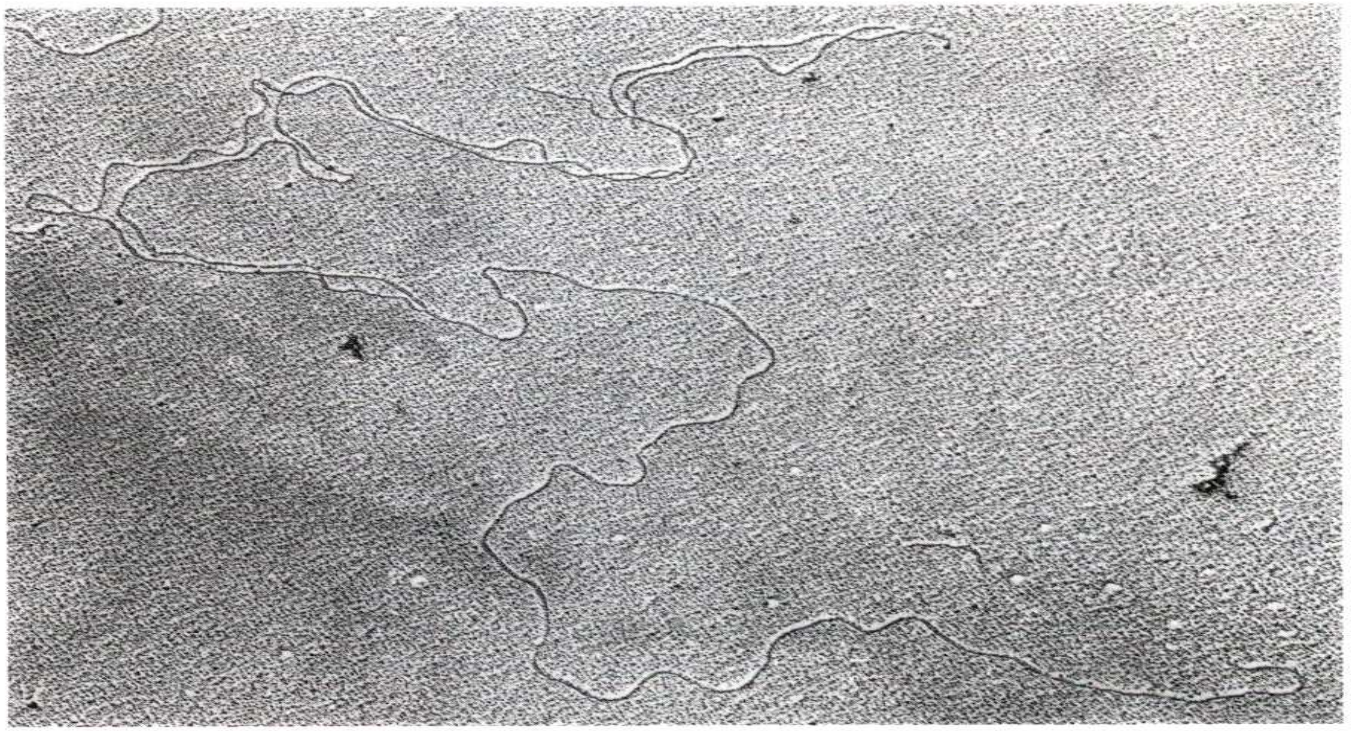
Fyzická struktura mitochondrialní DNA rostlin

via rolling circle DNA replication

(from Freifelder, 1983, Molecular Biology)



(a)



(b)

Figure 8-39
(a) Rolling circle or σ replication. Newly synthesized DNA is shown in red. (b) An electron micrograph of a rolling circle of phage λ DNA isolated from phage-infected *E. coli*. The length of the branch is 15.2 μm. (Courtesy of Marc Better.)

Kódovací potenciál genomu rostlin

***In organello* protein synthesis indicates 30-50 proteins encoded by most plant mitochondrial genomes**

Complete sequence of *A. thaliana* mitochondrial genome identified **57 genes**

respiratory complex components
rRNAs, tRNAs, ribosomal proteins
cytochrome c biogenesis

Plant mitochondrial genomes **do not encode a complete set of tRNAs**

mit encoded tRNAs of native (mitochondrial origin)
mit encoded tRNAs from imported plastid genome
missing tRNAs are nuclear encoded and imported into mitochondria to complete the set

42 orfs in *A. thaliana* mit genome that might be genes

A. thaliana mitochondrial gene density (1 gene per 8 kb) is lower than the nuclear gene density (1 gene per 4-5 kb)!

Jak mitochondrie regulují expresi jaderných genů.

Příklad alternativní oxidázové dráhy v mitochondriích a ROS.

Mitochondrial regulation of plant nuclear genes

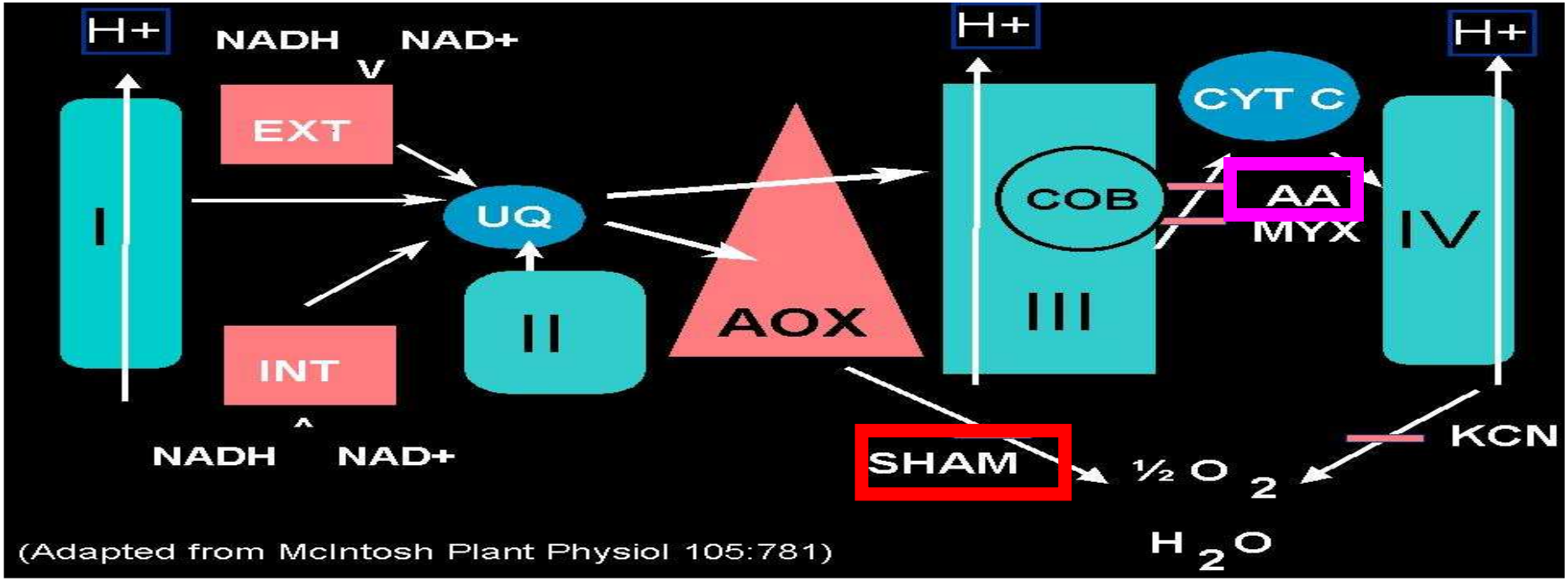
Plant mitochondrial respiratory electron transfer chain includes an alternative pathway for electron flow

Single subunit alternative oxidase (AOX) – je rezistentní ke kyanidu! A je inhibována Salicylhydroxamovou kyselinou (SHAM).

Encoded by a nuclear gene (*aox*)

Bypasses two of three sites for H+ transfer coupled to ATP synthesis

Transcription of nuclear *aox* is upregulated when electron flow through the cytochrome pathway is disrupted by the inhibitor antimycin A (AA)



Jak mitochondrie regulují jadernou genovou expresi.

NtAI genes (Maxwell et al. Plant J 29:267)

Nuclear genes up-regulated in response to AA, including ***aox***

Seven additional genes identified by differential mRNA display, most associated with stress responses

acc oxidase	glutathione S transferase
Sar8.2	cysteine protease
pathogen-induced lipase	SA-induced glucosyl transferase

Also induced by reactive oxygen species (ROS) (eg H_2O_2)

Induction is **blocked by antioxidants** such as flavones

Of all inducers, AA is the most rapid. This implicates **mitochondria as the site coordinating ROS signaling in the plant cell**

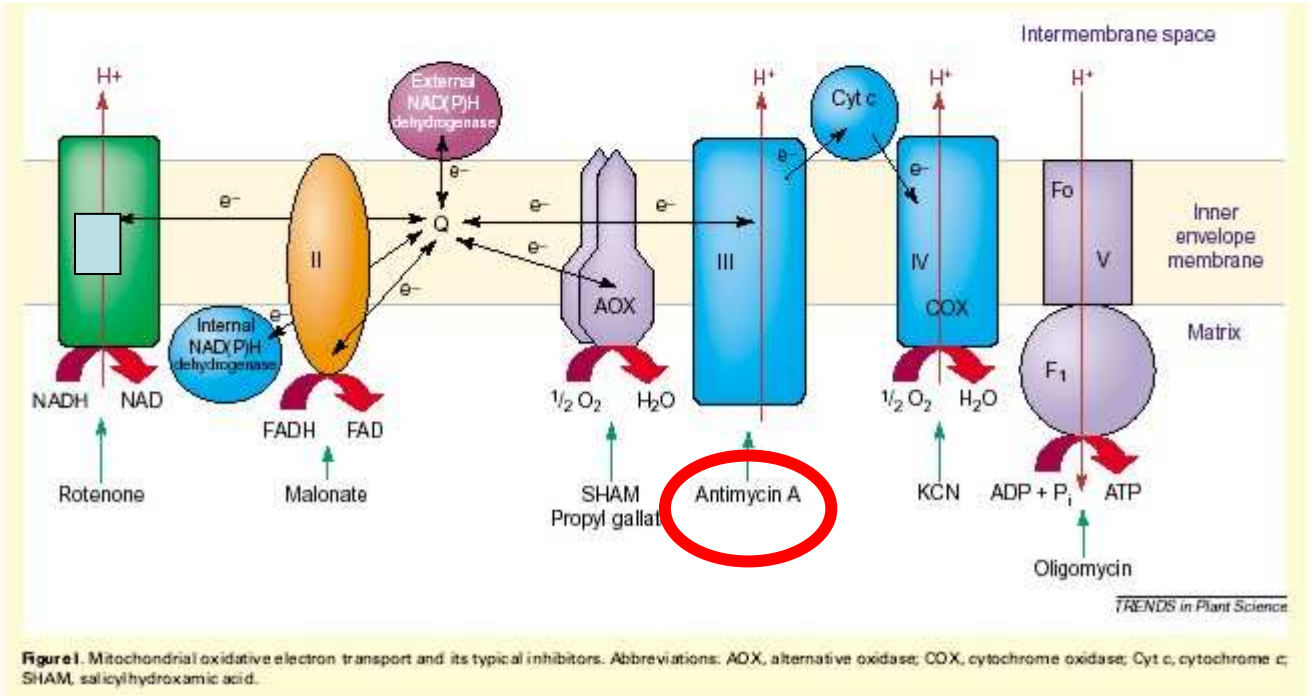
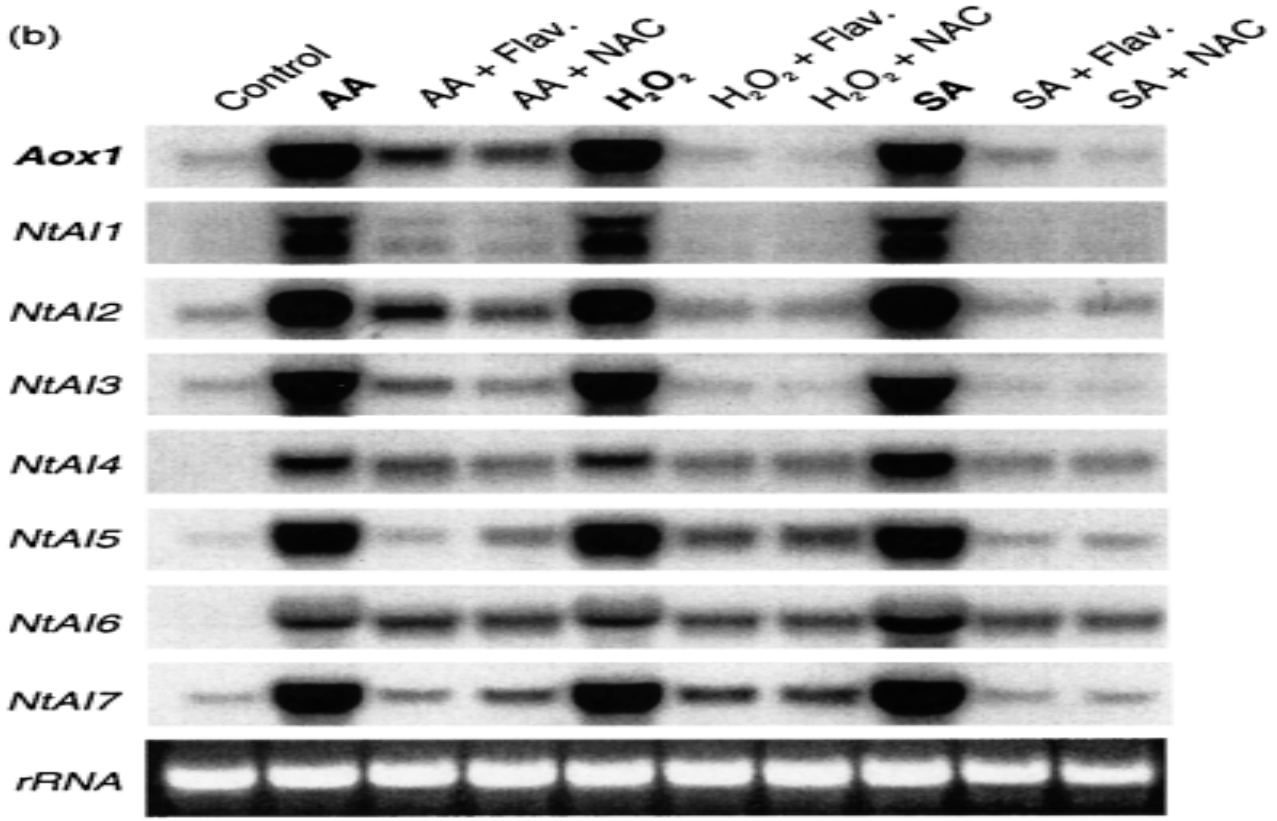


Figure I. Mitochondrial oxidative electron transport and its typical inhibitors. Abbreviations: AOX, alternative oxidase; COX, cytochrome oxidase; Cyt c, cytochrome c; SHAM, salicylhydroxamic acid.

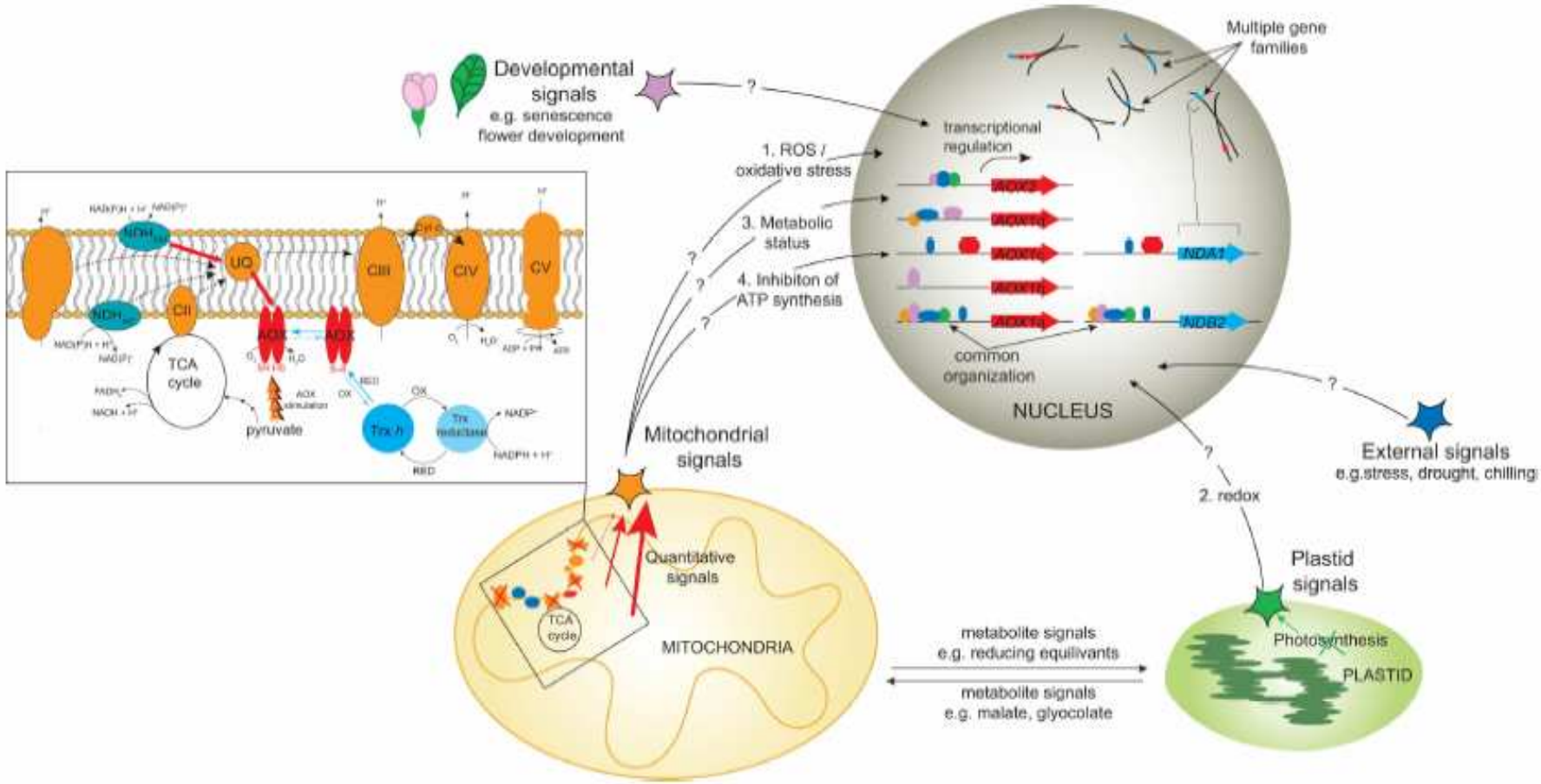
Mitochondrial regulation of plant nuclear genes

NtAI genes (Maxwell et al. Plant J 29:267)



NAC - N-acetylcystein a flavon = antioxidanty; AA = antimycin A

Figure 3. Antioxidants lower intracellular ROS levels and inhibit gene induction.
(a) Effects of antioxidant addition on AA, H_2O_2, and SA-dependent accumulation of intracellular ROS in tobacco suspension cells. ROS levels were measured 4 h after AA (5 µm), H_2O_2 (5 mm) and SA (1 mm) addition with and without preincubation for 45 min with N-acetylcysteine (25 mm) or flavone (1 mm). Data represent means ± SD for three experiments.
(b) Effect of the antioxidant treatment described above on the AA-, H_2O_2-and SA-dependent expression of *Aox1* and the *NtAI* genes.



This diagram depicts the potential signalling pathways that influence the expression of *AOX* in a gene-specific manner. Signals originating within the cell from mitochondrial or plastidic function interact with external signals from organ, developmental or environmental stimuli to activate expression of AOX. These inputs are processed via the promoters of genes where transcription factors involved in these signal transduction pathways converge to activate gene expression. The components involved in transducing the signals which ultimately result in altered expression are unknown. These pathways may interact to affect the magnitude and/or Aox gene induced. Abbreviations: CI = complex I, CII = complex II, CIII = complex III, CIV = Complex IV, CV = Complex V, AOX = alternative oxidase, NDH = alternative type II NAD(P)H dehydrogenase, INT = internal, EXT = external, ROS = reactive oxygen species.

Editování mRNA v mitochondriích.

