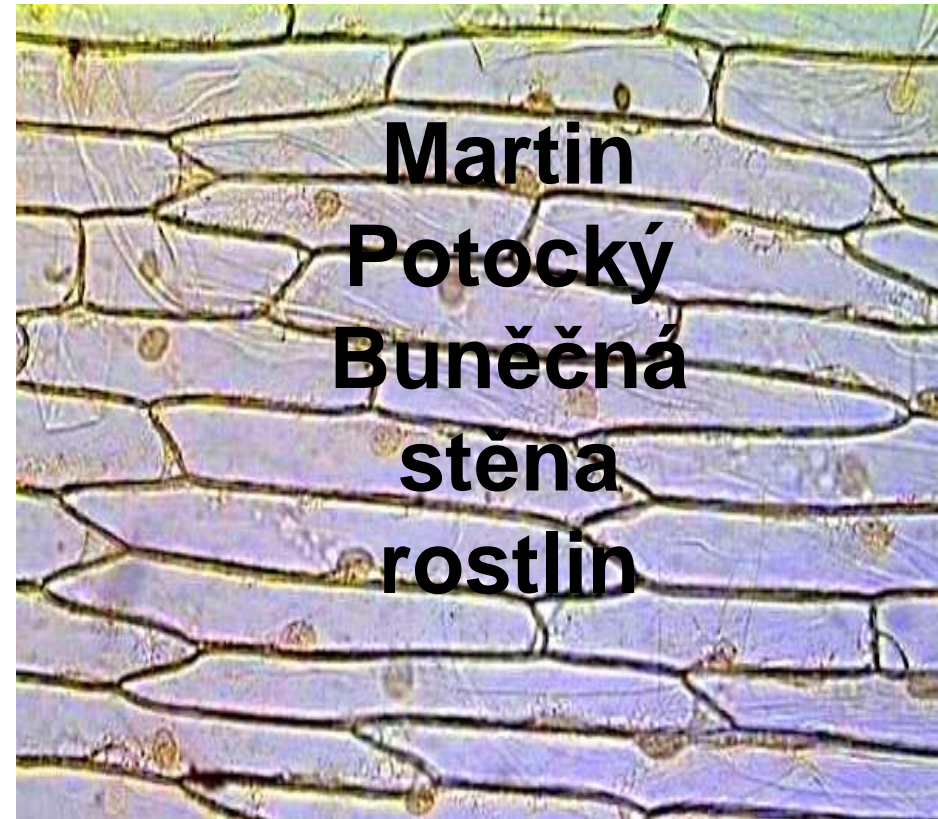


PINK
FLOYD
THE
WALL



**Martin
Potocký
Buněčná
stěna
rostlin**

Obsah

- Buněčná stěna jako integrální část rostlinné buňky
- Sacharidy jako základní stavební složky BS
- Makromolekuly v BS
- Struktura buněčné stěny, typy
- Biosyntéza BS
- Buněčný růst a BS
- Diferenciace buněk, primární a sekundární BS
- Význam BS pro člověka, biotechnologie atd...

Obsah

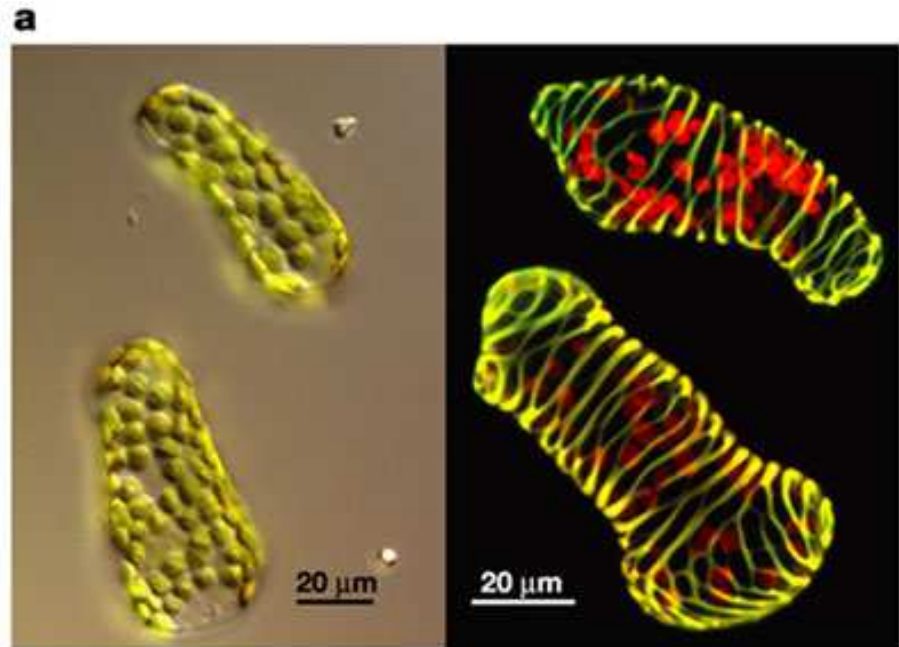
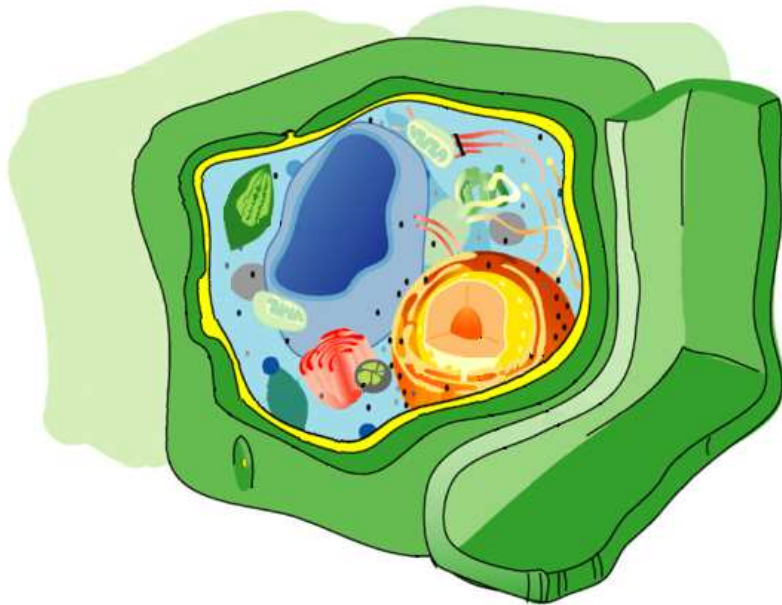
- **Buněčná stěna jako integrální část rostlinné buňky**
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- Význam BS pro člověka, biotechnologie atd...

- Buněčná stěna lépe vystihuje svébytnost suchozemských rostlin než plastidy a fotosyntéza.
- Je aspektem celé rostlinné buňky, ani syntéza celulózy se nedá pojmout jako jednoduchá enzymatická reakce.

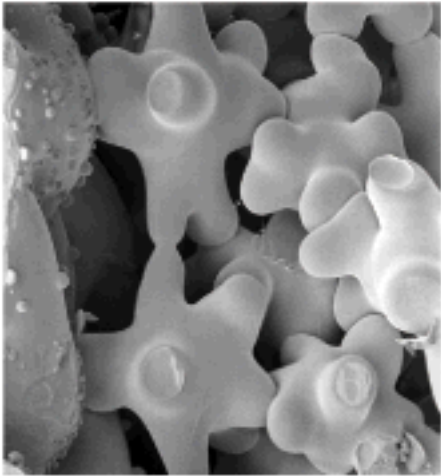
Funkce buněčné stěny

- Určuje vnější **tvar** a **růst** buňky
- Tvoří mechanické **struktury** v rámci celé rostliny
- Podílí se na funkční **specializaci** buněk a **vývoji** rostliny
- Tvoří **ochranu** před prostředím a patogeny
- Slouží jako **zásobárna**
- Zprostředkovává **komunikaci** – mezibuněčnou i z okolí dovnitř buňky
- Účastní se **rozpoznávání** vlastní/cizí

Buněčná stěna je živá organela! (většinou)

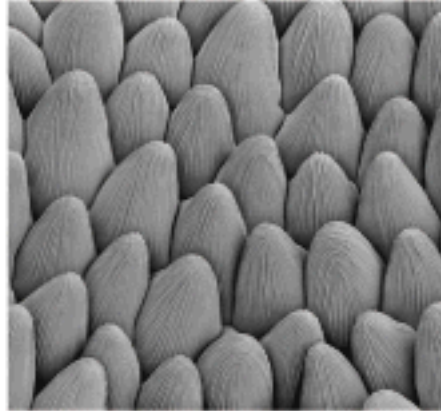


(A)



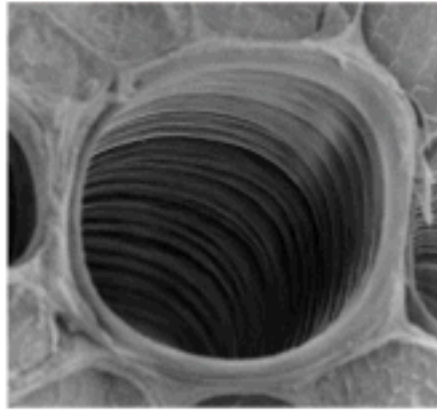
Listový houbový parenchym

(B)



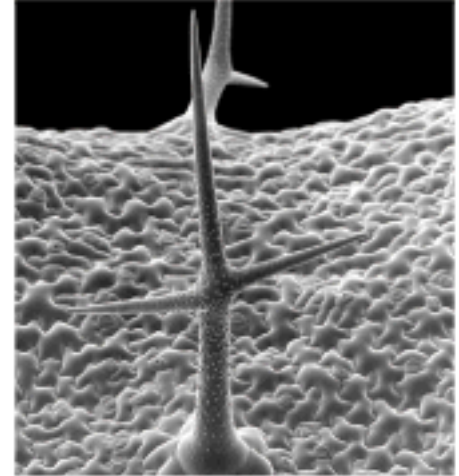
korunní plátek hledíku

(C)



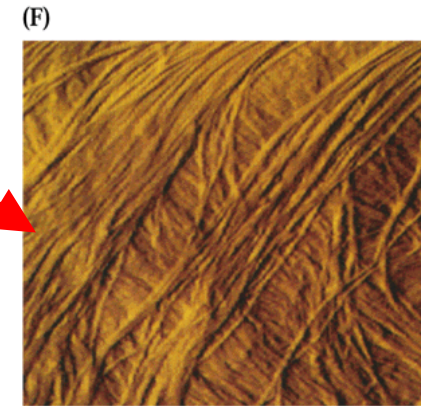
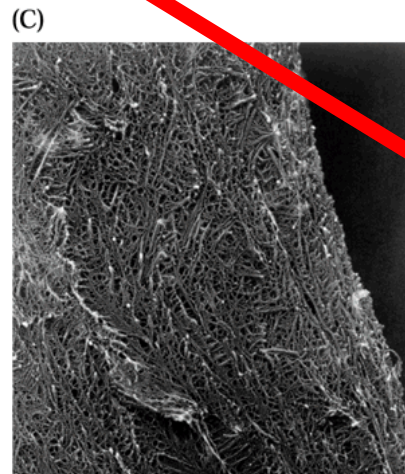
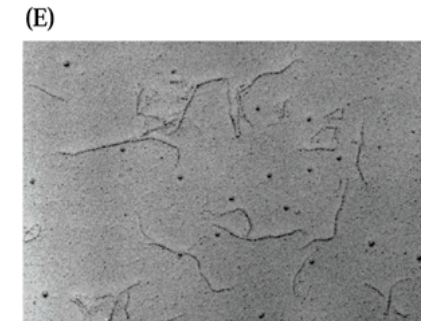
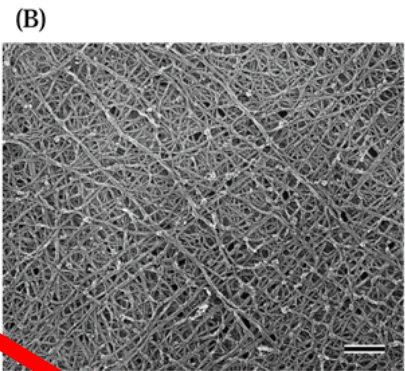
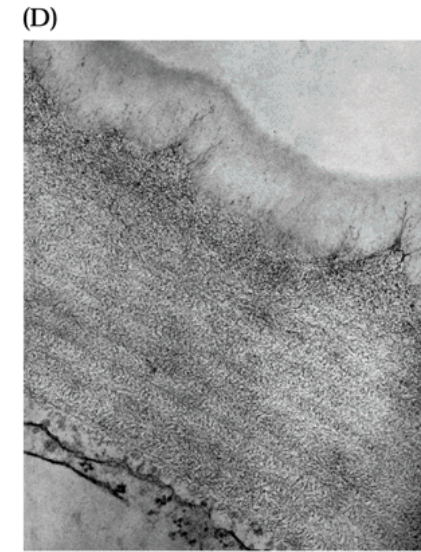
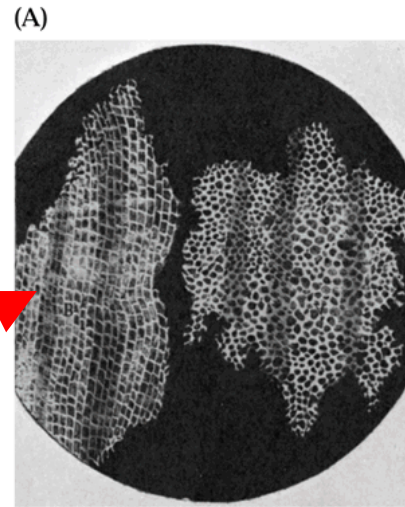
tracheida = xylem

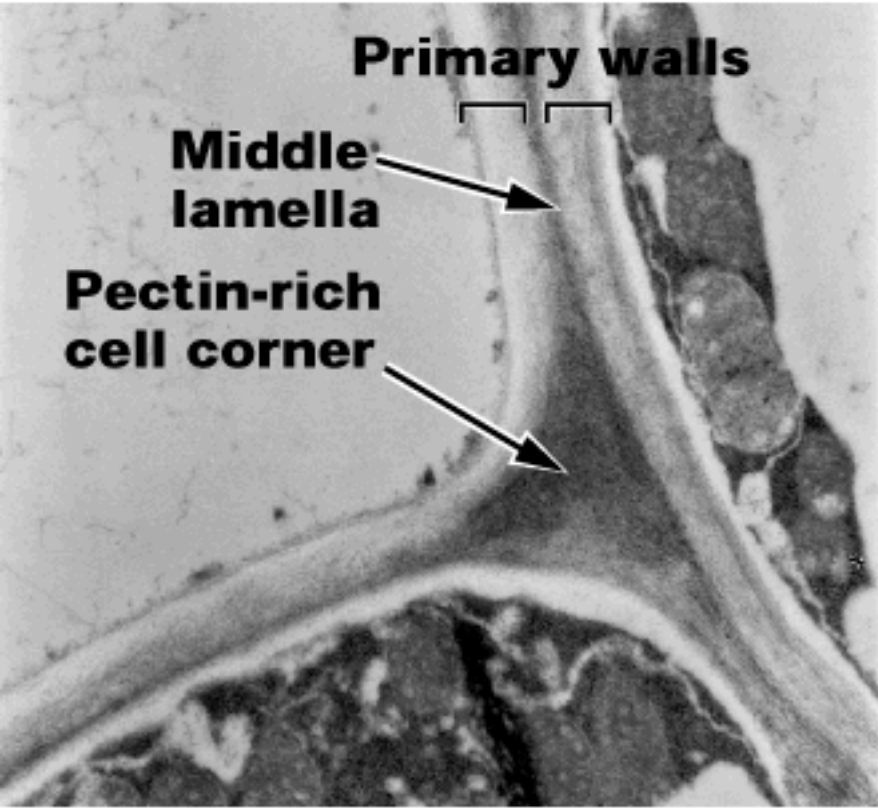
(D)



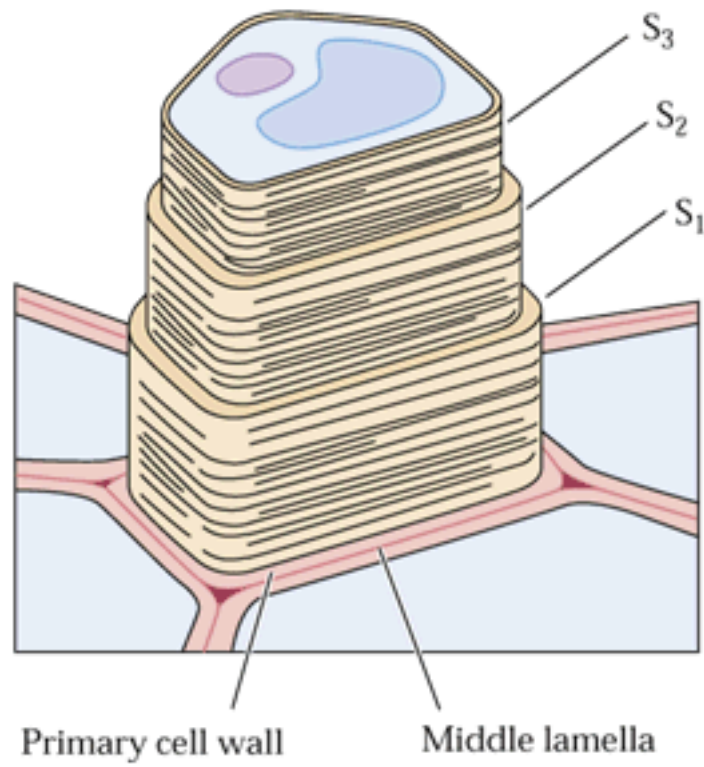
listový trichom

Od Roberta Hooka
k "atomic force
microscopy"

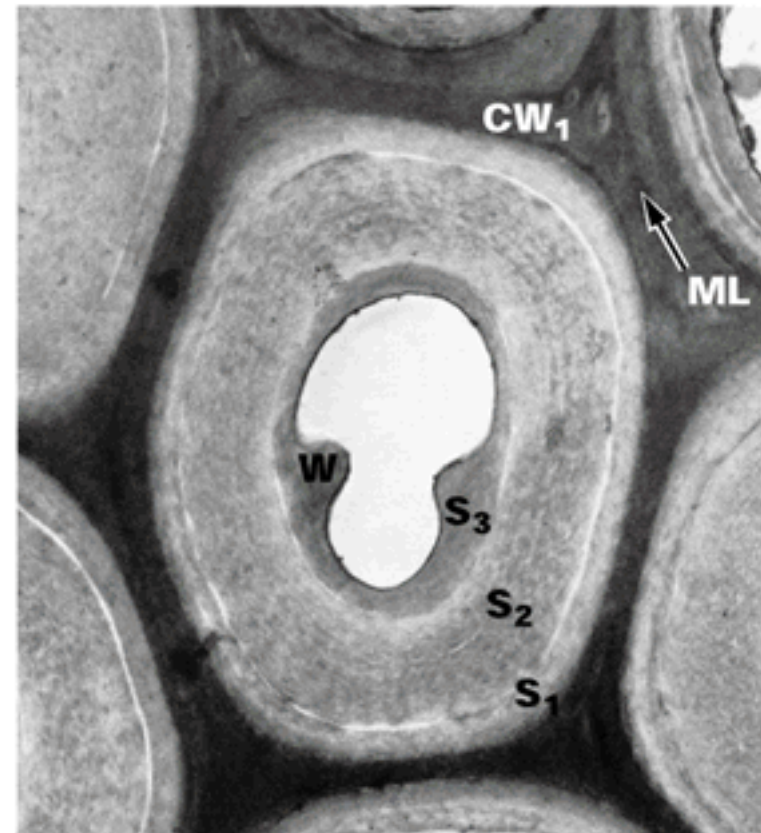




(A)



(B)



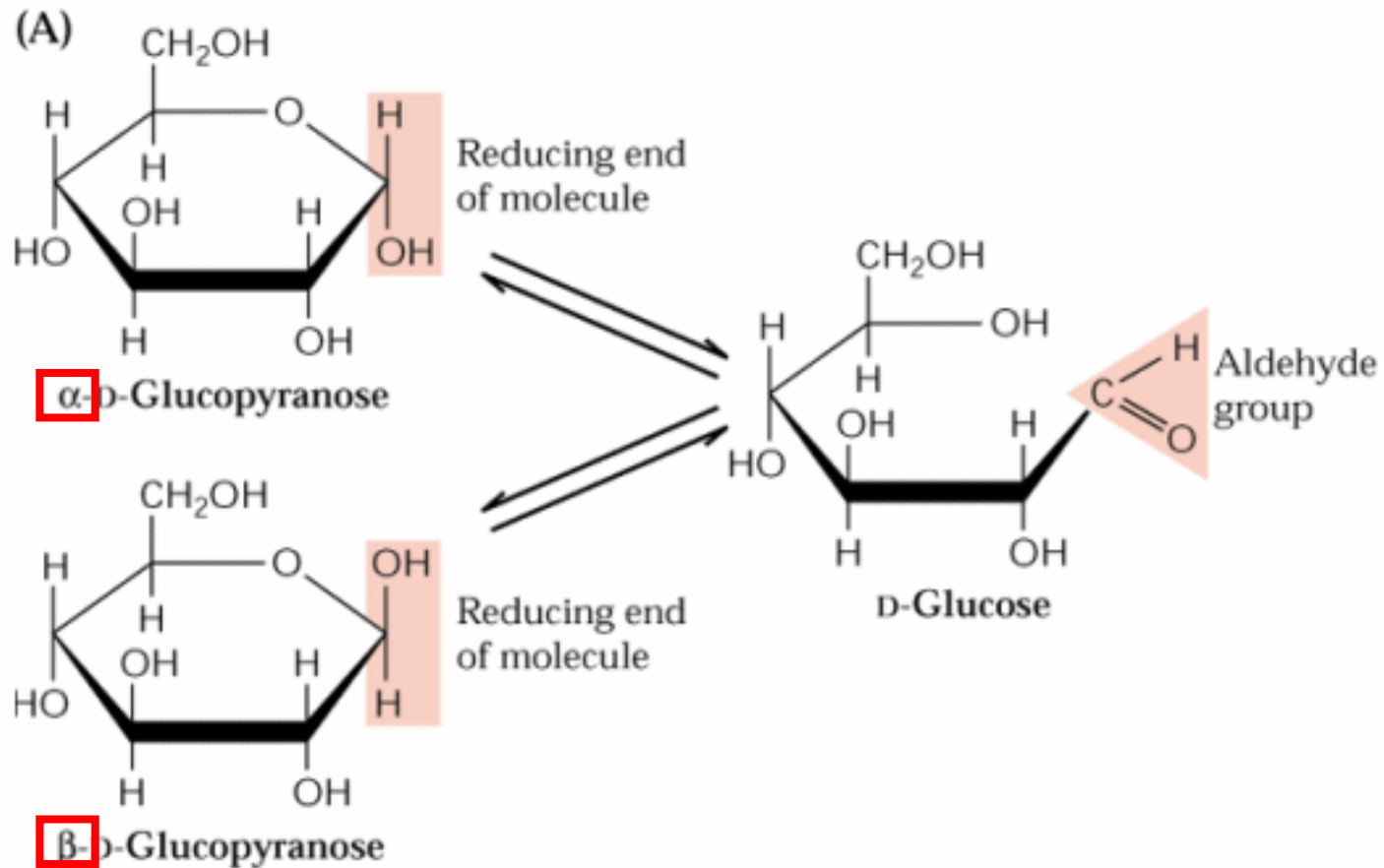
Složky buněčné stěny

- **Polysacharidy**
 - Kalóza
 - Celulóza
 - Hemicelulózy (zvl. XyG a GAX, MGL)
 - Pektiny (HG, RG-I a -II)
- **Proteiny**
 - Extensiny, expansiny, AGPs, GRPs, PRPs
 - Endoglukanasy, XET/XTH

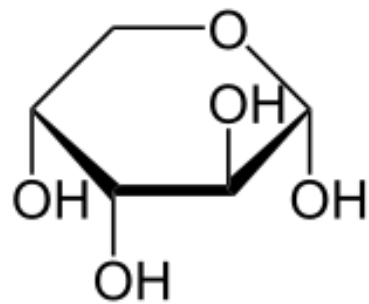
Obsah

- Buněčná stěna jako integrální část rostlinné buňky
- **Sacharidy jako základní stavební složky BS**
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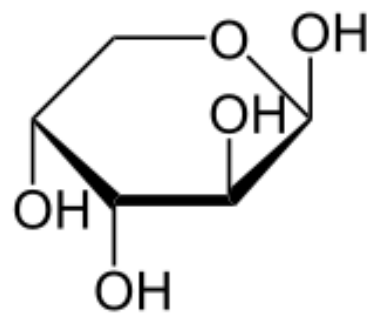
Stavební kameny stěny



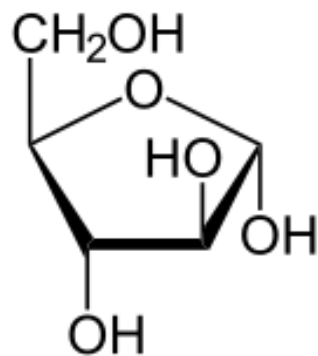
C1 – anomerní uhlík (karbonyl)



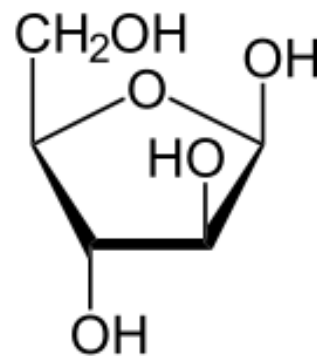
α -D-Arabinopyranose



β -D-Arabinopyranose

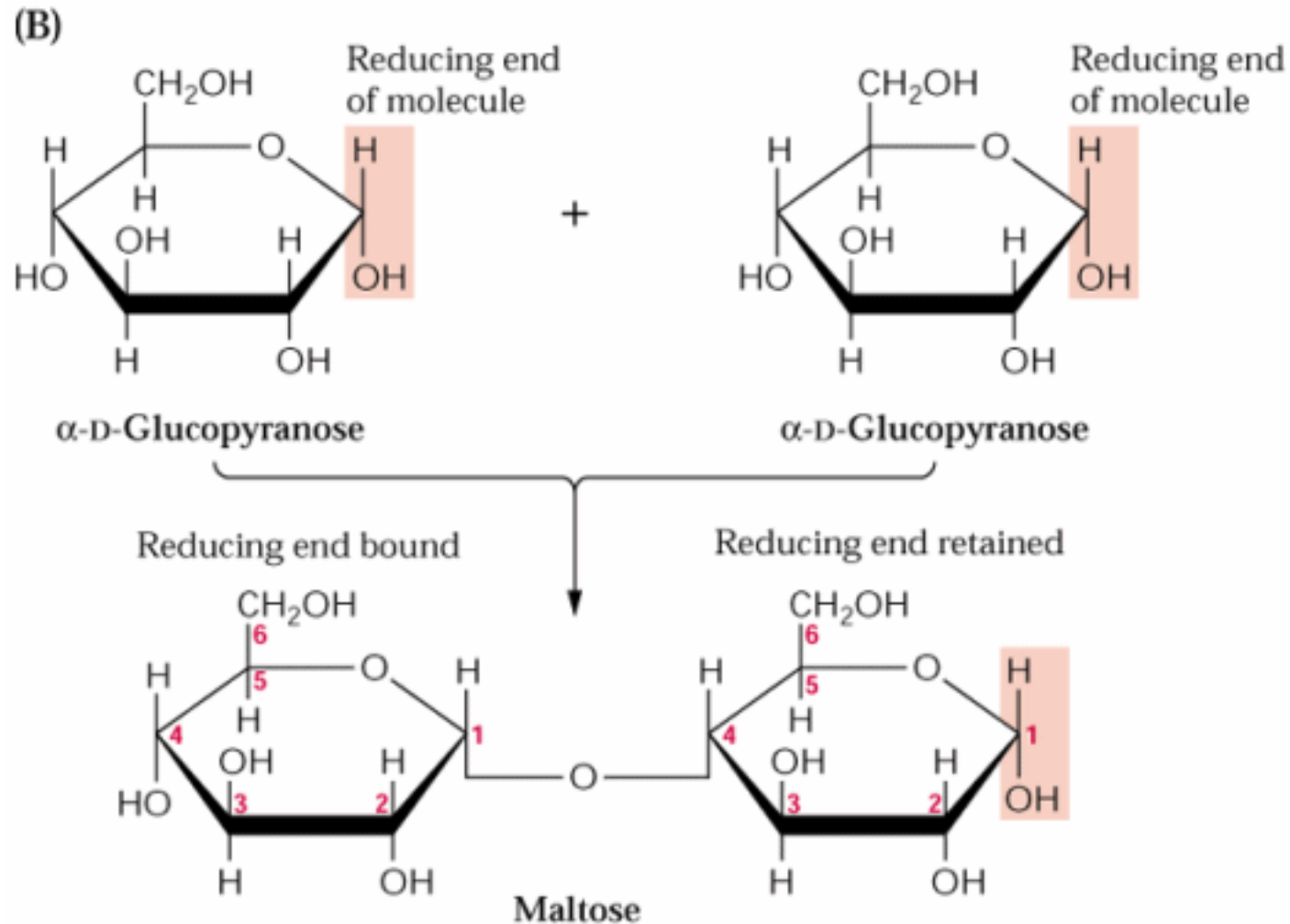


α -D-Arabinofuranose

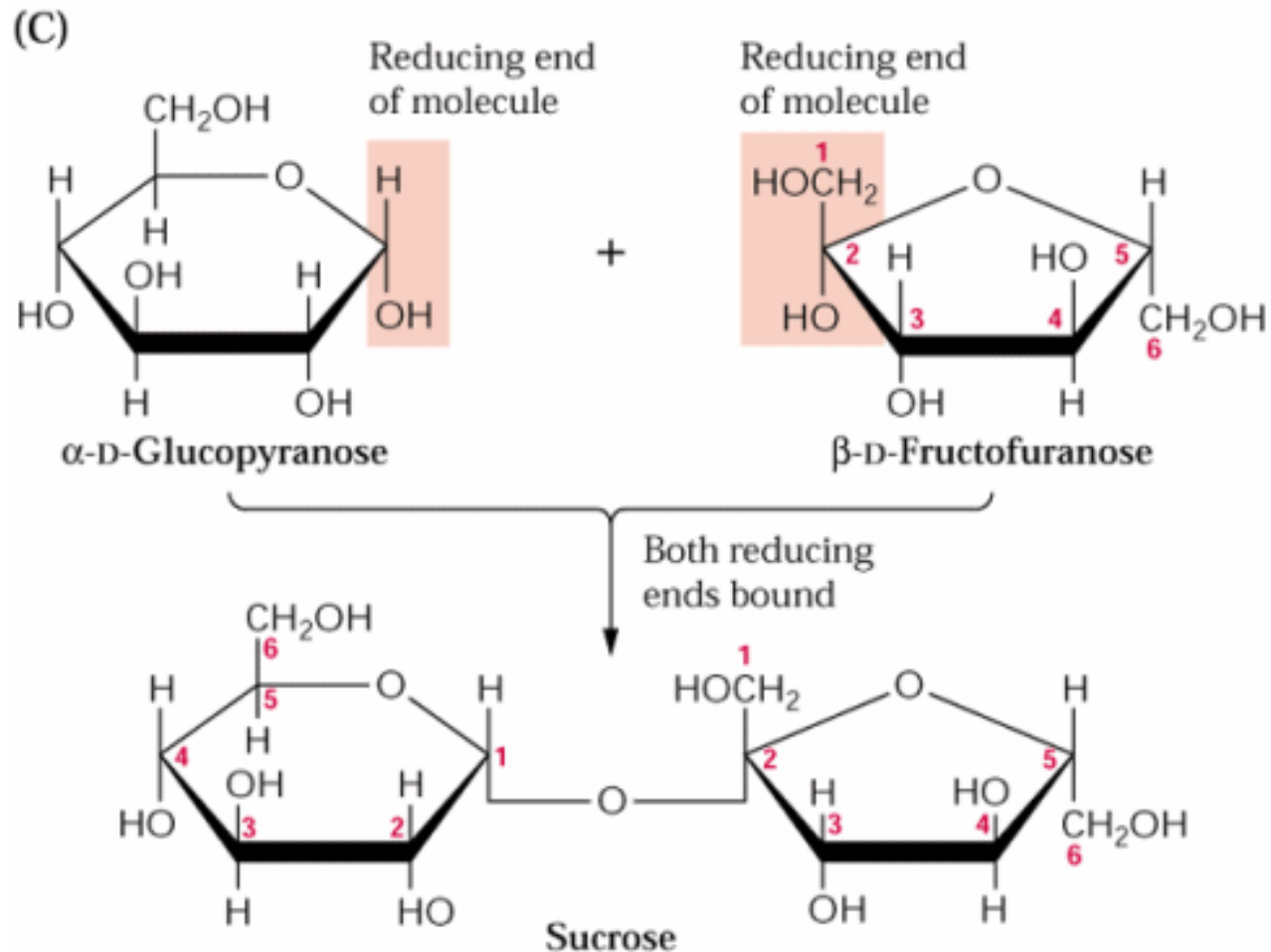


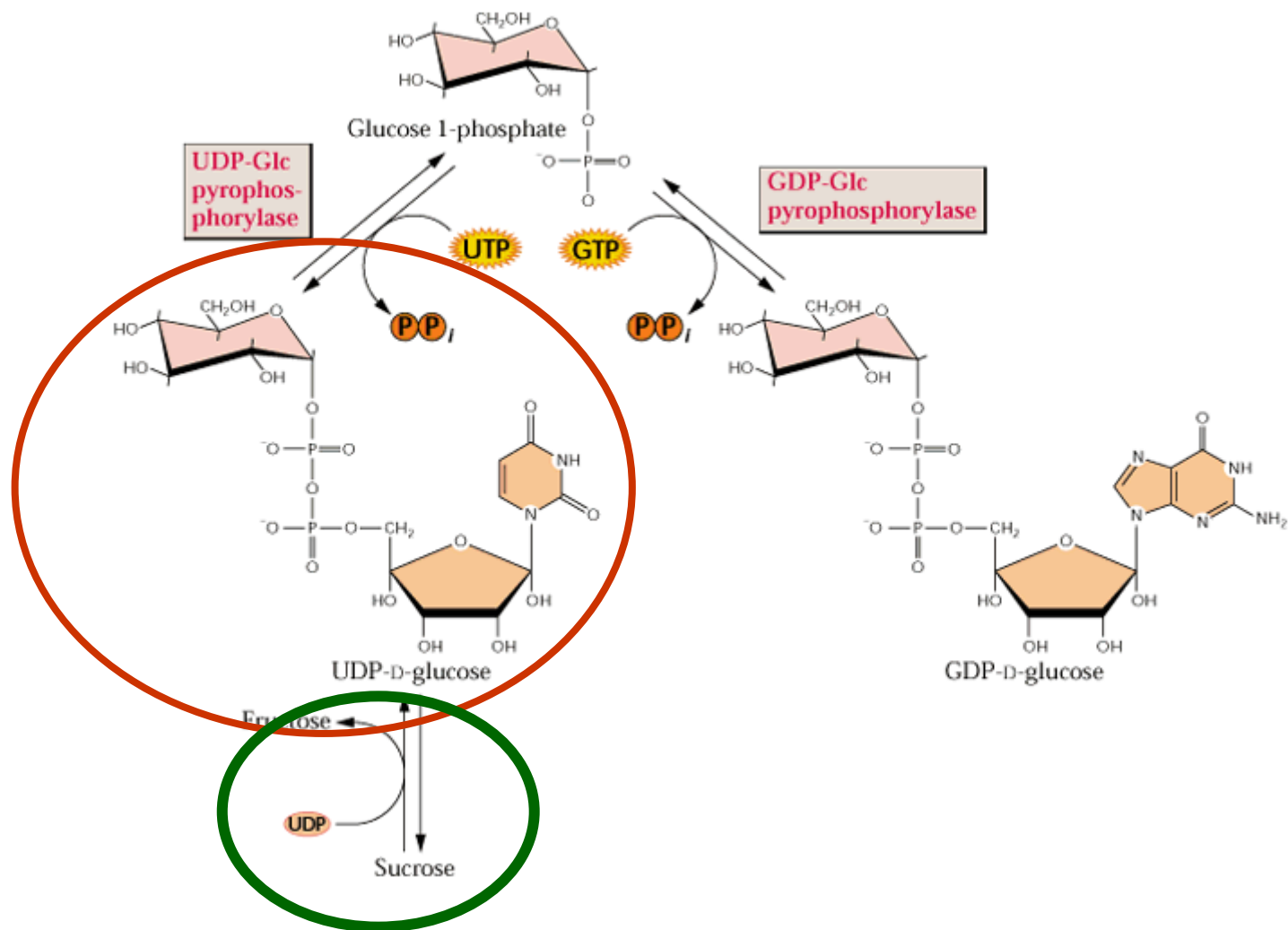
β -D-Arabinofuranose

Maltóza je redukující c.