0.5 až 5% pozic bývá modifikováno.

celkově jsou editovány stovky pozic – kol. **450 x** pro Arabidopsis

(pro připomenutí v plastidech je to jen 19x)

Editování mitochondriální RNA

Evidence for the importance of cis-guiding sequences in plant mitochondrial RNA editing

Editing of recombinant or rearranged mitochondrial genes

Recombination breakpoint immediately 3' to an editing site in rice *atp6* did ***not*** abolish editing

Recombination breakpoint seven nucleotides 5' to an editing site in maize *rps12* did abolish editing

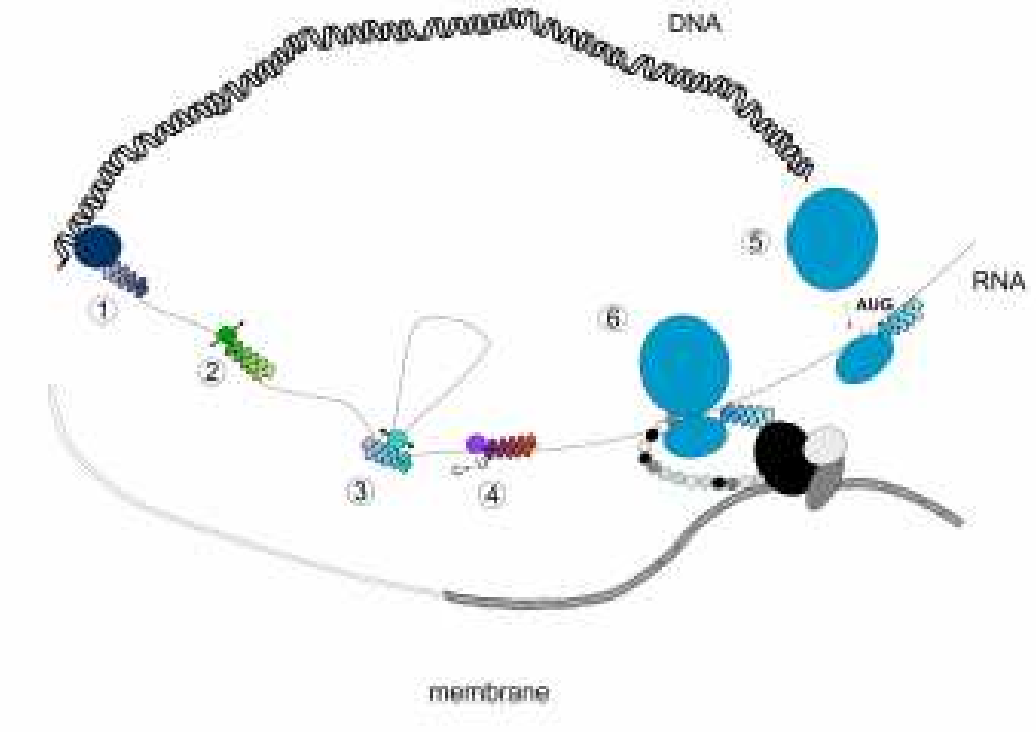
Recombination breakpoint 21 nucleotides 5' to an editing site in maize *rps12* did ***not*** abolish editing

Electroporation of genes into isolated mitochondria, followed by isolation of mitochondrial cDNA

Editing of mutated *coxII* gene demonstrated sequences from –16 to +6 required for editing

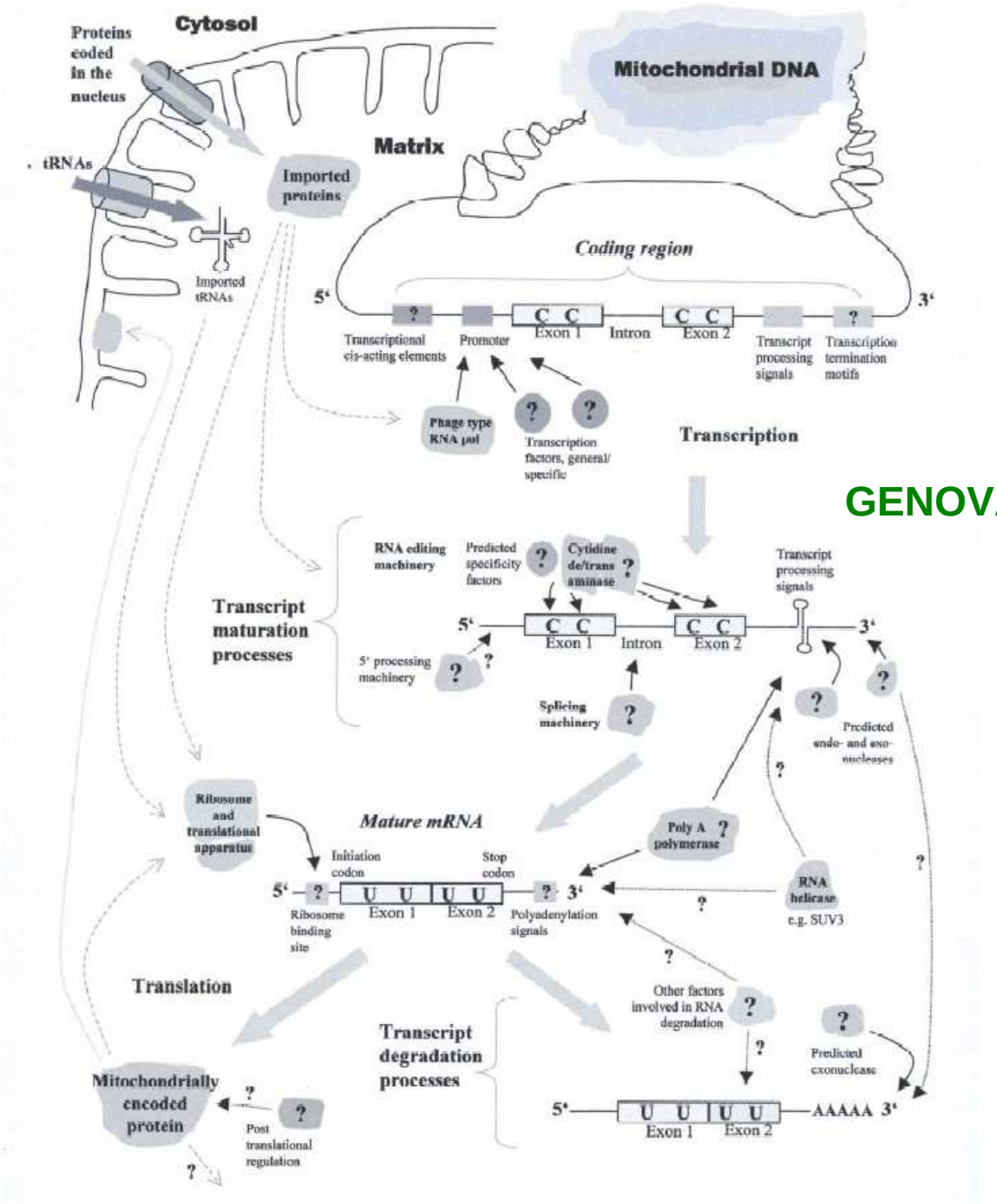
mechanismus opět DEAMINACE s C na U (viz. PLASTIDY)

Pentatricopeptidové bílkoviny (PPR) hrají roli v každém kroku genové exprese v mitochondriích včetně editování a cms.



PPR proteins have been found associated with every known stage of gene expression between transcription and translation. 1: PPR proteins have been found associated with the mitochondrial RNA polymerase; 2: PPR proteins have been implicated in RNA cleavage; 3: PPR proteins have been implicated in splicing; 4: PPR proteins have been implicated in editing; 5: PPR proteins are strongly thought to play a role in translation initiation; 6: PPR proteins are thought to be associated with ribosomes and to in some cases to tether the translation machinery to the mitochondrial inner membrane to facilitate insertion of newly synthesized polypeptides into the correct complex. Figure reproduced from Andres et al. (2007).

IMPORT



GENOVÁ EXPRESE

Import bílkovin do mitochondrií

Recognition of proteins destined for mitochondria

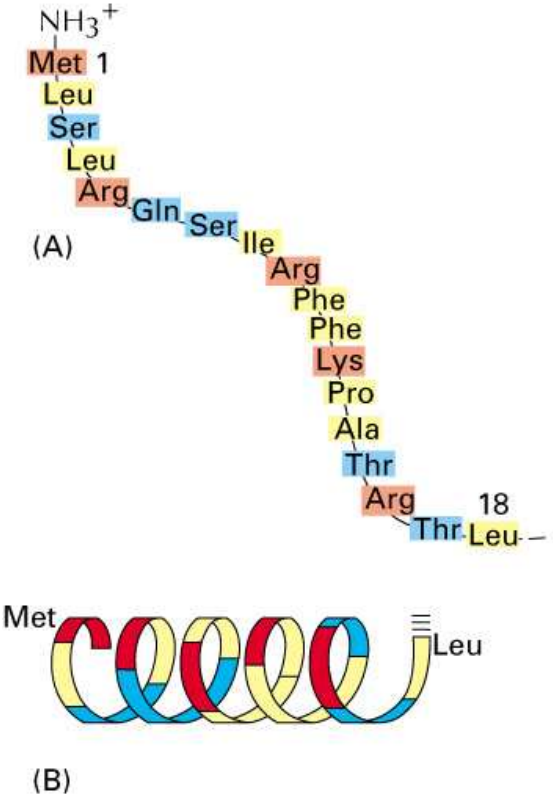
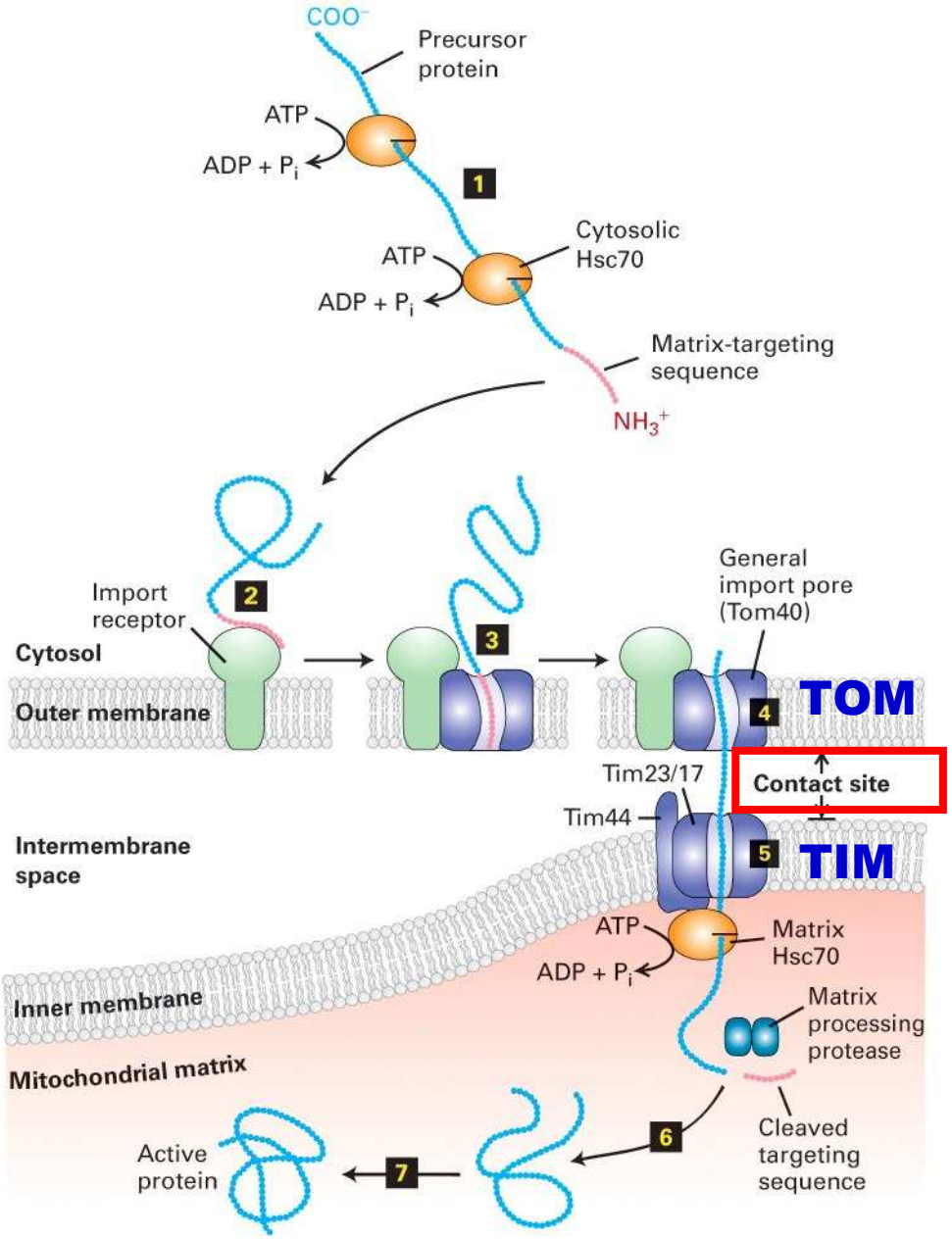


Figure 12–23. Molecular Biology of the Cell, 4th Edition.

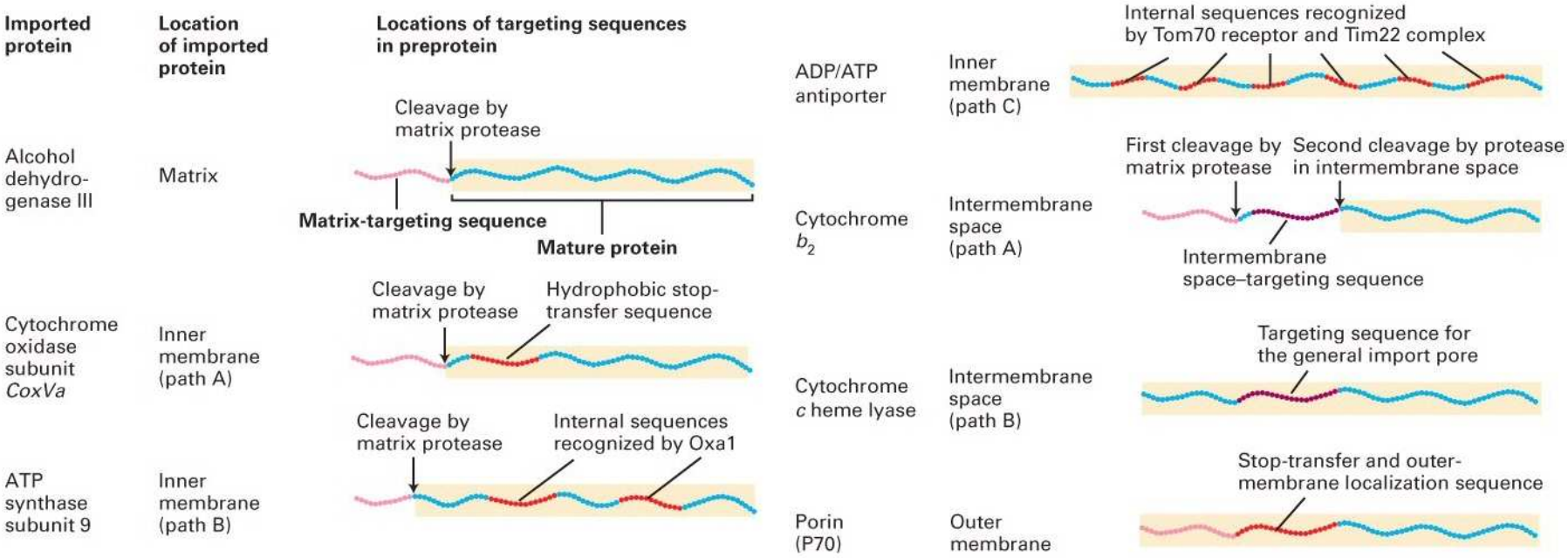
- This involves both terminal signal sequences and internal signal patches
- In some cases proteins for import are packaged with chaperones, especially Hsp70, but in other cases it would appear that the fully-folded protein is imported intact.
- There are two major recognition sites on the outer membrane, one recognising fully folded proteins, the other proteins associated with chaperones

N´term. Lokalizační značka pro import do mitochondrií se nazývá také presekvence. Často jsou to pozitivně nabité α-helixy. (Zvl. u membránových bílkovin je ovšem AA signál pro import často uvnitř bílkoviny.)



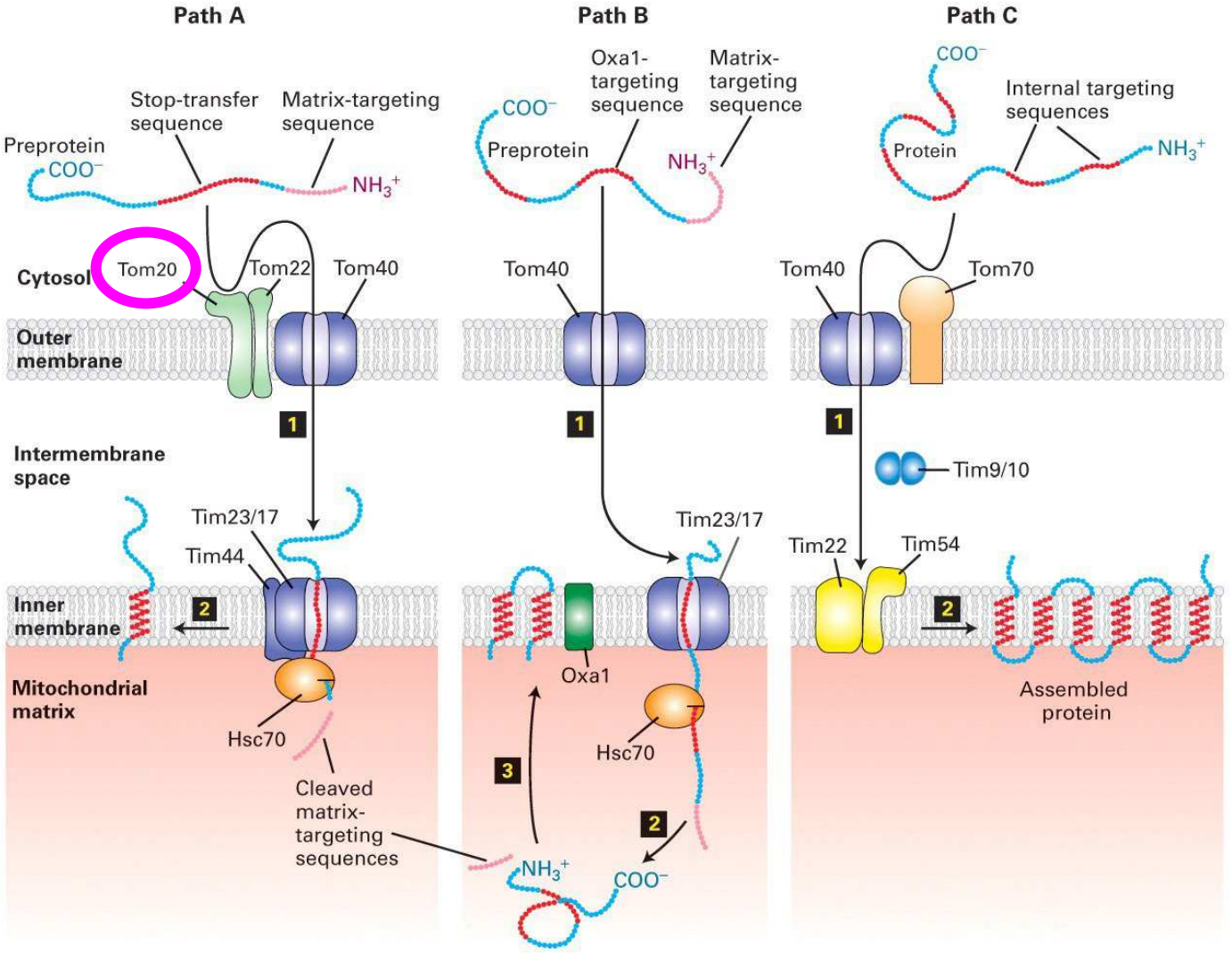
Entry of proteins into mitochondria, general principles

„Targetting“ do mitochondrií
příklady lokalizačních „značek“



Targetting of proteins to mitochondria involves **both internal and terminal signal sequences** and signal patches

Import into mitochondria



TOM20 příklad evoluční konvergence mezi živoč. a rostlinami.

U rostlin TOM70 schází a také recept. dom. na TOM22 – mají místo toho **TOM9 a další možné receptory včetně plastidového typu „TOC64-V“=mtOM64.**

- Transfer across the outer membrane does not require ATP hydrolysis directly but obviously energy is needed as the proteins are moving up a concentration gradient. The energy is probably provided by ATP hydrolysis in binding and releasing Hsp70

The Transfer - Inner membrane complex

1) It must recognise the signal on peptides projecting from the TOM complex
2) It must guide these through the inner membrane and then pull the rest of the molecule through.

- The matrix targetting signal must now be removed. This is carried out by a special Matrix processing peptidase, sometimes assisted by a second enzyme, the mitochondrial intermediate peptidase

Role Vm a energie

- Movement of proteins through the pore depends on the maintenance of the potential difference across the membrane. This is normally app. 200 mV which is equivalent to 400,000 V/cm
- The key role in dragging the remainder of the proteins through the membrane is played by the mitochondrial form of Hsc70. Transfer requires ATP hydrolysis. Three mechanisms have been suggested. The ratchet mechanism proposes that transfer is by Brownian motion with Hsc70 binding preventing back movement. The molecular motor model proposes that the conformational change of mtHsc70 results in the protein being pulled across the membrane

Mechanics of Movement

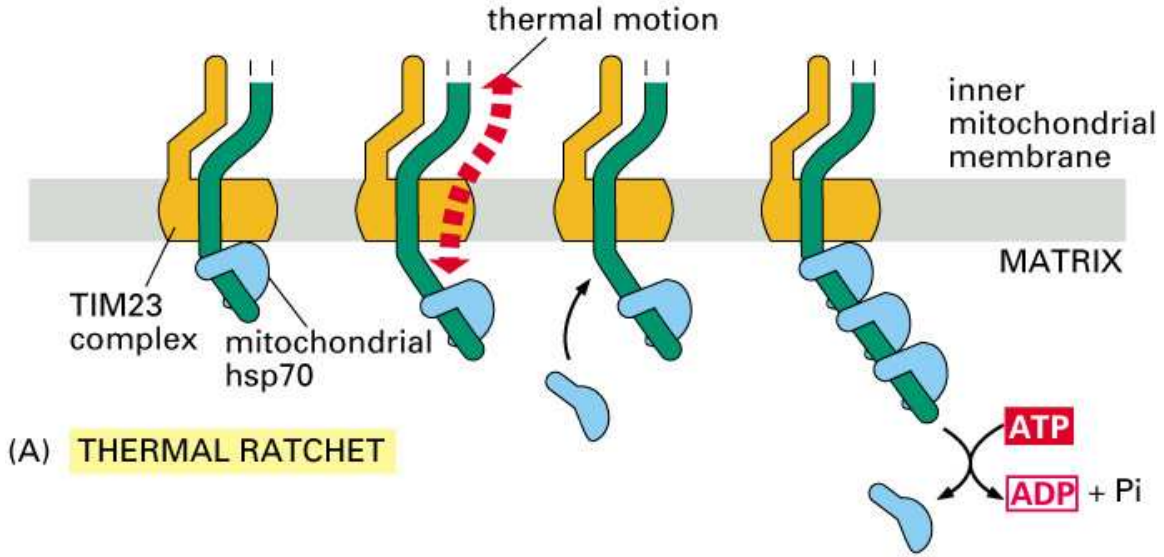


Figure 12–28 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

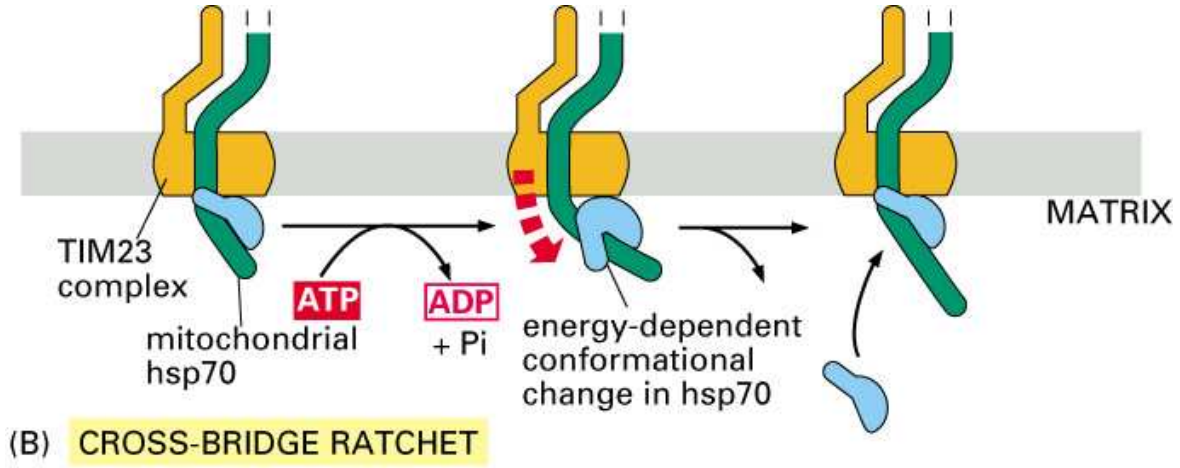


Figure 12–28 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

- Importy do mitochondrií a plastidů spolu souvisejí – **ALE JSOU POUZE ANALOGICKÉ**.

- Při endosymbióze sinice už v buňce byla mašinerie pro import do mitochondrií.

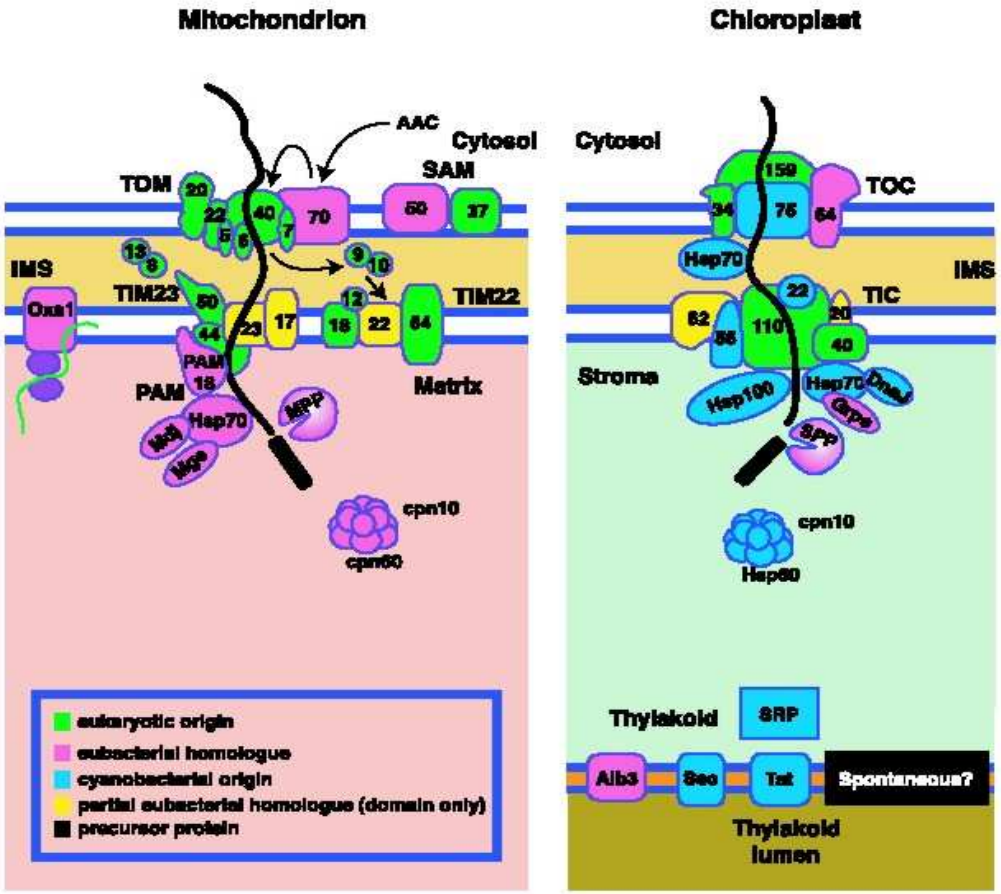


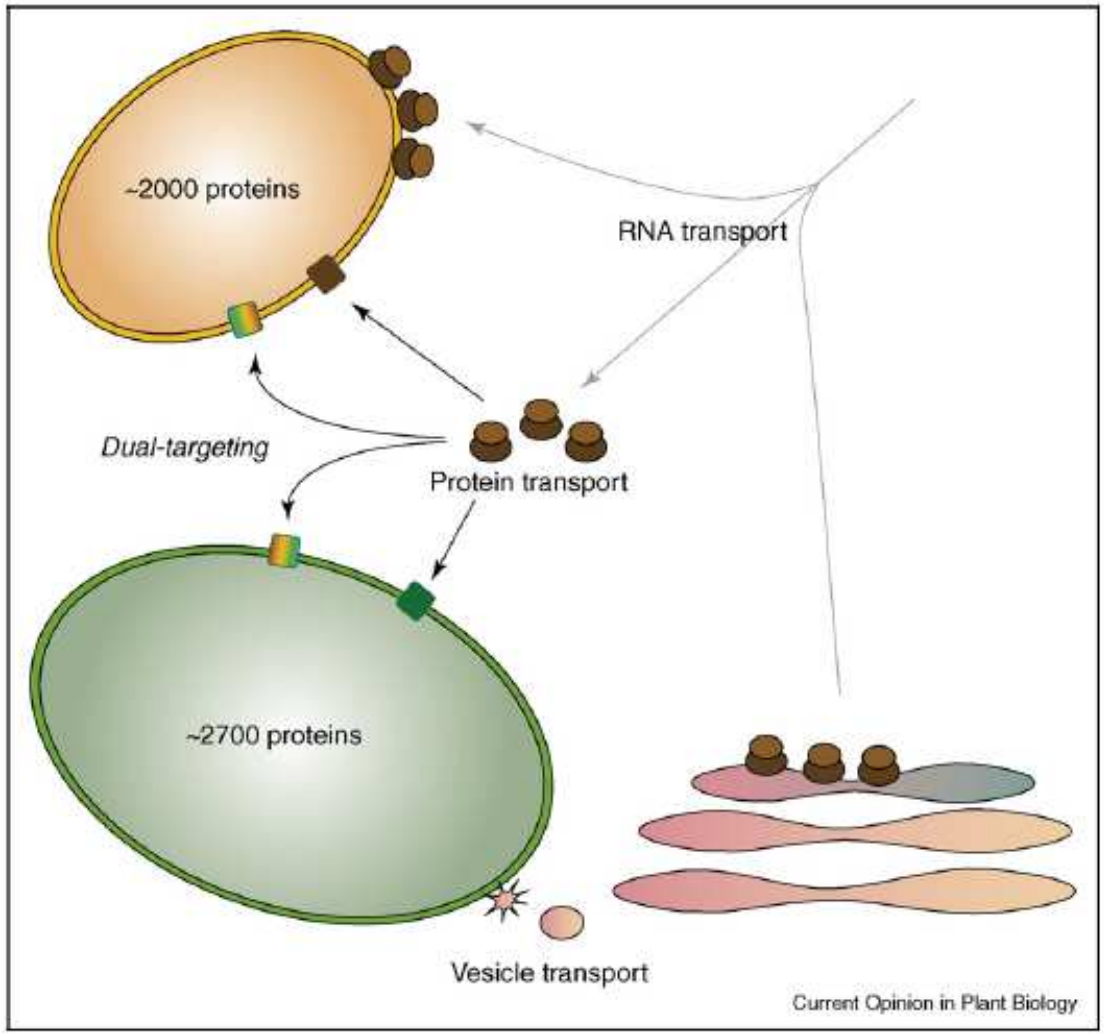
Fig. 3. Origins of mitochondrial and plastidal protein translocases. More than 25 mitochondrial translocases have been identified (**left**). TOM, translocase of the outer mitochondrial membrane; SAM, sorting and assembly machinery; TIM, translocase of the inner mitochondrial membrane; PAM, presequence translocase-associated motor; MPP, mitochondrial processing peptidase. Numbers correspond to component name and size (kD). The specific TIM22 pathway used by AAC and some eukaryotic-specific membrane proteins is indicated by black arrows. More than 15 translocases are identified in chloroplasts (**top right**) and thylakoids (**bottom right**). TOC, translocase of the outer chloroplast envelope; TIC, translocase of the inner chloroplast envelope; SPP, stromal processing peptidase; SRP, signal-recognition particle-dependent pathway; Sec, Sec-dependent pathway; Tat, Twin-arginine translocase; the black box depicts a spontaneous membrane protein insertion pathway. IMS, intermembrane space; colors indicate the possible origins of translocases determined by BLAST (*49*) searches with *Saccharomyces cerevisiae* mitochondrial translocases and *Pisum sativum* plastid translocases as input, except for cpn10 and SPP, where *Arabidopsis thaliana* homologs were used. Sources for mitochondrial translocases, (*29*, *41*, *47*, *52*, *53*); sources for plastid translocases, (*30*–*32*, *46*).

Import bílkovin do mitochondrií je podobný importu do plastidů i když jejich evoluční historie je různá - a obě tyto dráhy spolu souvisejí.

DVOJITÉ CÍLENÍ

„Dual targeting“ – podvojné adresování. Některé bílkoviny nesou lokalizační N-term.signál pro lokalizaci jak do mitochondrií, tak do plastidů(příp.peroxisomů). Např. jedna ze 3 NEP fág.podob. RNApol., AtZn-metalloproteáza (pro preseq./trans.pept. Odštěpování), 17x AAc-tRNA synt. . Jde bud´o nespecifický trans./pres. peptid či o dva „lokalizační“ peptidy tandemově za sebou.

Také UTRs na mRNA mají zde svou roli.



Multiple routes to organelles. Recent evidence has suggested novel routes into mitochondria and chloroplasts in addition to the canonical route via posttranslational import through receptors that are specific to each organelle. Co-translational import, whose specificity is perhaps influenced by RNA transport, needs to be investigated [2]. The occurrence of similar receptor proteins on both organelles might suggest an explanation for widespread dual-targeting [14]. An unknown number of proteins reach plastids via the secretory pathway [52**,53], presumably via vesicle fusion with the organelle.

TOM je dosti rostlinně specifický, zato TIM je jasně dalekosáhle homologní kvasinkovému.

Adresování do organel je ovšem silně závislé na diferenciačním/vývojovém stavu buňky – tj. (ne)-přítomnosti trans-regul. bílkovin.

SAMČÍ PYLOVÁ STERILITA

- **SAMČÍ PYLOVÁ STERILITA U ROSTLIN SOUVISÍ S NESTABILITOU MITOCHONDRIÁLNÍ DNA.**

Cytoplasmic male sterility (CMS)

- Controlled by mitochondrial genes
- Maternally inherited
- Used for hybrid production in many crops
  - Onion, carrot, cabbage
  - Corn, sorghum, pearl millet, sunflower, sugar beets

FERTILNÍ

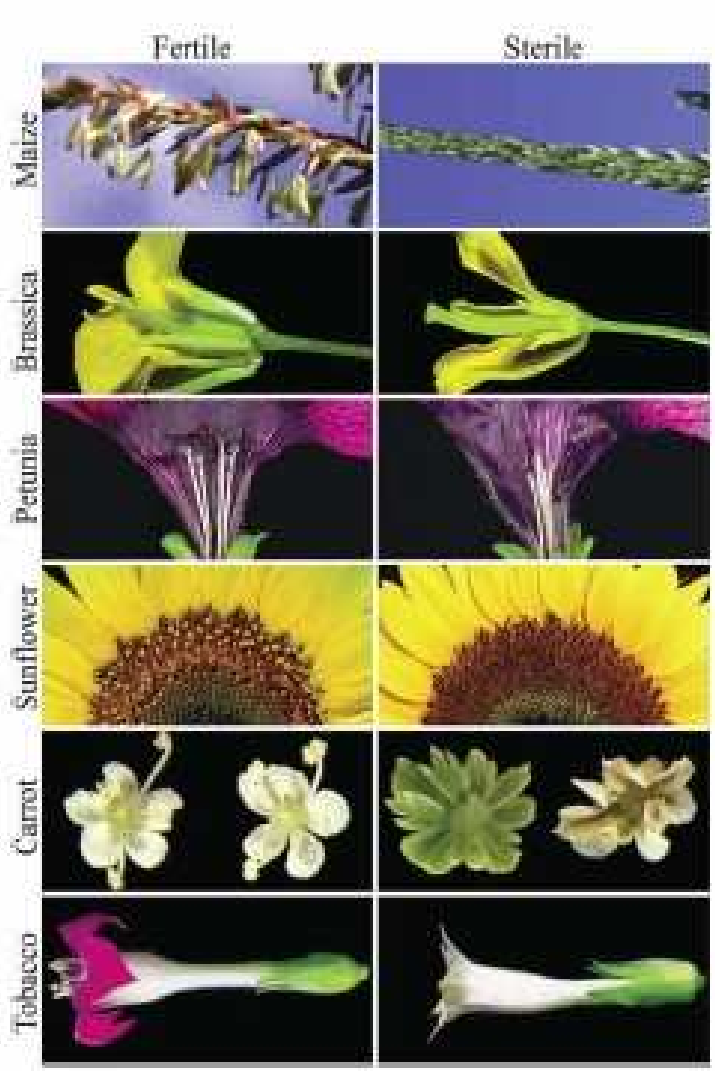


Figure 2. Floral Morphology in CMS and Fertile Plants.

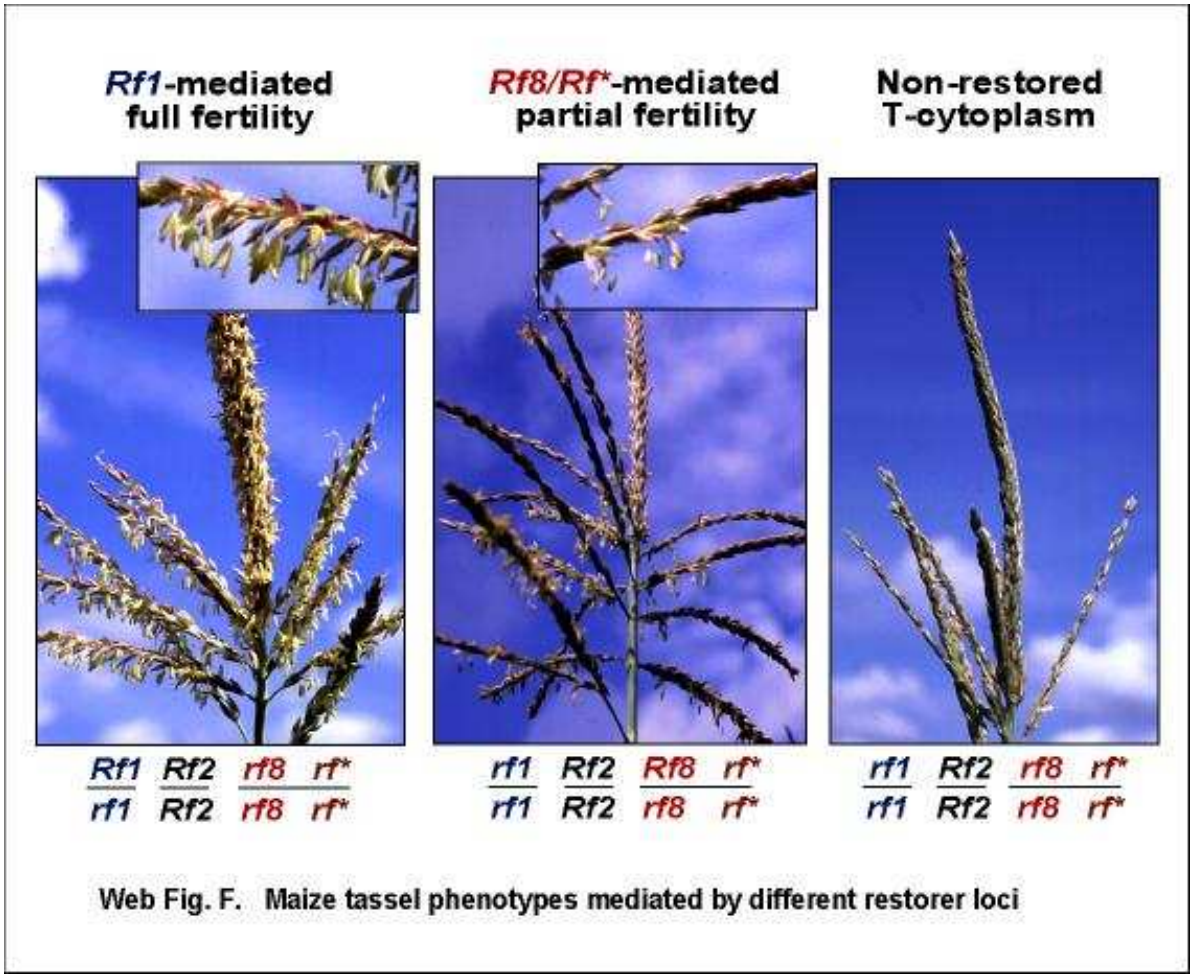
For maize, fertile lines exhibit exserted anthers, unlike the CMS-T line. For *Brassica*, flowers containing Ogura cytoplasm exhibit altered stamen morphology. For petunia, CMS and fertile flowers are indistinguishable except for degenerating anthers and a lack of pollen in the sterile line. For sunflower, flowers in CMS lack the pollen evident on the wild-type flower. For carrot, stamens in CMS have been converted to petal- or bract-like structures. For tobacco, CMS flowers have no exserted stamens, which are fused with carpels. Photographs courtesy of G. Brown (*Brassica*), P. Simon (carrot), R. Wise (maize), and K. Glimellius (tobacco).

cms STERILNÍ

Restorer genes
„obnovovací“ geny

- Nuclear genes (**Rf**) can restore male fertility (One locus restorer system)
  - CMS, rfrf is male sterile
  - CMS, Rfrf is male fertile
  - CMS, RfRf is male fertile
  - N, rfrf is male fertile
  - N, Rfrf is male fertile
  - N, RfRf is male fertile

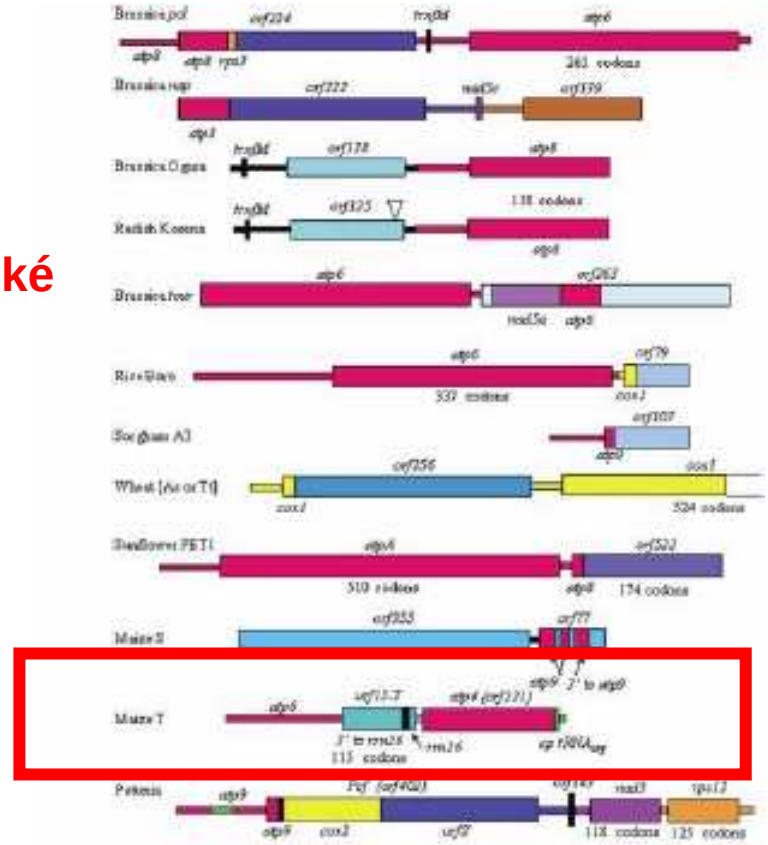
Fertility restoration in maize



Web Fig. F. Maize tassel phenotypes mediated by different restorer loci

Příprava hybridního osiva – využití heterose

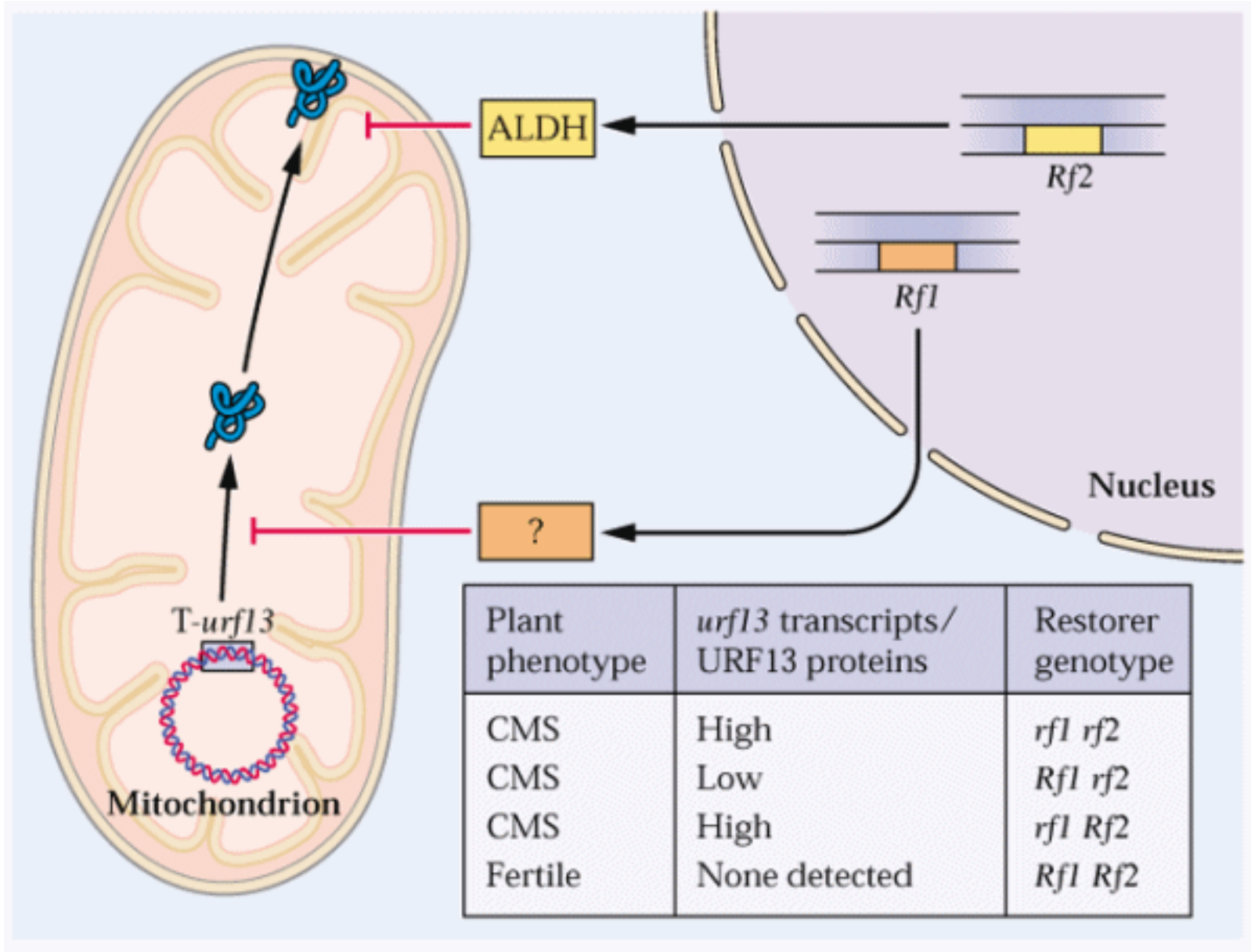
Hybridní/chimerické ORF jako příčina CMS.



„cytoplasma“ TEXAS *T-urf13*

Figure 1. Chimeric Genes Associated with CMS.