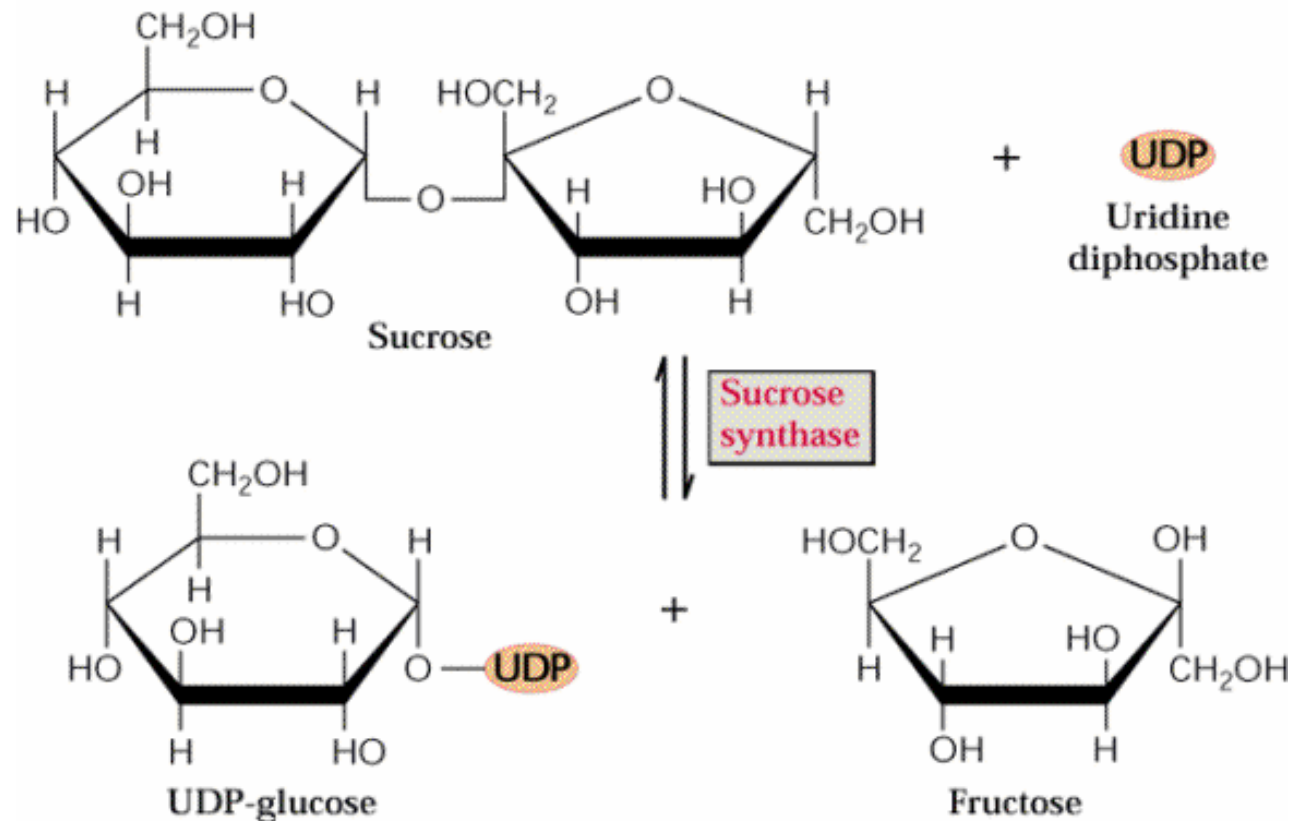
Sacharóza je neredukjící c.



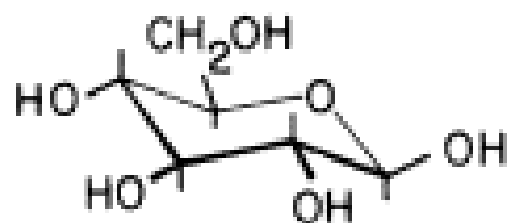


uridin di-fosfo glukóza = UDPG

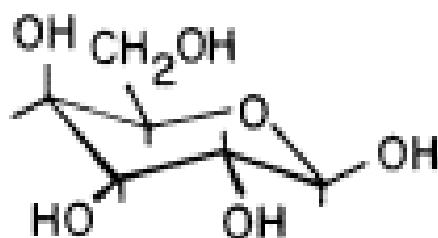
Sukróza syntáza – dodává UDPG pro syntézu celulózy i kalózy



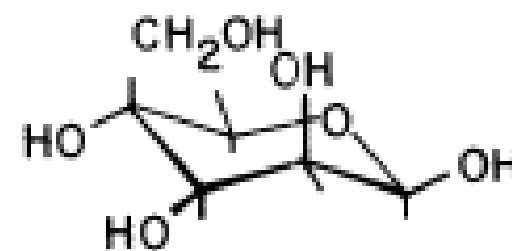
Nejhojnější monosacharidy BS



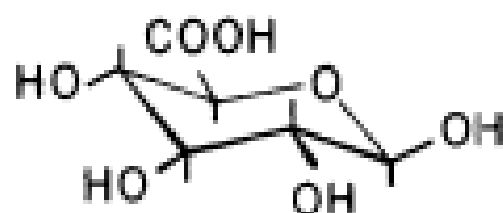
β -D-Glucose **G**



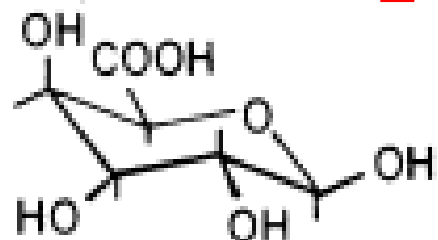
β -D-Galactose **L**



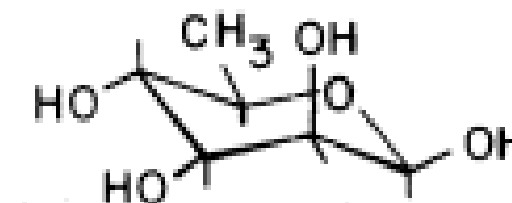
β -D-Mannose



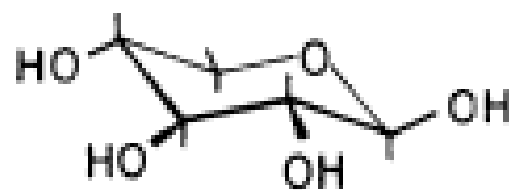
β -D-Glucuronic acid



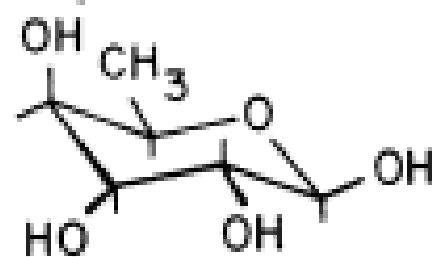
β -D-Galacturonic acid



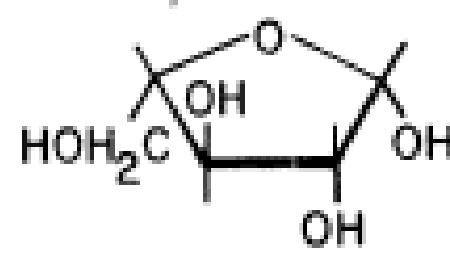
β -L-Rhamnose



β -D-Xylose **X**

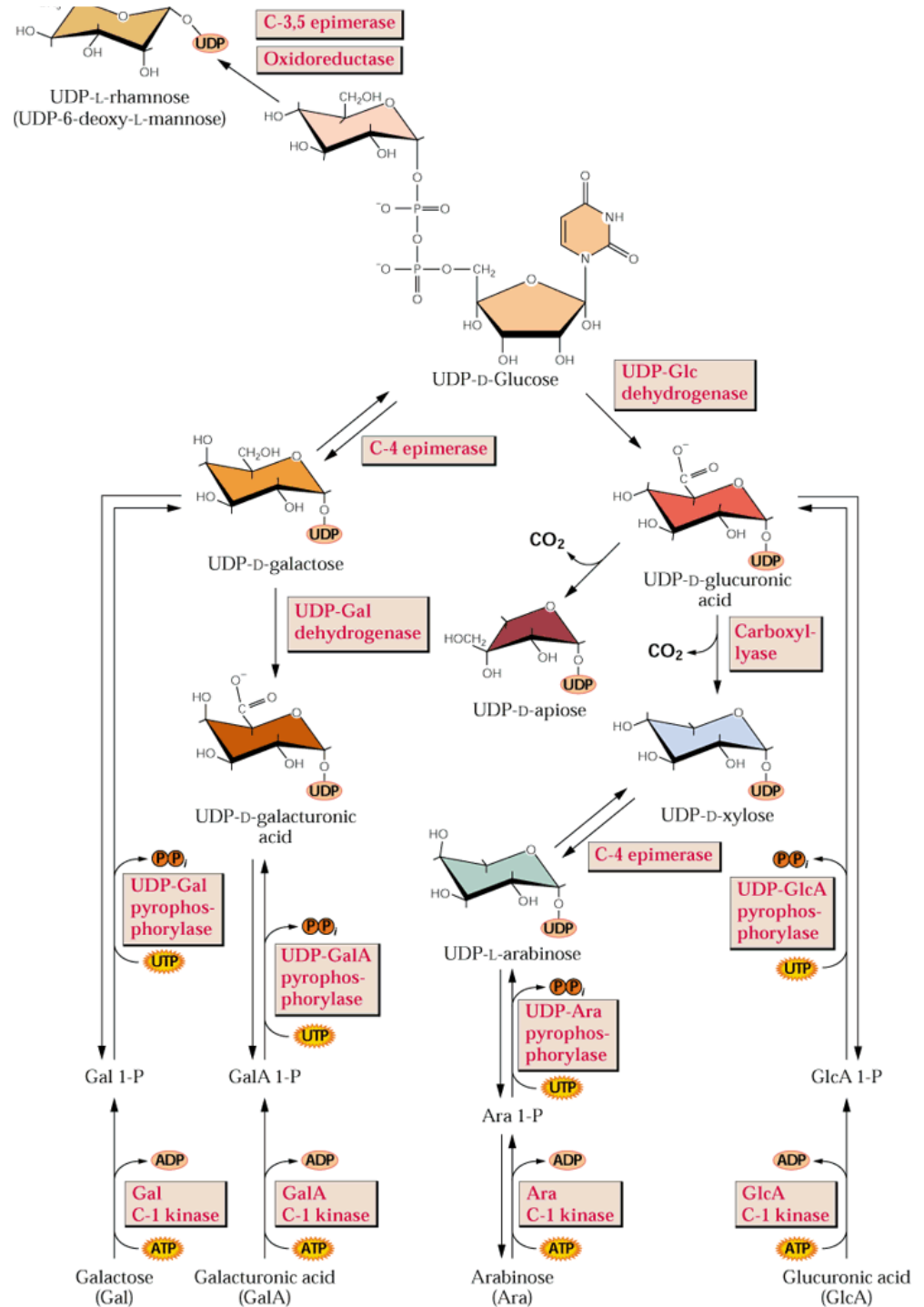


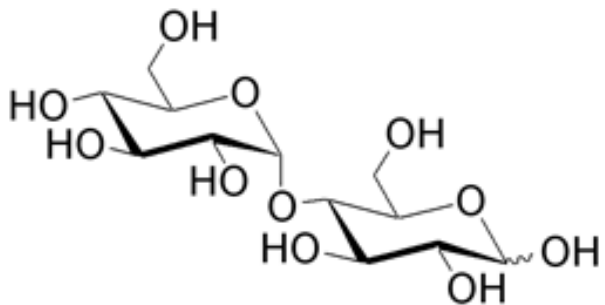
β -L-Fucose **F**



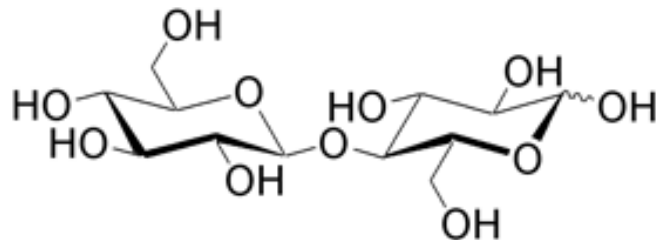
β -L-Arabinose **A**

biochemie cukrů

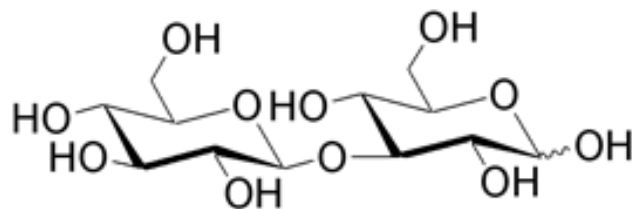




maltosa (vazba α 1-4) (škrob)



cellobiosa (vazba β 1-4) (celulóza)



laminaribiosa (vazba β 1-3) (kalóza)

Obsah

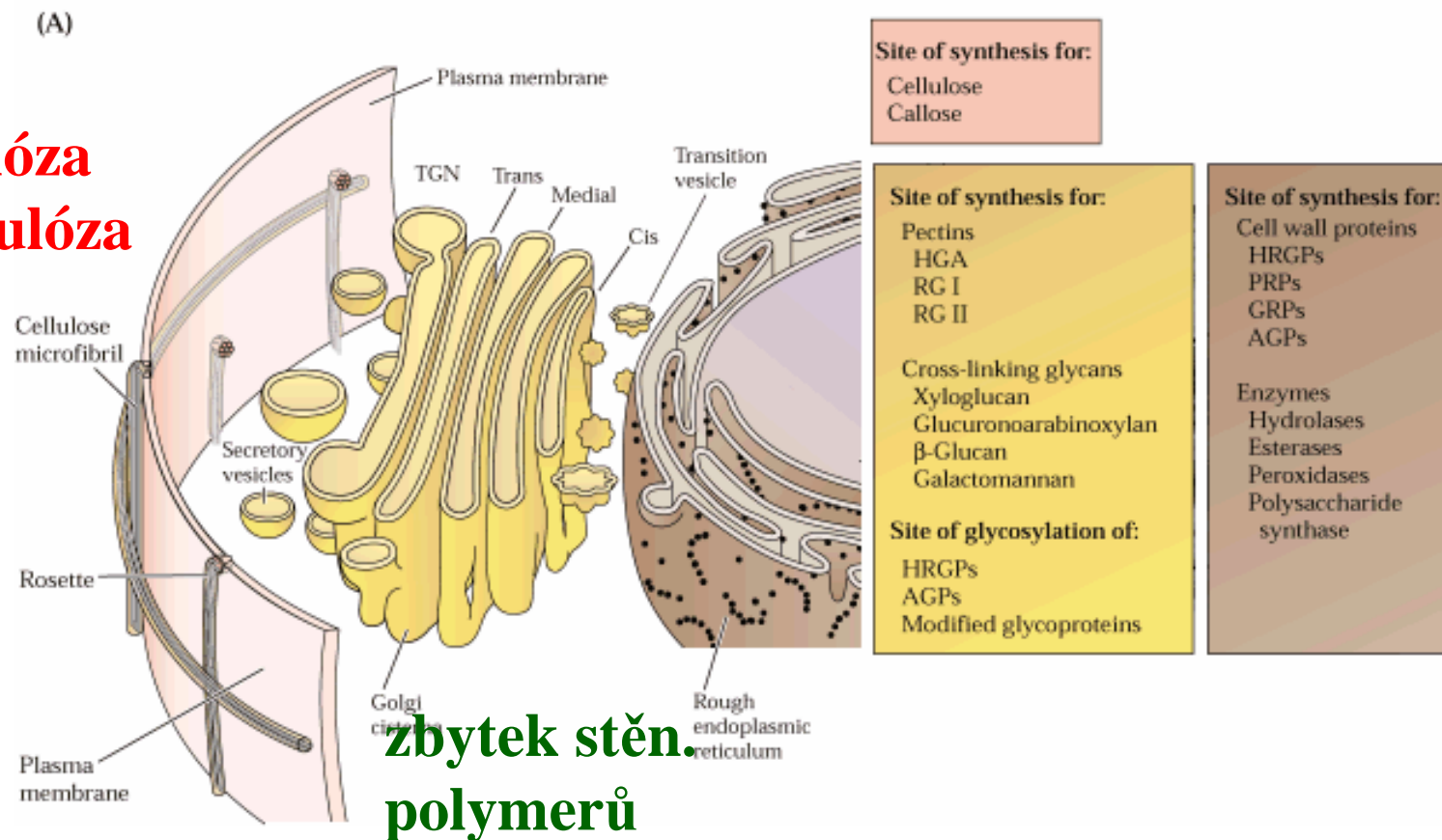
- Buněčná stěna jako integrální část rostlinné buňky
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- **Proteiny**
 - Extensiny, expansiny, AGPs, GRPs, PRPs
 - Endoglukanasy, XET/XTH

Lokalizace syntézy složek buněčné stěny

kalóza
celulóza



Kalóza - Zrození stěny

β -1,3-glukan

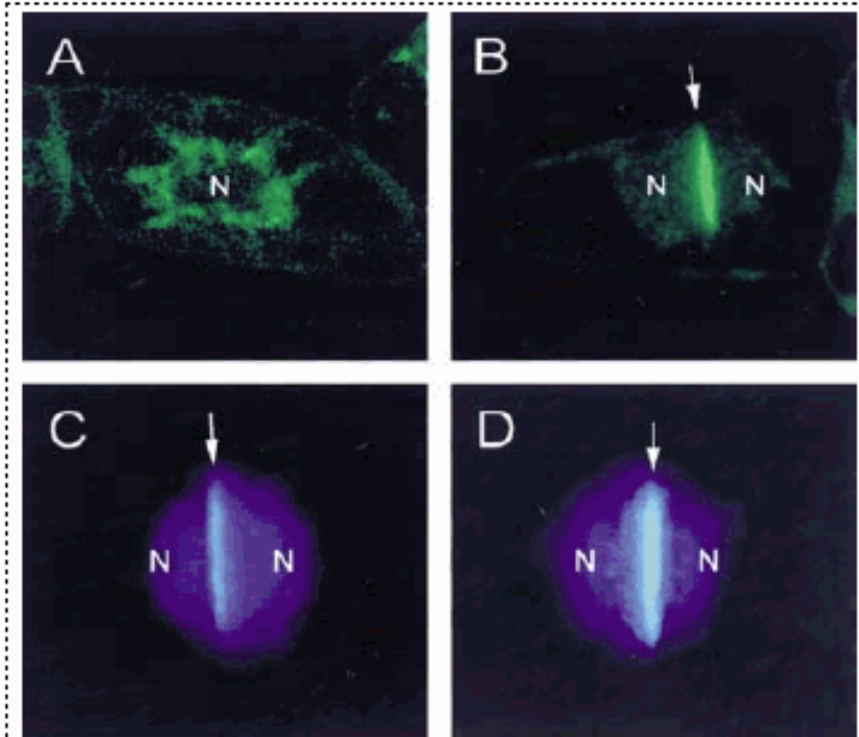
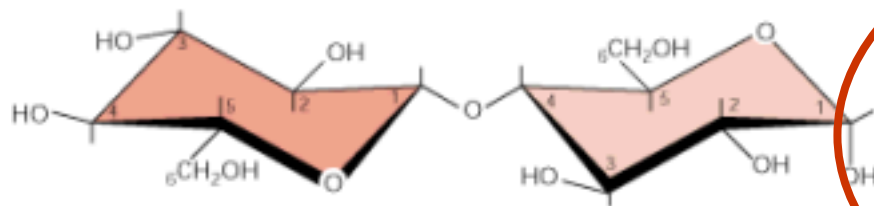
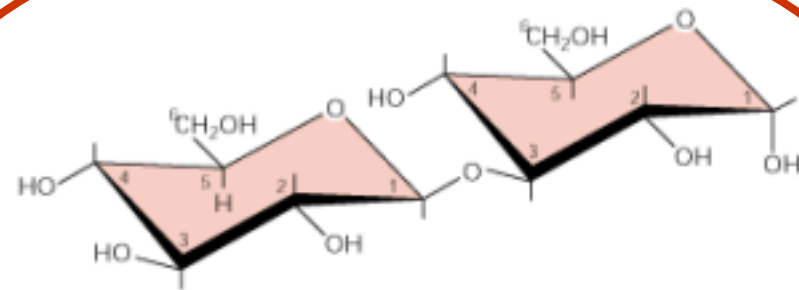


Figure 2. Subcellular localization of AtCalS1 and deposition of callose on the cell plate. *Arabidopsis* CalS1 was fused with GFP and expressed in tobacco BY2 cells under the control of the CaMV 35S promoter (Hong *et al.*, 2001a). A, B. Fluorescent images of transgenic BY-2 cells expressing the GFP-CalS1 fusion protein at the G1 stage of cell cycle (A) and during cytokinesis (B). C, D. Callose deposition in the cell plate of control (C) and transgenic cells over-expressing GFP-CalS1 (D). The cells stained with aniline blue and 4',6-diamidino-2-phenylindole (DAPI) were photographed with a fluorescence microscope with a UV filter set. Arrows indicate the cell plate. N, nucleus.

- Tvoří se při poranění
- Při infekci patogenem
- Důležitá součást BS pylu
- Při cytokinezi se ukládá do rostoucí přepážky



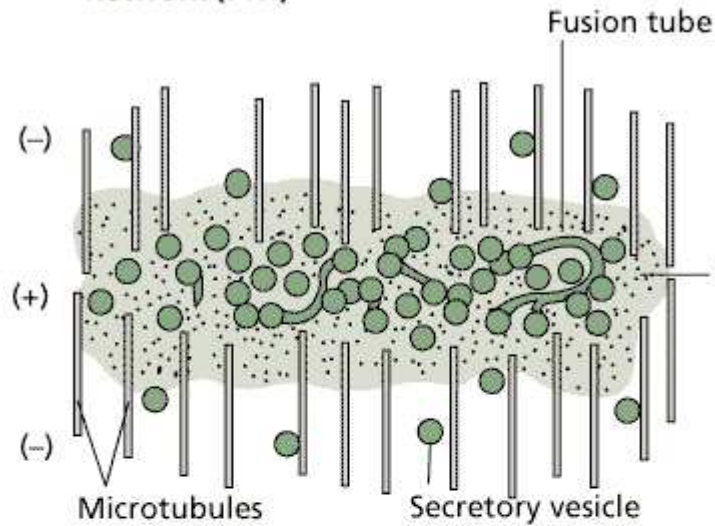
Cellobiose
(β -D-Glucosyl-(1 \rightarrow 4)-D-glucose)



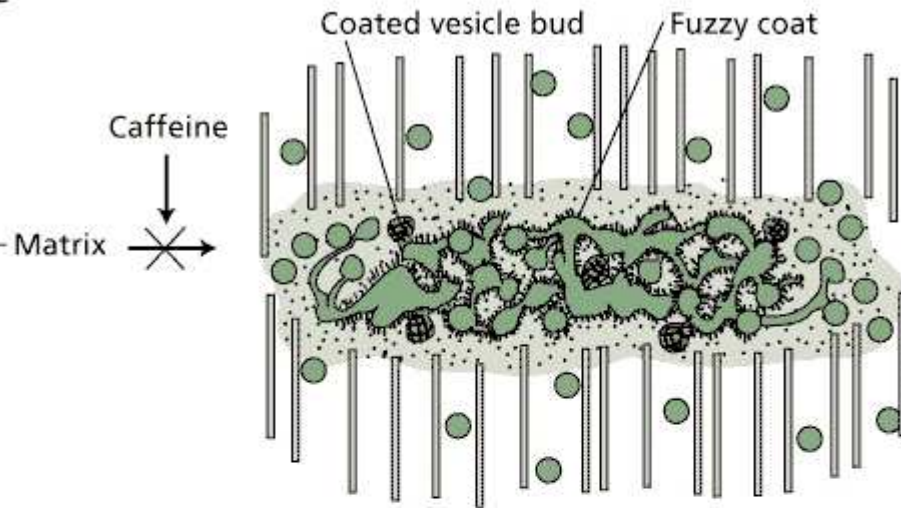
Laminaribiose
(β -D-Glucosyl-(1 \rightarrow 3)-D-glucose)

FRAGMOPLAST

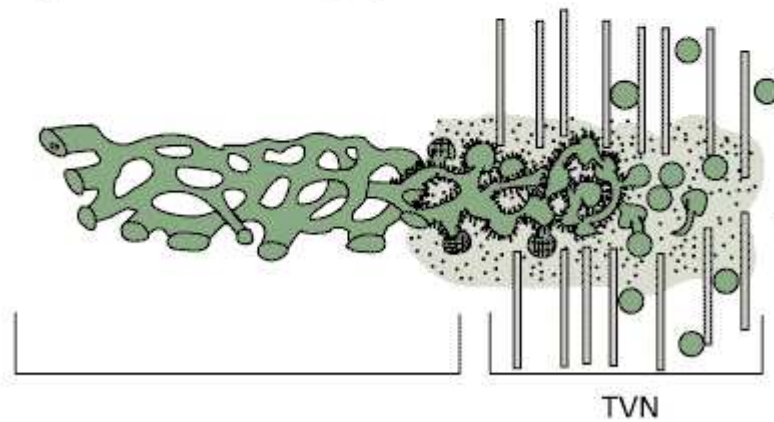
(A) Free vesicles and fusion tube-generated network (FTN)



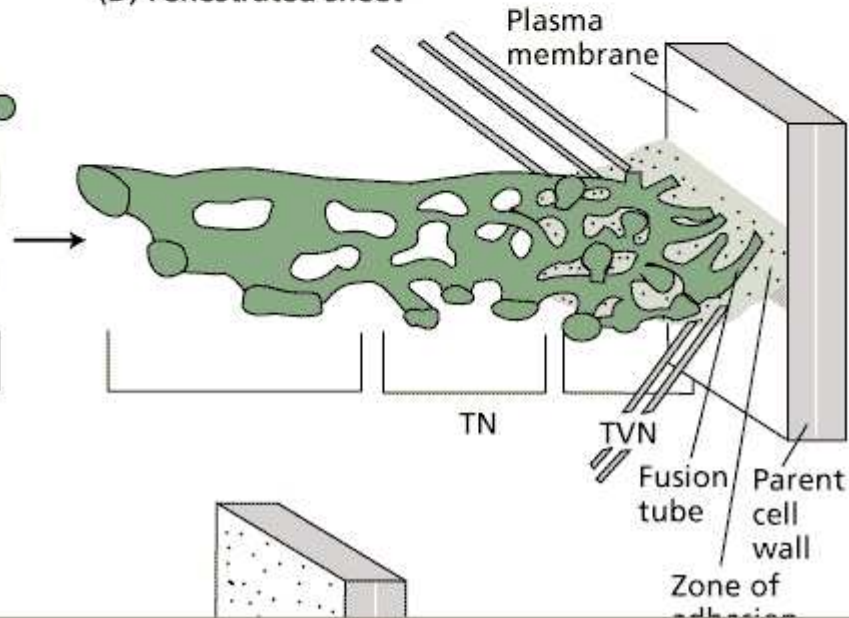
(B) Tubulo-vesicular network (TVN)



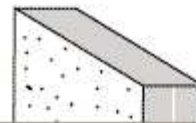
(C) Tubular network (TN)



(D) Fenestrated sheet

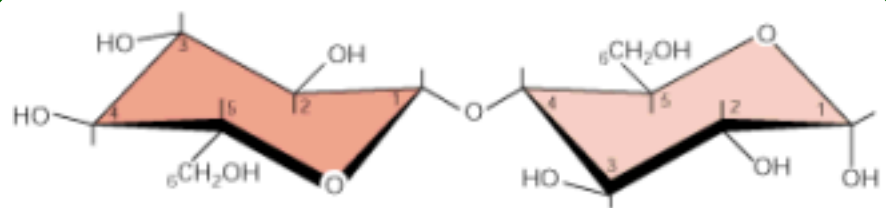


(E) New cell walls

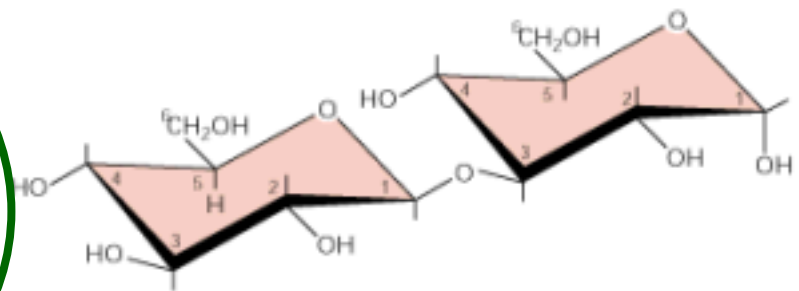


Celulóza

- Celulóza je nejrozšířenějším bio-polymerem na zeměkouli.
- Který je druhý?
- A třetí??