*Orf*s are listed by the current convention of number of codons, except for loci named otherwise to be consistent with historical convention (*urf13* encodes a 13-kD protein; *pcf* indicates petunia CMS-associated fused gene; *orf522* encodes 522 nucleotides rather than 522 codons). References are cited in the text. Red indicates genes for subunits of ATP synthase. Shades of blue indicate unknown reading frames within CMS-associated regions. Shades of yellow indicate genes for subunits of cytochrome oxidase. Orange indicates ribosomal protein genes. Shades of brown indicate conserved unidentified reading frames found in multiple vascular plant mtDNAs. Green indicates chloroplast-derived sequences. Additional details of the sequences can be found in references cited in the text.



Plant phenotype	*urf13* transcripts/ URF13 proteins	Restorer genotype
CMS	High	*rf1 rf2*
CMS	Low	*Rf1 rf2*
CMS	High	*rf1 Rf2*
Fertile	None detected	*Rf1 Rf2*

Rf potlačují jejich neblahé účinky chimerické bílkoviny.

PPR=pentatricopeptid repeat. U „RESTORERU“

Rf

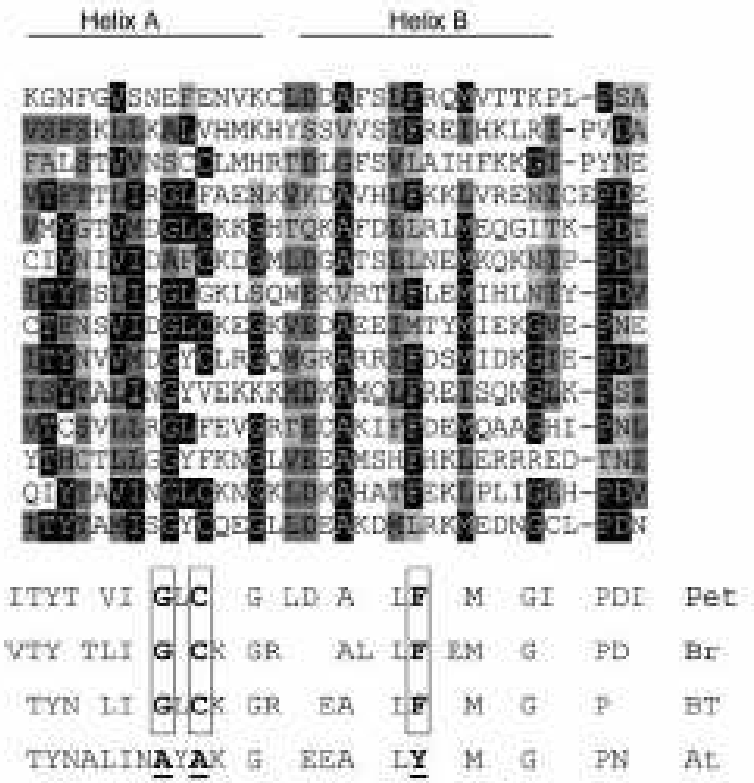


Figure 3. The PPR Motif and Nuclear Genes That Modify Mitochondrial Gene Expression.

(A) Alignment of the 14 motifs in the predicted petunia Rf protein. Each PPR was identified by MEME software (Bailey and Elkan, 1994). Identical amino acids are shown as black boxes with white lettering; at least 7 of the 14 motifs must have an identical or highly similar amino acid for the residue to be included in the petunia Rf consensus sequence. Similar amino acids are shown in dark gray and weakly similar residues are in light gray. The locations of the two predicted anti-parallel α-helices are indicated (Small and Peeters, 2000).

(B) Below the petunia Rf PPR motif alignment are shown the consensus sequences of the PPR motifs found in petunia Rf, *Brassica*/radish Rfo, and PPR8-1, the putative Boro rice restorer. The three Rf consensus sequences differ consistently at three residues (boxed) compared with those residues (underlined) in the consensus of 1303 PPR motifs analyzed in Arabidopsis by Small and Peeters (2000).

- **EVOLUČNÍ VÝZNAM CMS**
- **SSS = „substoichiometric shifting“**
- **podpora cizosprašování**

- MITOCHONDRIE HRAJÍ SPOUŠTěCÍ ROLI V PCD TAKÉ U ROSTLIN.

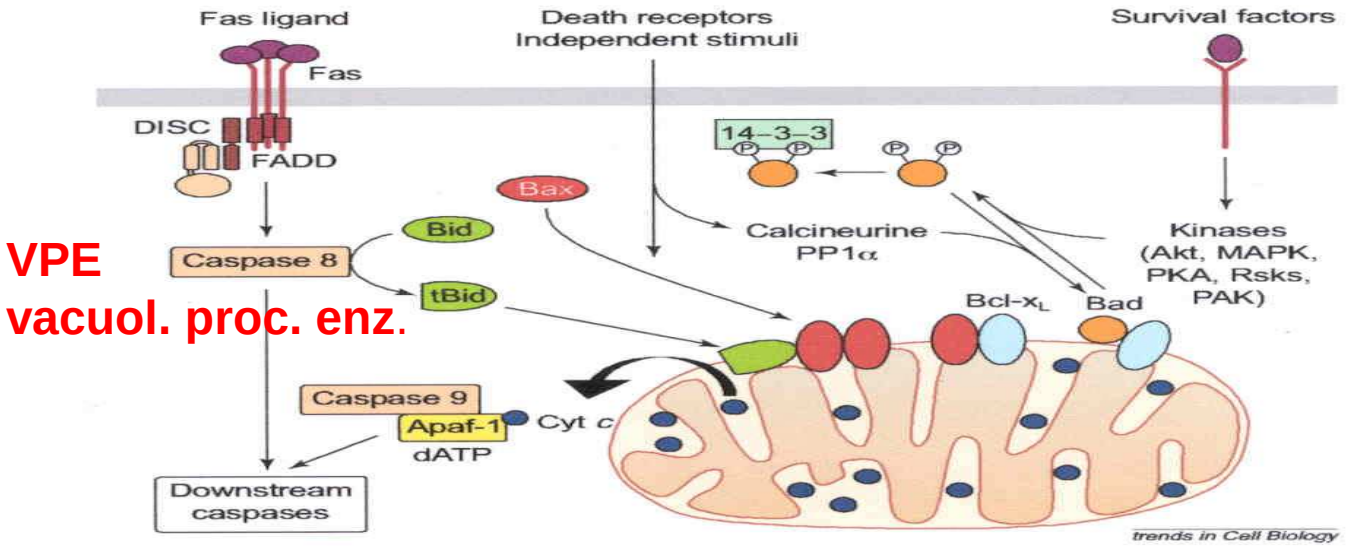
Aktivace PCD mitochondriálními signály také u rostlin.

A regulated pathway of cell death important in normal development and disease and defense responses in plants and animals

ALE KLÍČOVOU ROLI HRAJE u rostlin VAKUOLA A UTOFAGICKÁ DRÁHA

In animals, release of mitochondrial signaling molecules, especially the electron carrier cytochrome *c*, activates the cell death machinery, which is already in place. ***This is not regulation of gene expression!***

A role for mitochondria in plant PCD is becoming evident, although the nature of the signaling molecules is not yet known



VPE
vacuol. proc. enz.

Figure 3. Figure 2. Many death signals converge onto mitochondria and are mediated through members of the Bcl-2 protein family called `BH3-only' proteins, such as Bid and Bad. Caspase 8 then cleaves Bid, whose C-terminal fragment (tBid) translocates to mitochondria, where it activates Bax or Bax-like proteins and results in cytochrome-*c* (cyt *c*) release. Once in the cytosol, cytochrome *c* activates caspase 9 by binding to Apaf-1 and dATP.

from Desagher and Martinou Trends Cell Biol 10:369

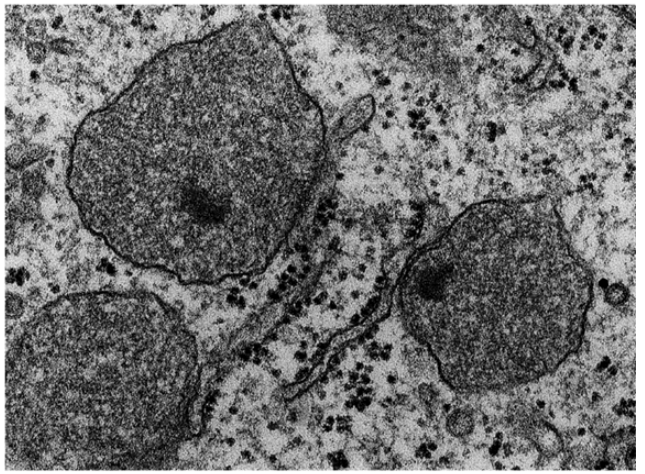
Peroxisomy

Peroxisomy

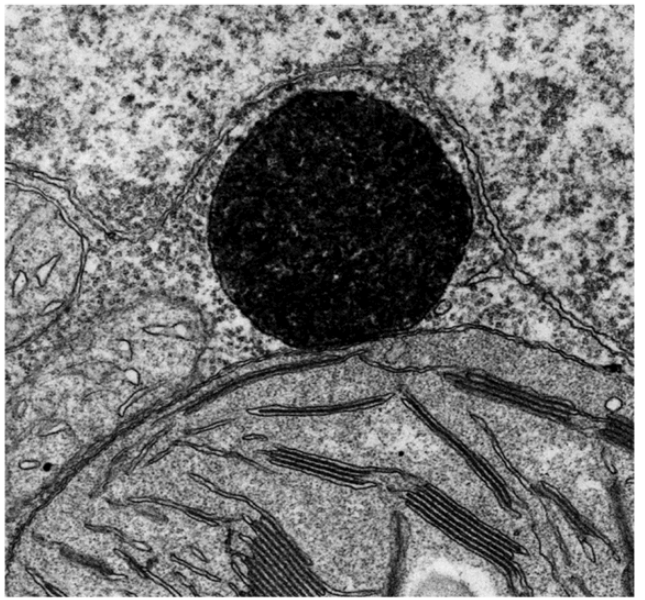
strukturně jednoduché, ale funkčně rozmanité organely (specializace podle pletiv)

Peroxisomes are a type of microbody. Microbodies are cell organelles bounded by a single membrane and are used for a variety if different processes. For example, peroxisomes contain enzymes which produce hydrogen peroxide (and have the means for destroying it). In addition, plants have glyoxysomes which contain the enzymes of the glyoxylate cycle and yeasts have a variety of microbodies including ones involved in methanol oxidation.

(A)



(B)



A) peroxisomy asociované s ER

B) vizualizovaná kataláza (tmavě) v peroxisomu

Typy peroxisomů

peroxisomy vždy obsahují enzym katalázu – štěpení peroxidu vodíku (marker peroxisomů)

detoxifikace *reactive oxygen species* (ROS)

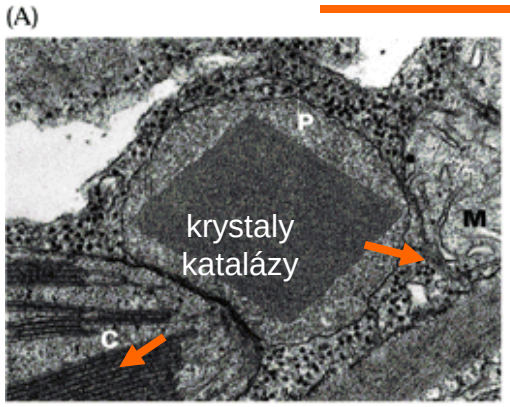
- **LISTOVÉ PEROXISOMY** – fotorespirace u C3 rostlin
- **GLYOXYSOMY** – β-oxidace mastných kyselin a glyoxalátový cyklus (klíčení semen)
- **GERONTOSOMY** – katabolismus lipidů v odumírajících listech
- **URIKOSOMY** – v hlízkách nodulujících bobovitých rostlin, specializované na dusíkatý metabolismus (při fixaci N_2): oxidativní odbourávání guaninu (přes kyselinu močovou na allantoin za současné produkce H_2O_2)

peroxisomy jsou možná pozůstatkem po prapůvodní respirační organele funkční před vznikem mitochondrií - **??? to je velmi málo podložená spekulace.**

Vlastnosti peroxisomů

	Plants	Human hepatocytes
Shape	spherical, ovoid, tubular, square, irregular	ovoid
Internal Structure	amorphous, paracrystalline	amorphous
Size	0.1-2.0 μm	0.5 μm
No. per cell	1-1000	100
% vol of cell	0.1-80%	1%

Funkce peroxisomů



Key

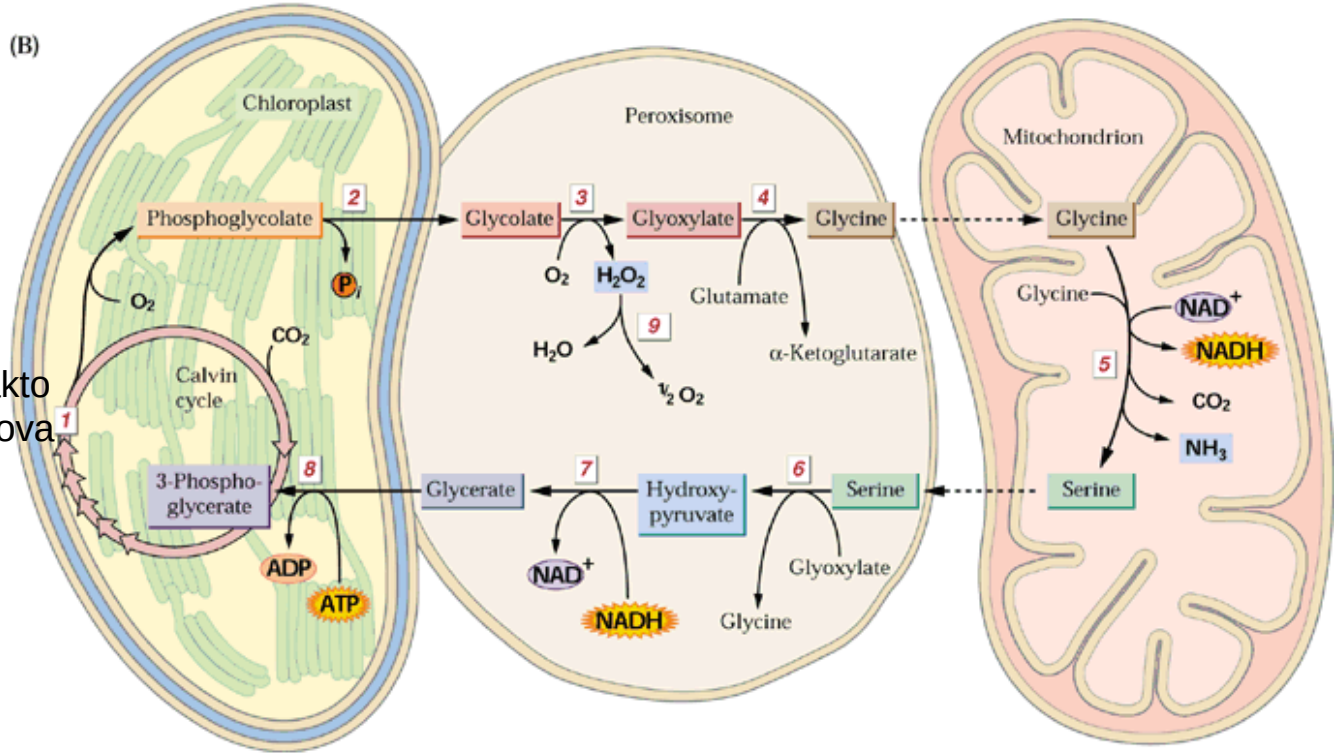
1. Ribulose-1,5-bisphosphate carboxylase/oxygenase
2. Phosphoglycolate phosphatase
3. Glycolate oxidase
4. Glutamate:glyoxylate aminotransferase
5. Glycine decarboxylase and serine hydroxymethyl transferase
6. Serine:glyoxylate aminotransferase
7. Hydroxypyruvate reductase
8. Glycerate kinase
9. Catalase

Fotorespirace:
moc světla, málo CO_2

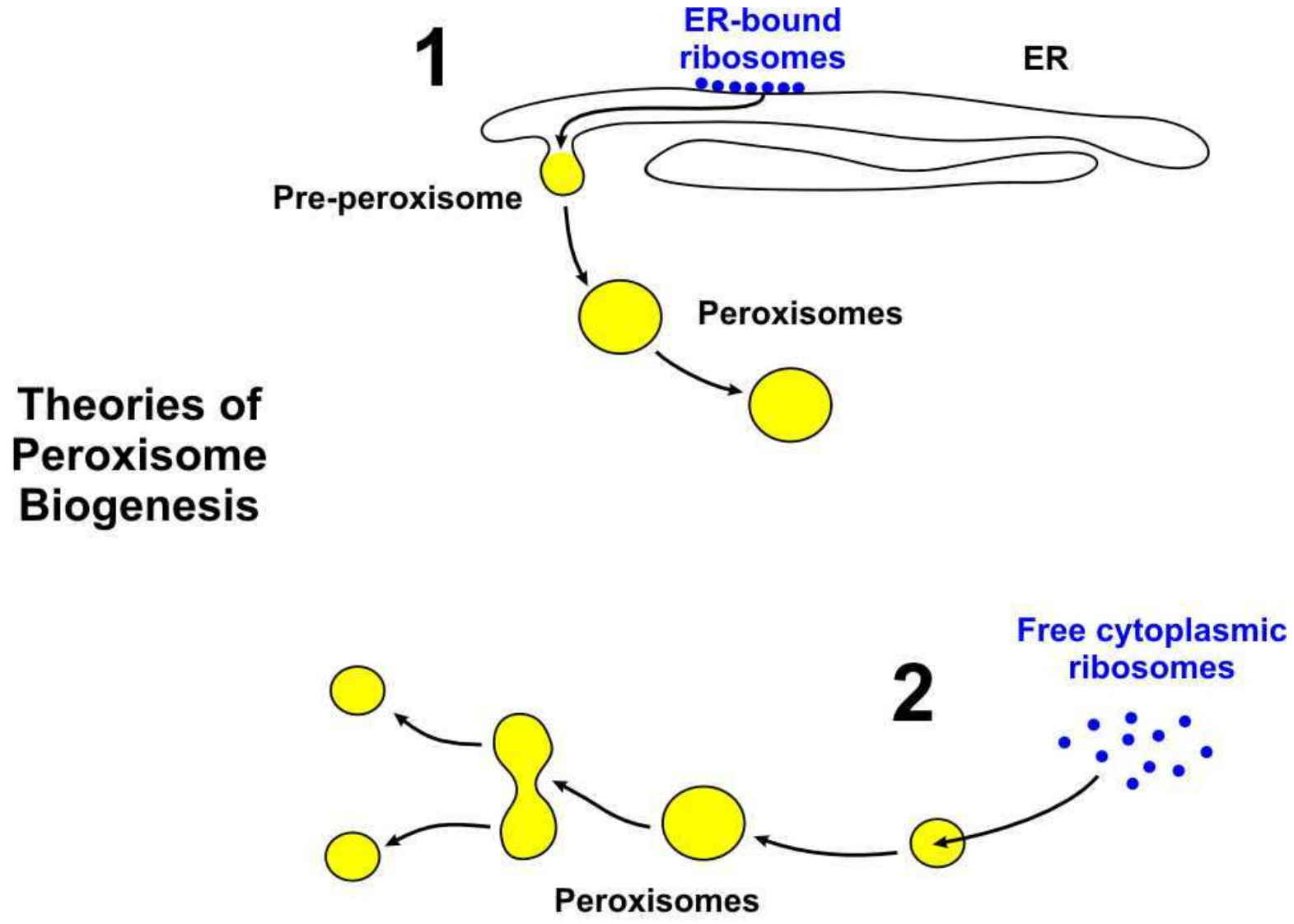
místo
2x 3-fosfoglycerátu
vzniká
1x 3-fosfoglycerátu a
1x 2-fosfoglykolát

fosfoglykolát
= produkt
fotorespirace

až 75 % jeho produkce se takto vrátí do Calvinova cyklu



Biogeneze peroxisomů



Biogeneze peroxisomů

tři modely:

A) vznik ze specifických částí ER

B) dělením stávajících perixosomů + import všech proteinů z cytoplasmy

C) dělením stávajících perixosomů + import membr. proteinů z ER a proteinů matrix z cytoplasmy

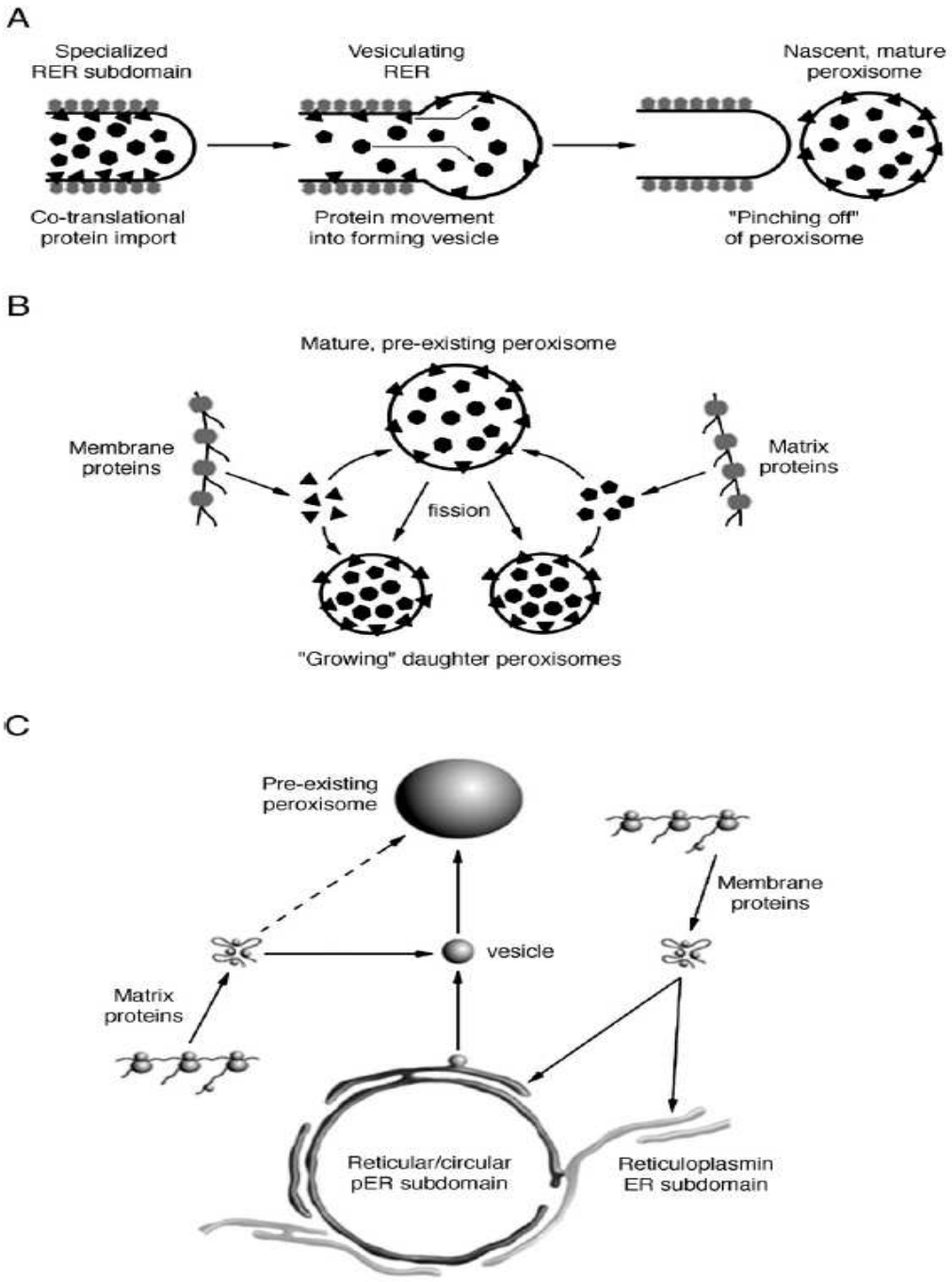
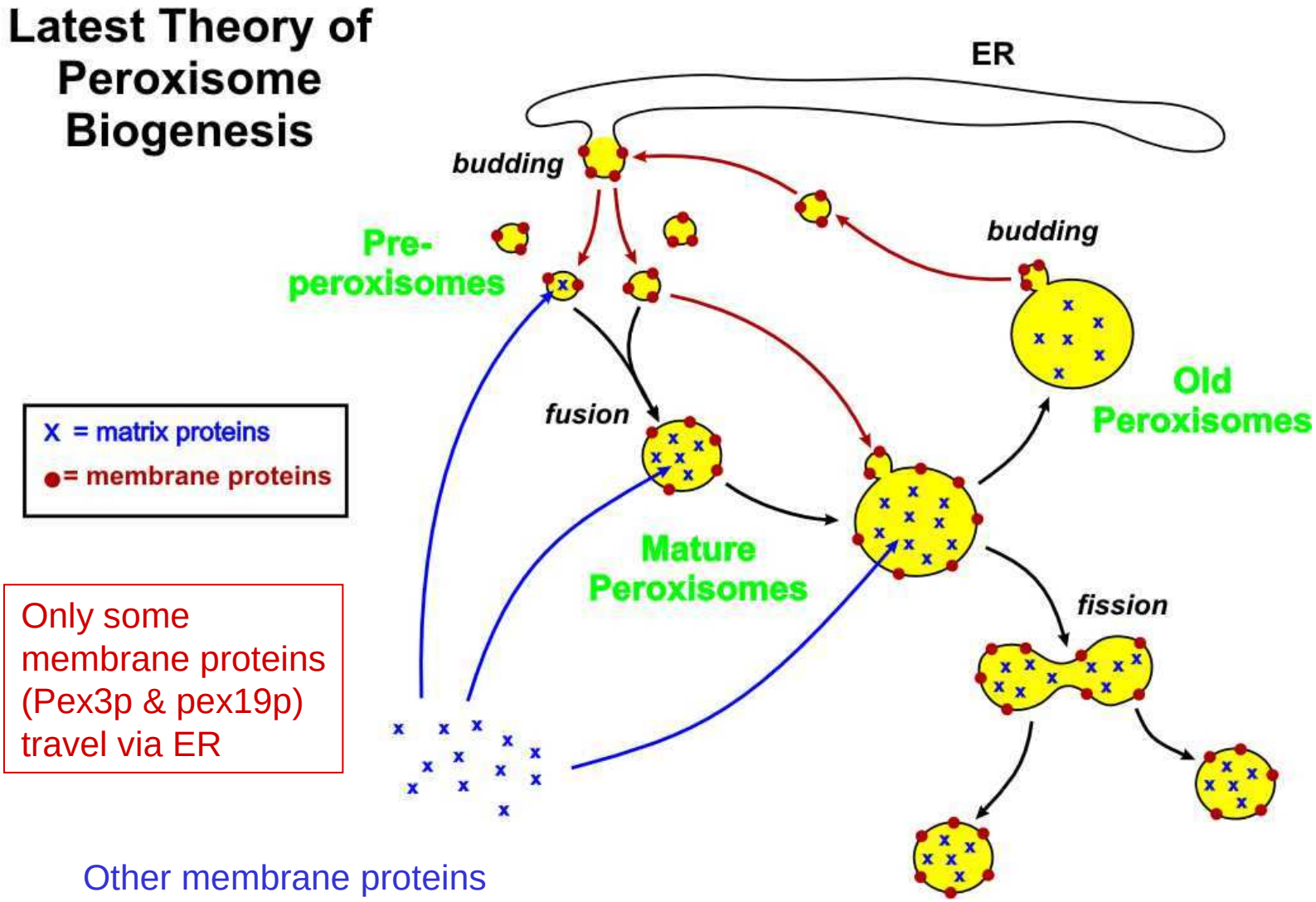


Fig. 1. Early prevailing models for peroxisome biogenesis. (A) The ER vesiculation model. Peroxisomal membrane (solid triangles) and matrix (solid polygons) proteins are synthesized on bound polyribosomes and co-translationally inserted into either the membrane or lumen of a specialized region of the rough ER (RER). Thereafter, both sets of nascent proteins move (somehow) into an expanding smooth membrane vesicle that, under some unknown influence, pinches off from the ER to produce a nascent functional, mature peroxisome. Adapted from Beevers [60]. (B) The autonomous peroxisome growth and division model. Peroxisomal membrane (solid triangle) and matrix (solid polygons) proteins are synthesized on free polyribosomes in the cytosol and sort (post-translationally) directly to pre-existing peroxisomes and the new (daughter) peroxisomes. Therefore, the pre-existing peroxisomes are envisaged to grow and undergo fission (division) to form new peroxisomes, which also grow via protein acquisitions. Alternatively in the case of interconnected peroxisomes, formation of new (daughter) peroxisomes bud from the contorted peroxisomal compartment called the "peroxisomal reticulum" (not shown). Adapted from Lazarow and Fujiki [61]. (C) The ER semi-autonomous peroxisome model. Peroxisomal membrane and matrix proteins are synthesized on free polyribosomes in the cytosol and interact with molecular chaperones prior to their post-translational sorting. Nascent PMPs (such as APX) sort from the cytosol either directly to the reticular/circular pER subdomain or first to the "general" reticuloplasmin-containing ER (subdomain?) and then to the pER. In the latter case, the mechanism responsible for sequestering PMPs into pER is considered equivalent to that described in the "privileged site budding" model for cargo protein sorting from the ER lumen or membrane into specific subdomains or "privileged sites" where COPII vesicles are formed [112]. Transport of PMPs from the pER seems to involve vesicles that are subsequently sorted to pre-existing peroxisomes. Matrix proteins also could sort indirectly (possibly to vesicles), or in some instances directly (dashed line), to pre-existing peroxisomes. Reproduced from Mullen et al. [47] with permission of the American Society of Plant Biologists.

Biogeneze peroxisomů



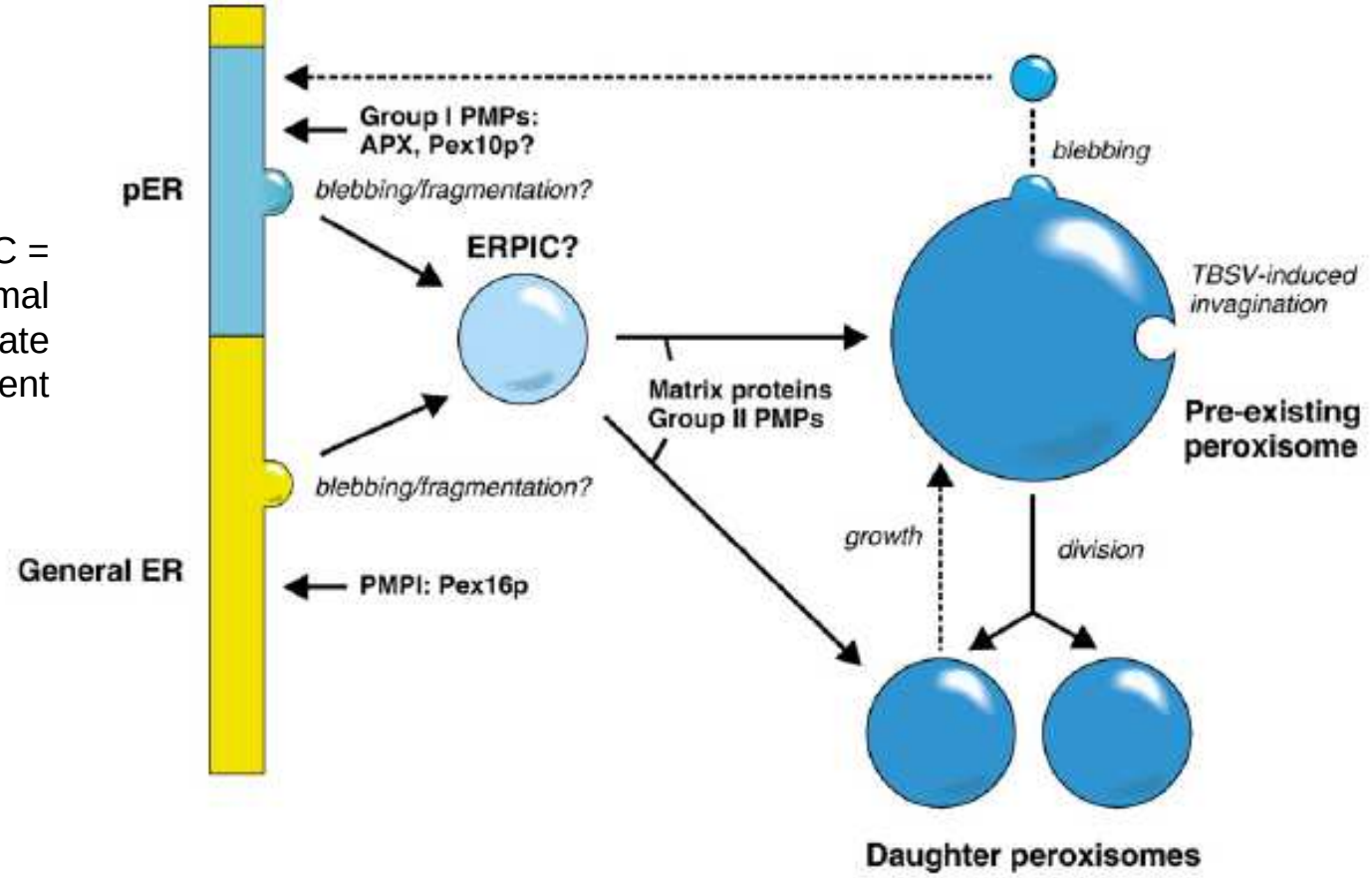
Only some membrane proteins (Pex3p & pex19p) travel via ER

Other membrane proteins imported like matrix proteins

PEROXINY – proteiny biogeneze peroxisomů

Semiautonomní model peroxisomu u rostlin

ERPIC = ER-peroxisomal intermediate compartment



existuje i retrográdní cesta z peroxisomu do ER: tombus +RNA viry (TBSV) se replikují v peroxisomech a některé proteiny se pak transportují do ER

Review

The ER-peroxisome connection in plants: Development of the "ER semi-autonomous peroxisome maturation and replication" model for plant peroxisome biogenesis

Robert T. Mullen[a], [*] **and Richard N. Trelease**[b], [*]

[a]**Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON, Canada N1G 2W1**

[b]**School of Life Sciences, Arizona State University, PO Box 874501, Tempe, AZ 85287-4501, USA**

Received 3 June 2006; revised 25 August 2006; accepted 6 September 2006. Available online 14 September 2006.

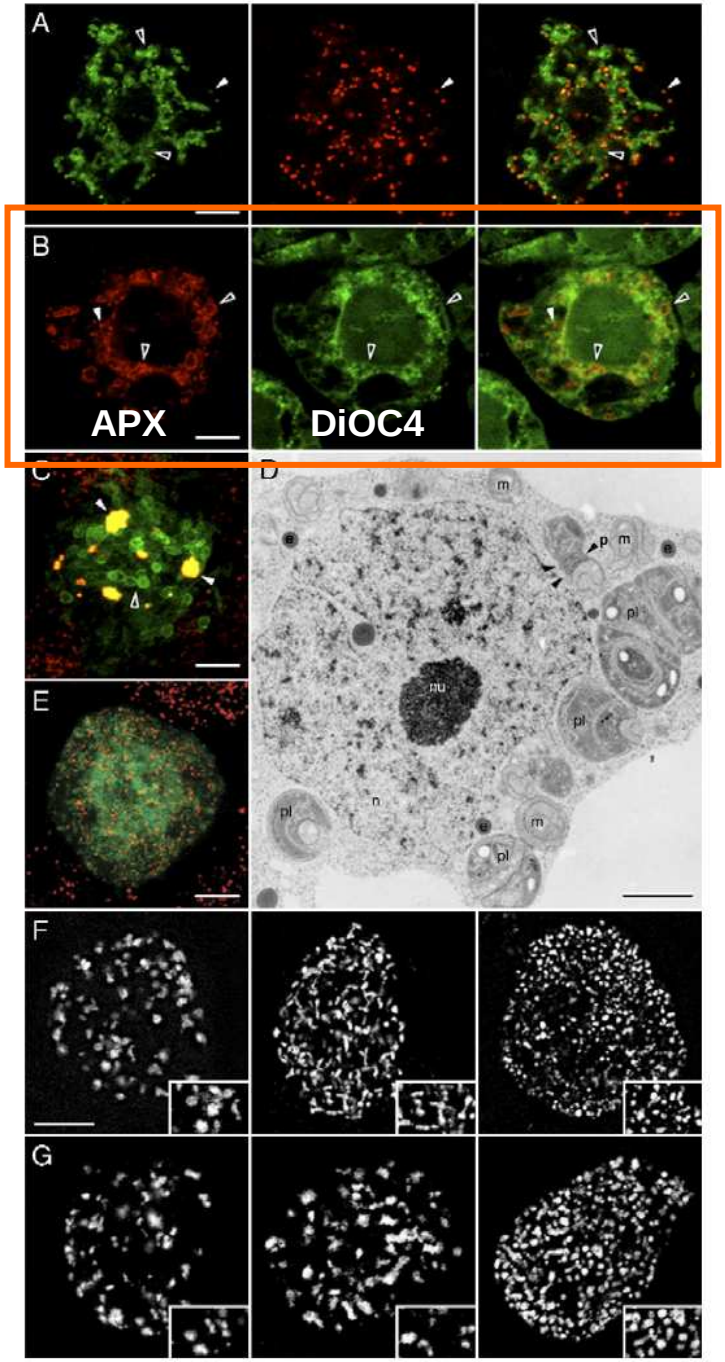
Abstract

The perceived role of the ER in the biogenesis of plant peroxisomes has evolved significantly from the original "ER vesiculation" model, which portrayed co-translational import of proteins into peroxisomes originating from the ER, to the "ER semi-autonomous peroxisome" model wherein membrane lipids and post-translationally acquired peroxisomal membrane proteins (PMPs) were derived from the ER. Results from more recent studies of various plant PMPs including ascorbate peroxidase, PEX10 and PEX16, as well as a viral replication protein, have since led to the formulation of a more elaborate "ER semi-autonomous peroxisome maturation and replication" model. Herein we review these results in the context of this newly proposed model and its predecessor models. We discuss also key distinct features of the new model pertaining to its central premise that the ER defines the semi-autonomous maturation (maintenance/assembly/differentiation) and duplication (division) features of specialized classes of pre-existing plant peroxisomes. This model also includes a novel peroxisome-to-ER retrograde sorting pathway that may serve as a constitutive protein retrieval/regulatory system. In addition, new plant peroxisomes are envisaged to arise primarily by duplication of the pre-existing peroxisomes that receive essential membrane components from the ER.

Keywords: Biogenesis; Endoplasmic reticulum; Peroxisome; Plant; Protein trafficking; Organelle

Abbreviations: APX, ascorbate peroxidase; BFA, brefeldin A; BY-2, bright yellow-2; CAT, chloramphenicol acetyltransferase; COPII, coat protein II; $DiOC_6$, 3,3′-dihexyloxacarbocyanine idodine; ESCRT, endosomal sorting complex required for transport; ER, endoplasmic reticulum; ERPIC, ER-peroxisome intermediate compartment; GFP, green fluorescent protein; HA, hemagglutinin; p33, 33-kDa replication protein; 92-kDa RNA-dependent RNA polymerase; pER, peroxisomal ER; Pex, peroxin; PMP, peroxisomal membrane protein; pMVB, peroxisomal multivesicular body; PTS, peroxisome targeting signal; TBSV, tomato bushy stunt virus; TMD, transmembrane domain; YFP, yellow fluorescent protein

Semiautonomní model peroxisomu u rostlin

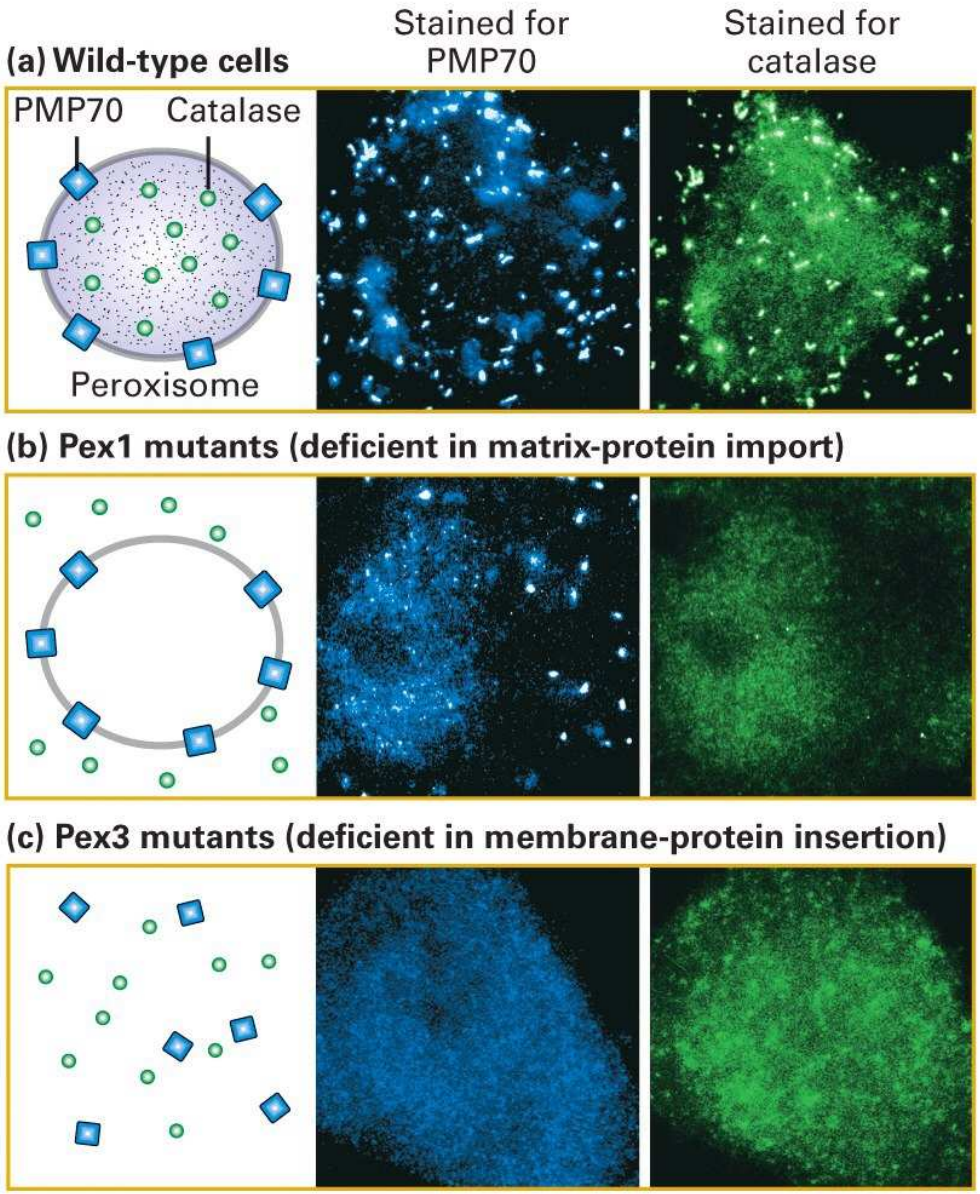


APX = askorbát peroxidáza
(perox. membr. protein)

DiOC4 = marker membrány ER

Fig. 2. Characterization of pER and peroxisome division in plant cells. (A) Confocal representative immunofluorescence images (z-sections) of transiently-expressed HA-APX (green, left panel) and endogenous peroxisomal matrix catalase (red, middle panel), as well as the overlay (right panel), in an individual transformed tobacco BY-2 cell. The solid arrowhead indicates one of the numerous obvious co-localizations of HA-APX with endogenous catalase in individual (punctate) peroxisomes; open arrowheads indicate examples of the localization of HA-APX (but not catalase) in reticular and circular pER. Reproduced from Mullen et al. [47] with permission of the American Society of Plant Biologists. (B) Confocal representative (immuno)fluorescence images (z-sections) of transiently-expressed HA-APX (green, left panel) and $DiOC_6$-stained ER membranes (red, middle panel), as well as the overlay (right panel), in a transformed BY-2 cell. The solid arrowhead indicates an example of HA-APX localized to an individual (punctate) peroxisome; open arrowheads indicate obvious co-localizations of HA-APX and $DiOC_6$ in reticular/circular structures considered to be pER. Reproduced from Mullen et al. [47] with permission of the American Society of Plant Biologists. (C) Representative confocal immunofluorescence projection overlay image of transiently-expressed CAT-APX localized to aggregated globular peroxisomes (yellow, solid arrowheads) and putative circular pER (open arrowheads) in a transformed BY-2 cell. This is a merged (overlay) image of expressed CAT-APX (green) and endogenous peroxisomal catalase (red). Note the distribution of endogenous catalase in non-aggregated (punctate) peroxisomes in the neighboring non-transformed BY-2 cells. Reproduced from Mullen et al. [48] with permission of SpringerLink Publishing. (D) Transmission electron micrograph of a BY-2 cell transiently-expressing GFP-APX. Individual peroxisomes indicated with solid arrowheads were identified by their single boundary membrane. Note the presence of numerous homo- and heterotypic aggregates of plastids, mitochondria, and peroxisomes within this cell. e, electron-dense bodies; p, peroxisome; pl, plastid; n, nucleus; nu, nucleolus. Reproduced from Lisenbee et al. [50] with permission Blackwell Munksgaard. (E) Representative confocal (immuno)fluorescence projection overlay image of transiently-expressed monomeric GFP(A_{206}K)-APX localized to individual (punctate) peroxisomes and reticular pER in a transformed BY-2 cell. This is a merged (overlay) image of autofluorescent expressed GFP(A_{206}K)-APX (green) and endogenous peroxisomal catalase (red). Note the distribution of non-aggregated (punctate) peroxisomes and reticular pER in this GFP(A_{206}K)-APX transformed cell compared to the aggregated (globular) peroxisomes and circular pER in the CAT-APX transformed cell shown in (D). Reproduced from Lisenbee et al. [50] with permission of Blackwell Munksgaard. (F and G) Representative confocal immunofluorescence projection images of myc-tagged Arabidopsis Pex11p isoforms a (myc-Pex11a) (F—top row of three images) and e (myc-Pex11e) (G—bottom row of three images) expressed transiently in individual Arabidopsis suspension cultured cells for 5 h (left panels), 24 h (middle panels) or 45 h (right panels). The insets show enlarged views of the myc-Pex11p isoform-bearing peroxisomes within the corresponding micrographs. In row F, note that compared to the peroxisomes in the myc-Pex11a transformed cell at 5 h, the peroxisomes at 24 h are more elongated, whereas at 45 h they are mostly spherical and clearly more numerous. In row G, note in contrast that peroxisomes in cells expressing myc-Pex11e do not elongate between 5 and 24 h, but are mostly spherical and clearly more numerous at 45 h. Numerical analyses of the peroxisomes in these myc-Pex11a and myc-Pex11e transformed cells revealed that the number of organelles actually doubled (duplication) over the 45-h time period [17]. Reproduced from Lingard and Trelease [18] with permission of The Company of Biologists. Bars in (A–C and E)=10 μm; Bar in (D)=2 μm; Bar in (F)=5 μm.