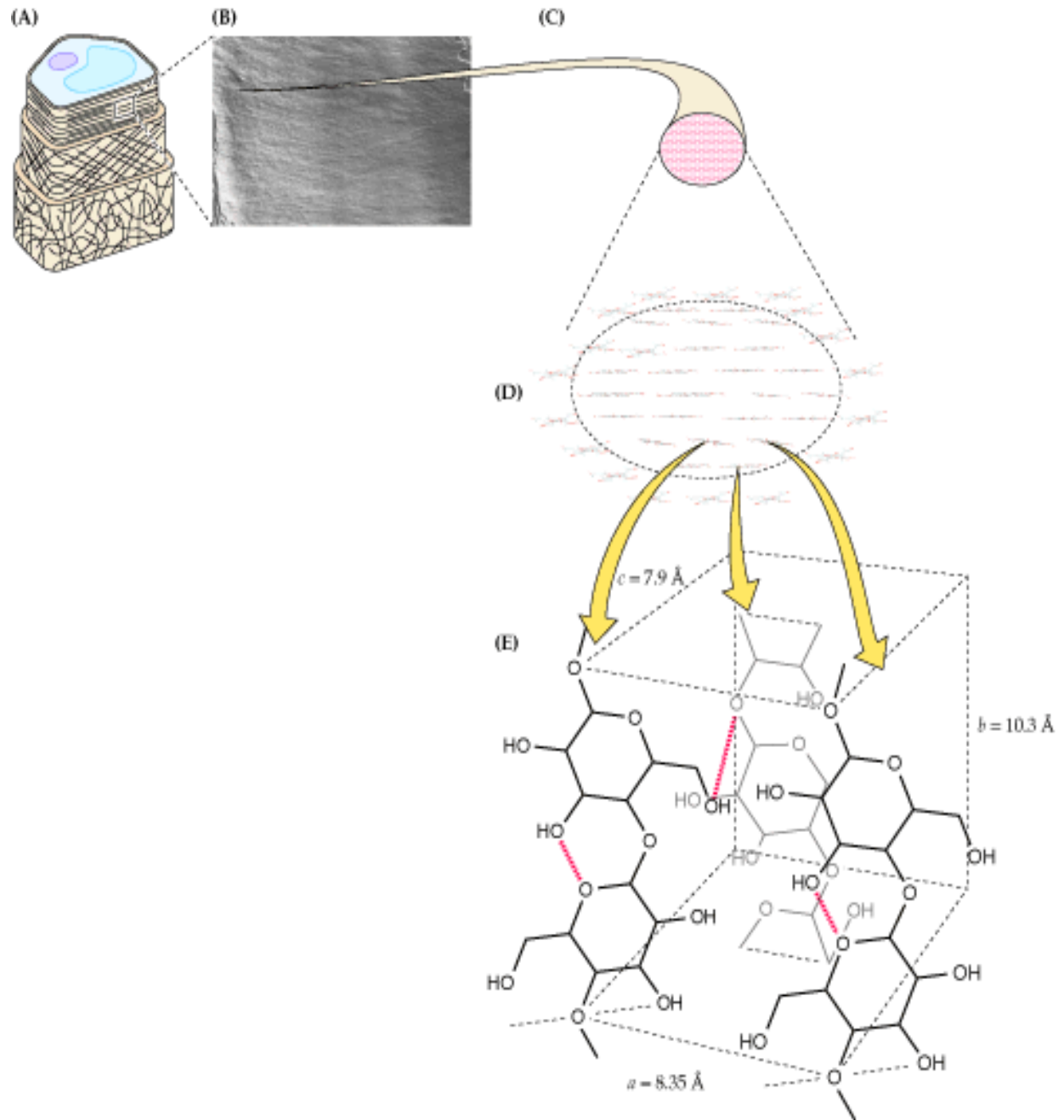


Cellobiose
(β -D-Glucosyl-(1 \rightarrow 4)-D-glucose)



Laminaribiose
(β -D-Glucosyl-(1 \rightarrow 3)-D-glucose)

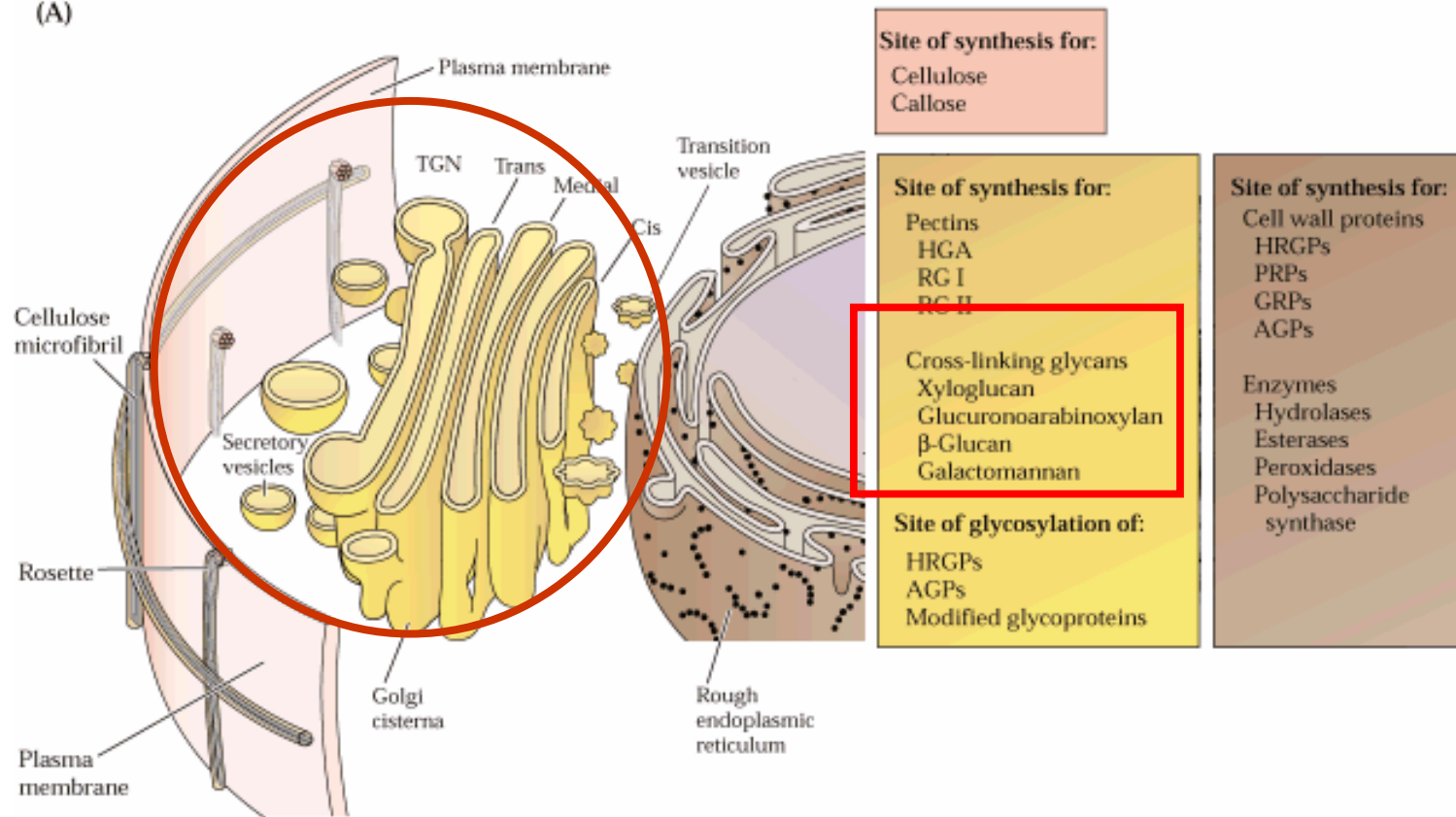
Mikrofibrila
celulózy asi 3 nm
silná je tvořena 30
až 36
individuálními
řetězci a může při
počtu 14 000 glc
jednotek
dosáhnout délky
7 μ m.



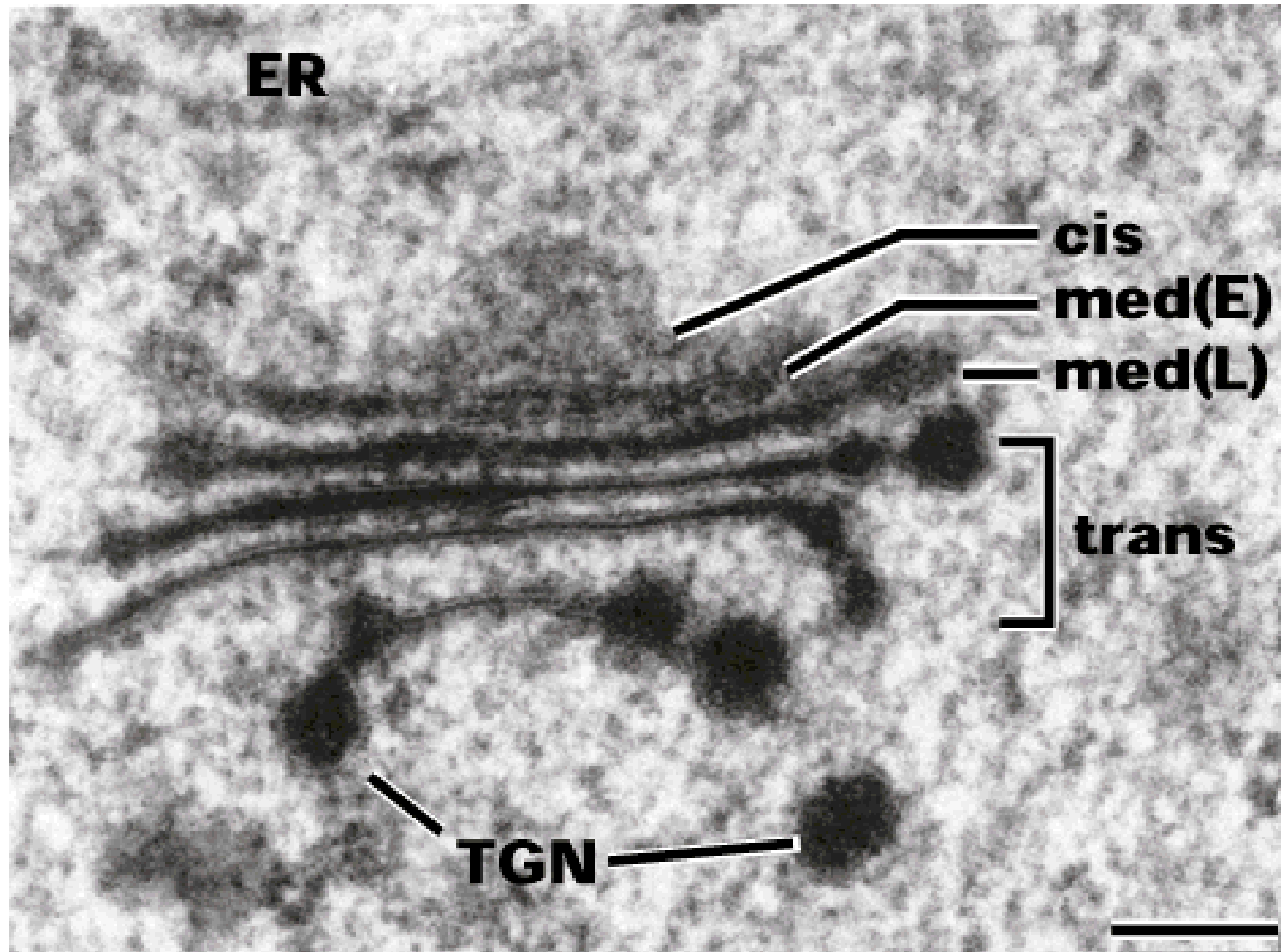
Hemicelulózy

- Xyloglukany = I
- Glukuronoarabinoxylany = II

(A)



(C)

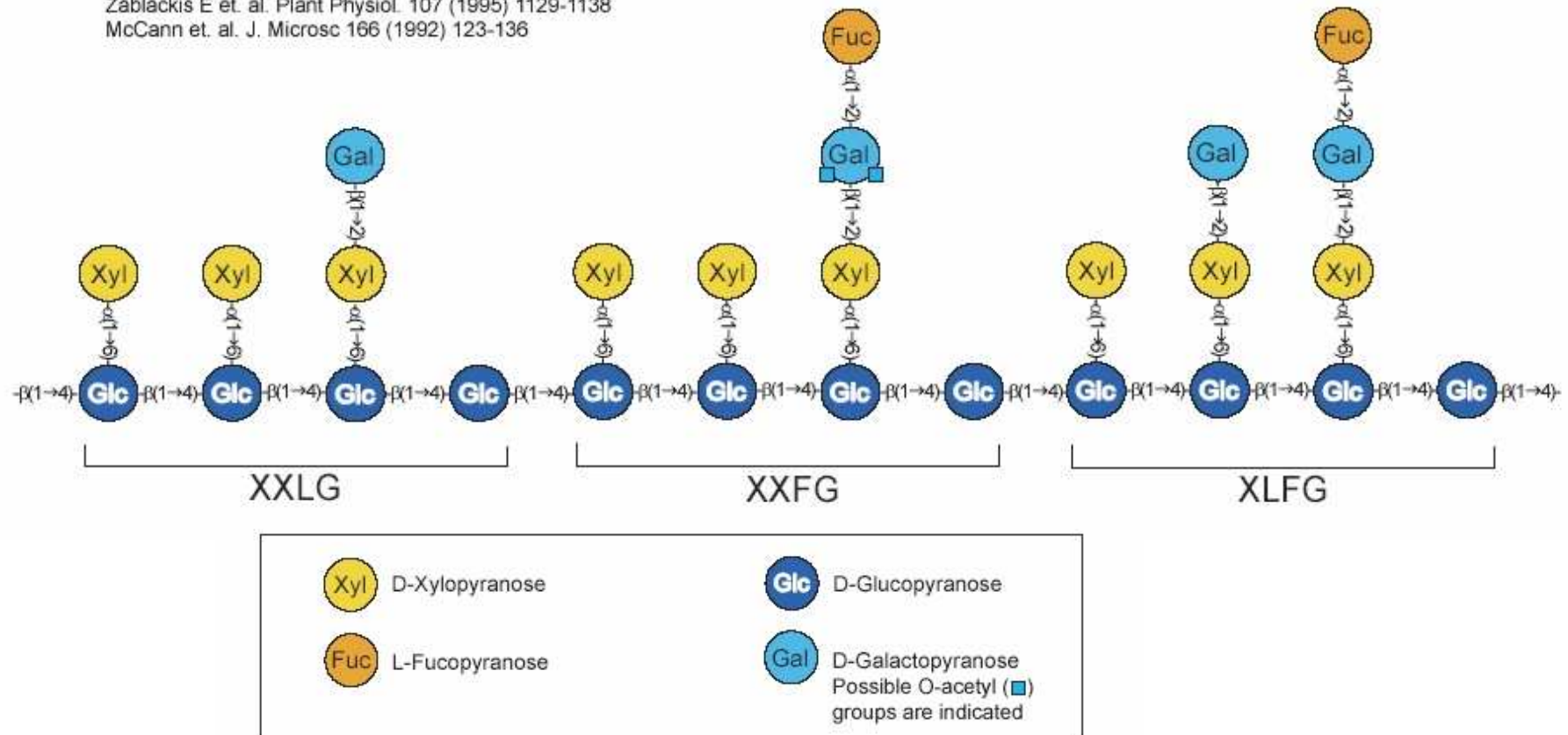


Xyloglukan (XG) - I.

Figure S1. Xyloglukan.

Xyloglukan is composed of subunits of XXXG, XXLG, XXFG, and XLFG in most plant families. Xyloglucans derived from Solanaceous plants and immature podaceous plants contain XXGG subunits and may have small amounts of XGGG and GXGG subunits. Individual xyloglukan molecules range in length from 30 - 400 nm which correspond to roughly 15 - 200 Glc residues. Acetylation?

Vincken JP *et al.* Plant Physiol 114 (1997) 9-13
 Zablackis E *et al.* Plant Physiol. 107 (1995) 1129-1138
 McCann *et al.* J. Microsc 166 (1992) 123-136

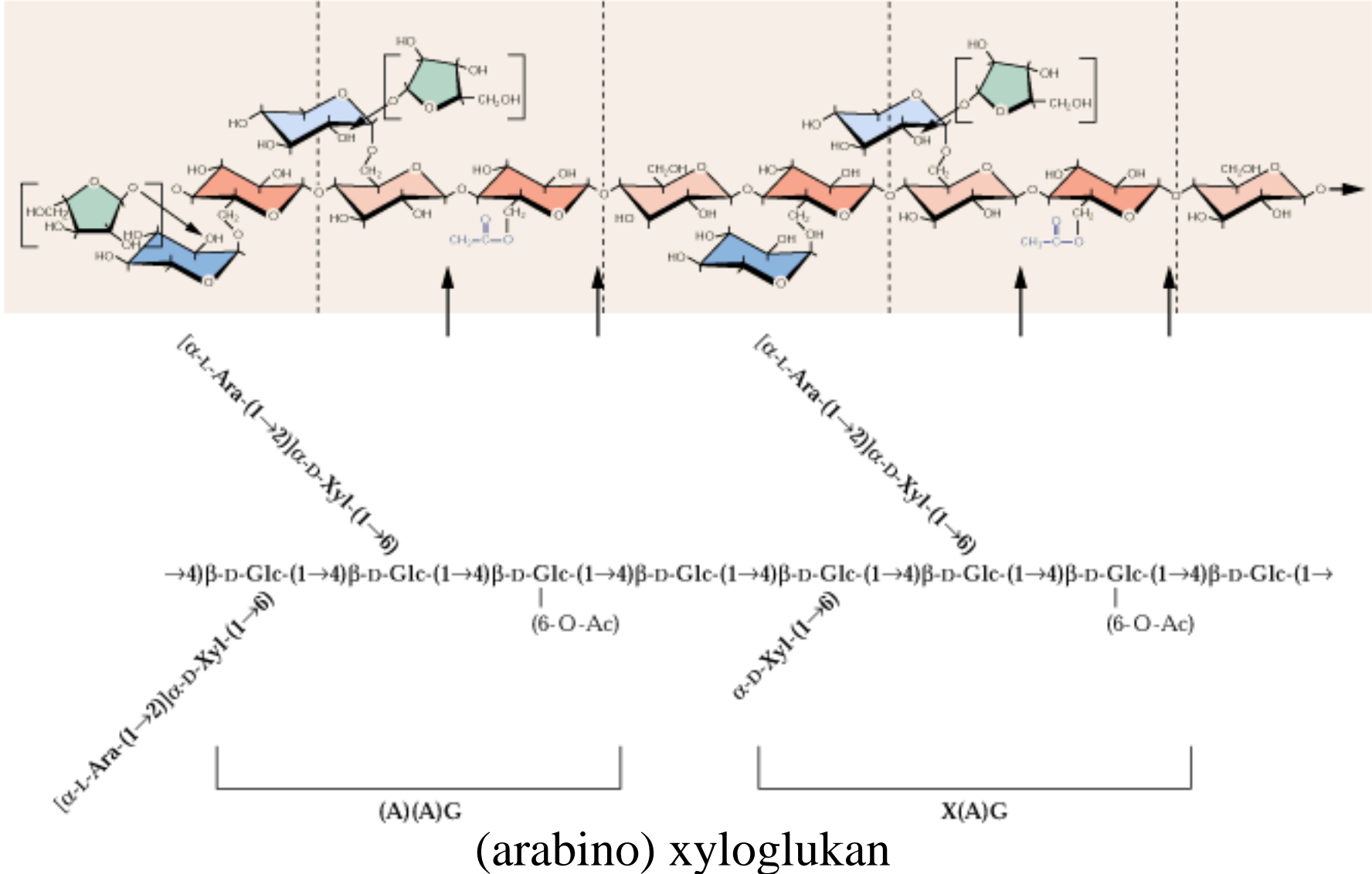


(fukogalakto) xyloglukan

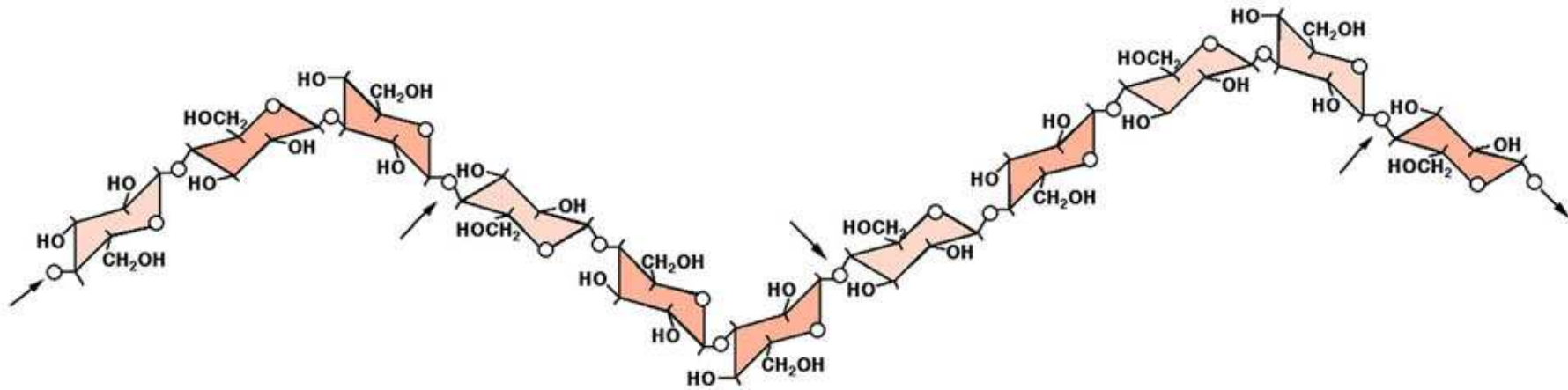
Xyloglukan II. – *Solanaceae*

(B)

Solanaceous (arabino)xyloglucans



Xyloglukan (XG) - *Poales*



Mixed-linkage (1→3),(1→4)-β-D-glucan

Glukuronoarabinoxylan (GAX)

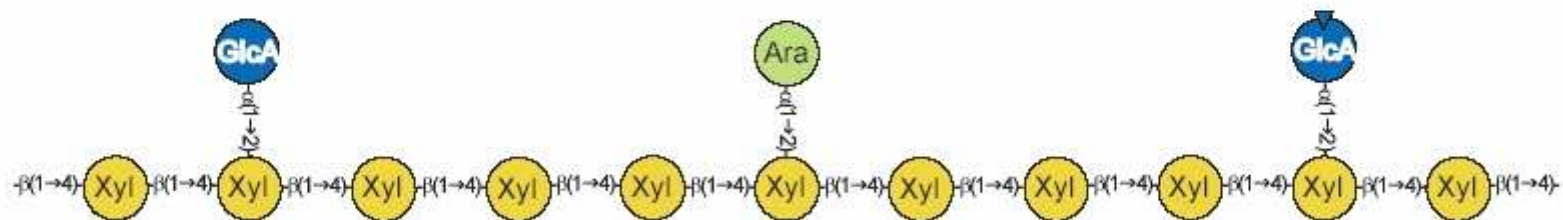
Figure S2. Glucuronoarabinoxylan.


Glucuronoarabinoxylan (GAX) accounts for roughly 5% of the dicot cell wall. Approximately 25% of the Xyl residues are substituted with either Ara, GlcA, 4-O-methyl glcA, or--in some plant families--ferulic acid.

DP?


Zabackis E et. al. Plant Physiol. (1995) 107 1129-1138

Darvill et al. Plant Physiol. (1980) 66 1135-1139



 D-Xylopyranose

 L-Arabinofuranose

 D-Glucopyranuronic acid,
also with 4-O-methyl groups (▼)

PEKTINY

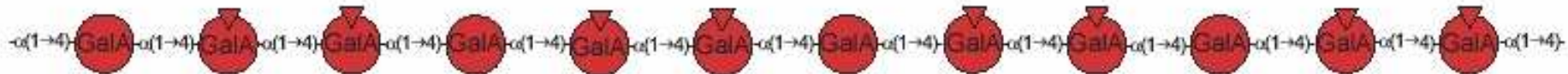
Pektin – homogalacturonan (HGA)

Ca²⁺ cross-link

Figure S5. Homogalacturonan.

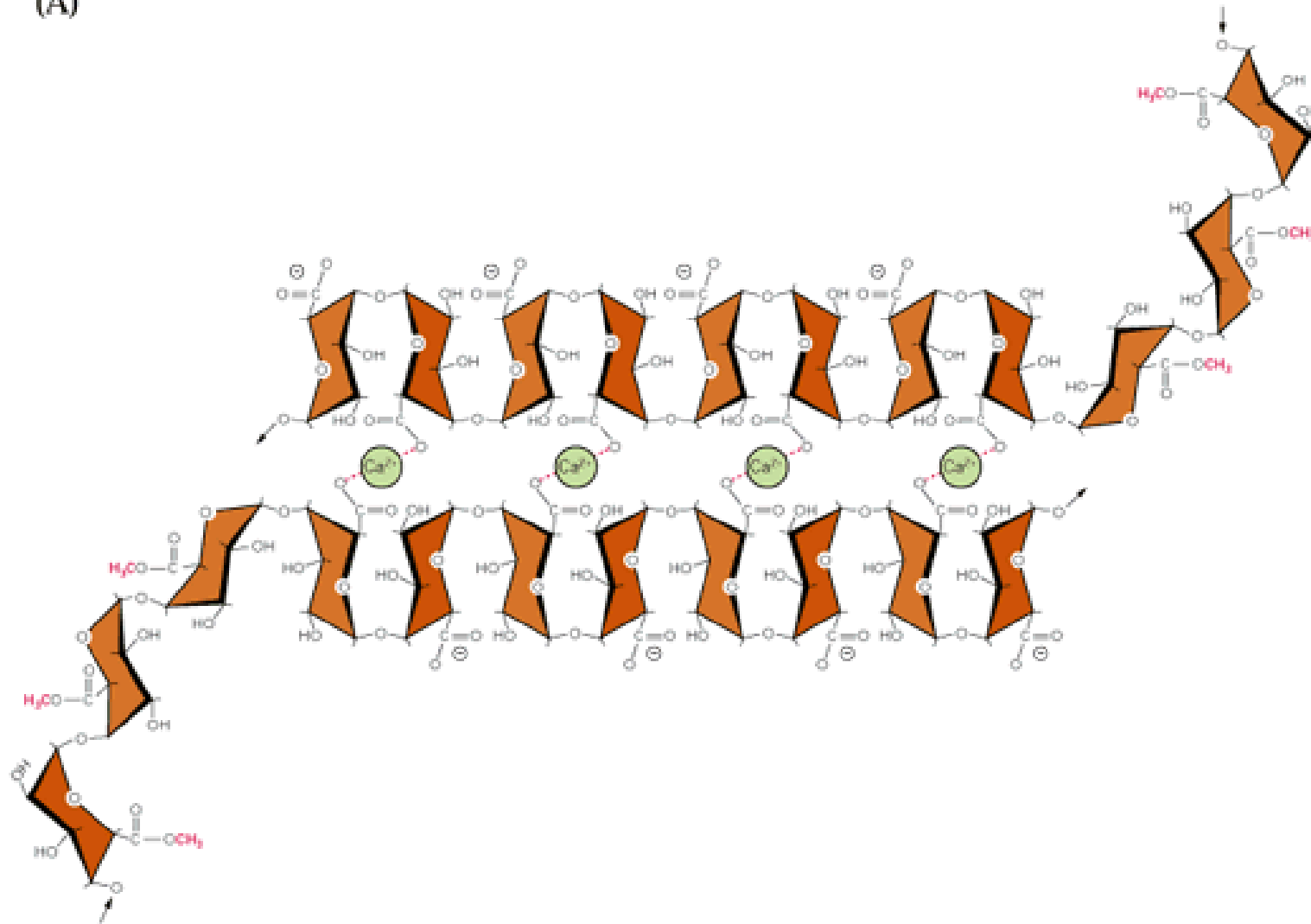
Homogalacturonan deposits in the cell wall in a highly methyl-esterified form and is subsequently de-esterified by pectin methyl esterases (PME's) to a varying degree. In some plant families, GalA residues may be O-acetylated at C2 or C3. Degrees of polymerization have been reported that range from 5 to 200.

Vincken JP *et al.* Plant Physiol 132 (2003) 1781-1789
Ridley BL *et al.* Phytochemistry 57 (2001) 929-967
Willats WGT *et al.* J. Biol. Chem 276 (2001) 19404-19413
Mohnen D *Comp. Natl. Prod. Chem.* 3 (1998)????



D-Galactopyranuronic acid, some are methyl esterified (▼)

(A)



Zvýšená hustota vápenatých můstků – větší tuhost stěny

Pektin

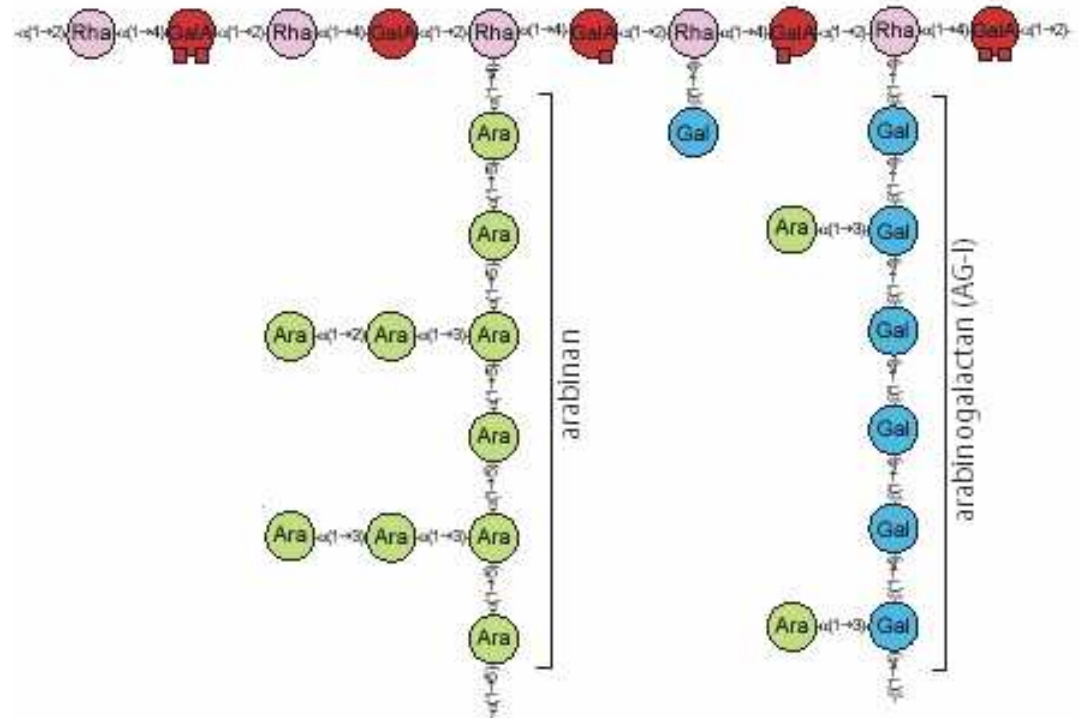
Rhamnogalakturona





RG-I



Figure S3. Rhamnogalacturonan I (RG-I).

The backbone can be composed of more than 100 $[\rightarrow 2)\text{-}\alpha\text{-L-Rhap-(1}\rightarrow 4)\text{-}\alpha\text{-D-GalpA-(1}\rightarrow 3)]$ -disaccharide units. Between 20% to 80% of the rhamnosyl residues carry side chains, depending on the source. These side chains can be single unit $[\beta\text{-D-Galp-(1}\rightarrow 4)]$ or arabinogalactan I with variable chain length. In smaller amounts, $\alpha\text{-L-Fucp}$, $\beta\text{-D-GlcpA}$, 4-O-Me GlcpA and ferulic and coumaric acid may also be present. It is also suggested that homogalacturonan and xylogalacturonan are attached to RG-I.

Vincken JP et al. Plant Physiol 132 (2003) 1781-1789
 Ridley BL et al. Phytochemistry 57 (2001) 929-967



	D-Galactopyranuronic acid may contain acetyl groups (■) at O2 and O3		L-Rhamnopyranose
	L-Arabinofuranose		D-Galactopyranose

	D-Galactopyranuronic acid may contain acetyl groups (■) at O2 and O3		L-Rhamnopyranose
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Pektin Rhamnogalakturonan RG-II

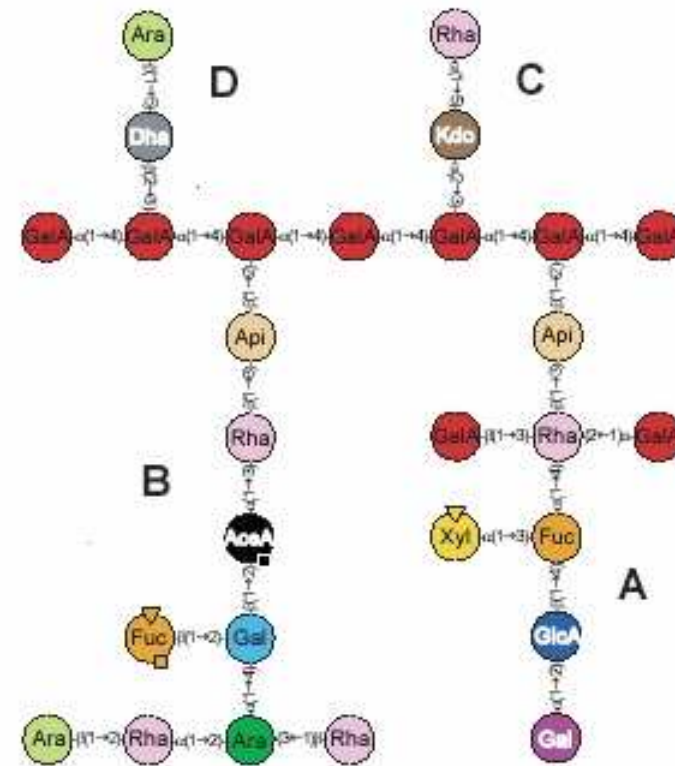
nejvíce kombinací
borátový cross-link

Borátový diester – přes apiózu

Figure S4. Rhamnogalacturonan II (RG-II).