Import proteinů do peroxisomů



matrixové a membranové proteiny vstupují do peroxisomů různými cestami

pex3 mutanti nemají peroxisomy

Import do matrix peroxisomů

peroxisome targeting signal = **PTS**
import do **MATRIX** peroxisomů

Dva hlavní typy
adresových sekvencí:

PTS1 – Ser-Lys-Leu (SKL) na C-konci,
není odštěpován

PTS2 – na N-konci, je odštěpován;
– u živočichů je vzácný, u rostlin docela častý

PTS1 (u At 182 genů)

1) Carboxy-terminal
2) Small (3 amino acids)
3) Consensus sequence = **S**-**K**-**L** (malé bazické aa)
4) Not cleaved following import

PTS2 (u At 74 genů)

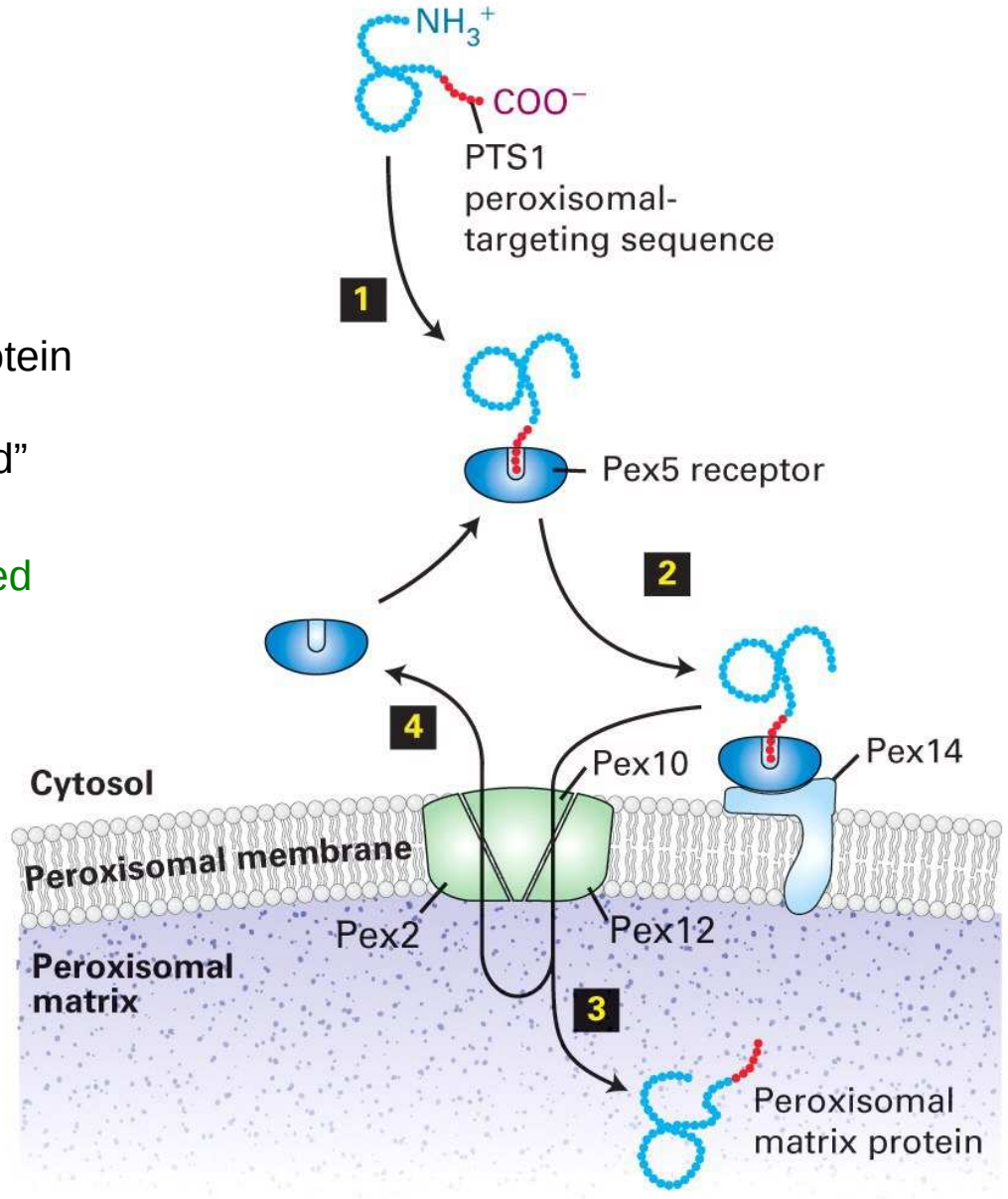
1) Amino-terminal or near the amino terminus
2) Medium sized (~9 amino acids)
3) Consensus sequence = **R**-**L**-(X)5-**H**-**I/L/F**
4) Cleaved following import (in mammals)

Import do matrix peroxisomů

The mechanism of PTS1 appears to involve binding of the SKL sequence to Pex5. This then interacts with a peroxisomal membrane protein called Pex14 forming a channel.

It is not clear whether Pex5 and the protein move together across the channel or whether the imported protein is “pushed” through.

In the peroxisome the protein is released and Pex5 is recycled with the help of Pex2, 10, and 12.



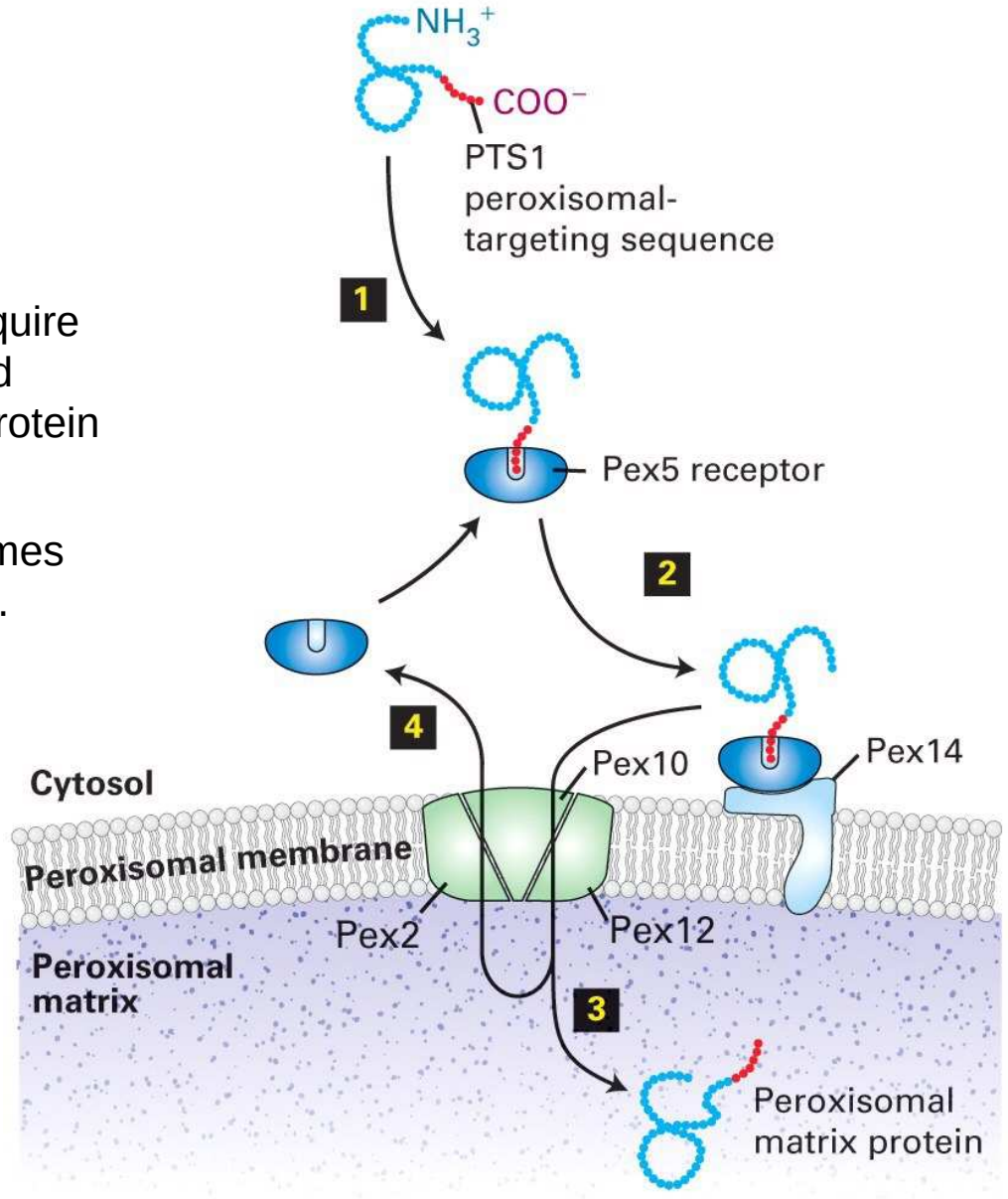
Import do matrix peroxisomů

Podmínky pro import do matrix:

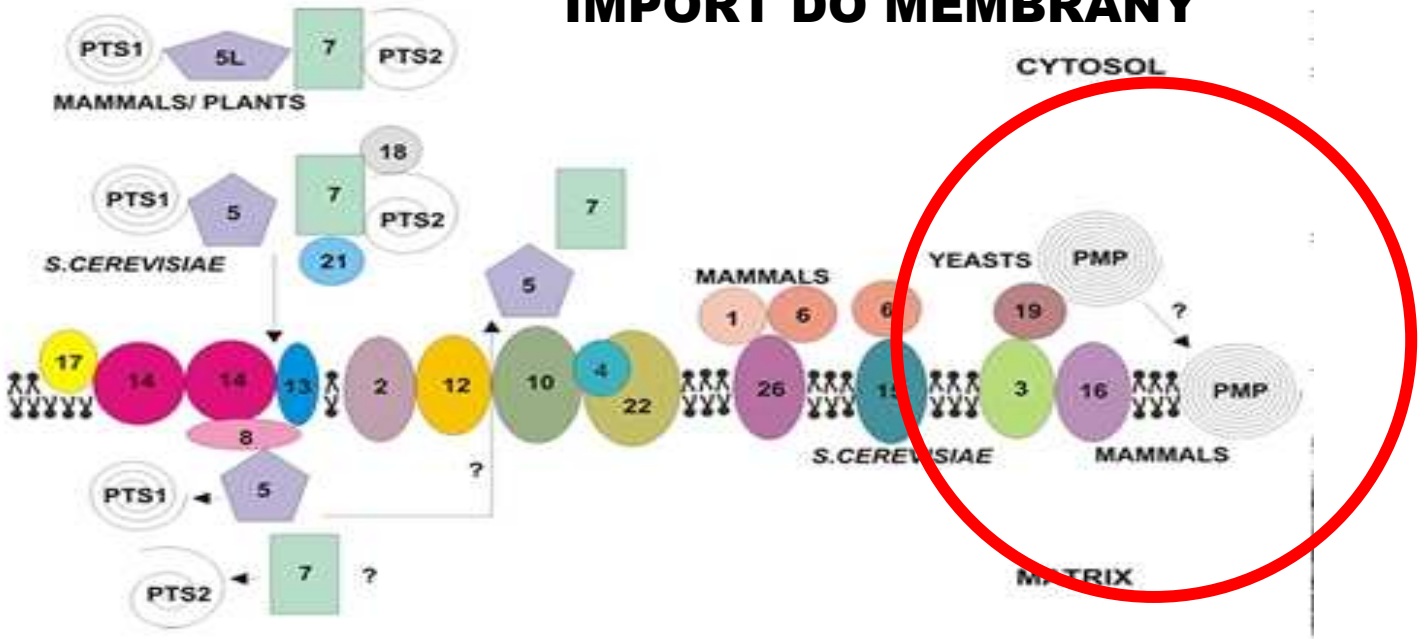
1) ATP hydrolysis is required for import.

2) Import into peroxisomes does not require unfolding of the protein chain (even gold particles conjugated to a peroxisomal protein can be imported).

3) Hsp70 is however needed and becomes bound to the exterior of the peroxisome.



IMPORT DO MEMBRÁNY



Schematic diagram of protein import into peroxisomes

Most peroxisome matrix proteins possess one of two targeting signals, a C-terminal tripeptide called PTS1 or an N terminal nonapeptide termed PTS2. These proteins interact with the cytosolic receptors PEX5 and PEX7 respectively. In mammals and plants the two pathways are coupled, as an isoform of PEX5 (PEX5L) is required as an accessory protein for PEX7. In bakers yeast the two pathways are separate with PEX7 having 2 unique accessory proteins, PEX18 and PEX21. PEX5 and PEX7 receptors with their cargoes dock at a complex in the peroxisome membrane comprised of PEX14, 13 (and 17 in baker's yeast). This docking complex is part of a larger complex 'the importomer' which includes the membrane proteins PEX2,10 and 12. By a mechanism that remains unknown, matrix proteins traverse the membrane, probably still associated with their receptor. PEX5 at least partially traverses the membrane and interacts with PEX8 on the trans side of the membrane. Subsequently cargo is unloaded and the receptors recycled, again by an unknown mechanism that involves PEX4 and 22. Insertion of peroxisome membrane proteins (PMPs) is less well understood but requires PEX19, which may function as a receptor/chaperone and PEX3. In mammals PEX16 is also involved in this process. (see Brown and Baker 2003 for further details).

Komunikace kompartmentů

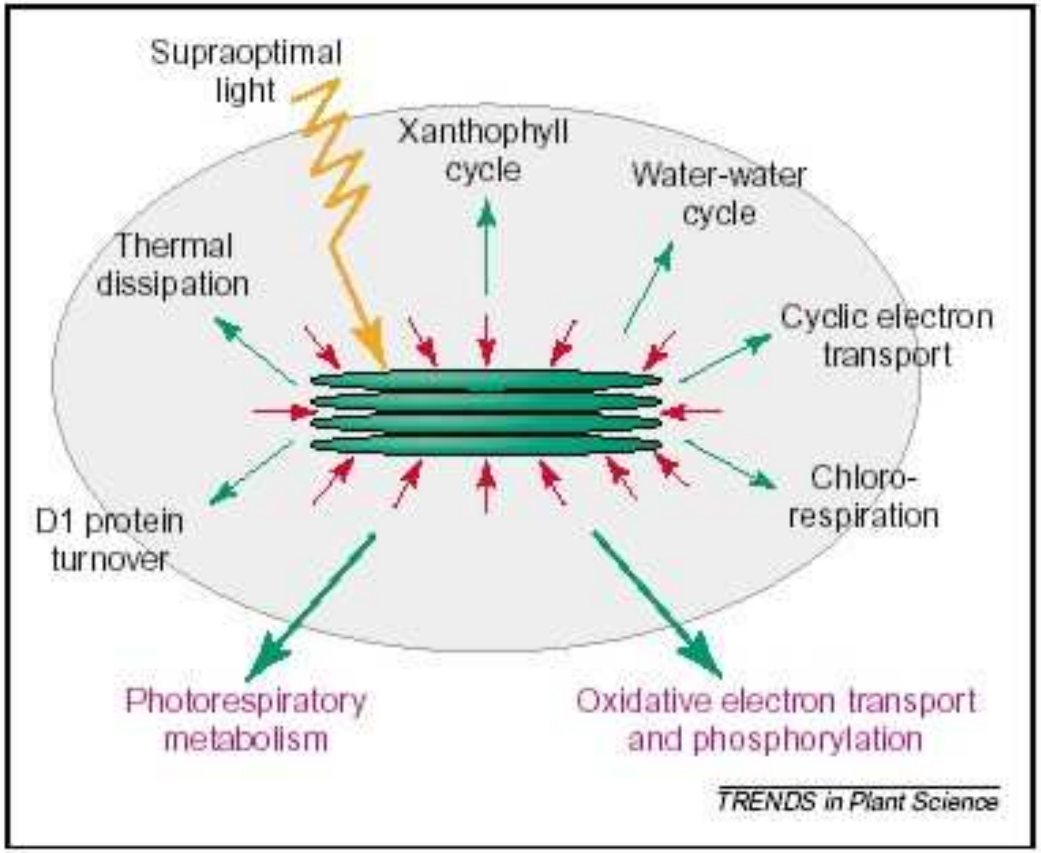
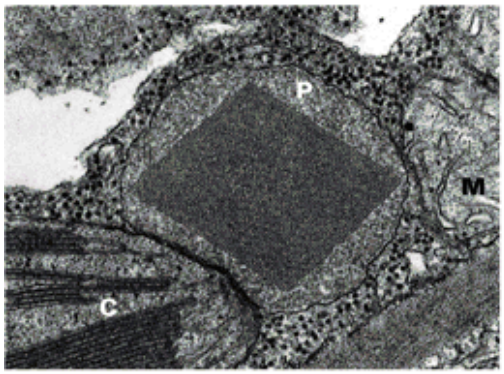


Figure 2. Protection of chloroplast thylakoid membranes against photoinhibition by different processes. The chloroplasts are vulnerable to photoinhibition under supraoptimal light levels or suboptimal CO_2 concentrations. The red arrows indicate the stress on chloroplast membranes. There are several mechanisms within the chloroplasts that protect them from overexcitation (green arrows): thermal dissipation, scavenging active oxygen species, cycling of electrons and repair of damaged proteins. Marked protection against photoinhibition is provided by photorespiration and oxidative electron transport, both of which are mediated by mitochondria. Thus, mitochondria play a significant role in protecting chloroplasts against photoinhibition.

Fotorespirace

(A)



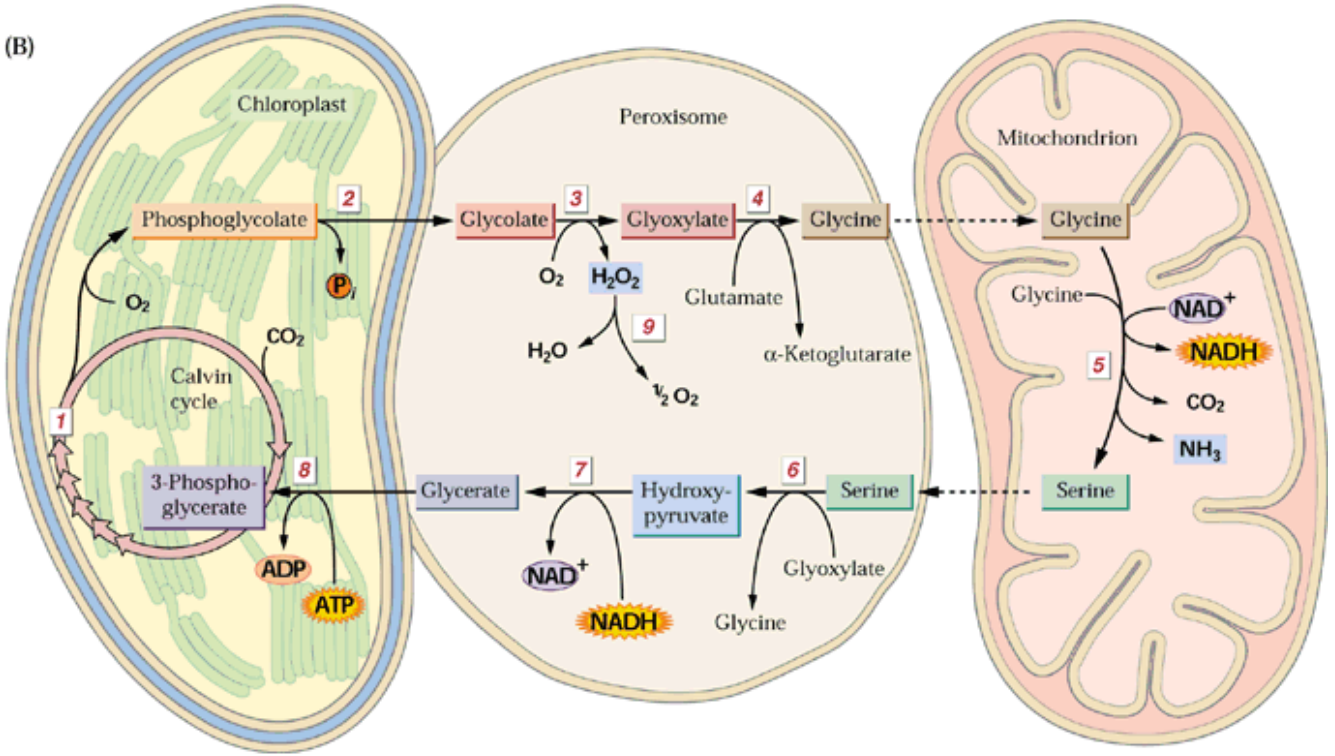
Key

1. Ribulose-1,5-bisphosphate carboxylase/oxygenase
2. Phosphoglycolate phosphatase
3. Glycolate oxidase
4. Glutamate:glyoxylate aminotransferase
5. Glycine decarboxylase and serine hydroxymethyl transferase
6. Serine:glyoxylate aminotransferase
7. Hydroxypyruvate reductase
8. Glycerate kinase
9. Catalase