Four side chains (A-D) are linked to a homogalacturonan backbone consisting of at least 7 $[\rightarrow 4]-\alpha\text{-D-GalpA}-(1\rightarrow)$ residues. The order and the linkages of the glycosyl residues in the side chains as well as the distribution of the side chains have not been unambiguously determined.

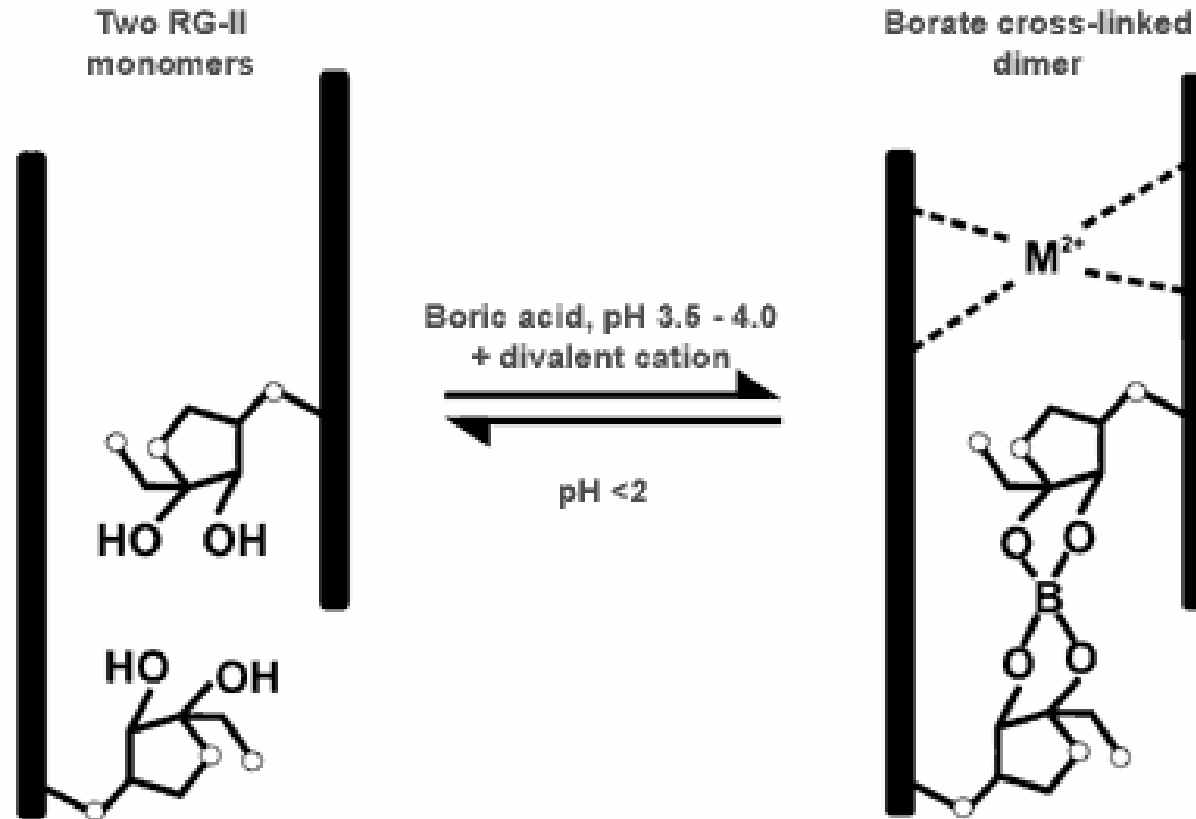
O'Neill MA et al. *Annu Rev Plant Biol* 55 (2004) 109-139
 Vincken JP et al. *Plant Physiol* 132 (2003) 1781-1789
 Ridley BL et al. *Phytochemistry* 57 (2001) 929-967
 Du Penhoat CH et al. *J Biomol NMR* 14 (1999) 253-271



D-Galactopyranuronic acid	D-Galactopyranose	L-Fucopyranose 2-O-methyl (∇) and O-acetyl (\square) groups are indicated	3-deoxy-D-lyxo-2- heptulopyranosylonic acid
L-Rhamnopyranose	L-Galactopyranose	2-O-methyl- D-Xylopyranose	3-deoxy-D-manno- octulopyranosylonic acid
D-Apiofuranose	L-Arabinofuranose	O-acetyl-L-Aceric acid	
D-Glucopyranuronic acid	L-Arabinopyranose		

Rhamnogalakturonan RG-II

nejvíce kombinací
borátový cross-link



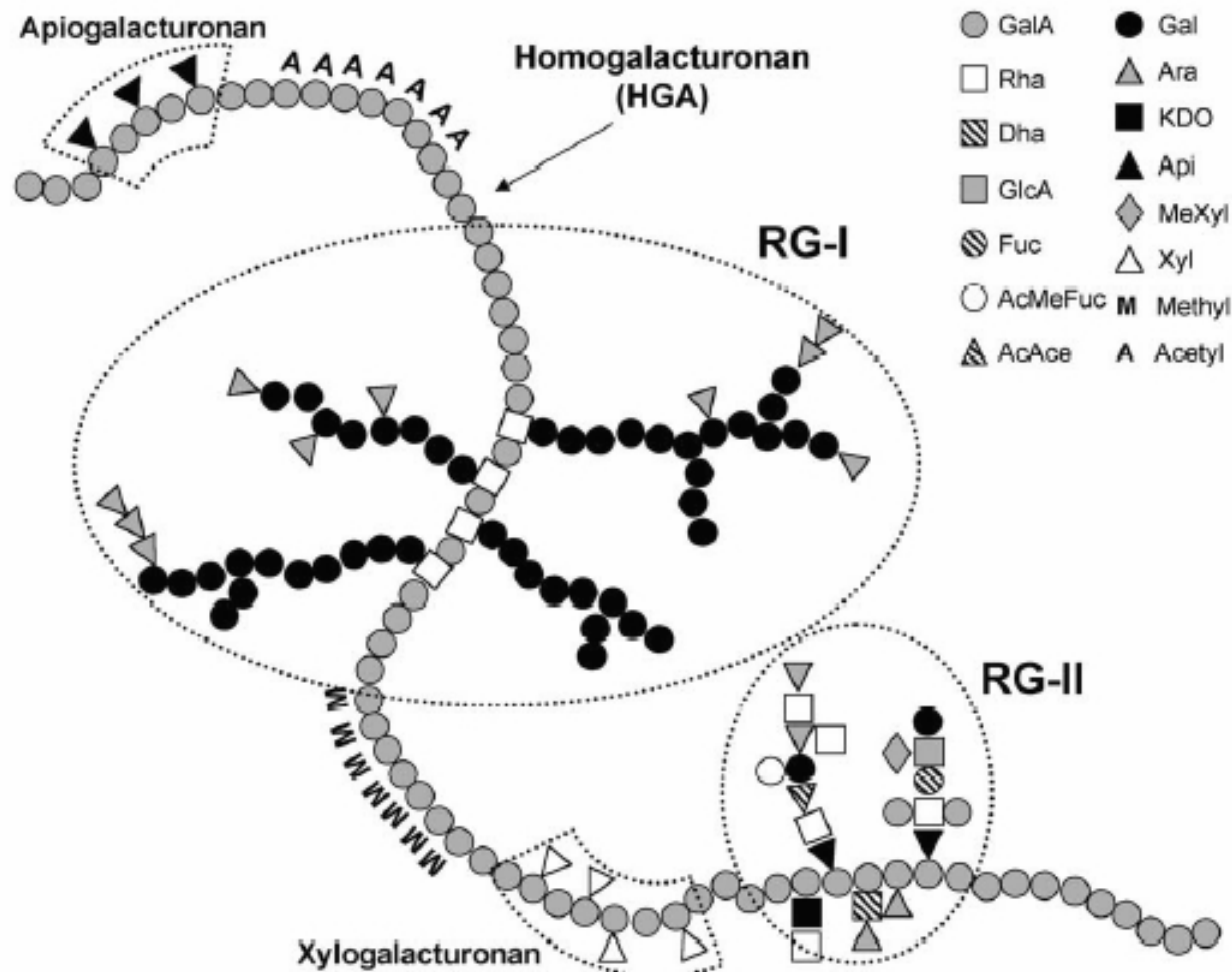
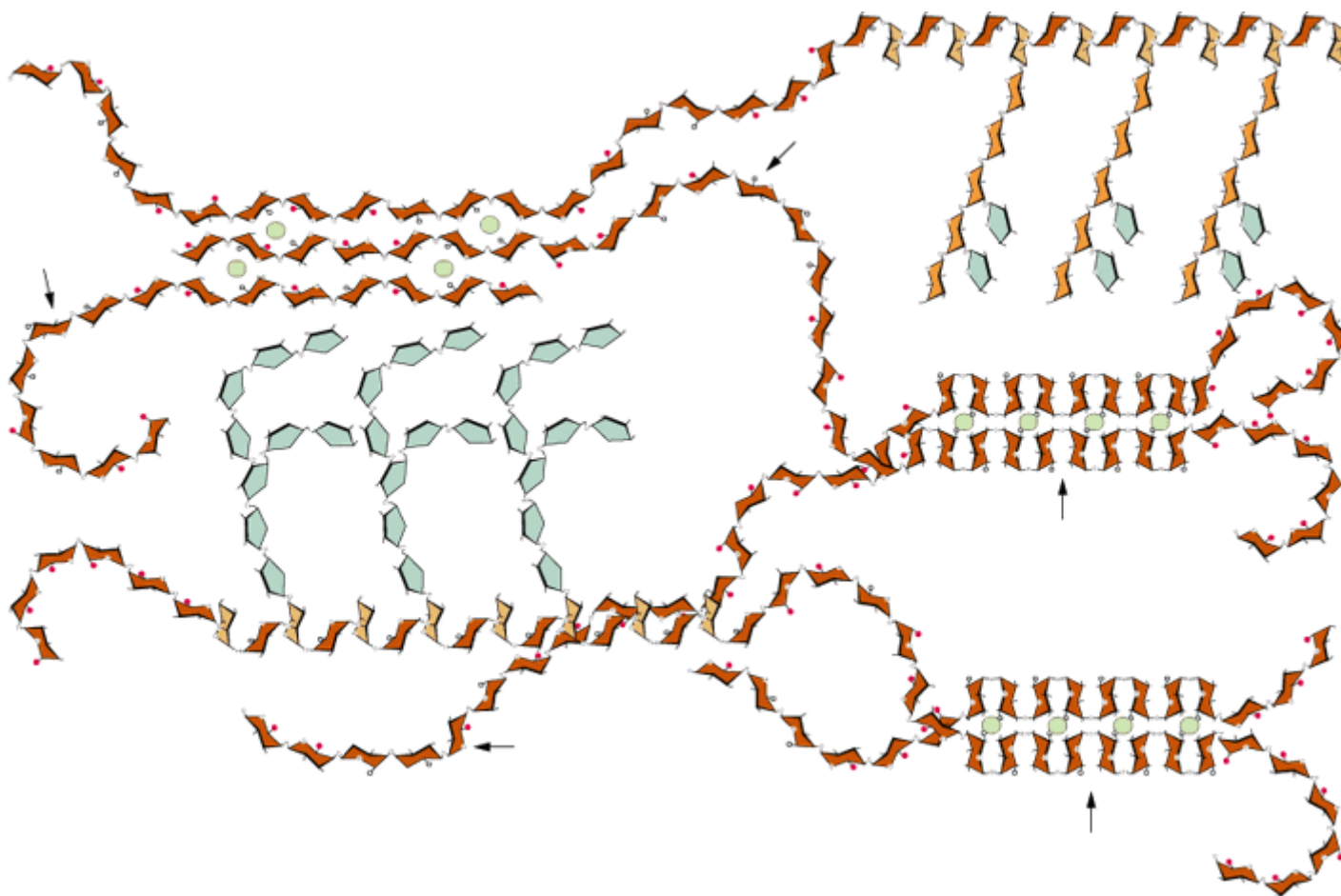


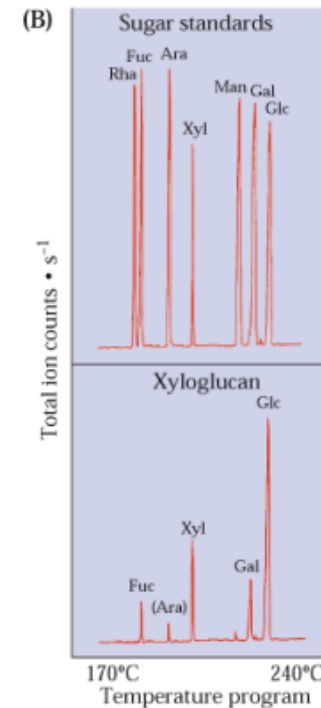
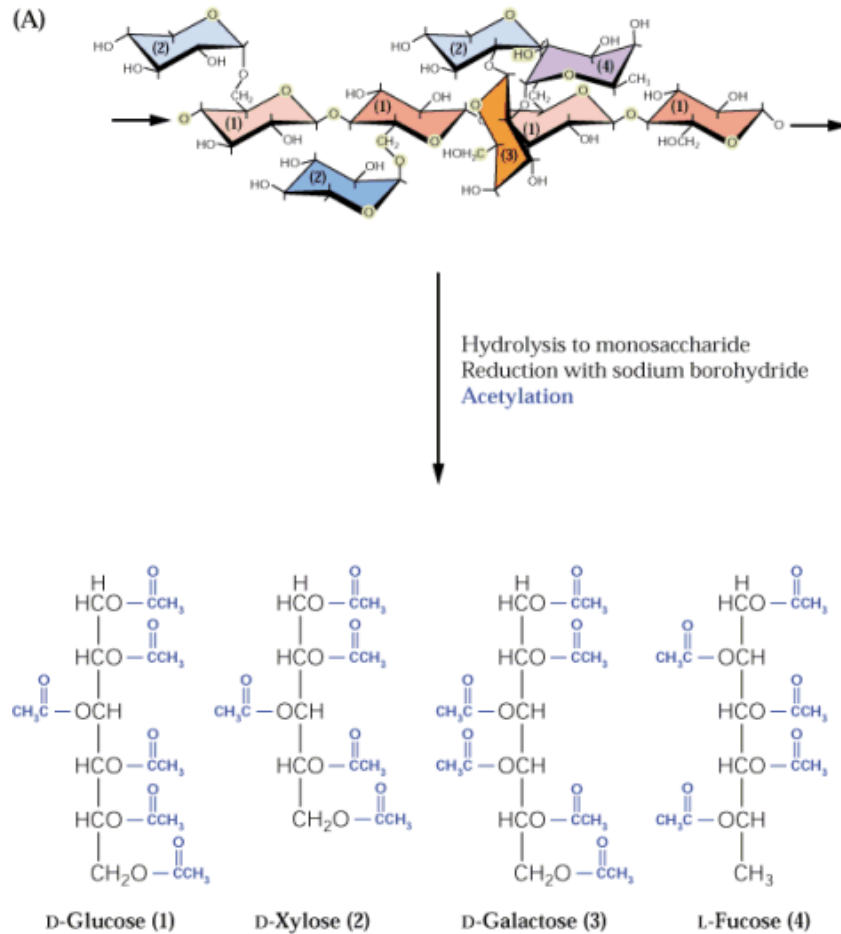
Fig. 1. Schematic representation of the “canonical” primary structure of pectins. For the sake of simplicity, the schematic representation of HGA, RG-I, and RG-II is given assuming that these three domains are covalently linked, although this point is not firmly established.

Pektiny ve stěně **mohou** být kovalentně propojeny.

Pektinová matrix ustavuje velikost „pórů“ ve stěně



Metody chemické analýzy b. stěny - mimořádně náročné



= Poměrné zast. cukrů.

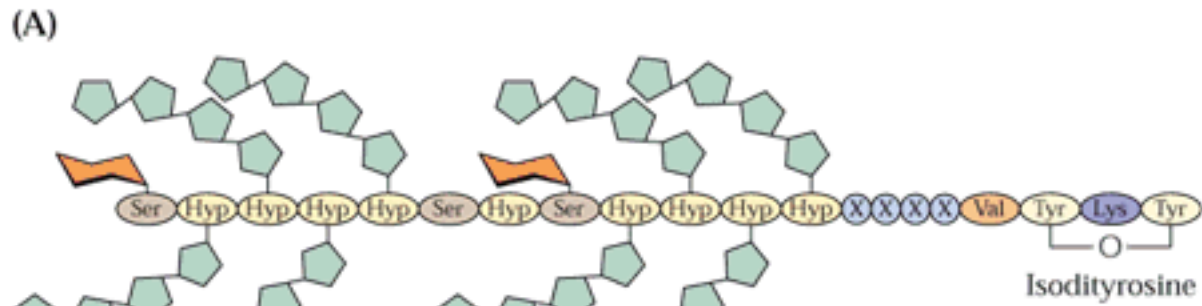
Při analýze struktury polysach. jsou nejprve modif. volné –OH skupiny – methyljodidem.

Dnes je velmi populární
neinvazivní Fourierova
transformační infračervená
(FTIR) mikrospektroskopie, která
je schopna kvantitativně
detekovat řadu substituentů (př.
karboxyl.kys., estery, fenoly,
amidy...)

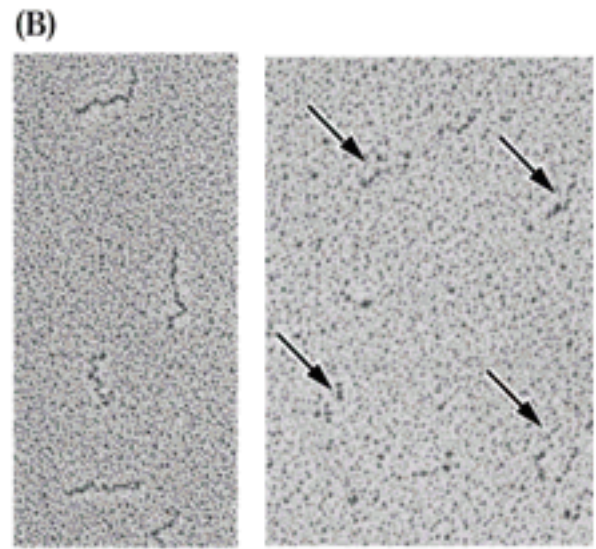
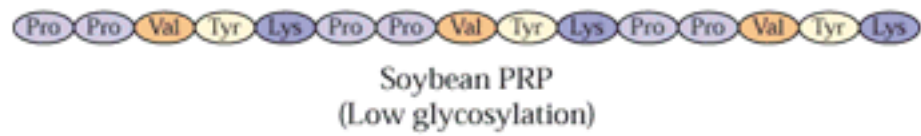
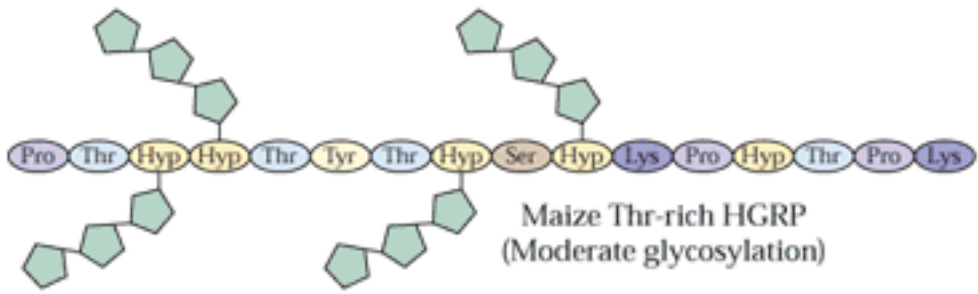
Stěnové strukturní bílkoviny

- Extensiny (a obecně HRGPs; hydroxyprolin bohaté glykoproteiny)
- PRPs (prolin bohaté proteiny)
- GRPs (glycin bohaté proteiny)
- AGPs (Arabinoproteoglykany)

Extensiny



příb. AGP (dále)

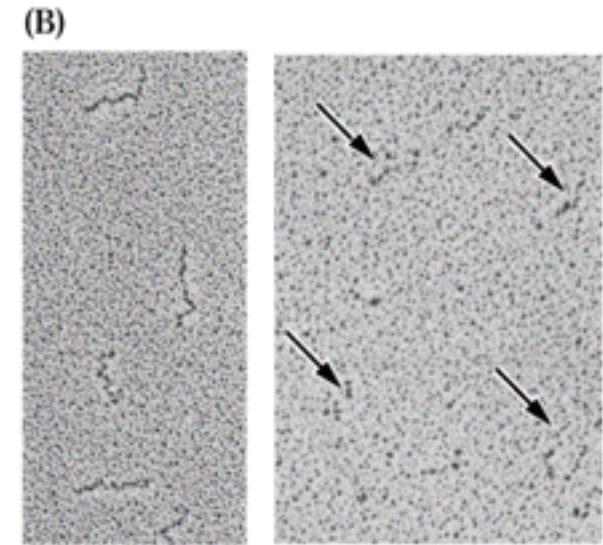
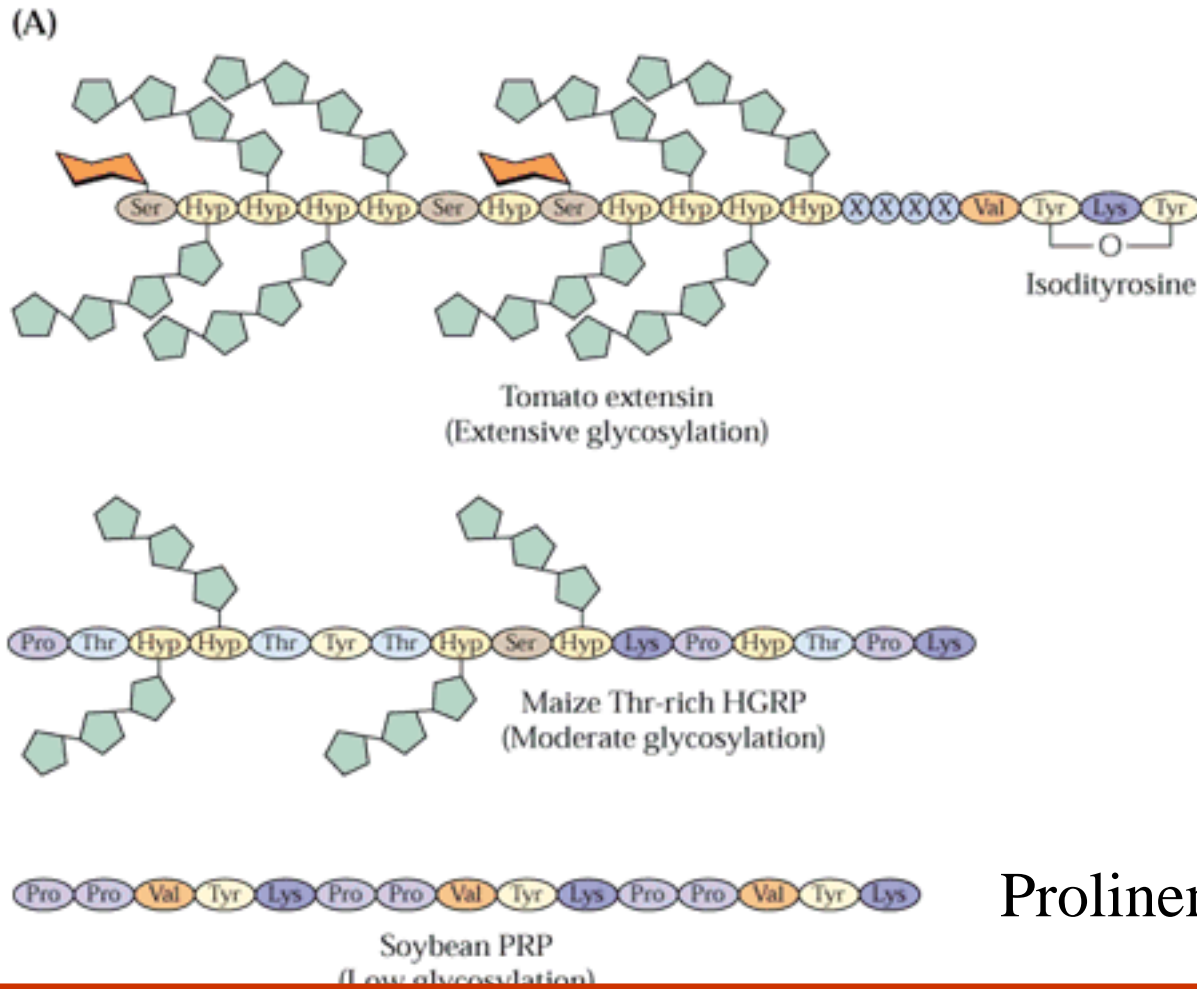


Odstranění arabinos z extensinu vede ke ztrátě tyčinkovitého tvaru.

Prolinem bohaté b.

Glycinem bohaté bílkoviny

Glycin Rich Prot.



Odstranění arabinos z extensinu vede ke ztrátě tyčinkovitého tvaru.

Prolinem bohaté b.

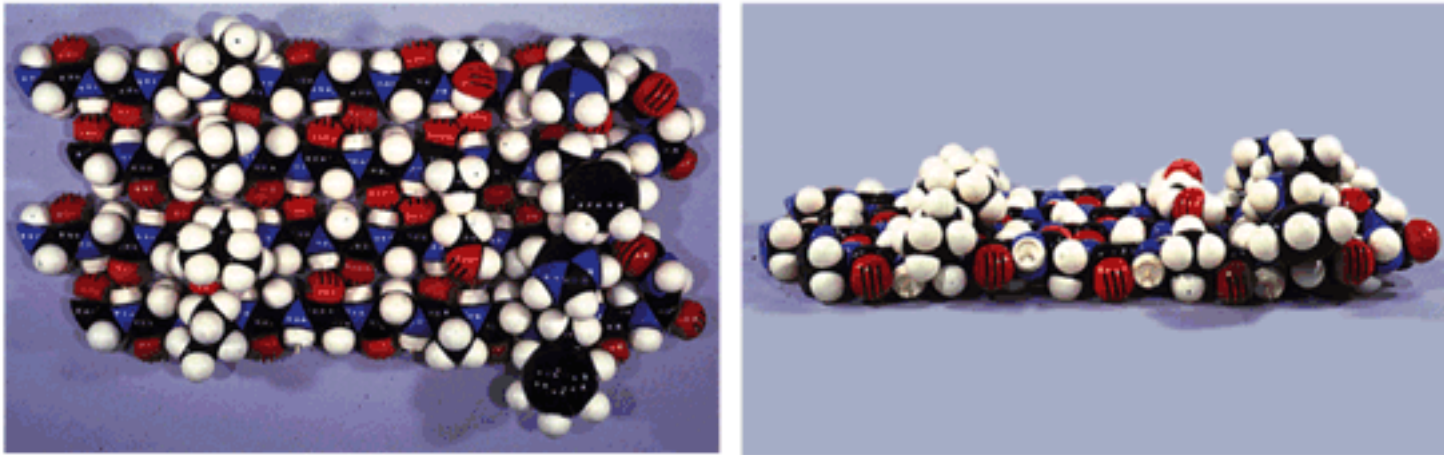
Glycinem bohaté bílkoviny

! [No glycosylation]

Petunia GRP
(Ser-Gly-His-Gly-Gly-Gly-Phe-Gly-Ala-Gly-Gly-Gly-Val)

GRP mohou tvořit β -list

(c)

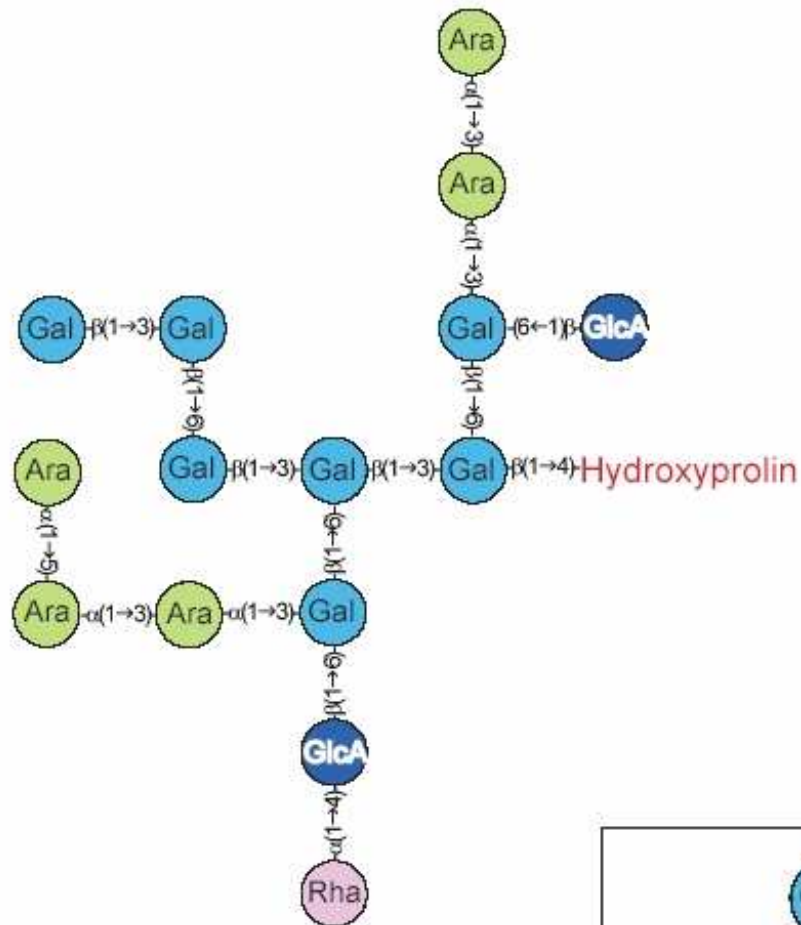


- GRP - důležití nositelé mechanických zátěží ve stěně ve zralém (tj. post-apoptotickém) xylému.

Figure S6. An Arabinogalactan protein (AGP) glycan.

Tan L *et al.* J Biol Chem 279 (2004) 13156-13165

The glycan is linked to hydroxyprolin of the peptide chain.



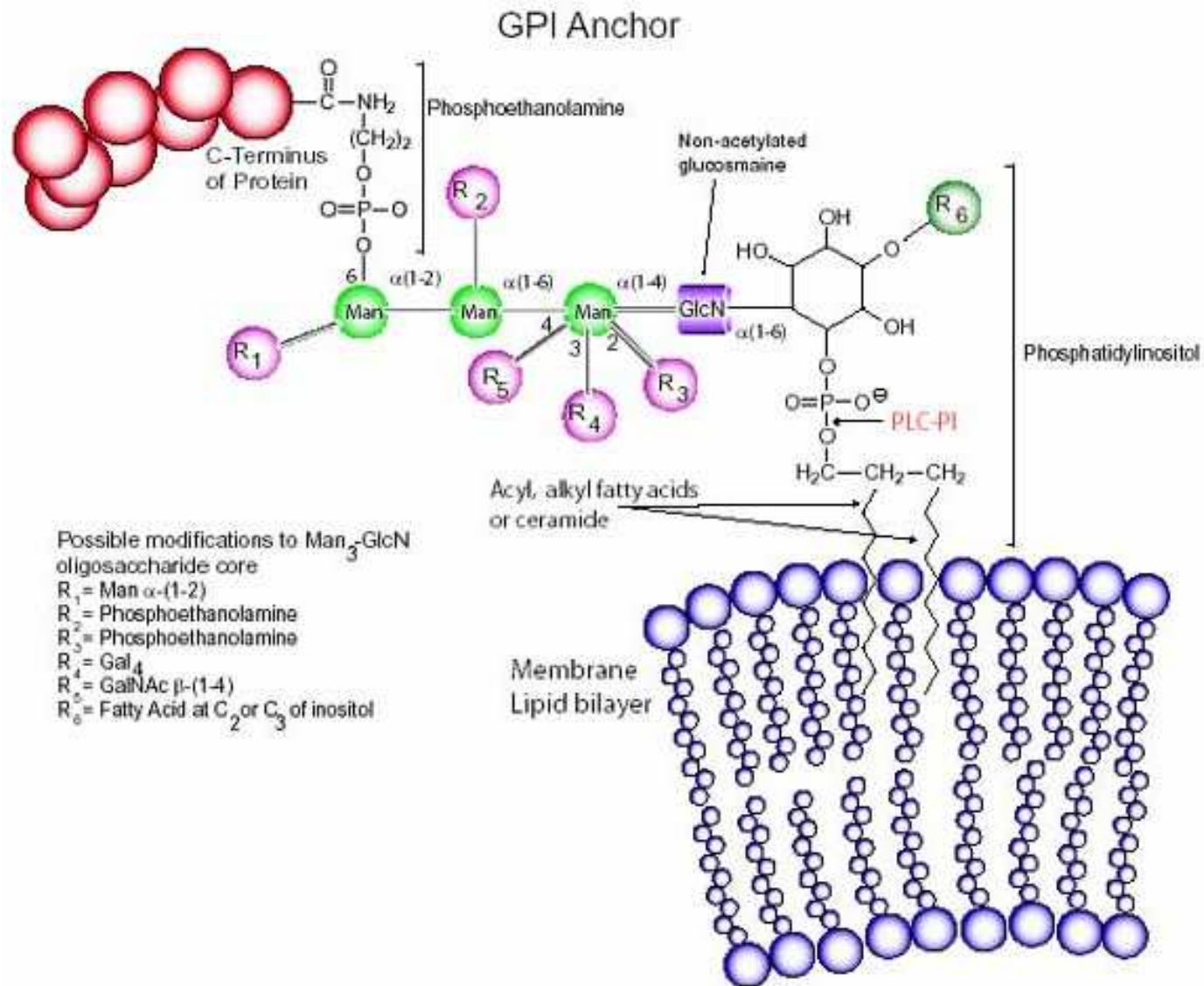
Většinou zakotveny GPI kotvou v membráně

(modifikace probíhá v ER katalyzována transamidázou; po odštěpení peptidu na C-konci je připojena glykosylfosfatidylinositolová kotva - inositolový ifosfolipid, s oligosacharidem substituovaným fosfoethanolaminem)

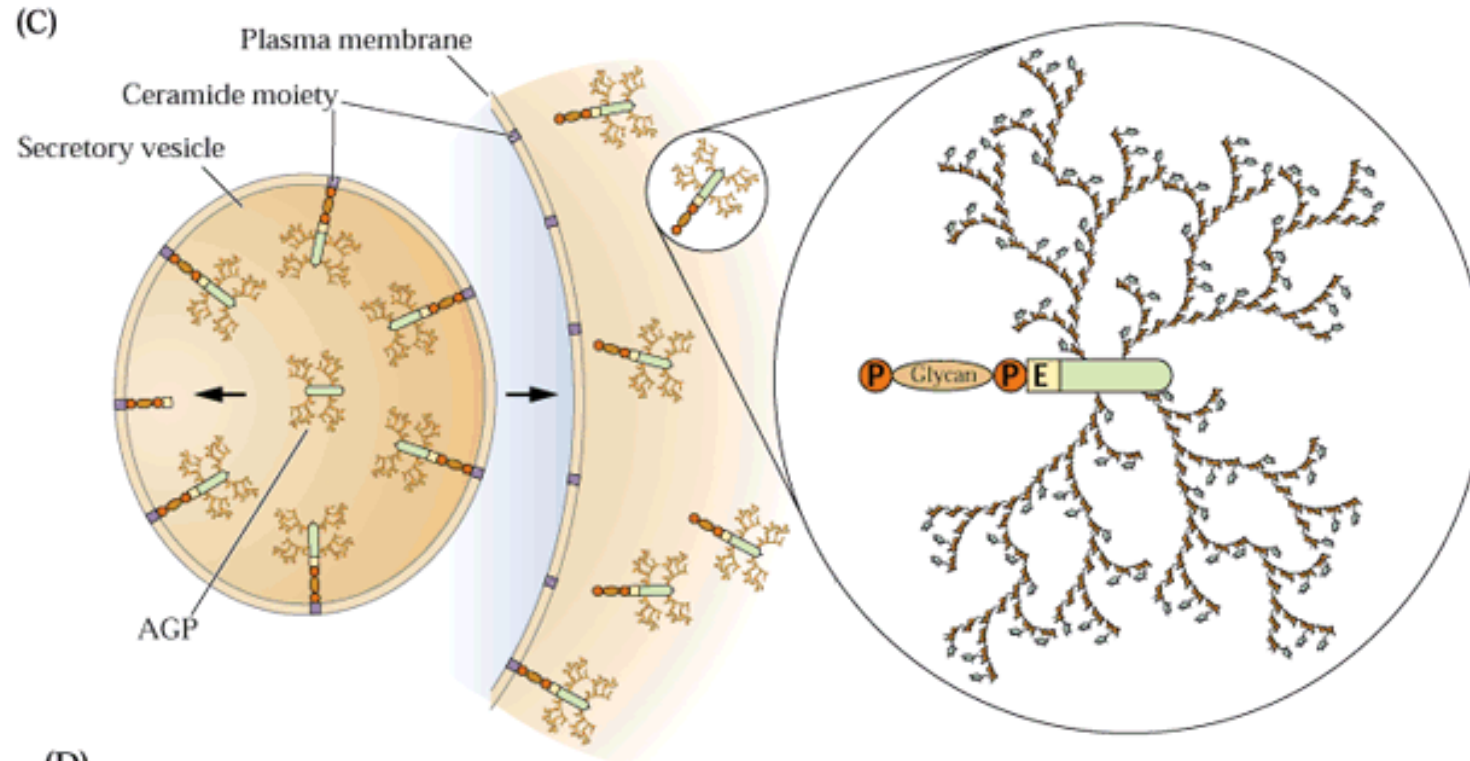
jako inhibitor vážící AGP - Yariv reagent (β -D-gal)₃



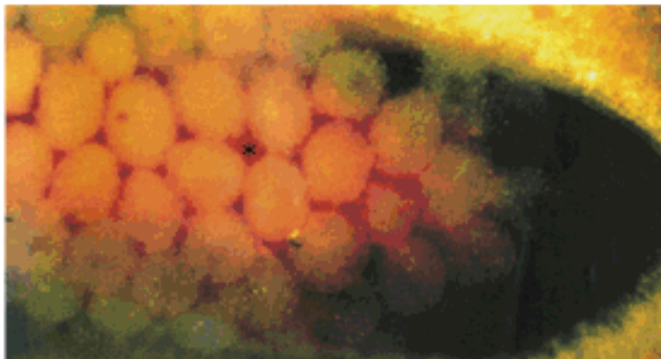
AGP kotví v membráně pomocí GPI



AGP kotví v membráně pomocí GPI



(D)

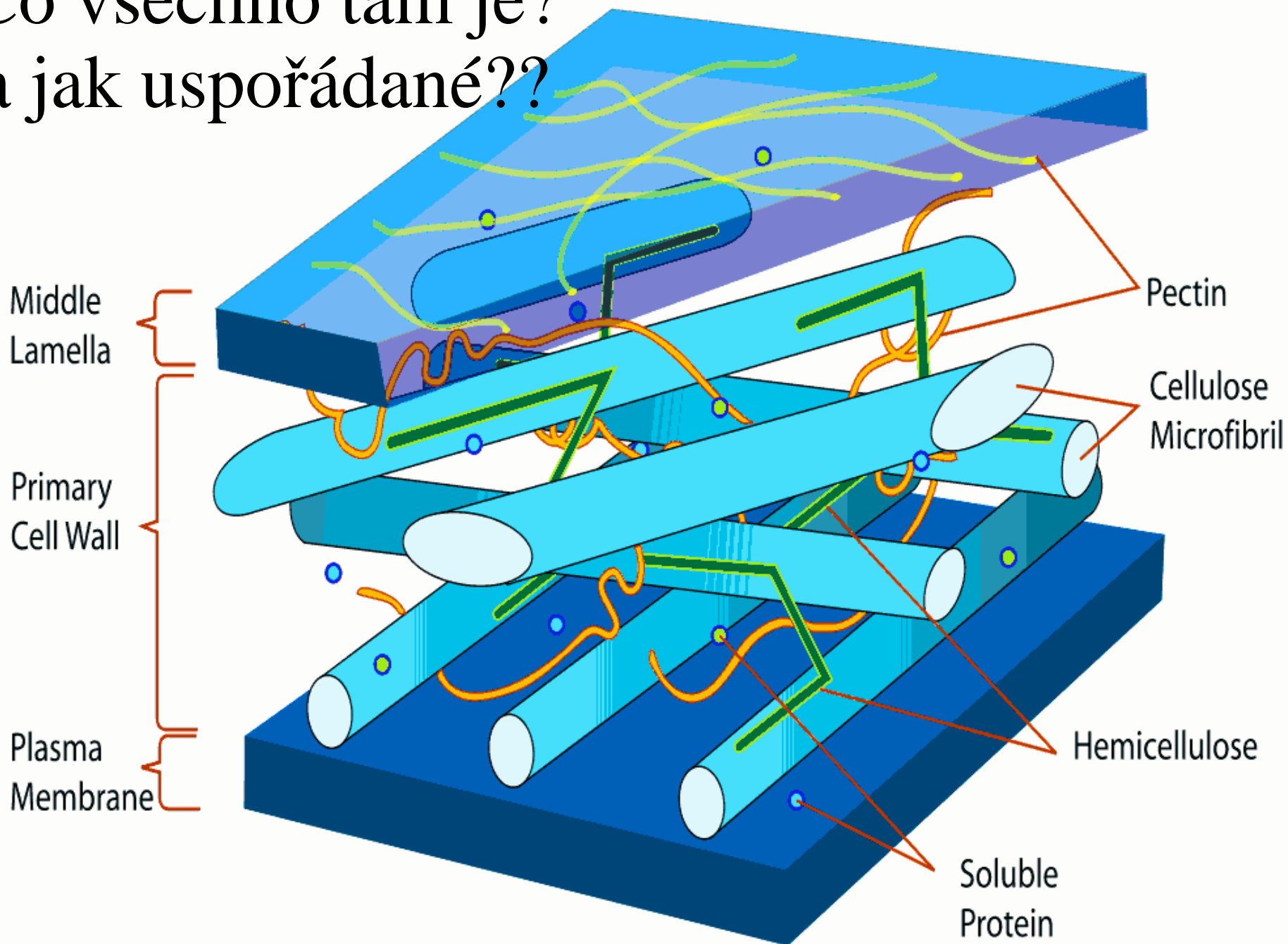


Převážnou část molekuly tvoří (poly)sacharidy

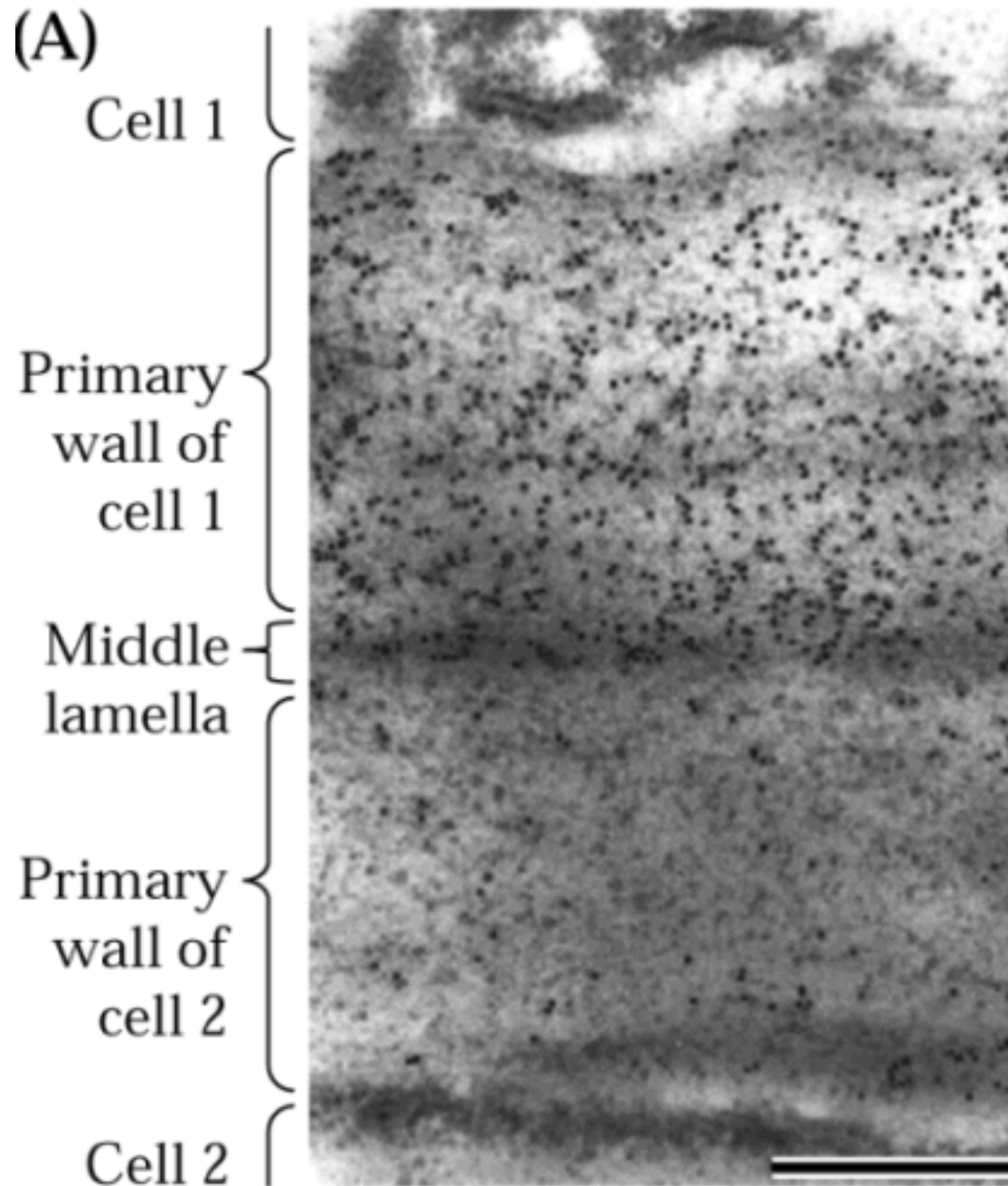
Obsah

- Buněčná stěna jako integrální část rostlinné buňky
- Sacharidy jako základní stavební složky BS
- Makromolekuly v BS
- **Struktura buněčné stěny, typy**
- Biosyntéza BS
- Buněčný růst a BS
- Diferenciace buněk, primární a sekundární BS
- Význam BS pro člověka, biotechnologie atd...

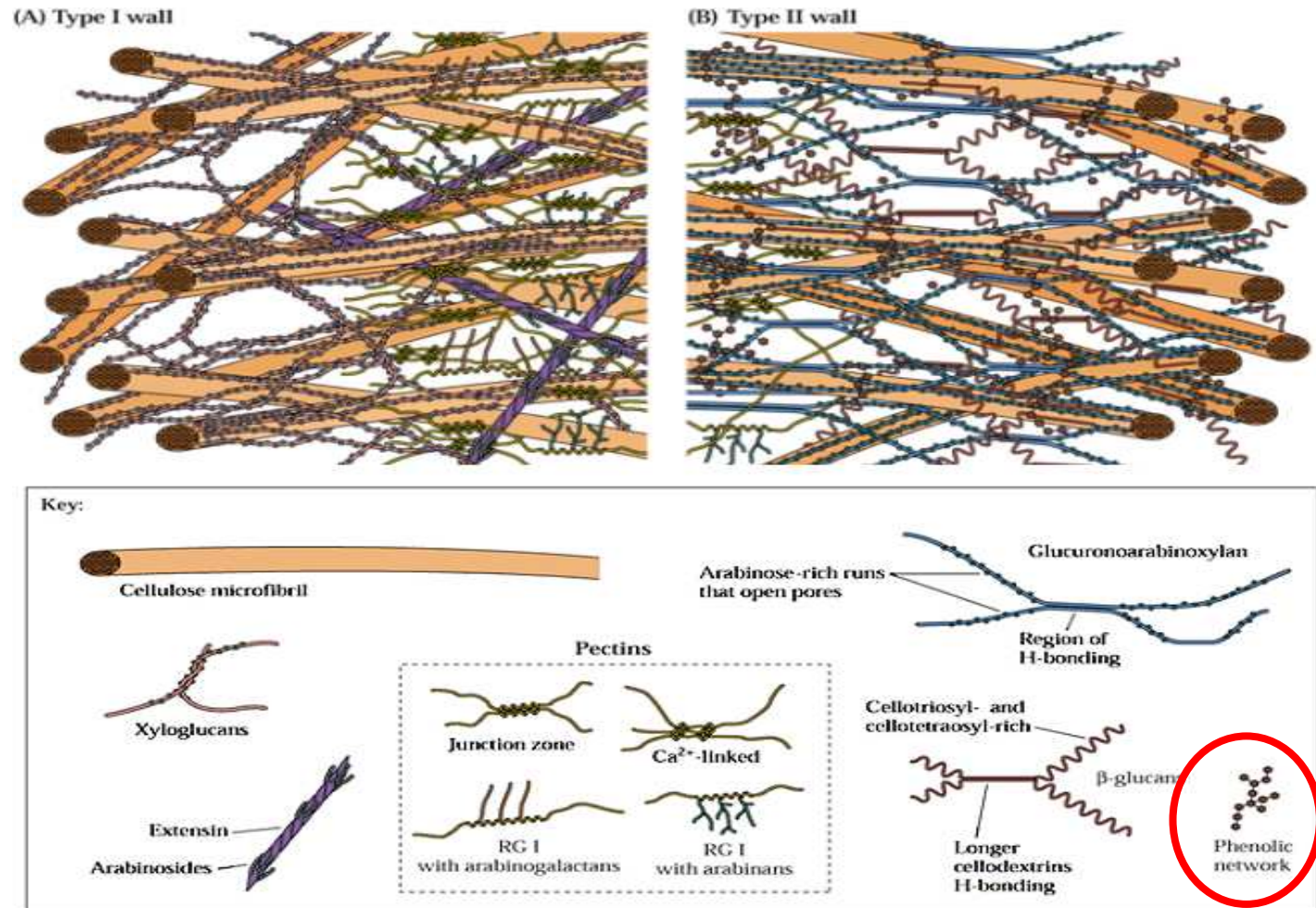
Co všechno tam je?
a jak uspořádané??



Perikarp
rajčete
JIM5
protilátka
=
deesterifikovaný
pektin



Stěny I. a II. typu



- I. – bohaté pektiny a proteiny; xyloglukany (**většina rostlin**)
- II. – chudé pektiny a proteiny; glukuronoarabinoxylany, bohaté křížové vazby přes fenolické molekuly – diferulát. (*Commelinoida*)

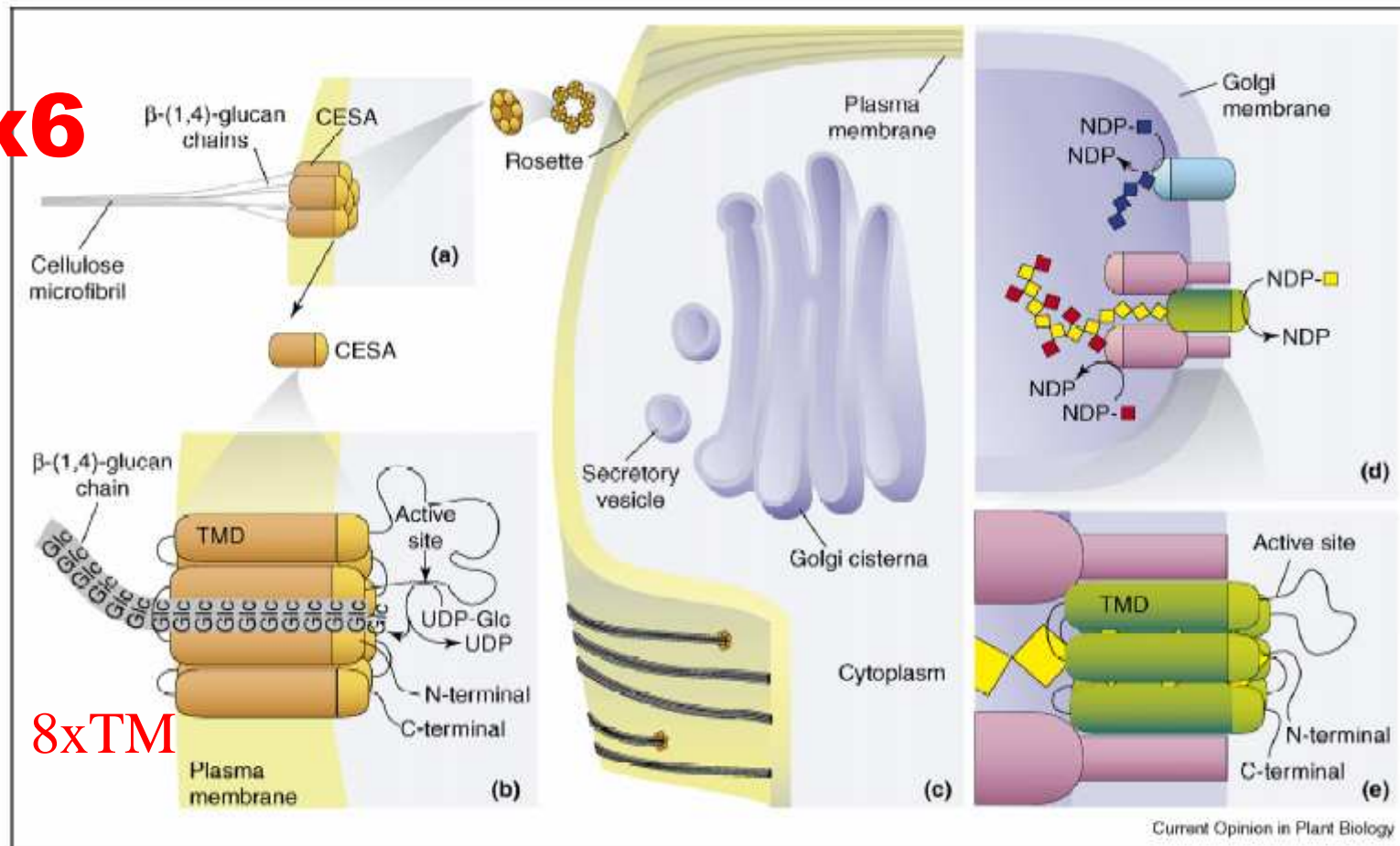
Obsah

- Buněčná stěna jako integrální část rostlinné buňky
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- Makromolekuly v BS
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- Význam BS pro člověka, biotechnologie atd...

Biosynéza celulózy

Celulóza syntáza
terminální komplex

6x6



8xTM

Schematic representation of hypotheses regarding wall polysaccharide biosynthesis. Wall polysaccharides are made in two cellular compartments. Cellulose and callose (not shown) are made at the plasma membrane. (a) Rosettes move in the plane of the membrane, guided by cortical microtubules, producing cellulose microfibrils in the wall that have same orientation as the microtubules in the cytosol [27**]. (b) It is thought that each hexameric rosette comprises six rosette subunits, and that each rosette subunit contains six CESA proteins, providing a total of thirty-six CESA proteins per rosette. Each CESA protein is predicted to span the membrane via eight transmembrane domains, with the N-terminus, the C-terminus and the active site facing the cytosol. The growing glucan chain is thought to move through a channel in the membrane to the wall, where it coalesces with other glucan chains to form a microfibril. (c) Matrix polysaccharides are synthesized in the Golgi before deposition into secretory vesicles that deliver them to the cell surface. The backbones of at least some hemicellulosic polysaccharides are synthesized by CSL proteins that show sequence similarity to the CESA proteins. (d) The topology of the CSL proteins is not known, but two possibilities are shown. If the CSL proteins use sugar nucleotides (NDP-□) present in the Golgi lumen, then the model shown in top part of (d) would apply. If the CSL proteins operate in the same way as the CESA proteins, then the model shown in the lower part of (d), and in an expanded view in (e), would be favored. The glycan synthases are thought to form complexes with glycosyltransferases that add side chains to the polymer (bottom part of [d]). Such organization in a complex might be especially important for the synthesis of polysaccharides such as XyG, which has a regular pattern of side-chain substitution. TMD, transmembrane domain.

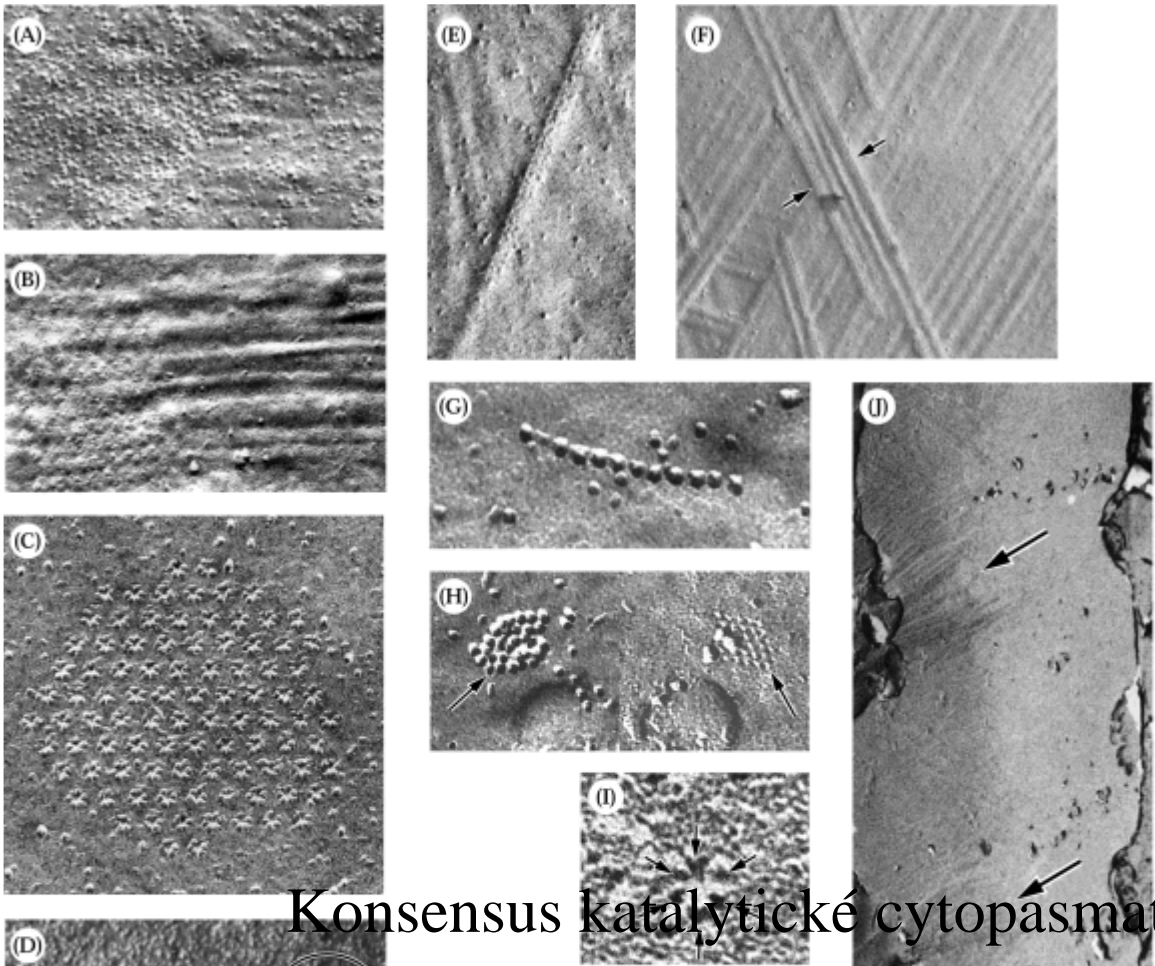
Terminální komplexy

Celulosa syntázy

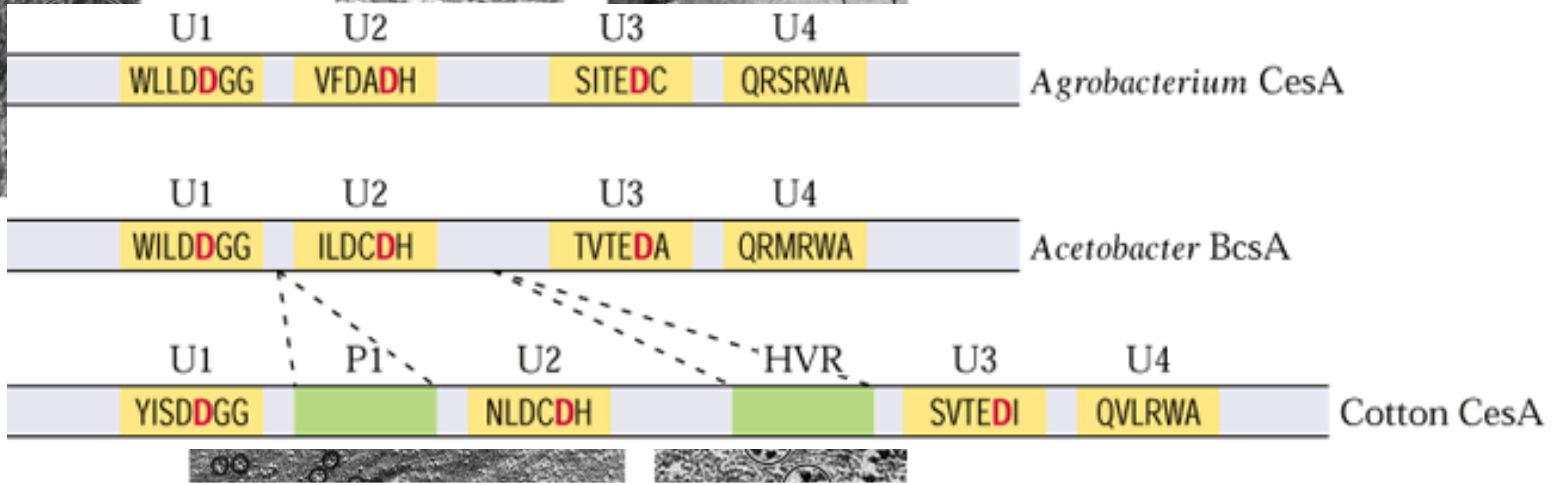
10x u A.t.

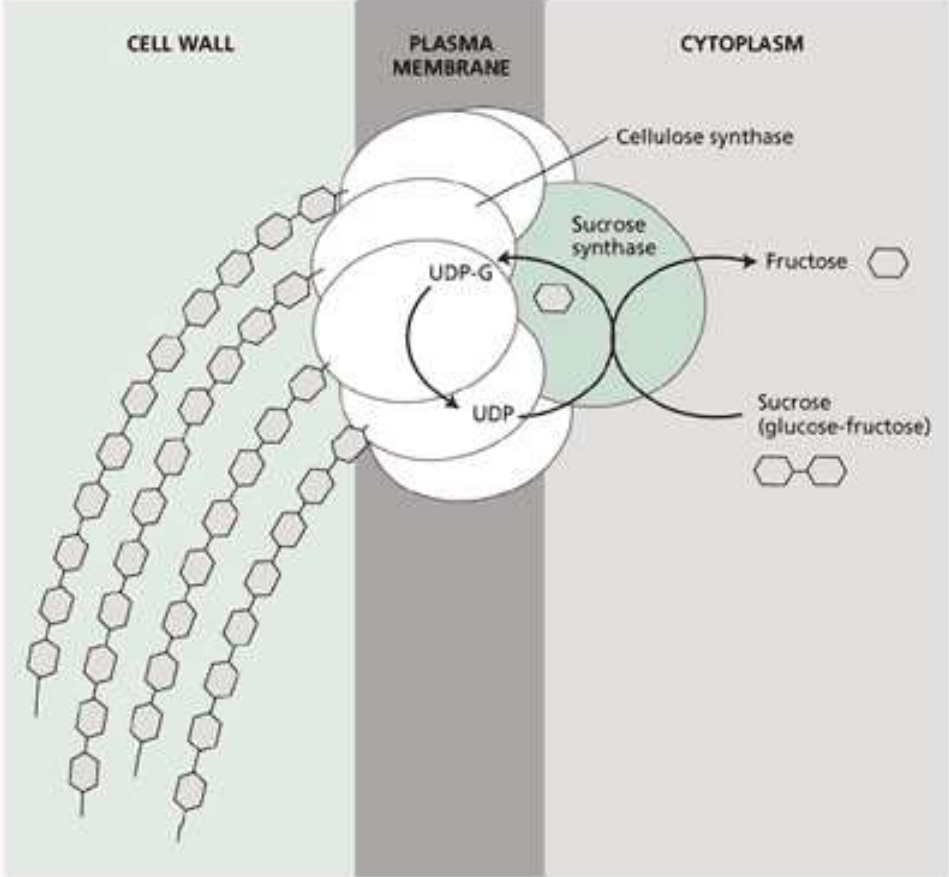
radial swelling (rsw1) - CesA1
procruste - CesA6

produkují méně celulosy a klesá
 anizotropie růstu
 mikrofibrily jsou
 disorganizovány, ale MT jako
 WT!