Fotorespirace:
moc světla, málo CO_2

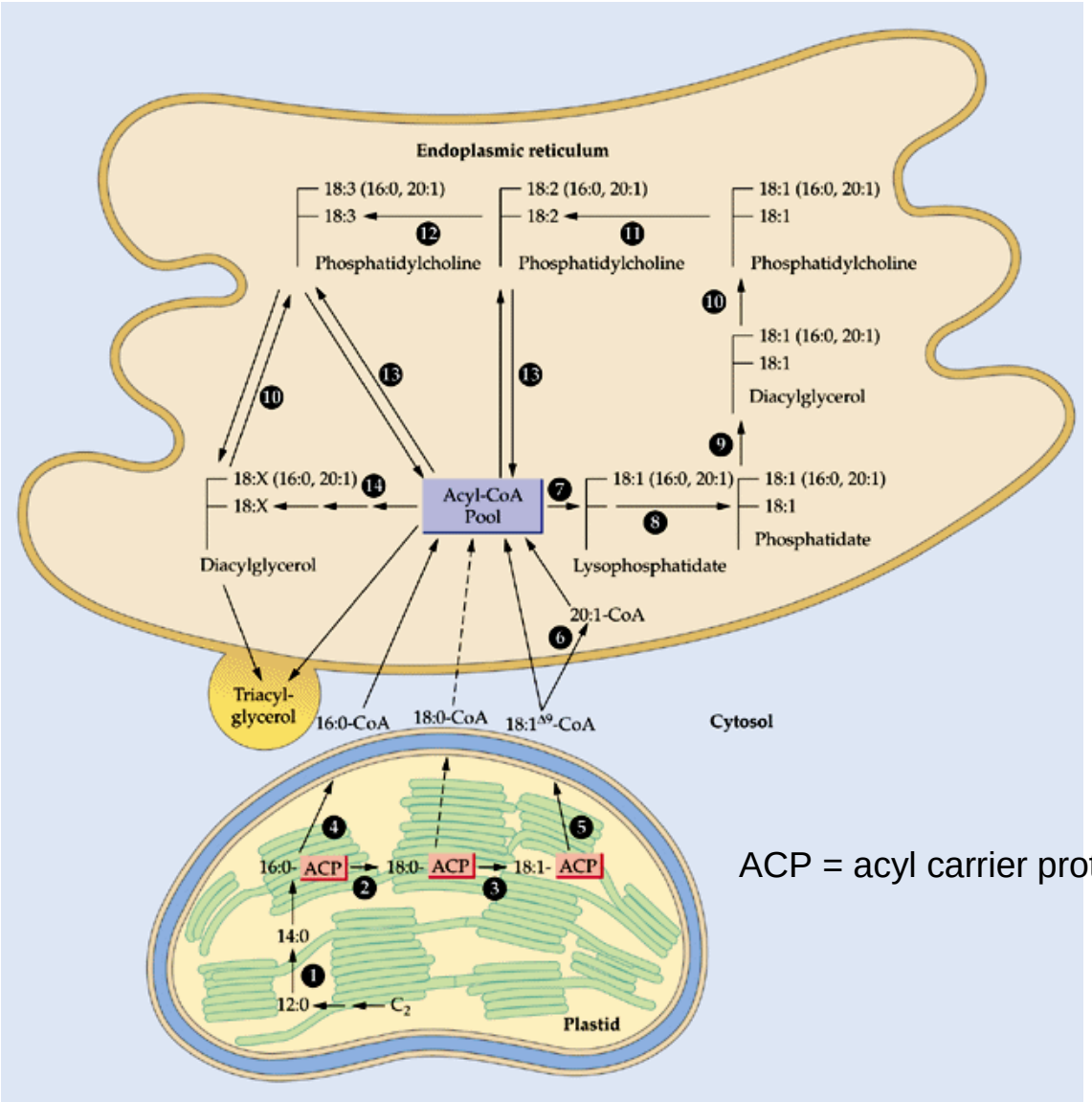
místo
2x 3-fosfoglycerátu
vzniká
1x 3-fosfoglycerátu a
1x 2-fosfoglykolát

(B)



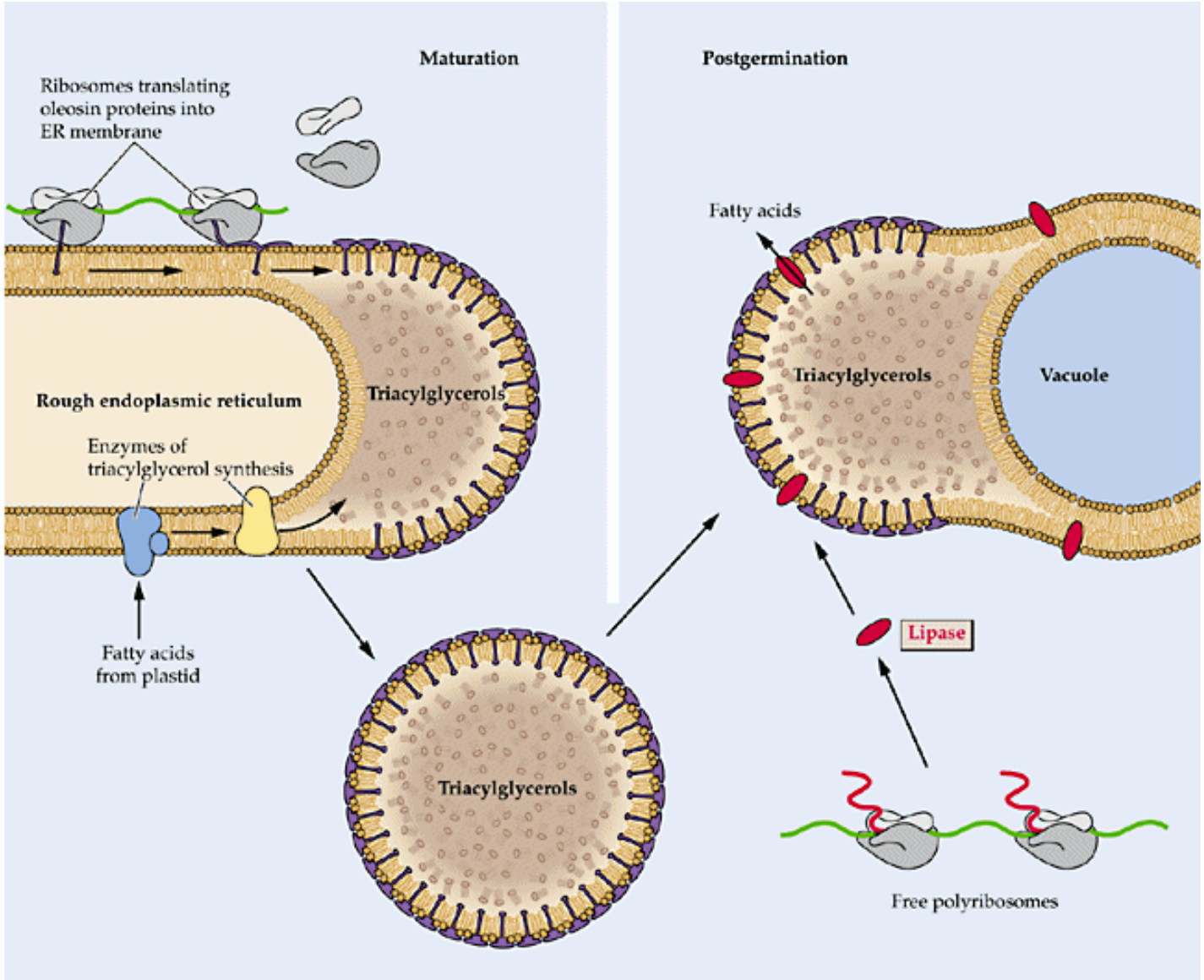
až 75 % produkce fosfoglykolátu se takto vrátí do Calvinova cyklu

Olejová tělíska – vznik a mobilizace

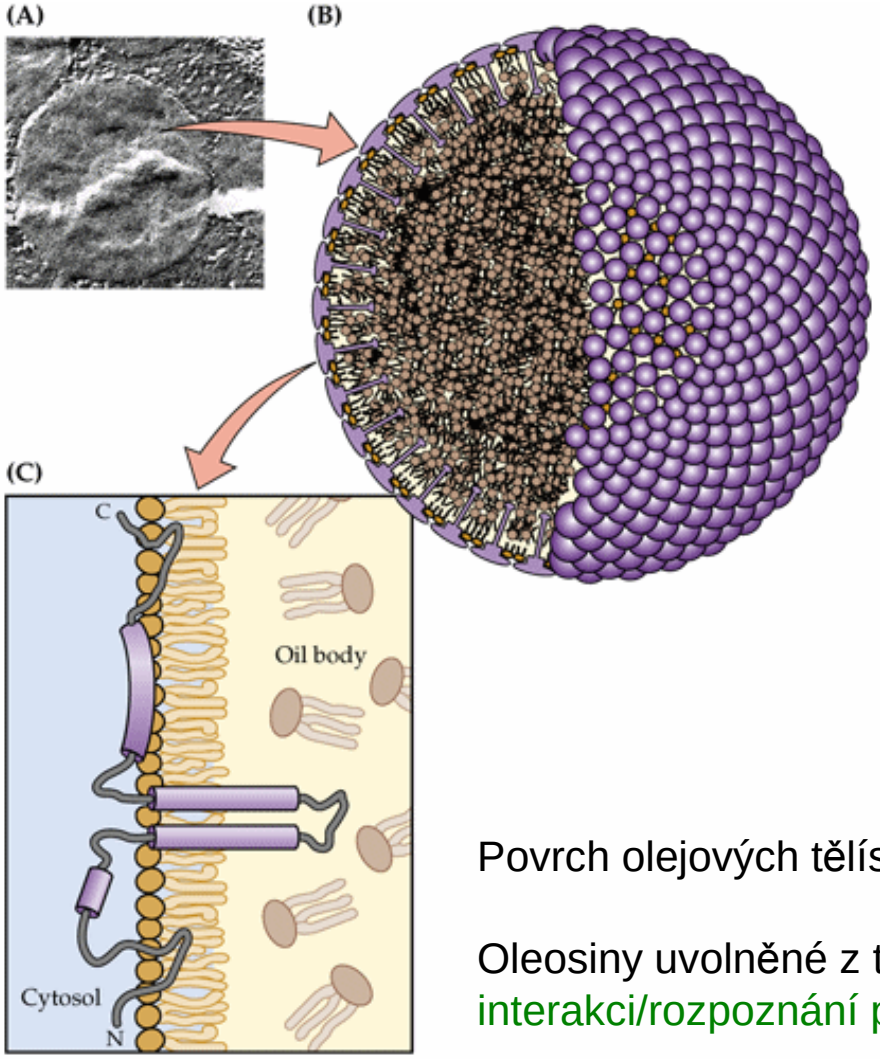


ACP = acyl carrier protein

Olejová tělíska – vznik a mobilizace



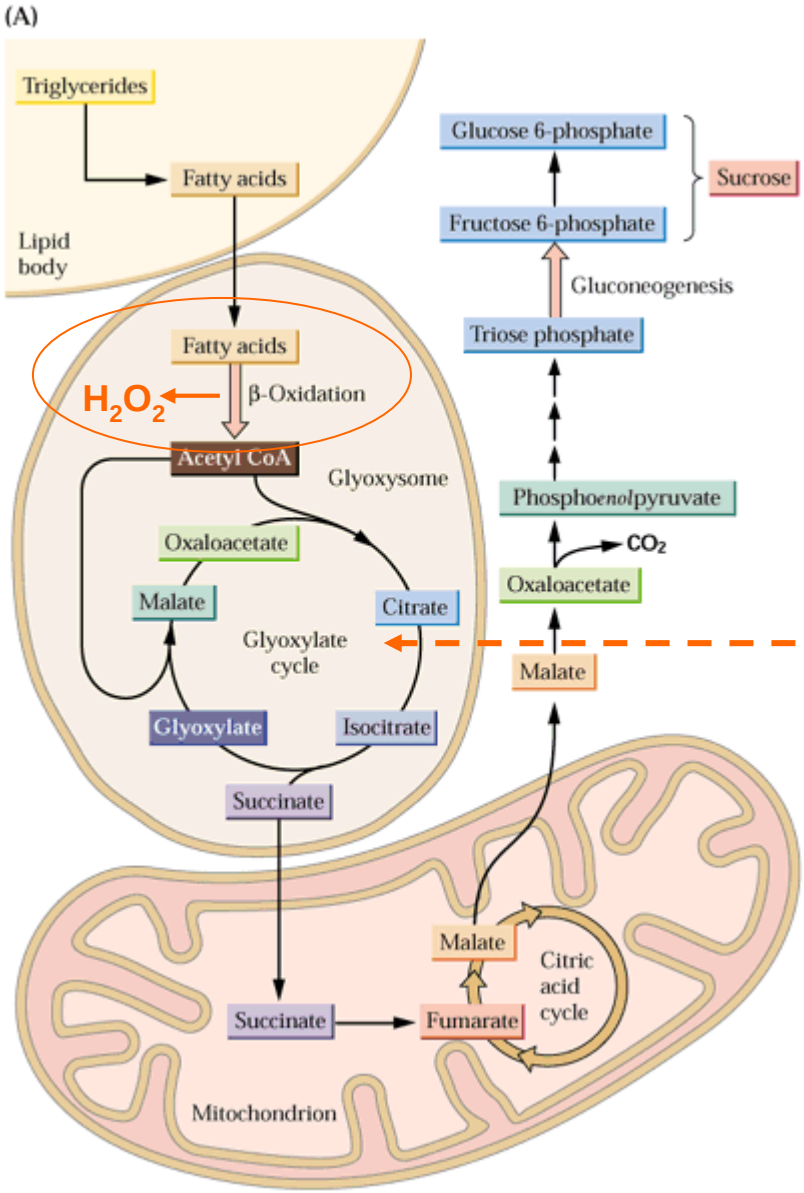
Olejová tělíska – vznik a mobilizace

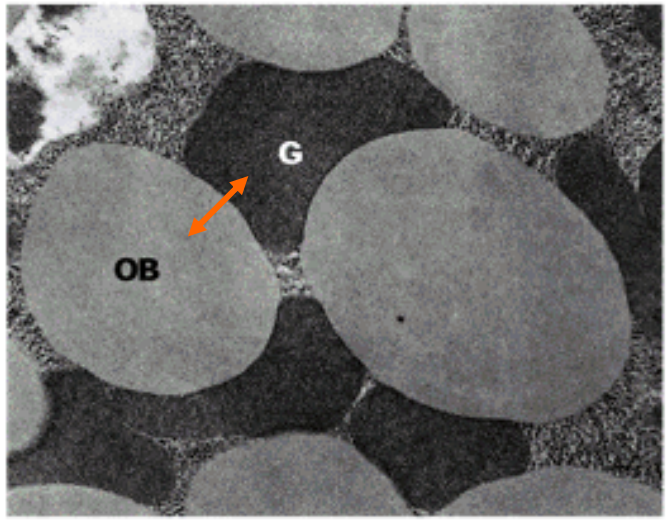


Povrch olejových tělísek stabilizujicí oleosiny.

Oleosiny uvolněné z tapeta mají důležitou roli při interakci/rozpoznání pylu a blizny.

Olejová tělíska a glyoxysomy





glyoxalátový cyklus

mobilizace zásobních tuků a glukoneogeneze
rostliny dokážou převádět tuky na cukry

Export malátu (kys.jablečné) z plastidů zajišťuje nepřímo „rozvod“ redukčních ekvivalentů do cytoplasmy a dalších organel

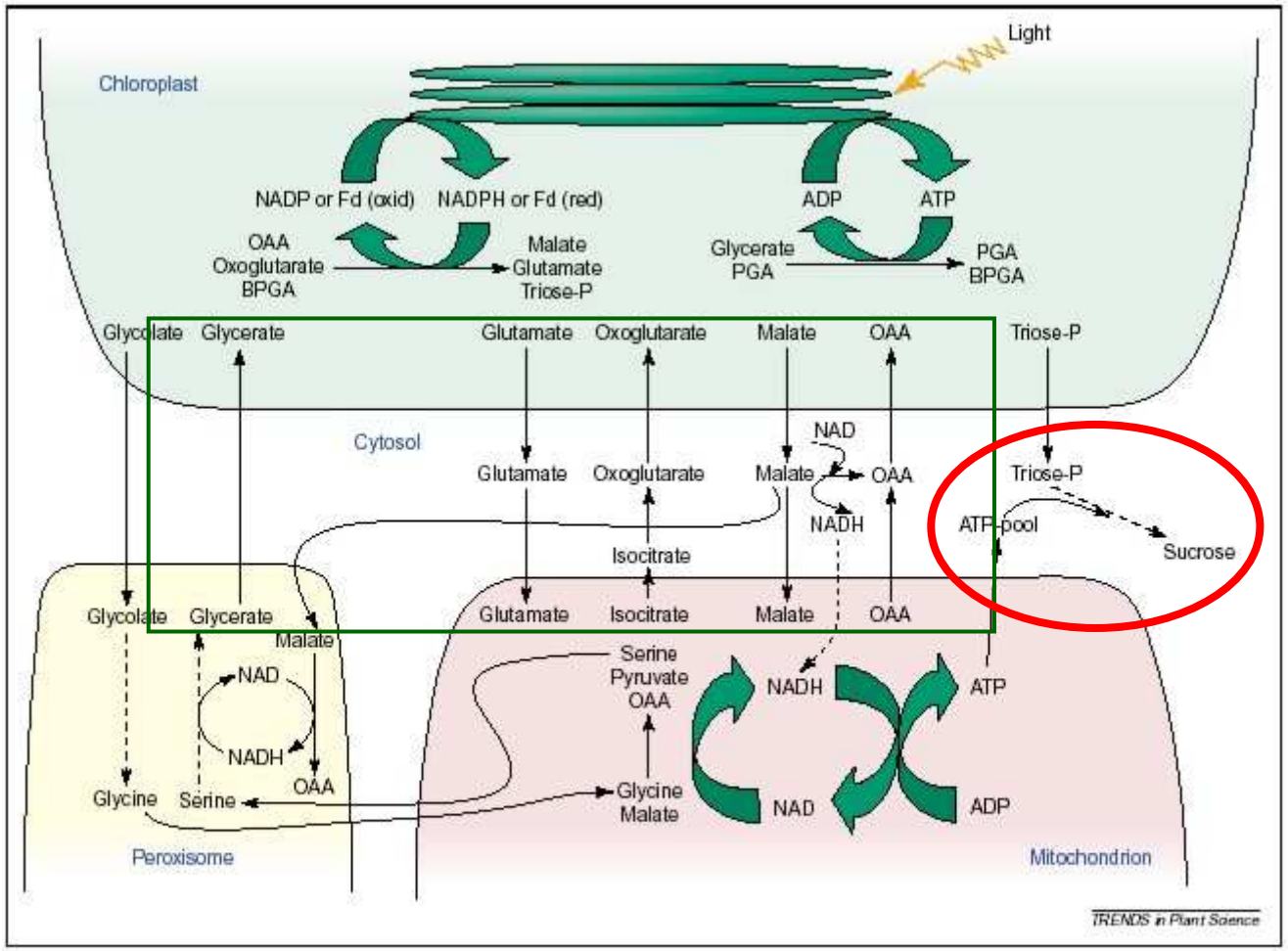


Figure 1. Use of reduced equivalents [NADPH or ferredoxin (Fd)] from chloroplasts. Mitochondria, cytosol and peroxisomes are common sinks for reduced equivalents. The dissipation of excess redox equivalents from chloroplasts is ensured by the export of glycolate, malate, glutamate and triose phosphate (triose-P). The arrows indicate the metabolite movement. The recycling of these four compounds involves mitochondria. Furthermore, mitochondrial ATP synthesis helps to sustain the formation of sucrose, an important end product of carbon assimilation. Abbreviations: BPGA, 1,3-bisphosphoglycerate; Fd(oxid), oxidized Fd; Fd(red), reduced Fd; OAA, oxaloacetate; PGA, 3-phosphoglycerate. The electron transport system of mitochondria is indicated in Box 1.

v cytoplasmě vládne relativně redukční prostředí

Shrnutí metabolické komunikace organel

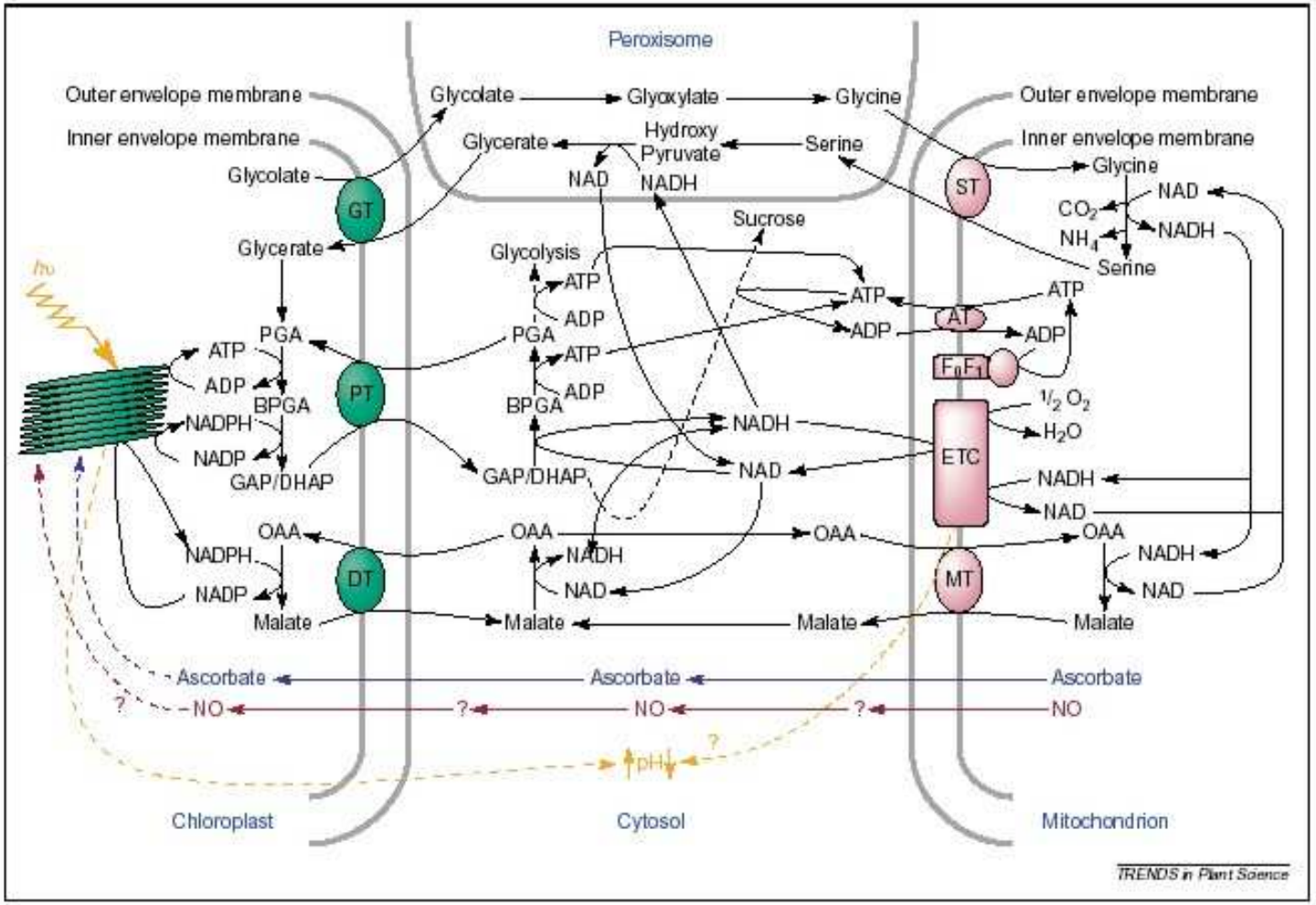


Figure 3. Biochemical cross talk between chloroplasts, mitochondria, cytosol and peroxisomes mediated by metabolites or other signals. Metabolite movement (indicated by arrows) is facilitated by the translocators (on the inner envelope membranes of chloroplasts and mitochondrial or porins (of peroxisomes). Such metabolite flux ensures the maintenance of the redox state and allows an efficient recycling of carbon and nitrogen across cellular compartments. The mitochondria are the most active organelles because they oxidize glycine, malate and even NADH through their electron transport chain (ETC) and synthesize ATP by the help of ATPase complex (F_0F_1). Only mitochondria can export ATP to cytosol using the adenylate translocator (AT). The photorespiratory reactions, which help chloroplasts to dissipate energy and NADPH, are also backed up by mitochondria. The translocators shown on chloroplast membrane are the dicarboxylate translocator (DT), glycolate–glycerate translocator (GT) and phosphate translocator (PT), whereas those on the mitochondrial membrane are the malate translocator (MT) and a putative glycine–serine translocator (ST). The other possible signals between the organelles include ascorbate, nitric oxide (NO) and cytosolic pH, but these phenomena are yet to be studied in detail. Abbreviations: BPGA, 1,3-bisphosphoglycerate; DHAP, dihydroxyacetone-3-phosphate; GAP, glyceraldehyde-3-phosphate; OAA, oxaloacetate; PGA, 3-phosphoglycerate.

Rostliny mutanta ječmene *albostrians*, kompenzují nefunkční mutantní plastidy aktivací mitochondrií.

Further evidence of a close association between chloroplast development and mitochondrial protein composition has come from work on mutants as well as experiments using chemical treatments to disrupt normal plastid development. The *albostrians* mutant of barley, for example, has ribosome-deficient plastids and this prevents synthesis of a range of chloroplast proteins. Affected leaf tissue is white and the plastids lack thylakoids and chlorophyll. Expression of mitochondrially-encoded genes was found to be affected, with enhanced expression of genes encoding cytochrome oxidase subunits (*coxII*, *coxIII*) and ATPase (*atpA*, *atp6*, *atp9*). It was shown, by crossing the mutants with wild-type barley, that the increase in transcript levels was due to the lack of chloroplast development and was not a result of the nuclear *albostrians* allele. This was also supported by the effect of bleaching by treatment with norflurazon, which led to impaired chloroplast development and also enhanced the level of mitochondrial transcripts. These studies indicate that plastid development can affect mitochondrial gene expression (Hedtke *et al.*, 1999).

C3 a C4 rostliny

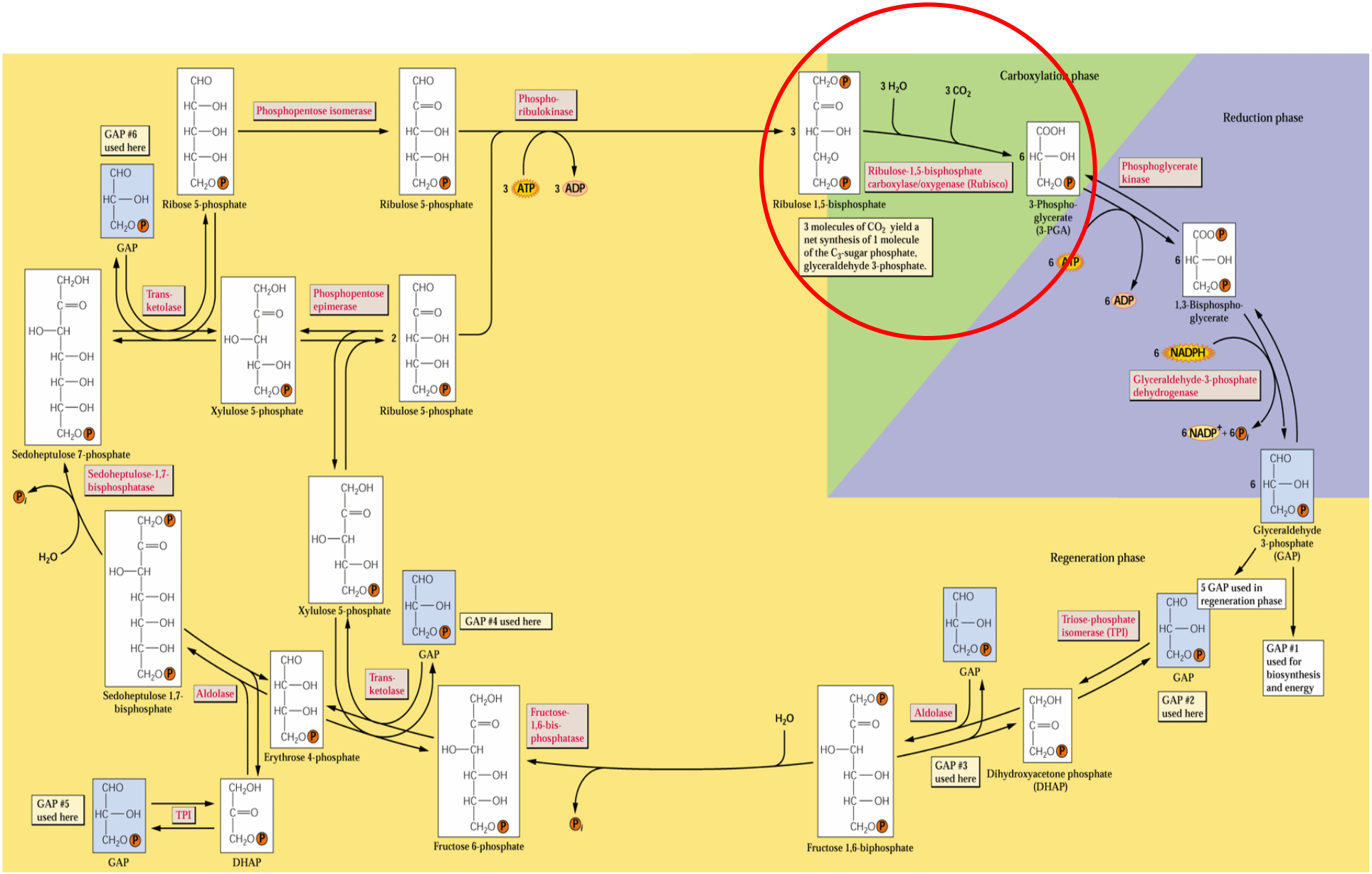
U C3

Vysoká teplota zvyšuje oxygenázovou aktivitu Rubiska proti carboxylázové.

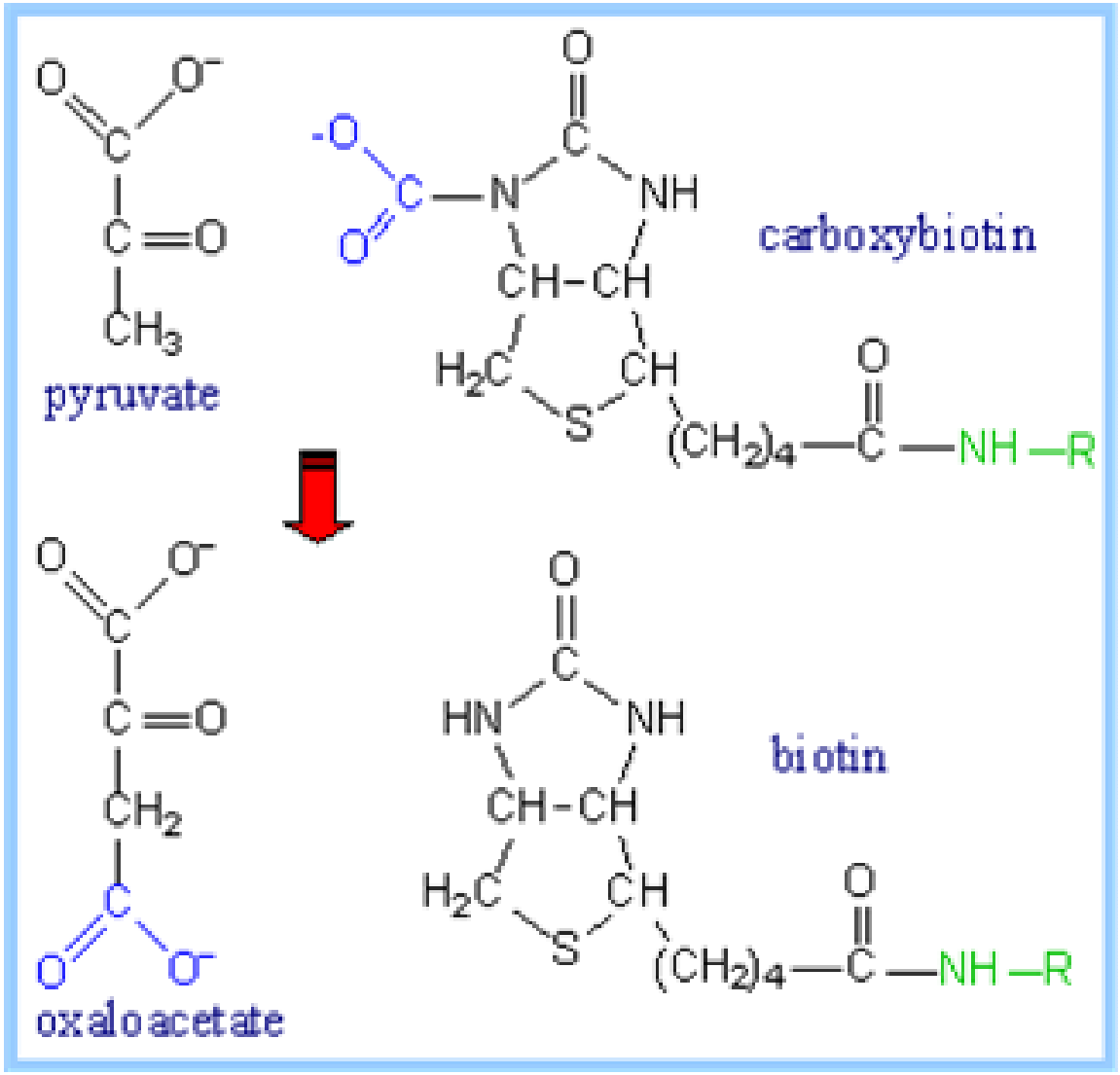
Při zvýšené teplotě se nepříznivě mění rozpustnost O_2 a CO_2.

Při zvýšené teplotě hrozí velké ztráty vody při zvýšené transpiraci.

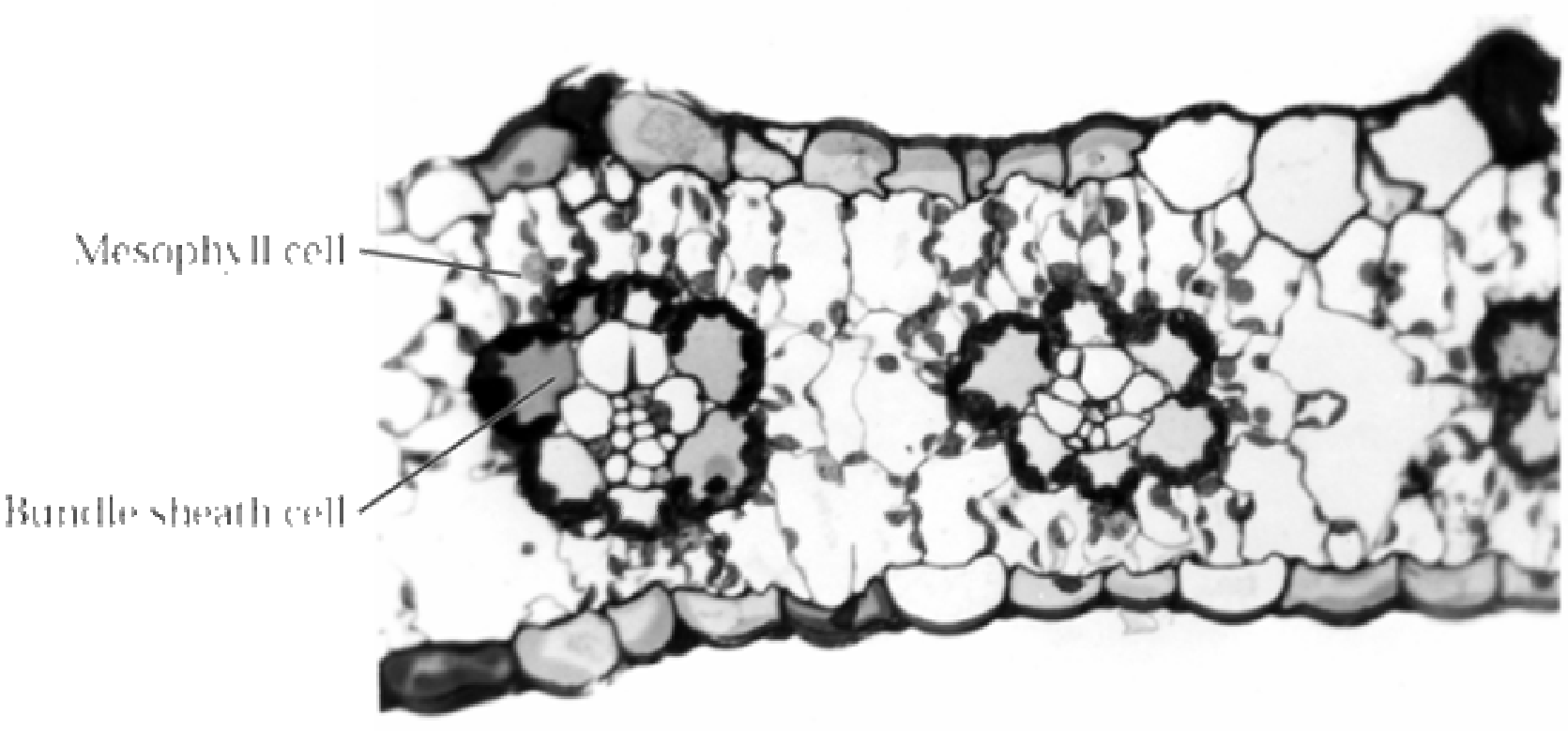
C3 rostliny


Carboxylation phase
Reduction phase
Regeneration phase
Ribose 5-phosphate
Phosphopentose isomerase
Ribulose 5-phosphate
Phospho-ribulokinase
3 ATP
3 ADP
3 H2O
3 CO2
Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco)
Ribulose 1,5-bisphosphate
3 molecules of CO2 yield a net synthesis of 1 molecule of the C3-sugar phosphate, glyceraldehyde 3-phosphate.
3-Phospho-glycerate (3-PGA)
Phosphoglycerate kinase
6 ATP
6 ADP
1,3-Bisphospho-glycerate
6 NADPH
Glyceraldehyde-3-phosphate dehydrogenase
6 NADP+ + 6 Pi
Glyceraldehyde 3-phosphate (GAP)
5 GAP used in regeneration phase
GAP #1 used for biosynthesis and energy
GAP #2 used here
GAP #3 used here
GAP #4 used here
GAP #5 used here
GAP #6 used here
GAP
Trans-ketolase
Xylulose 5-phosphate
Phosphopentose epimerase
Sedoheptulose 7-phosphate
Sedoheptulose-1,7-bisphosphatase
Pi
H2O
Sedoheptulose 1,7-bisphosphate
Aldolase
Erythrose 4-phosphate
Triose-phosphate isomerase (TPI)
Dihydroxyacetone phosphate (DHAP)
Fructose-1,6-bis-phosphatase
Fructose 6-phosphate
Fructose 1,6-biphosphate
TPI
DHAP

C4 rostliny



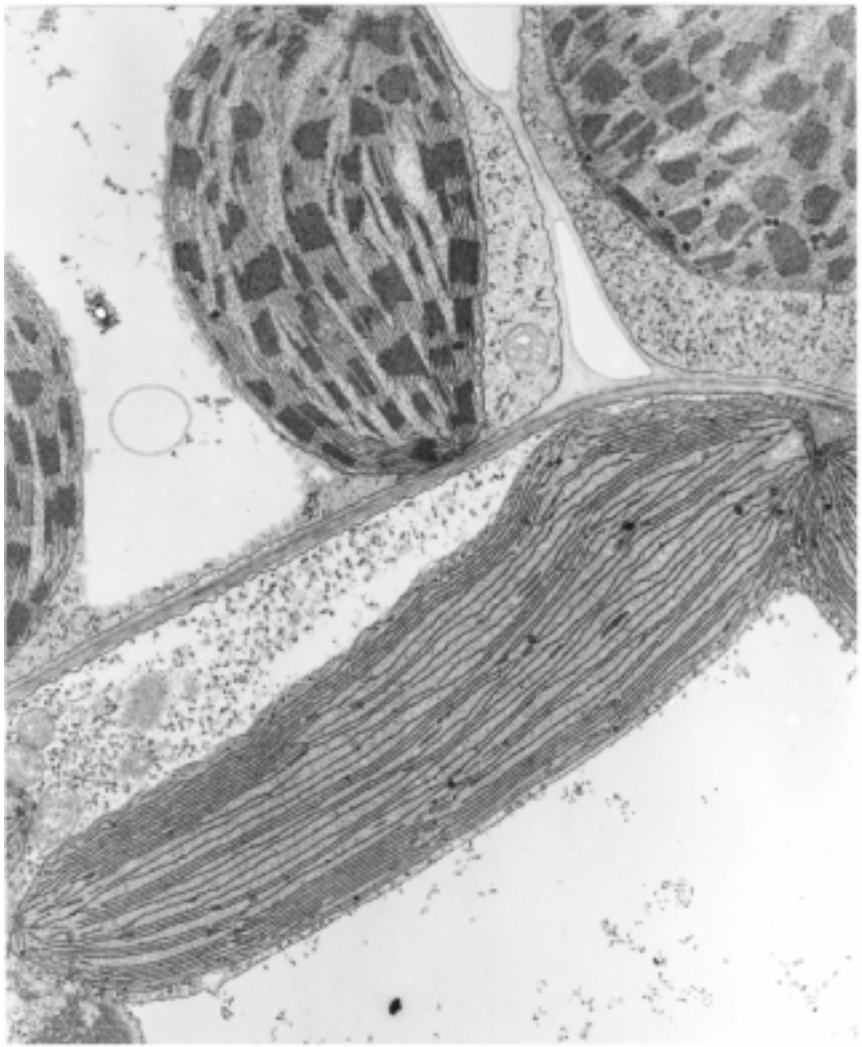
Věnčitá anatomie C4 rostlin



„KRANZ“/věnčitá anatomie – CO_2 je primárně zachycován v mezofylu karboxylací PEP na oxaloacetát

prostorová separace

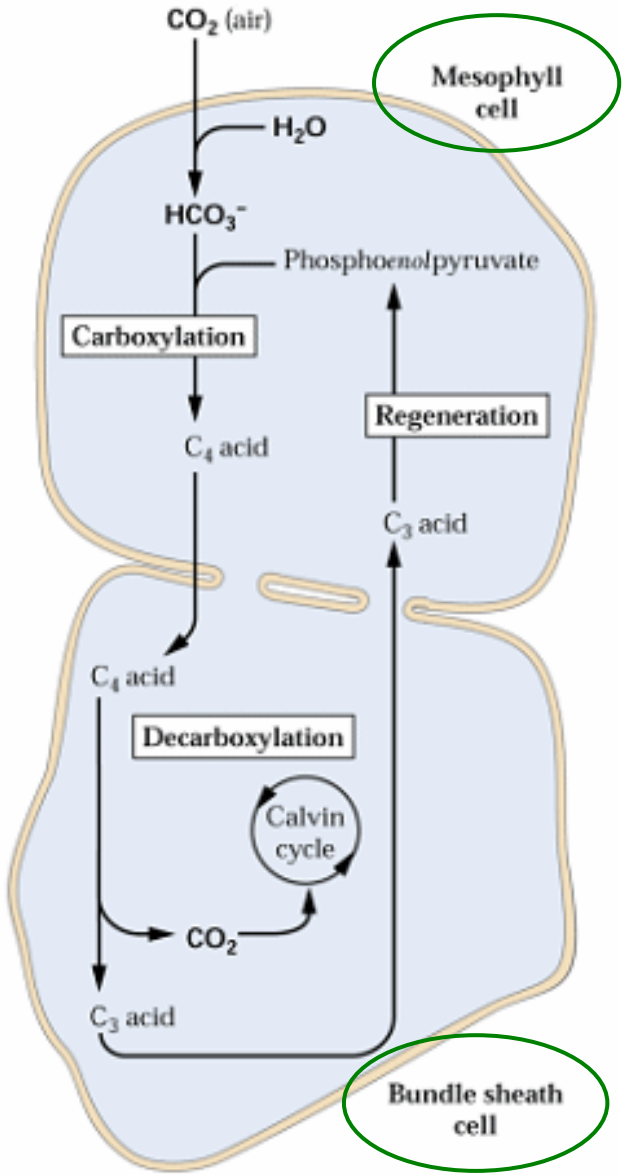
Věnčitá anatomie C4 rostlin

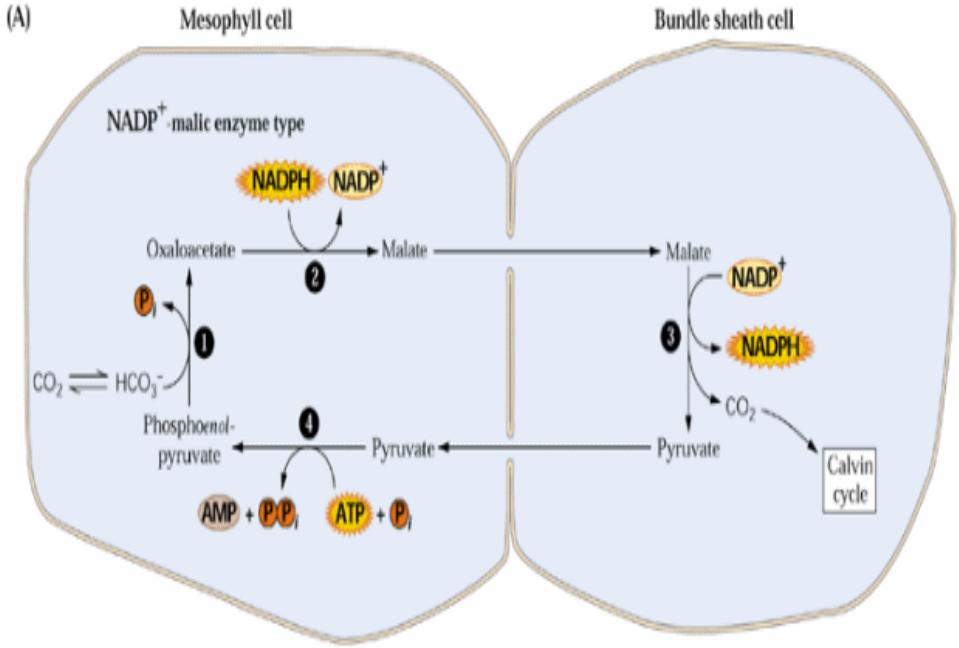


Mezofyl

Buňky pochev cévních svazků

Věnčitá anatomie C4 rostlin





1 = PEP karboxyláza

uvnitř rostliny je daleko vyšší poměr koncentrace CO_2/O_2 ve prospěch CO_2, fotorespirace je potlačená

CAM rostliny

Crassulacean Acid Metabolism

Časová separace
k příjmu CO2 dochází ve tmě – průduchy otevřené ve dne by znamenaly dehydrataci.