Konsensus katalytické cytoplasmatické domény CesA

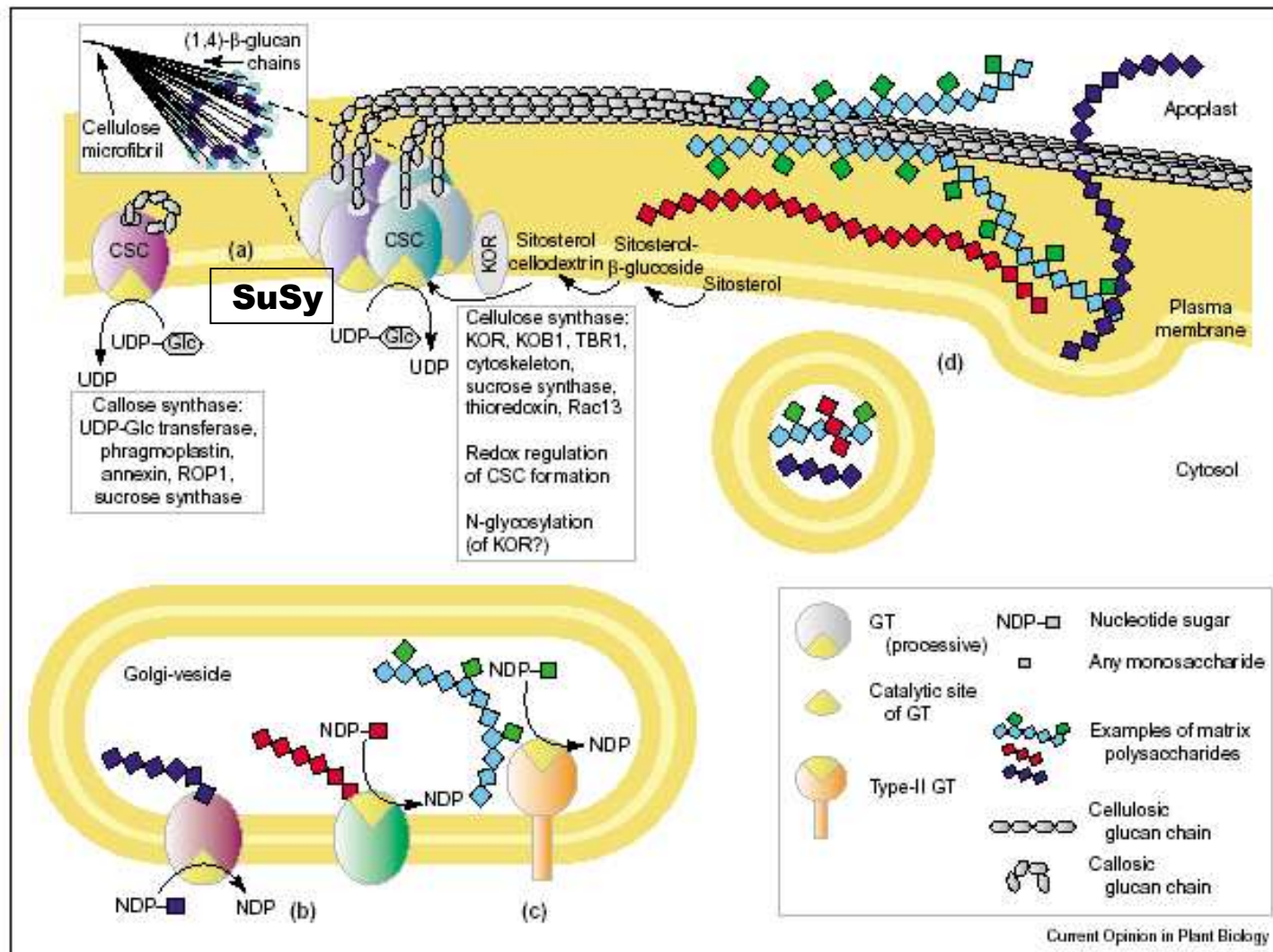




Vedle CesA podjednotek je
důležitá pro syntézu celulózy
také endoglukanasa/celulóza

KORRIGAN

a další dosud neznámé bílkoviny
např. COBRA, KOBITO, RIC



Synthesis of cell wall polysaccharides. Glycosyltransferases (GTs) utilize nucleotide sugars as donor substrates to generate polysaccharides. The metabolism of the nucleotide sugars themselves is described in detail elsewhere (G Seifert, this issue). **(a)** Cellulose and callose are synthesized at the plasmamembrane. Different CESA isoforms aggregate into higher-order rosettes (box in top left corner) to produce (1,4)- β -glucan chains that coalesce into cellulose microfibrils. Proteins that are known or suspected to interact [10] with the CSC, and mechanisms that are known to be important for cellulose synthesis and CSC activity, are shown in the central text box. The proposed pathway of primer synthesis for cellulose synthesis [24], which starts with sitosterol and requires KOR endoglucanase activity, is also depicted. CALS and proteins reported to interact with it (text box; [10]) are schematized on the left. **(b)** By contrast, matrix polysaccharides are synthesized within the Golgi apparatus. Depending on the topology of the catalytic sites, nucleotide sugars can be employed from the cytosol or the inside of the cisternae [13]. **(c)** Type-II GTs, which consist of a transmembrane stem and a catalytic domain, play a major role in decorating polysaccharides with side-chains. **(d)** The synthesized matrix polysaccharides are secreted by exocytosis into the apoplast, where they form highly ordered networks with cellulose microfibrils.

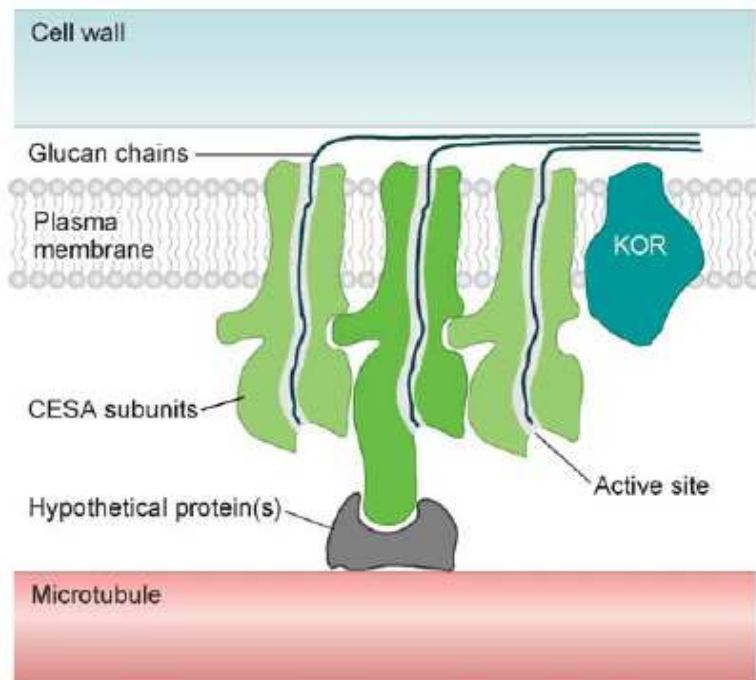


Figure 1

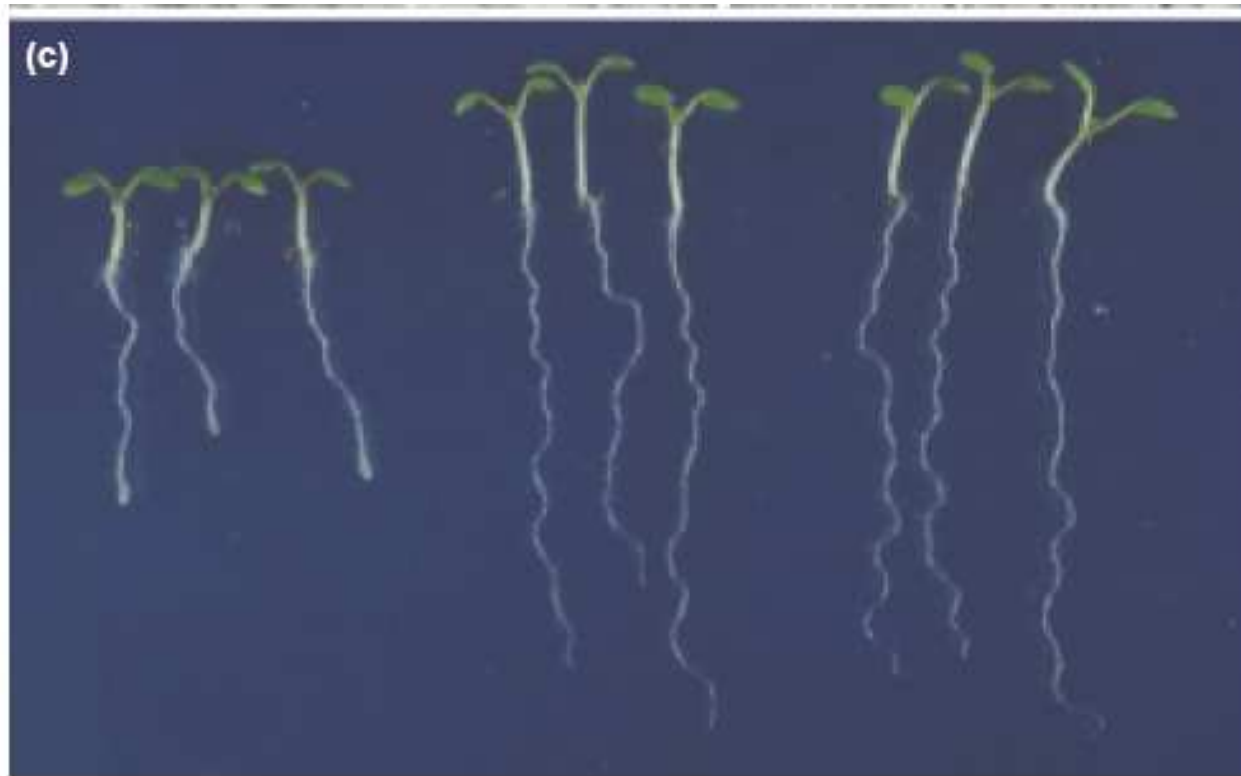
Schematic model of cellulose synthesis. Cellulose synthesis takes place in the plasma membrane. The plasma membrane is tightly appressed to the cell wall so that most of the cellulose synthase is in or below the plane of the membrane, which minimizes friction as the enzyme moves through the plasma membrane in response to elongation of the growing glucan chains by addition of glucan moieties from cytoplasmic UDP-glucose. The cellulose synthase complex is thought to contain as many as 36 CESA proteins, only a subset of which are illustrated. That three types of CESA proteins are required to form a functional complex suggested that different types of CESA proteins perform specific functions, such as interacting with the cortical microtubules.

Pro fungování TK je třeba **tří různých** paralogů/podjednotek CESA.

CESA 1,3 a 2/6 (I)– primární stěna

CESA 4,7 a 8 (II) vysoce transkripčně koordinovány – sekundární stěna.

***rsw1* = cesA1 – teplotně sensitivní
ts (radial swelling)**

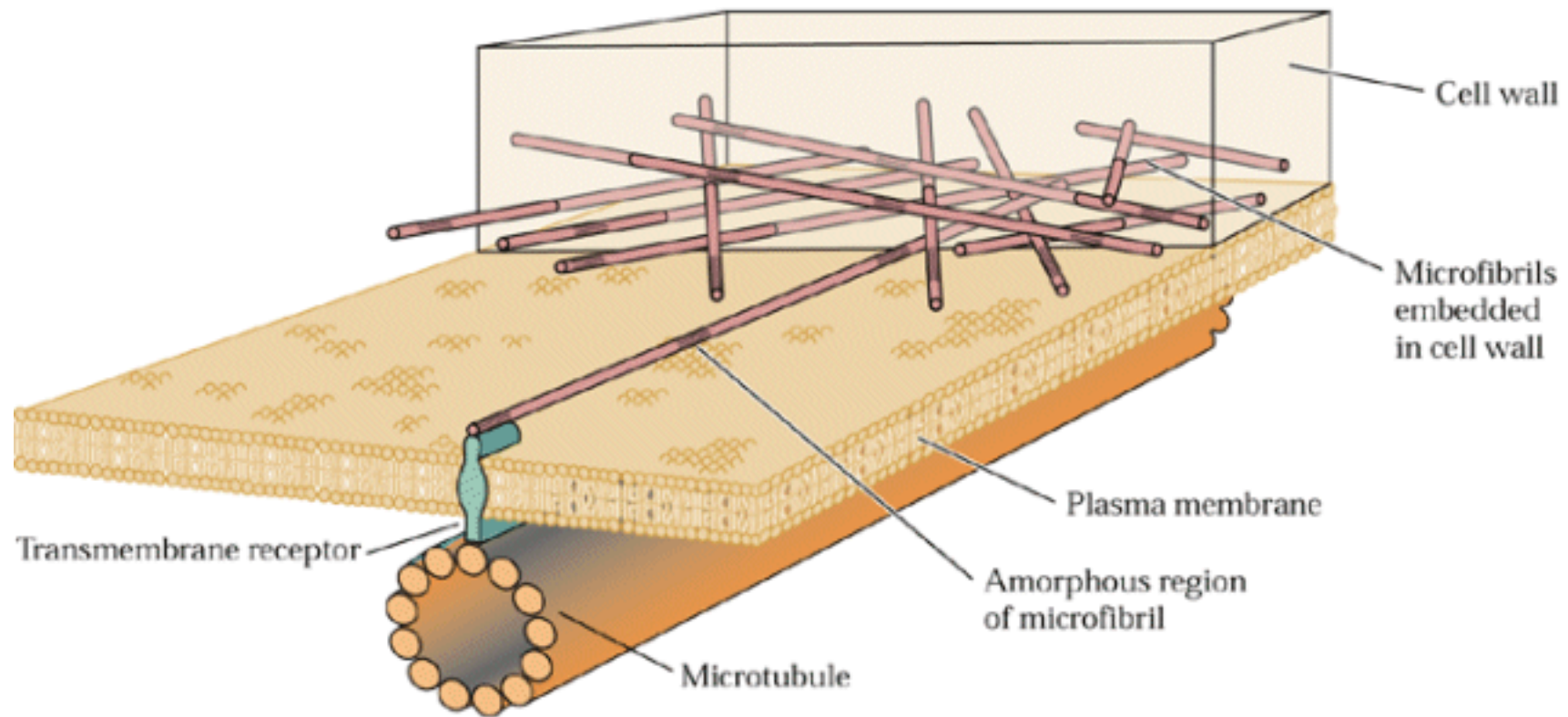


bar =100 nm. (c) The phenotypes of *rsw1* grown for five days at 21°C and two days at 31°C (left), of *rsw1* transformed with a wild-type copy of the *CesA1* gene (centre) and of Columbia wild type (right). (a and b, micrographs courtesy of Werner Herth, Zellenlehre, Heidelberg.)

30°C

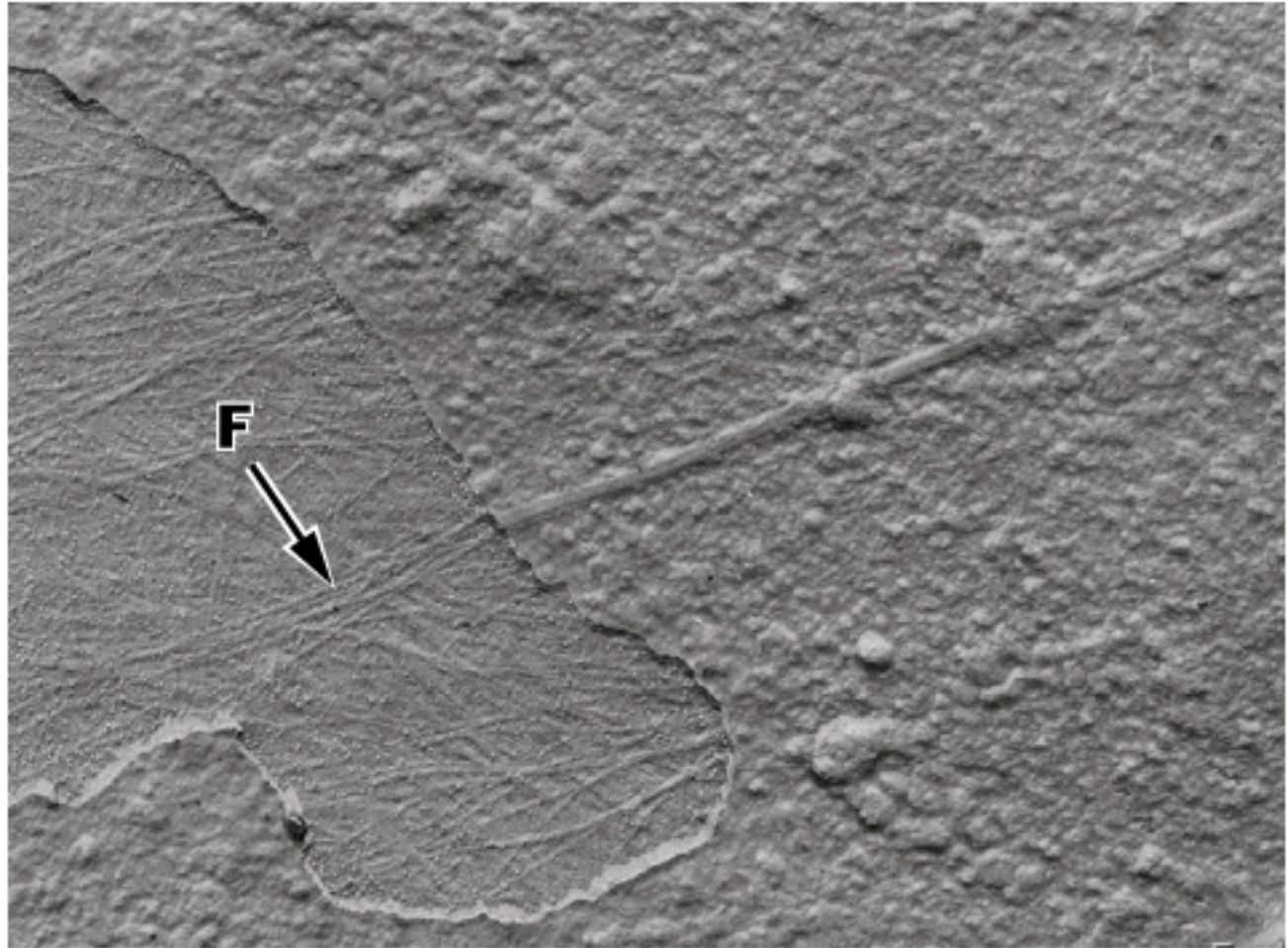
V restriktivní teplotě má mutant jen 50% celulózy ve srov. s WT|

Kortikální MT orientují ukládání mikrofibril celulosy

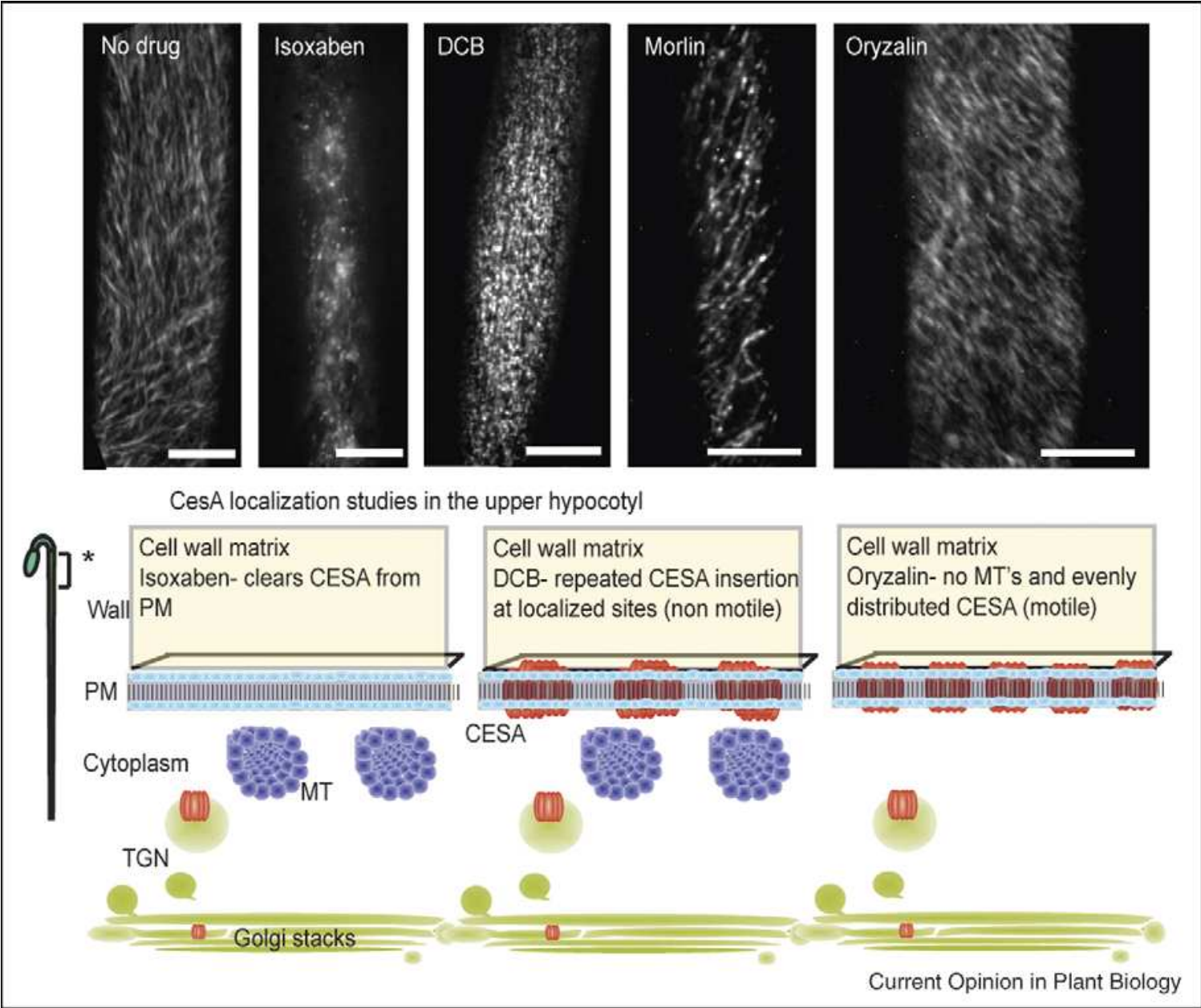


**Kolokalizace
MT s
mikrofibrilou**

(B)



- Jednou z možností jak interaguje (a „drží“ se) TK mikrotubulu je konformace spojená s torzním tlakem při syntéze mikrofibrily.
- Roli ovšem hrají také některé kinesiny – MTs motory



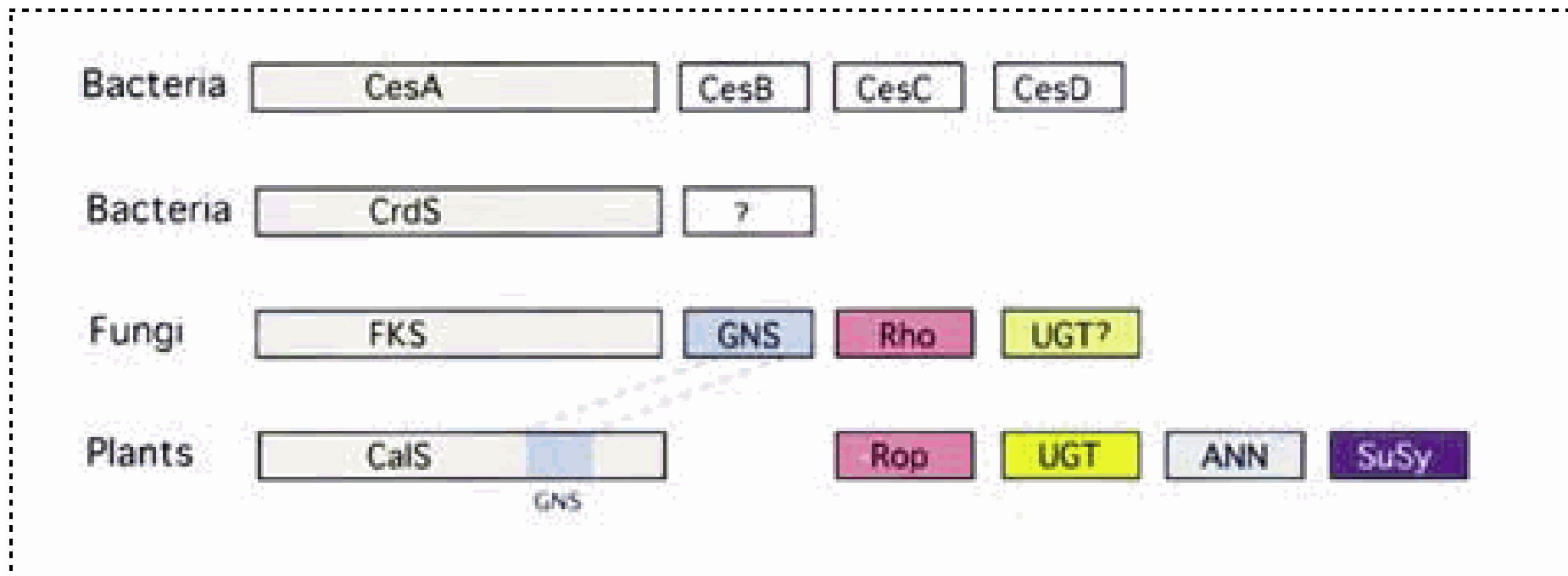
mor1 - 215kDa MAP

u ts mutanta se v restriktivní teplotě rozpadají kortikální MT a dochází ke ztrátě polarity, ale mikrofibrily orientovány jako u WT.

- *mor1* byl popsán nezávisle jako *gem1*
- mutace která vede k symetrickému dělení buněk při mikrosporové=1. pylové mitóze.

- Arabidopsis 10x CESA
- **Topol 18!!x CESA**

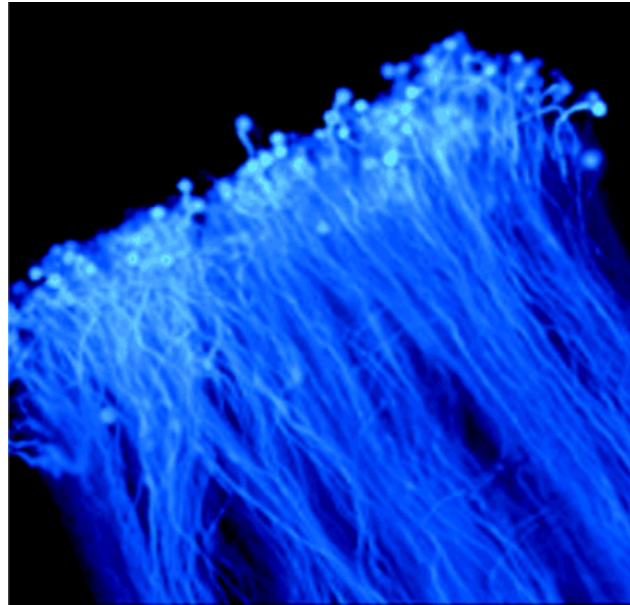
Kalosa syntázy



CalS je blízce příbuzná β -1,3,glukan syntáze kvasinky

- **12 x CalS** izozymů v *Arabidopsis*

Barveno "odbarvenou" (pH11) anilínovou modří - kalóza



Kalóza při rozmnožování

Také meicyty obaleny kalózou - tetrády

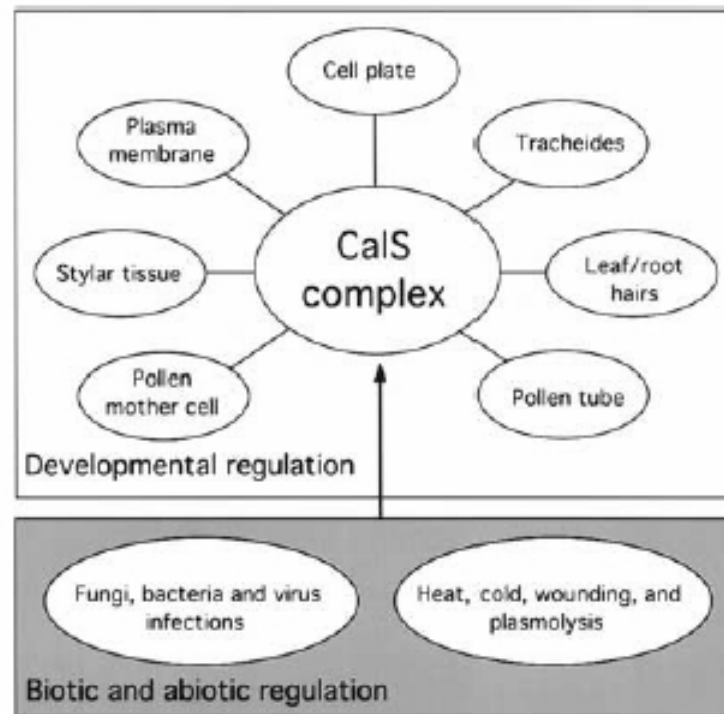
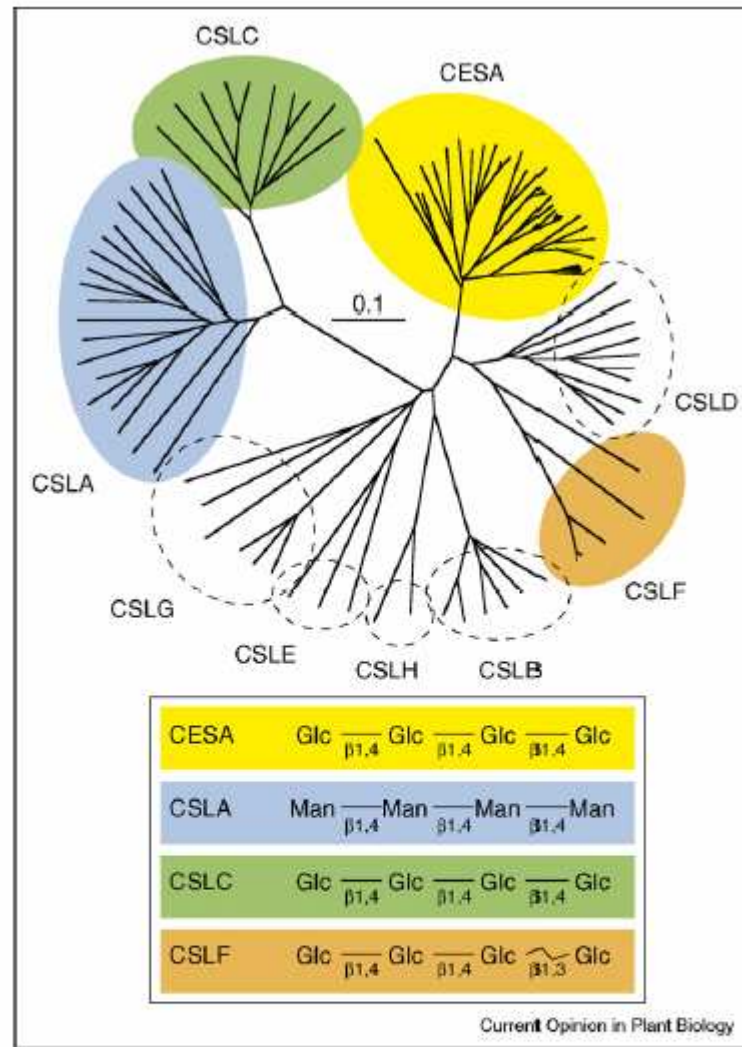


Figure 4. Callose synthases are present in many locations of the plant and their activities are regulated by developmental status and by biotic and abiotic signals.



The CSL hypothesis. Plant genomes contain CESA and homologous CSL protein sequences. A phylogenetic tree of these sequences was provided by Dr Todd Richmond (prepared as described at http://cellwall.stanford.edu/php/display_tree.php). CESA and CSL subfamilies are shown in ovals. The subfamilies shown in colored ovals contain one or more members whose enzymatic function has been demonstrated; the functions of members of subfamilies within dashed ovals are unknown. The corresponding structures of polysaccharide products of various subfamily members are indicated.

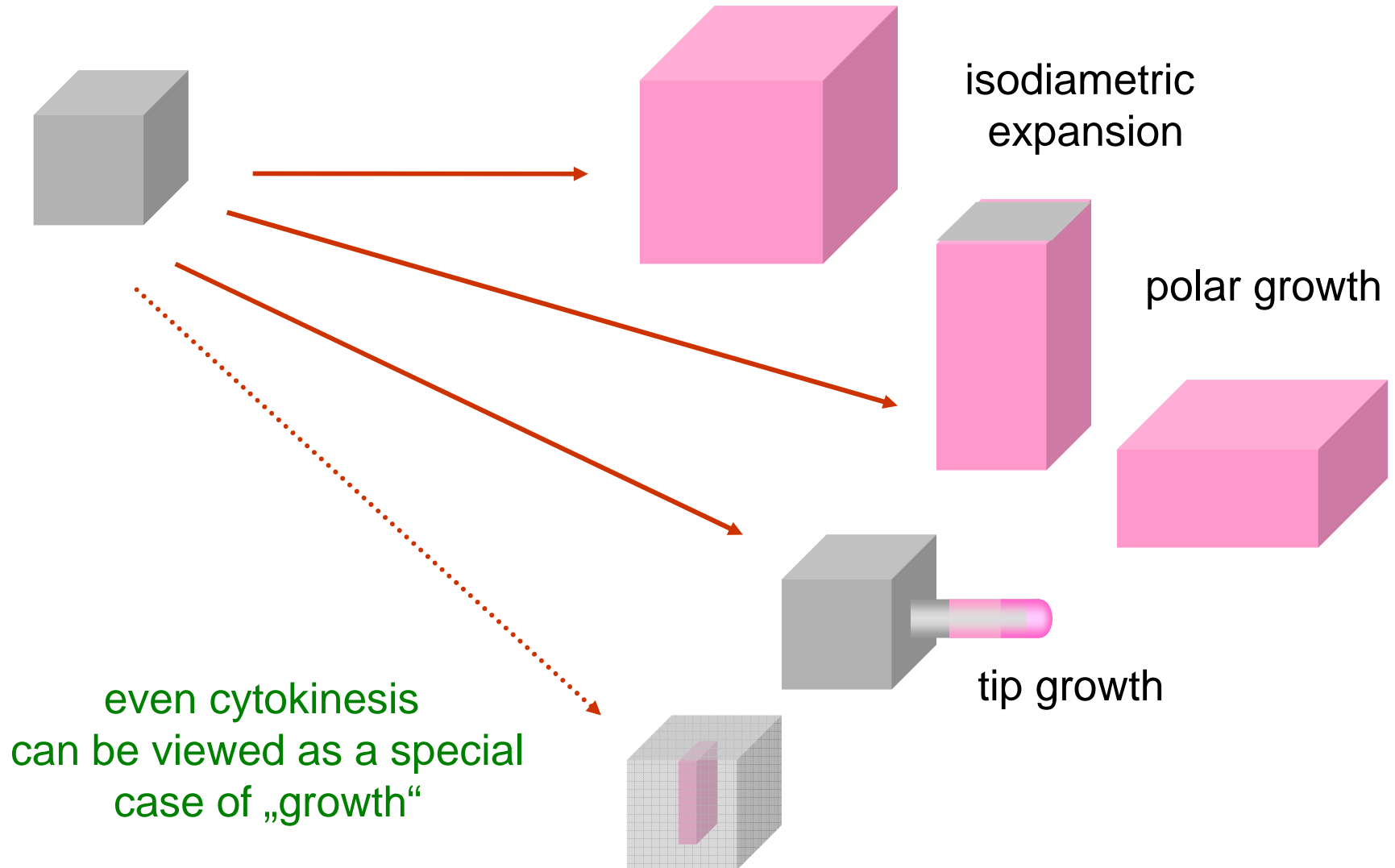
- Syntetizují CSL hemiceluózy podobně jako CESA celuózu, nebo funguje „bežící pás“ glykosyltransferáz?
- CSLA je glukomannansyntáza, ale
- XyG – buď CSLC nebo „bežící pás“ glykosyltransferáz?

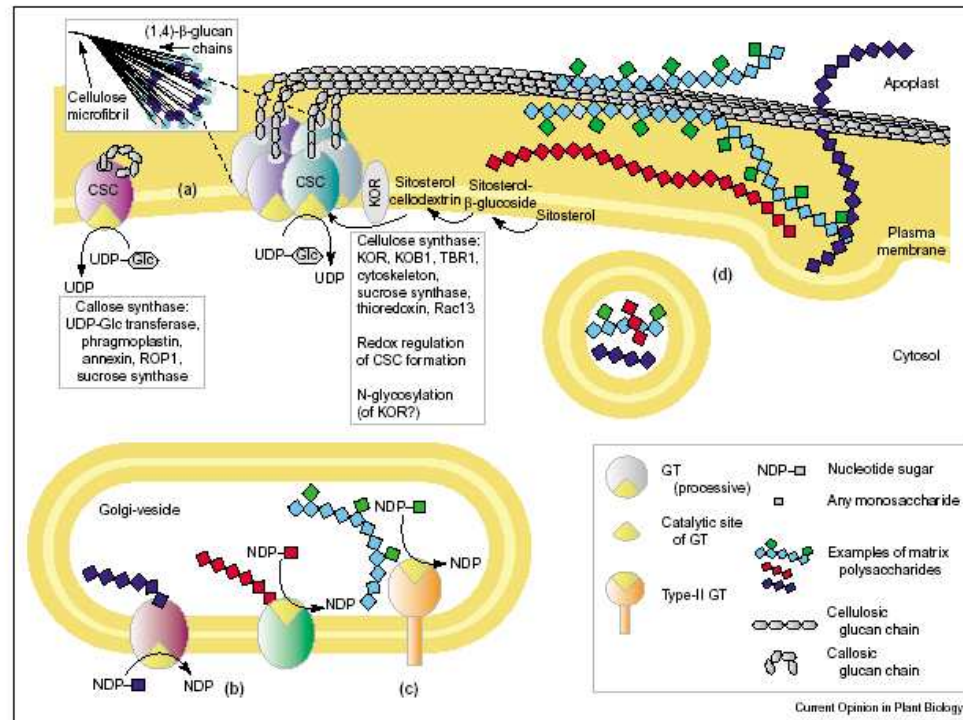
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- Diferenciace buněk, primární a sekundární BS
- Význam BS pro člověka, biotechnologie atd...

Jak to může fungovat všechno
najedou?

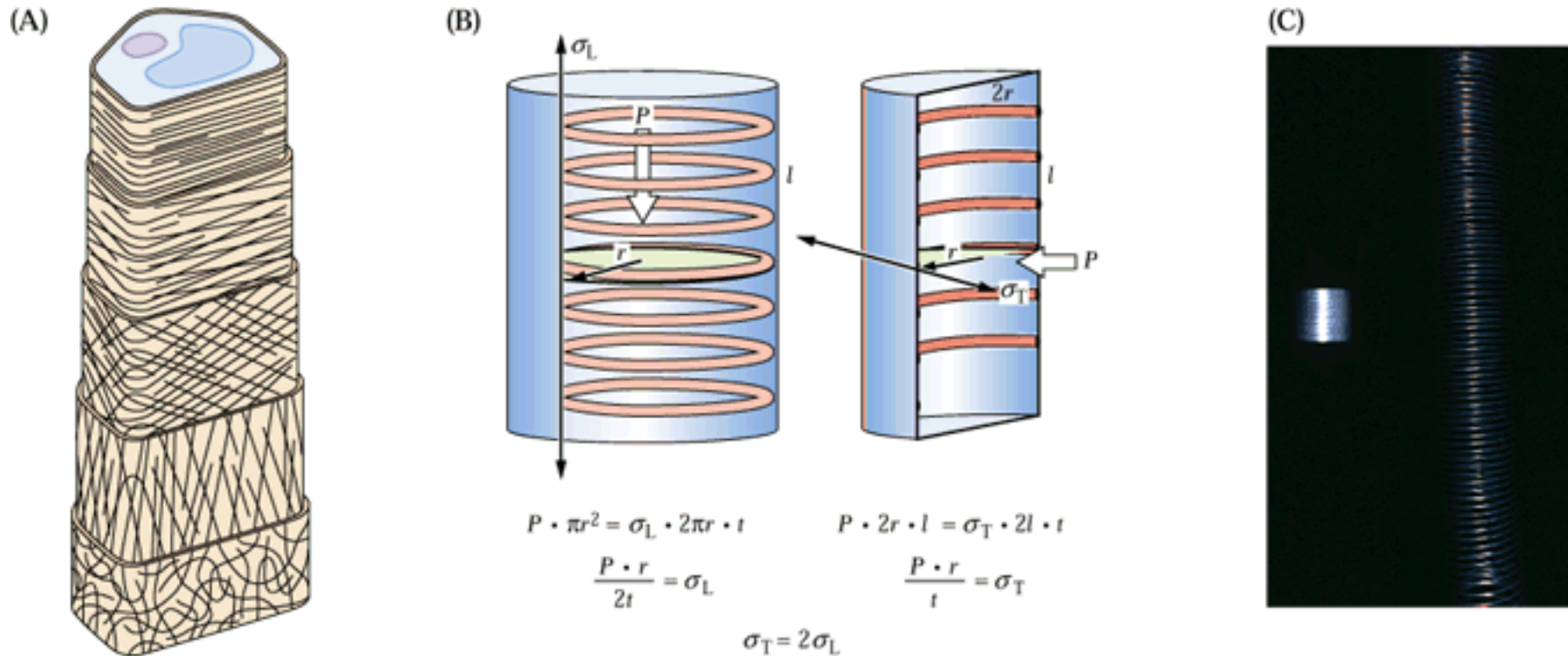
Modes of (plant) cell growth





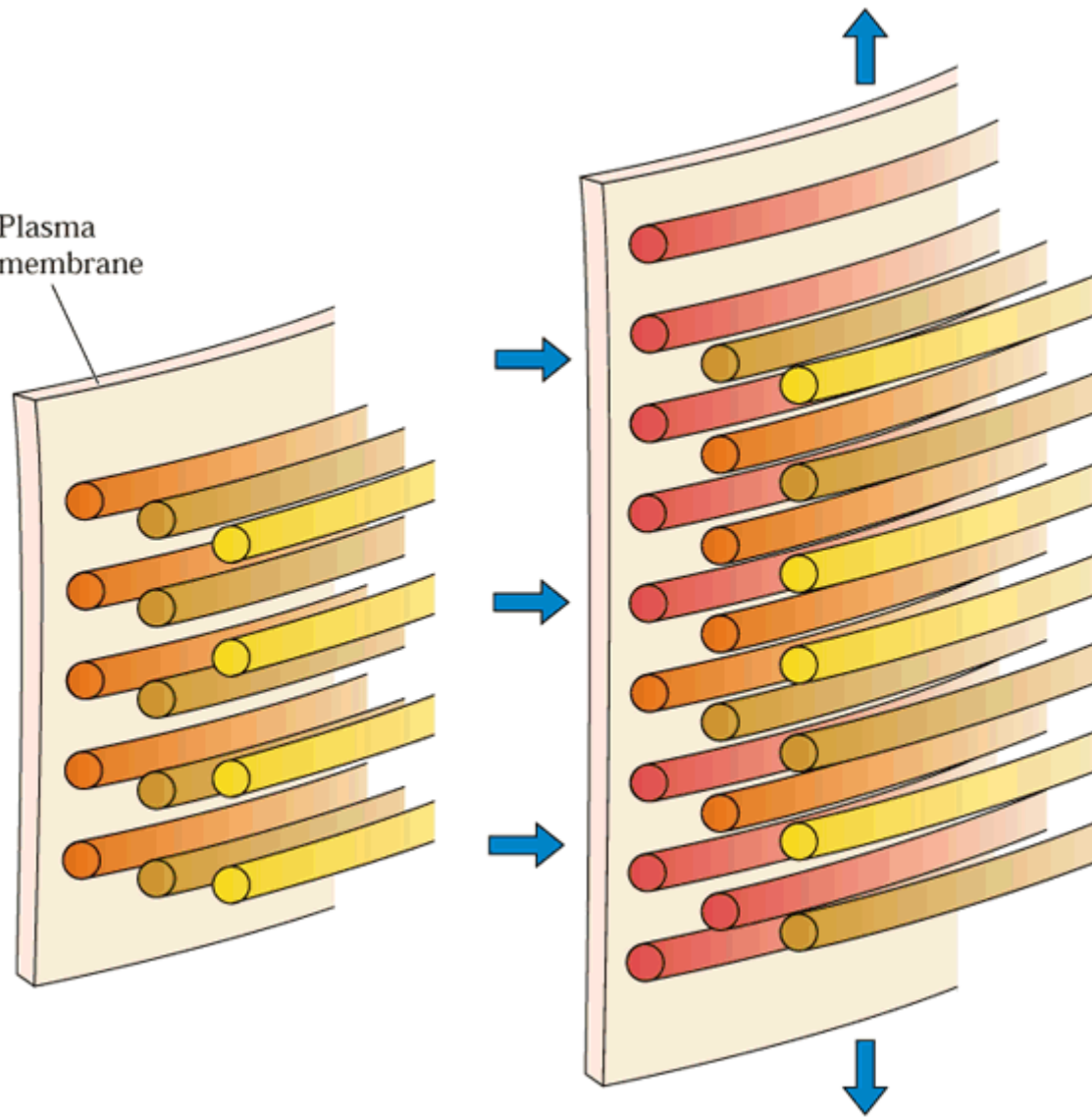
Synthesis of cell wall polysaccharides. Glycosyltransferases (GTs) utilize nucleotide sugars as donor substrates to generate polysaccharides. The metabolism of the nucleotide sugars themselves is described in detail elsewhere (G Seifert, this issue). **(a)** Cellulose and callose are synthesized at the plasmamembrane. Different CESA isoforms aggregate into higher-order rosettes (box in top left corner) to produce (1,4)-β-glucan chains that coalesce into cellulose microfibrils. Proteins that are known or suspected to interact [10] with the CSC, and mechanisms that are known to be important for cellulose synthesis and CSC activity, are shown in the central text box. The proposed pathway of primer synthesis for cellulose synthesis [24], which starts with sitosterol and requires KOR endoglucanase activity, is also depicted. CALS and proteins reported to interact with it (text box; [10]) are schematized on the left. **(b)** By contrast, matrix polysaccharides are synthesized within the Golgi apparatus. Depending on the topology of the catalytic sites, nucleotide sugars can be employed from the cytosol or the inside of the cisternae [13]. **(c)** Type-II GTs, which consist of a transmembrane stem and a catalytic domain, play a major role in decorating polysaccharides with side-chains. **(d)** The synthesized matrix polysaccharides are secreted by exocytosis into the apoplast, where they form highly ordered networks with cellulose microfibrils.

Musí fungovat metabolická koordinace mezi syntézou celulózy a syntézou a sekrecí! necelulózních složek buněčné stěny!

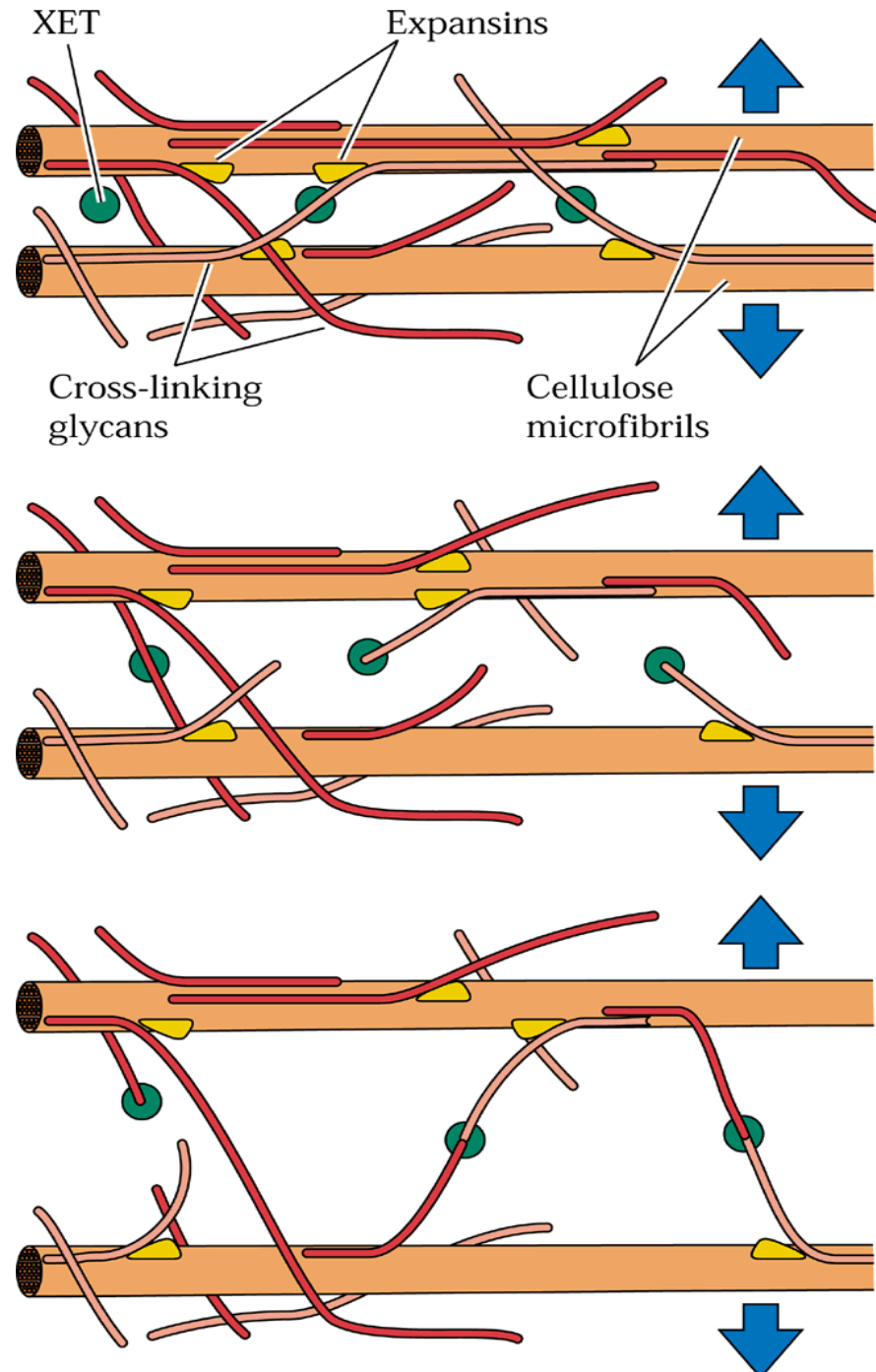


(A) Orientace mikrofibril se ve stěně během ukládání dalších vrstev a růstu mění – pův. představa o pasivním natahování (B) během dlouhivého růstu vzniká ve stěně velké tangenciální napětí (σ_T), které roste příp. až k několika stovkám MPa, při turgoru 1MPa (C) názorný model pružiny

Plasma membrane

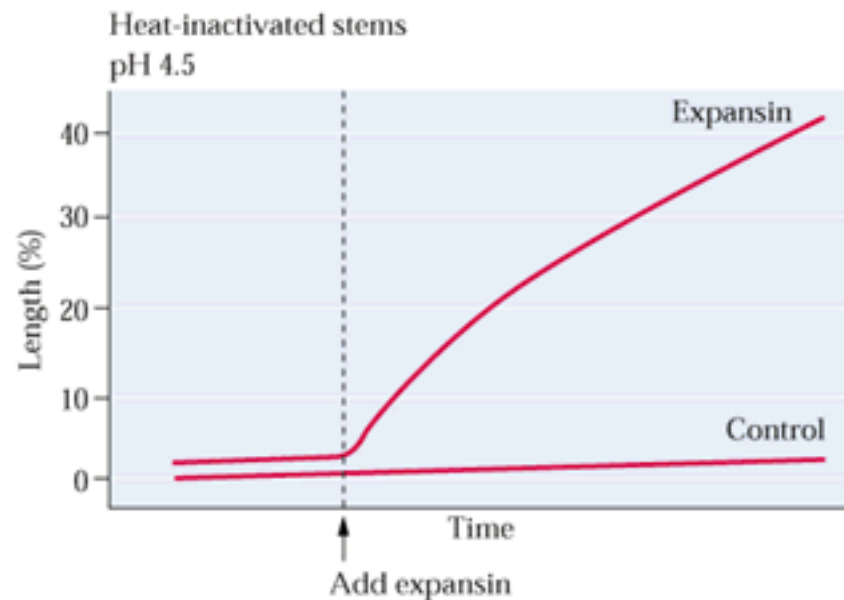
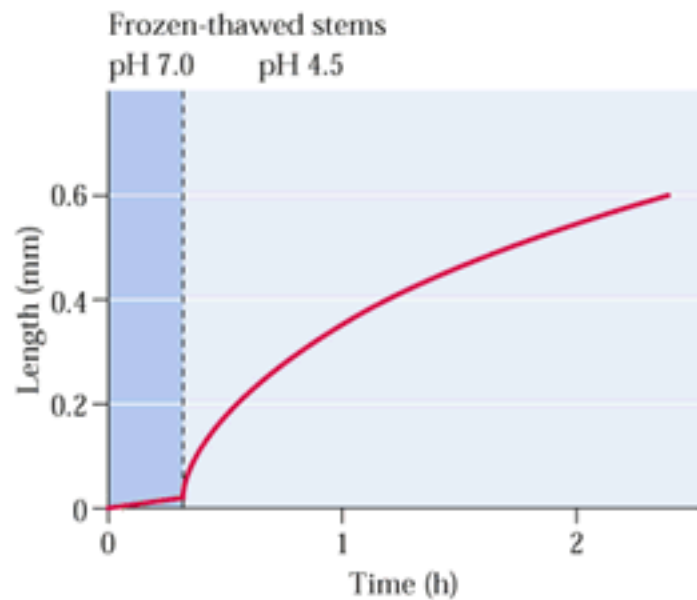
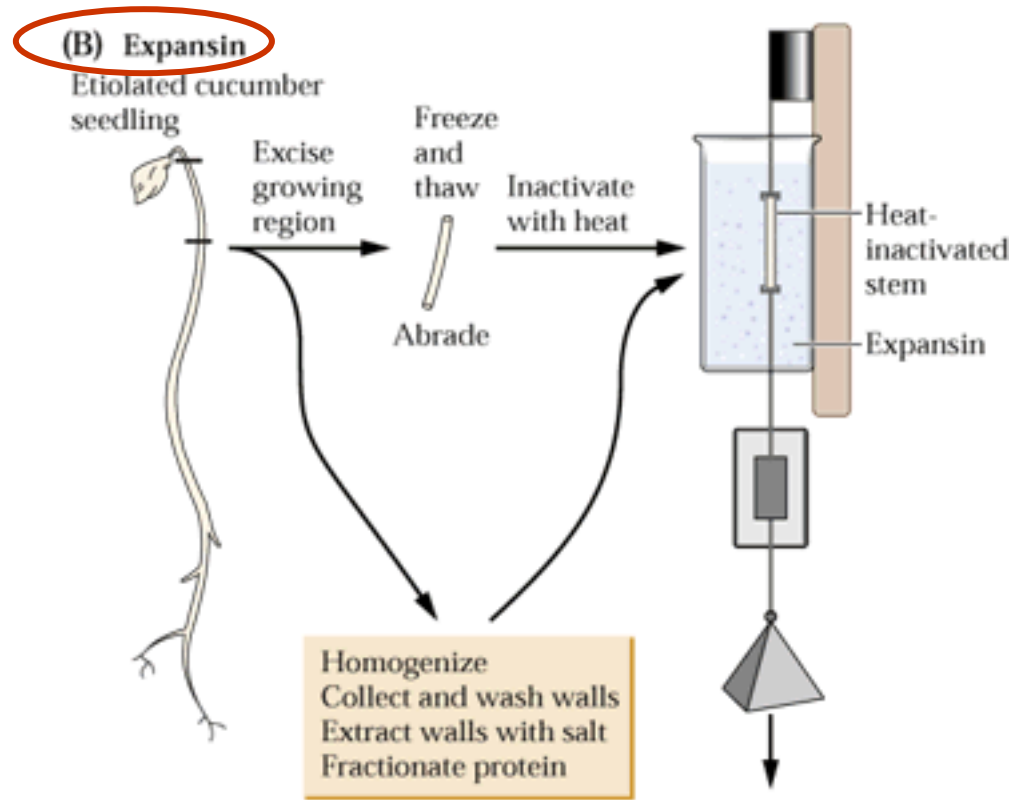
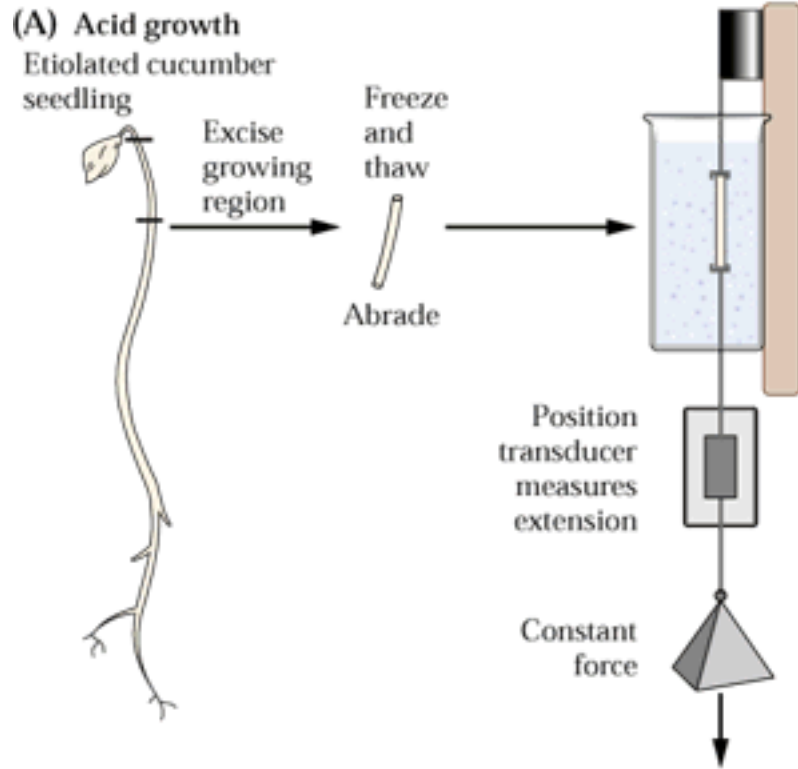


XET
xyloglukan
endotransglykosylasy
a
EXPANSINY

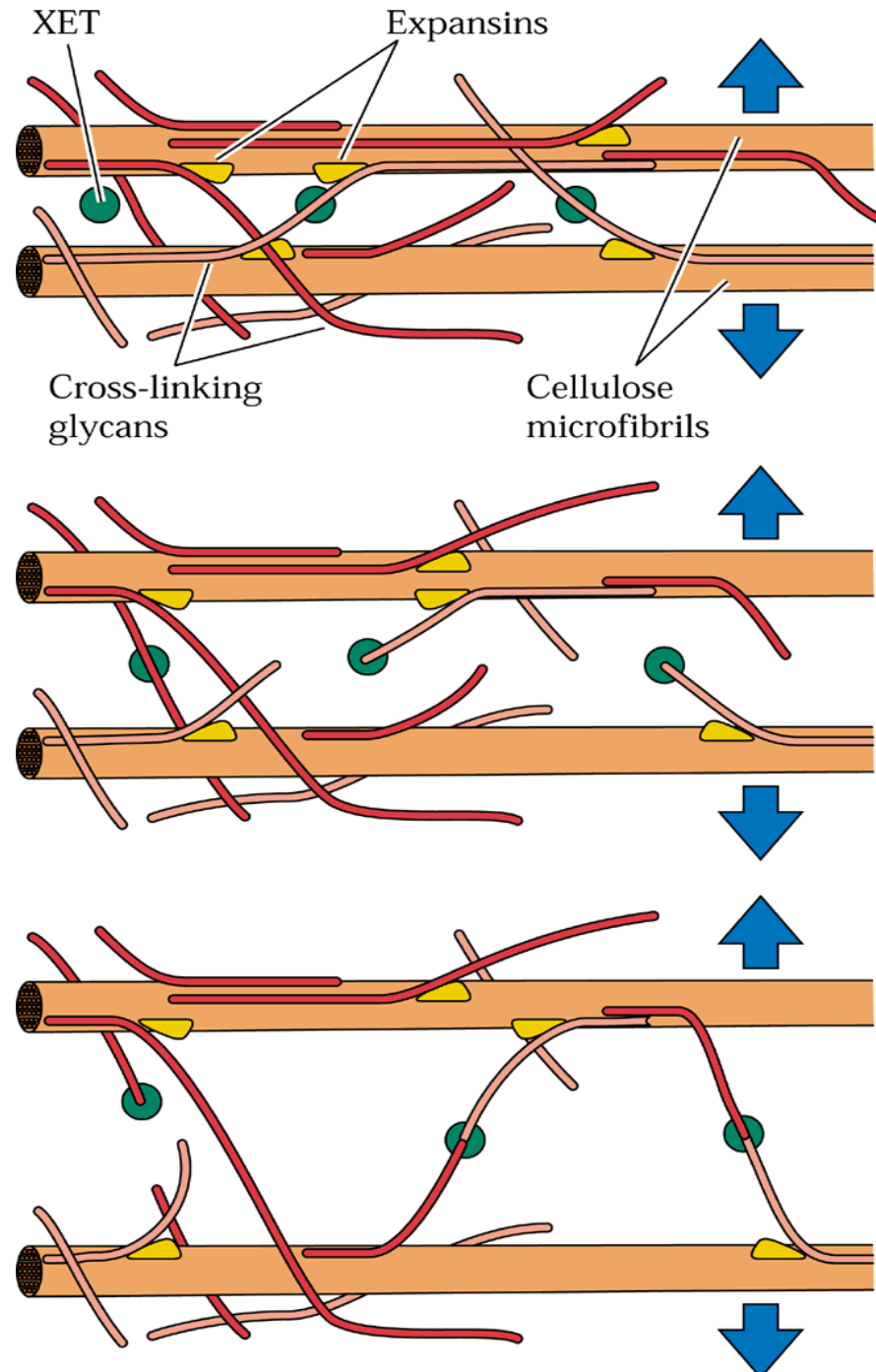


- **Expansiny jsou aktivovány snížením pH a hrají klíčovou roli v tzv. kyselém růstu indukovaném auxinem.**

(IAA aktivuje H⁺-P-ATPázy)



XET
xyloglukan
endotransglykosylasy
a
EXPANSINY



Stěnové XET/XTH – endotransglykosylázy/hydrolázy

- Nemění poměr mezi obsahem celulózy a XG.
- Pevnost a roztažnost zůstávají zachovány.
- Zřejmě zvyšují pevnost stěny (stále nejasné)

Stěnové **hydrolázy** – xyloglukanázy (**XG**) (hemicelulázy)

- Hydrolyzují nejen spojovací část, ale částečně i frakci vázanou na celulózu a mění chemické složení stěny.
- Působí zvýšenou tuhost, sníženou visko-elasticitu při zachování podélné extensibility.

Anti-intuitivní funkce stěnových enzymů.

- Zdá se, že hlavní funkcí XG je udržovat mikrofibrily celulózy od sebe – aby nekolabovaly do velkých shluků.
- XG působí tedy ve směru rozvolnění stěny.
- **Proto mohou glukonázy/hydrolázy zvyšovat!! pevnost/tuhost stěny, zatímco XET zvyšují viskoelasticitu při zachování mechanické pevnosti.**

Komplementarita účinků expansinů a XET.

- Expansiny oslabují uniaxiální pevnost, ale XET ne.
- XET katalyzují roztahování/přeskupování stěnové sítě za konstantního bi-axiálního napětí, zatím co expansiny ne.
- Proto často obě aktivity kolokalizují v místech růstu.

“Zacementování” BS po skončení růstu

- Type I BS
 - extensiny a jiné HRGPs
 - deesterifikace pektinů PME
- Type II BS
 - Thr bohaté proteiny
 - cross-linking fenolických látek

Hydroxylové radikály (.OH)
vznikající ve stěně činností PRX a
O₂ a NADH, Fentonovou reakcí
(Fe²⁺ a H₂O₂ =ROS) případně v
NOX, mohou **neenzymaticky**
štěpit stěnové polysacharidy – XG a
pektiny.

STÁLE NEJASNÉ !!!!!

Funkce **askorbátu** ve stěně úzce souvisí s tvorbou ROS (oxidative burst)

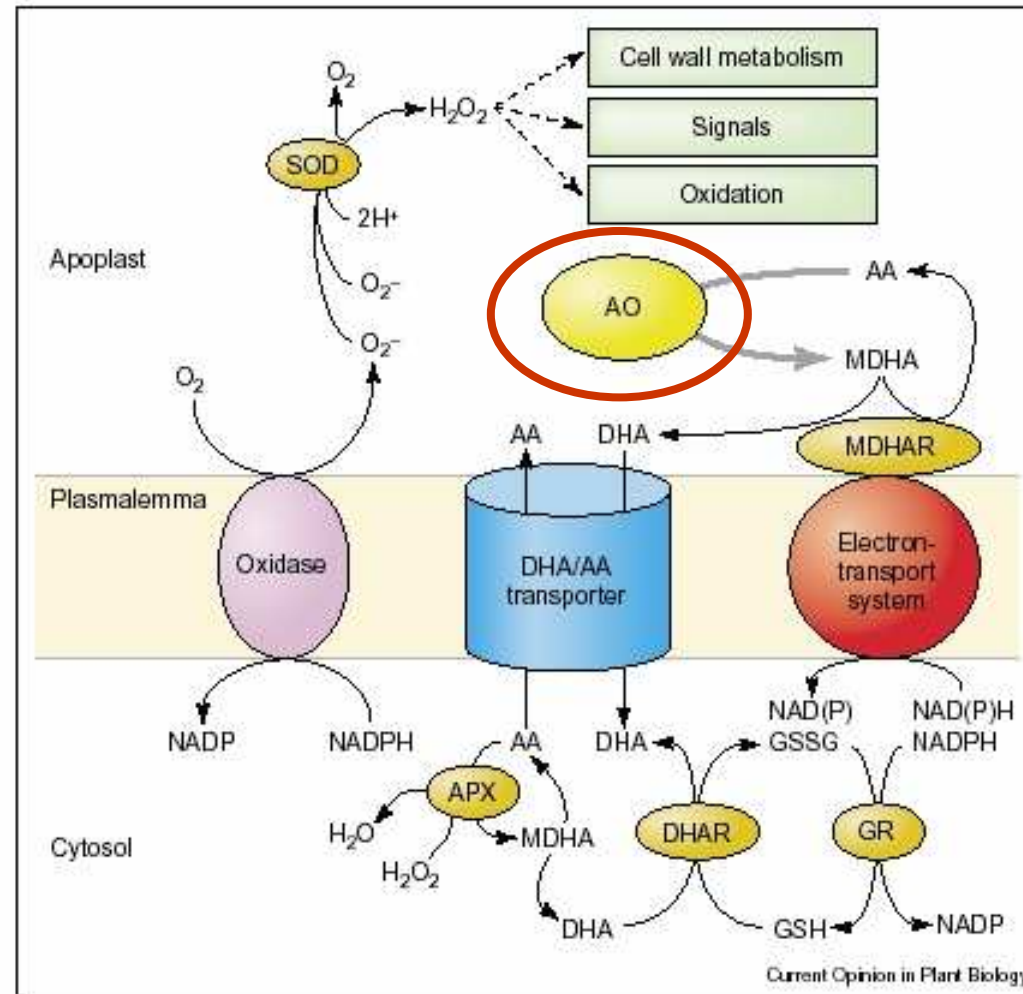
Je hlavním **antioxidantem stěny**. Jeho redoxní stav reguluje askorbát oxidáza (AO) a podílí se tak na regulaci redoxních poměrů v apoplastu a tím modifikuje aktivity receptorů a přenos signálů.

Askorbát ve stěně

Figure 1

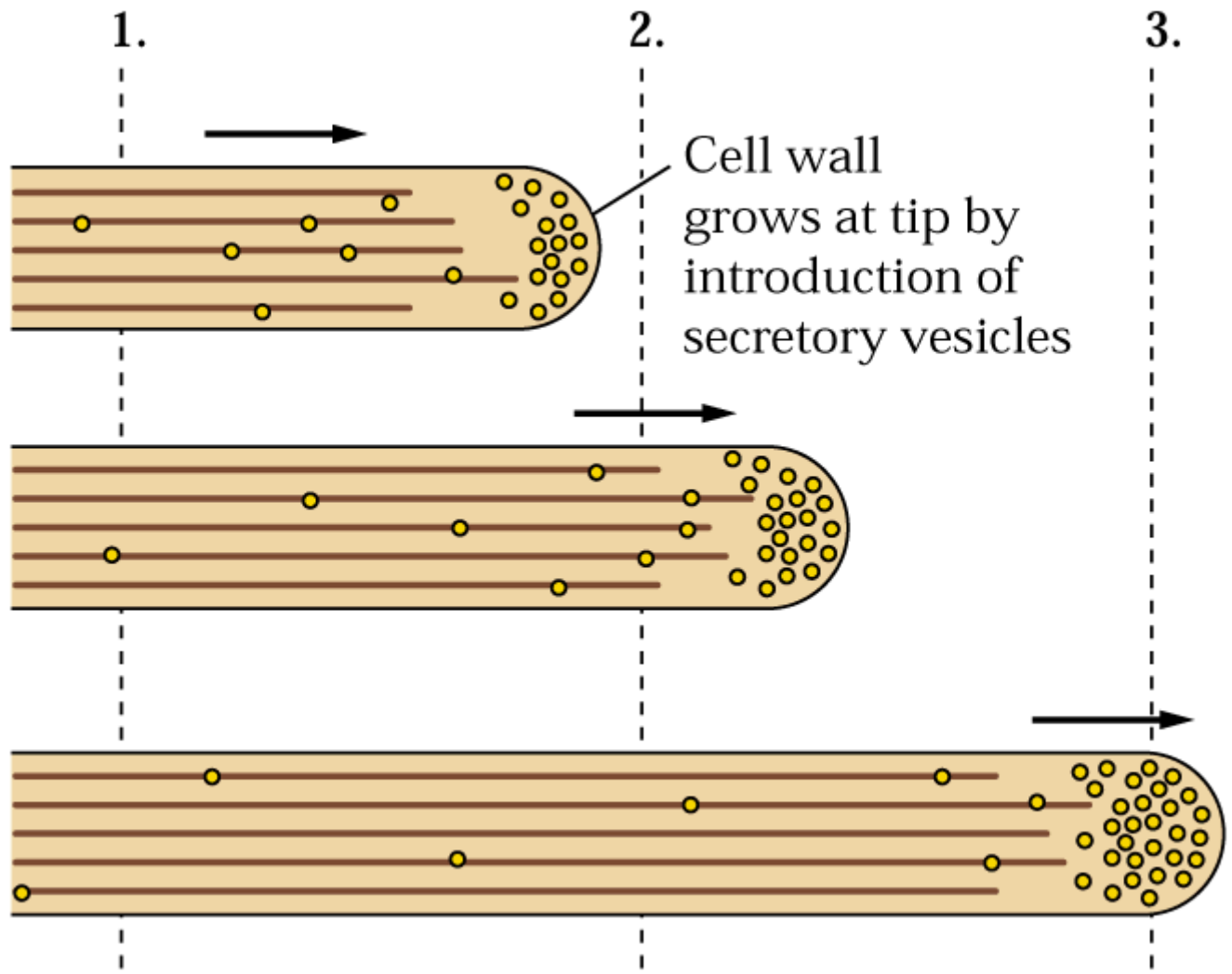
MDHAR - monodehydroaskorbát
reduktáza
MDHA-monodehydroaskorbát

Askorbát
oxidáza
hlavní
regulátor



Redox interactions across the plasmalemma involving the major apoplastic oxidants, superoxide and H₂O₂, and the antioxidant ascorbate. APX, ascorbate peroxidase; GR, glutathione reductase; GSSG, glutathione disulphide; SOD, superoxide dismutase.

- **Apikální růst**



Pylové láčky mají rostoucí špičku tvořenou převážně metylovanými pektiny.

V oblasti pod špičkou dochází k deesterifikaci (demetylaci) a pektiny jsou křížově propojovány (cross-link) Ca^{2+} .

kalóza

celulóza

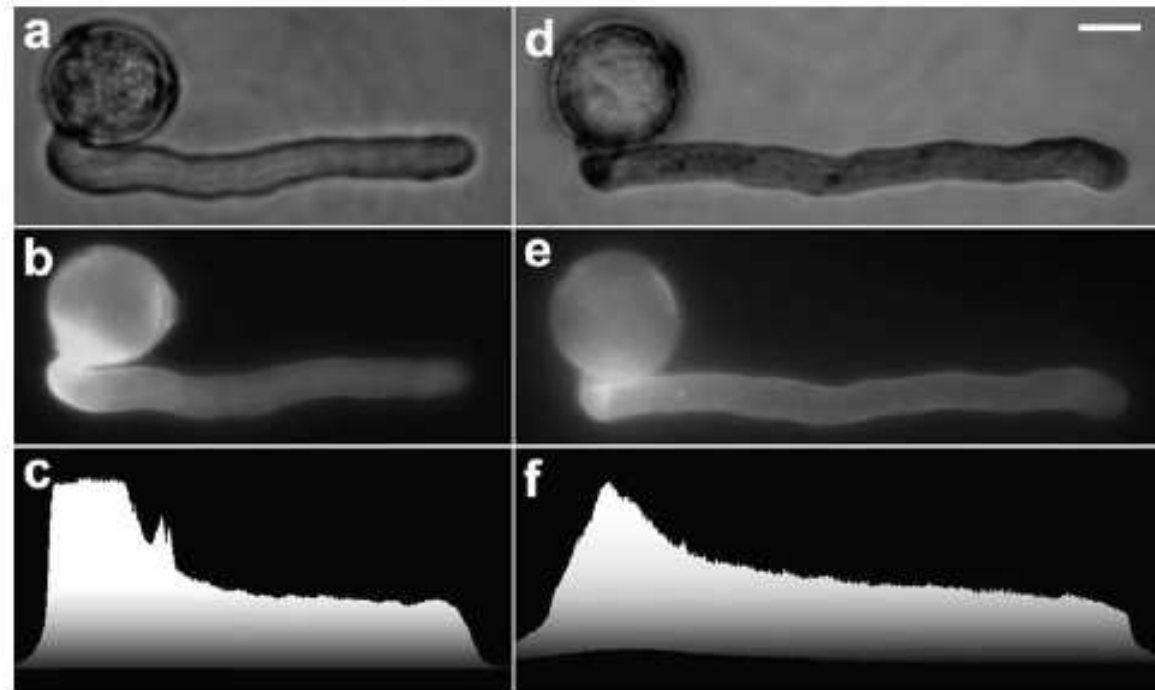


Fig. 3a-f Fluorescent labeling of cell wall components in *Solanum* pollen tubes and pollen grains. Pollen tubes were fixed and subsequently stained for callose with decolorized aniline blue (**a-c**) or for cellulose with calcofluor white (**d-f**). Bright-field images are provided for reference (**a,d**). Relative fluorescence intensity along the longitudinal axis was plotted with the Surface Plot function of ImagePro (**c,f**). The vertical axis represents unitless, relative grey values for the pixel with the highest brightness on the vertical line crossing the *x*-axis. The *x*-axis corresponds to the horizontal axis of the respective fluorescence micrograph. Label for both callose and cellulose was absent at the pollen tube apex. The intensity increased very gradually towards the distal portion of the tube. Bar = 10 μ m

Local stiffness and visco-elasticity of the pollen are affected by pectinase

To assess the role of pectin in the mechanical properties of the cell wall in a quantitative manner, local stiffness and visco-elasticity were measured using a micro-indentation device. Local deformations were induced at two positions on growing pollen tube: at the apex and at a distal position between 20 and 30 μ m from the apex. As shown earlier for other species (Gardner and Parre 2004), normally growing *Solanum*

oba začínají až za špičkou

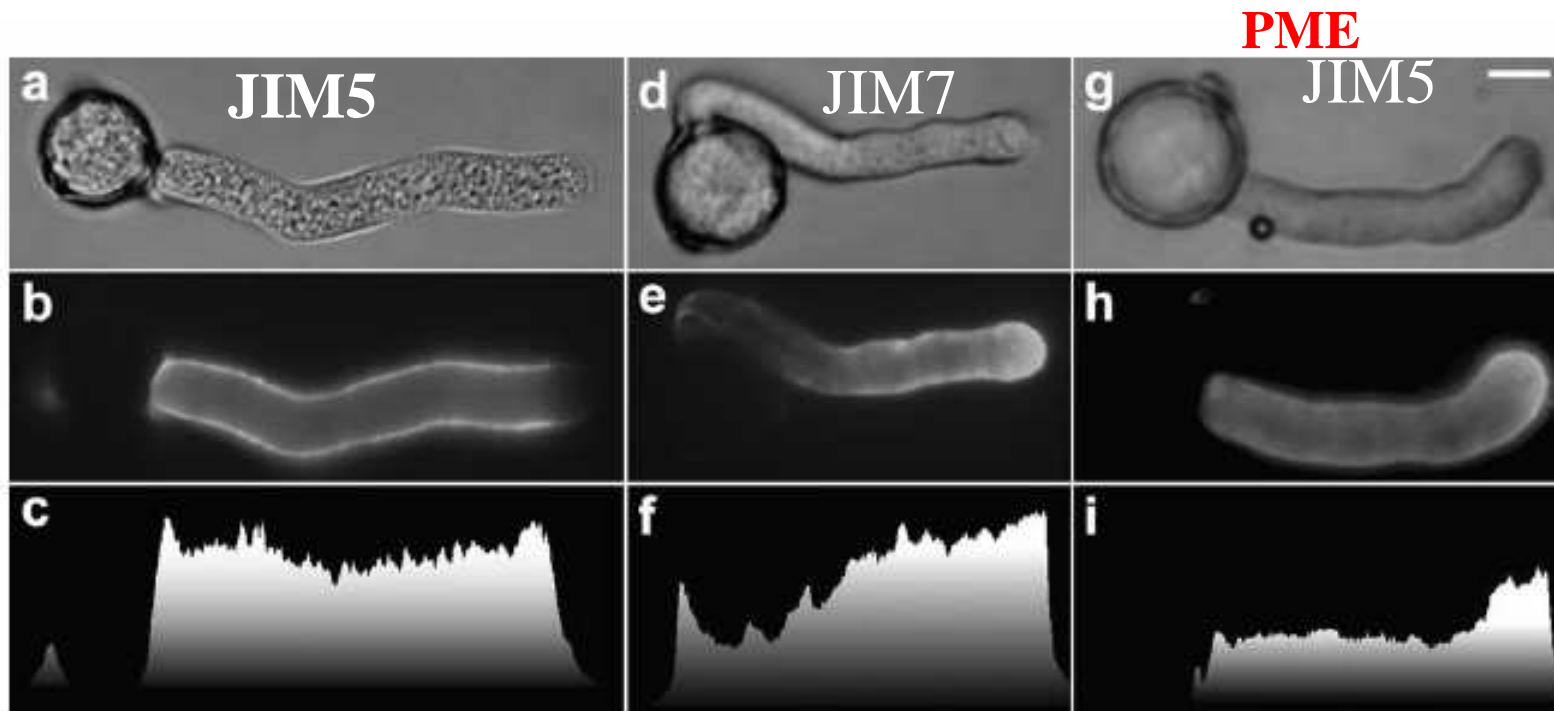


Fig. 4a-i Immunofluorescent label for pectins in *Solanum* pollen tubes and pollen grains. Pollen tubes were fixed and subsequently labeled for acidic pectins with JIM5 (a-c, g-i) or for methyl-esterified pectins with JIM7 (d-f). Label for acidic pectins was absent from the pollen tube apex; the distribution in the distal regions was rather homogeneous. Label for methyl-esterified pectins was highest at the apex and decreased considerably towards distal regions. The pollen tube in g-i was grown in medium containing 0.5 mg ml^{-1} PME to convert methyl-esterified pectins into the acidic variety, thus presumably allowing JIM5 to label the entire pectin population. Label intensity was highest at the apex, thus indicating that the total amount of pectin is highest in this region. Bar = $10 \mu\text{m}$

inhibit pollen tube growth. Our results indeed confirmed a negative effect on germination and tube growth for concentrations of 0.1 mg ml^{-1} and above (Fig. 7).

To demonstrate that de-esterification indeed rigidified the cell wall, we assessed pollen tube stiffness of PME-treated pollen tubes with the micro-indenter. We chose a PME concentration that was not completely inhibitory, but that resulted in an increase of immunolabel for acidic pectins at the apex. PME at 0.5 mg ml^{-1} was an appropriate concentration that fulfilled the requirement. JIM5 labeling of pollen tubes

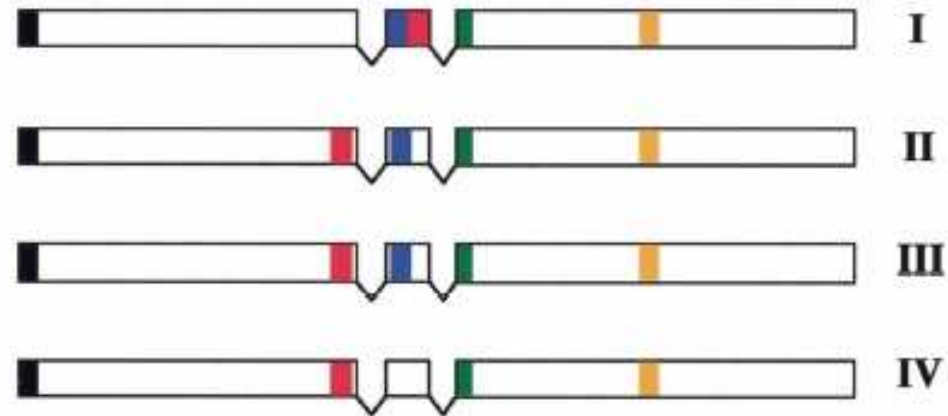
u g-i jsou pektiny na špičce demetylovány přidáním PME
JIM5 – deesterif. kys. p. ; JIM7 – metyl-esterifikované p.

Stěna jako senzor mechanických poměrů v buňce a okolí

"celulozoví" mutanti nejen hromadí kompenzačně pektiny, ale mají "signálně" aktivovanou dráhu ukládání ligninu.

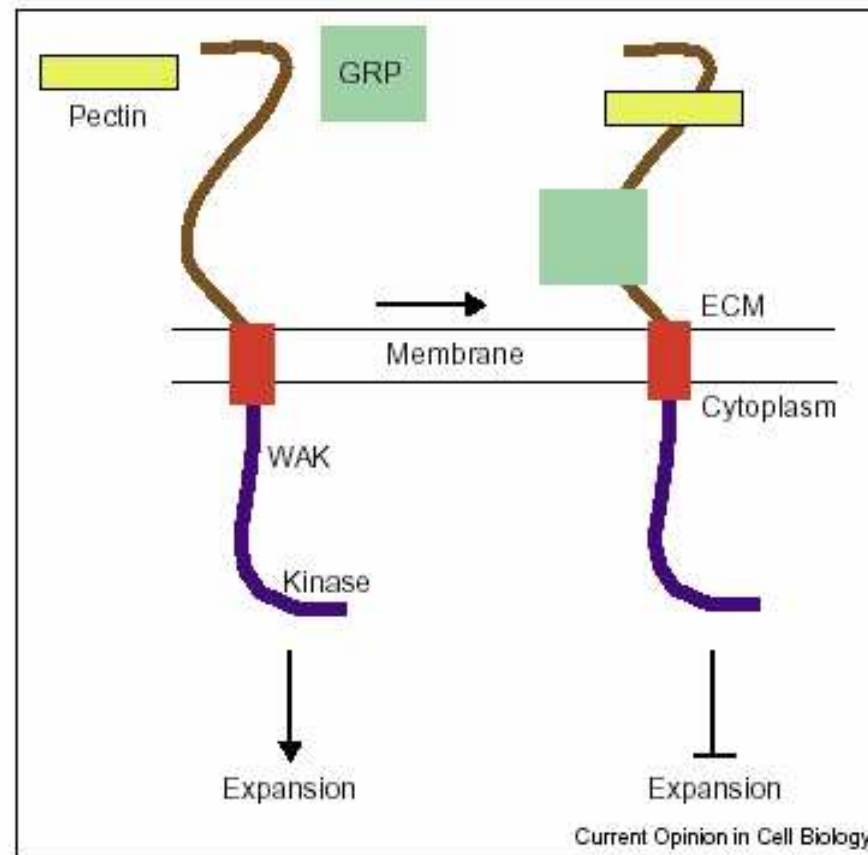
WAKs

Figure 1. Intron-exon structure is conserved between the *WAK* and *WAKL* genes. The diagram shows a standardized depiction of a *WAK* or *WAKL* gene from each of the four groups (I-IV). Exons are represented by boxes. Introns are represented as 'V's. Regions of each gene encoding functional domains are indicated with shaded boxes: N-terminal signal sequence (black), EGF2-like domain (red), calcium-binding EGF domain (blue), transmembrane domain (green), and Ser/Thr protein kinase active site (orange).



Wall Associated Kinases

Figure 2



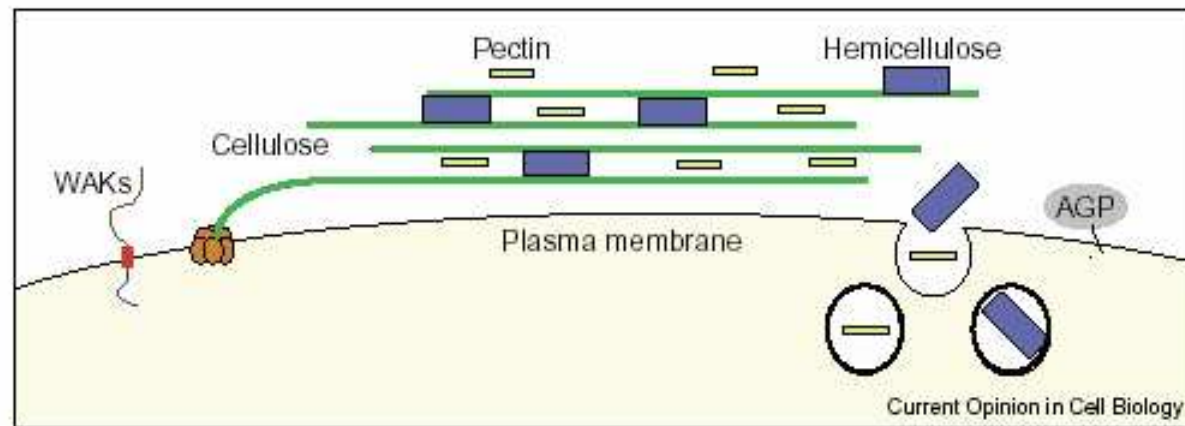
A model for how WAKs, pectins and GRPs regulate cell expansion. WAKs extend a EGF-containing domain into the ECM and can bind both pectin and GRP. Loss of WAK protein leads to a loss of cell expansion and GRP may negatively regulate WAK kinase activity, perhaps in conjunction with pectins.

N'

C'

WAKs interagují s pektiny (HGA) za přítomnosti Ca^{2+}

Figure 1



A cartoon for the biosynthesis of the plant cell wall. Cellulose microfibrils (green) are synthesized by cellulose synthase complexes (orange). Pectin (yellow), hemicellulose (blue) and other carbohydrates (not shown) are secreted through vesicles. Transmembrane WAKs, GPI-anchored AGPs, and possibly other proteins (not shown) may serve to link the ECM to the cell.

Obsah

- Buněčná stěna jako integrální část rostlinné buňky
- Sacharidy jako základní stavební složky BS
- Makromolekuly v BS
- Struktura buněčné stěny, typy
- Biosyntéza BS
- Buněčný růst a BS
- **Diferenciace buněk, sekundární BS, obrana**
- Význam BS pro člověka, biotechnologie atd...

- Buněčná diferenciace u rostlin = velmi často diferenciace ve složení b. stěny.

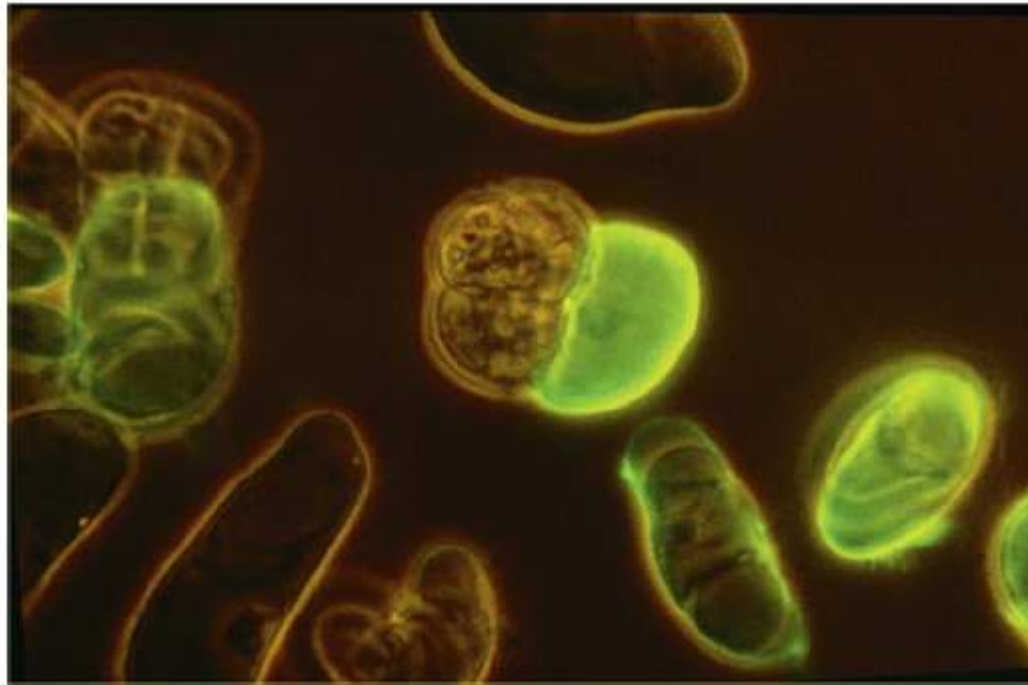
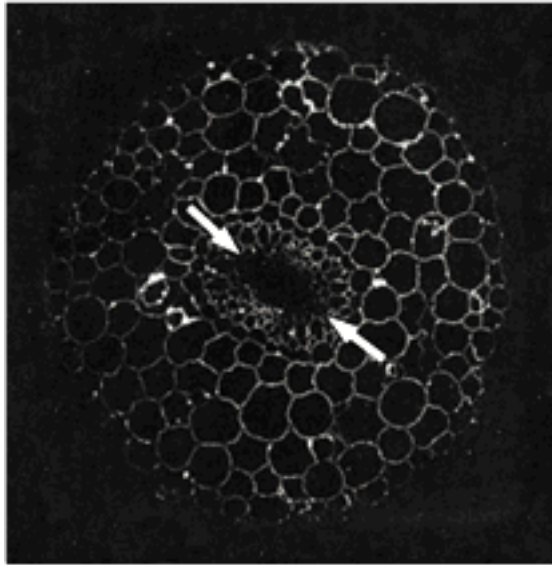


Figure 3

Carrot suspension cells can be divided in JIM8⁺ cells not undergoing somatic embryogenesis (*green*) and JIM8⁻ cells that form somatic embryos (*brown*). Removal of JIM8⁺ cells from the suspension blocks somatic embryogenesis (67).

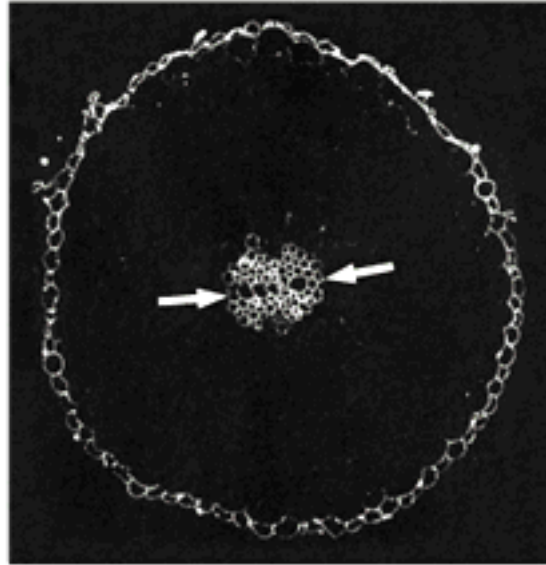
Asymetrické b. dělení – JIM8⁺ b. produkují pravděp. oligosach. signál uvolňovaný z AGPs. Kondiciované médium stačí k indukci u JIM8⁻ buněk.

(A)



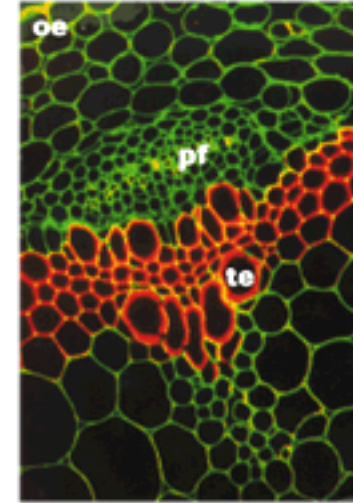
AGP spec. pro kortex

(B)



AGP spec. pro vaskul.a epid.

(C)



zel.: Met-pektin
žlut.: deesterif. pekt.

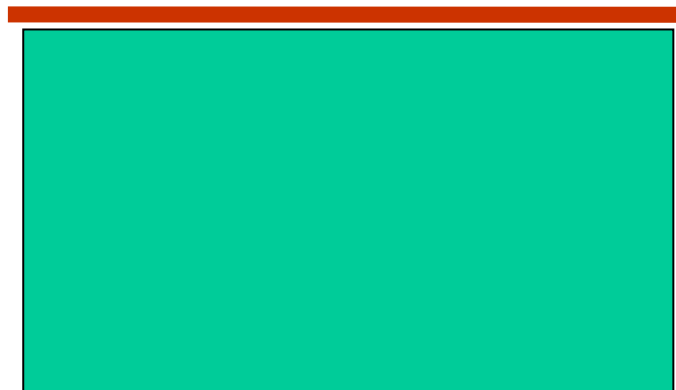
OVES kořen – A a B

Arabidopsis stonek – C, různě modifikované pektiny v téže buňce.

Buňky mají na svém povrchu
specializované mikrodomény
Buněčné Stěny =CW

- Př. Epidermální buňka

Povrch listu



Vnitřek listu

Rozdíly v
metabolismu
pektinů působí
rozdíly tuhosti
plodů

**Polygalakturonázy
a pektolyázy**

(A)



(B)

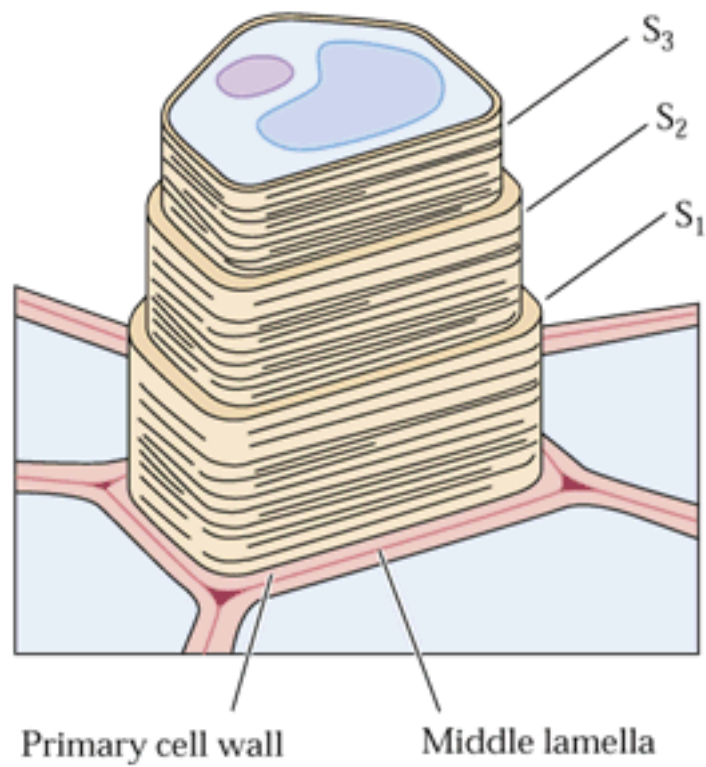


(C)

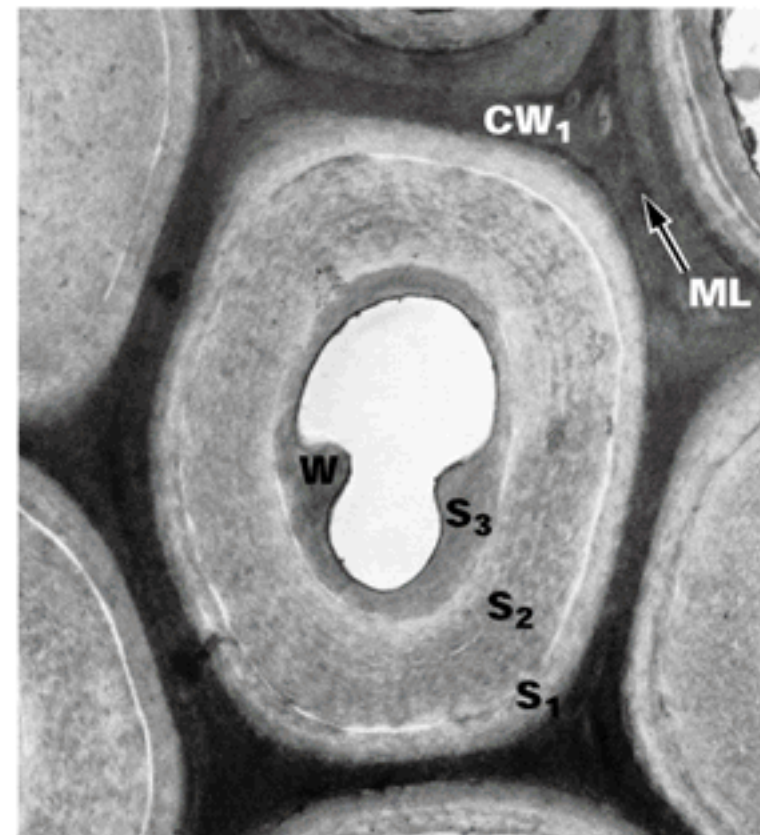


Sekundární buněčná stěna

(A)



(B)



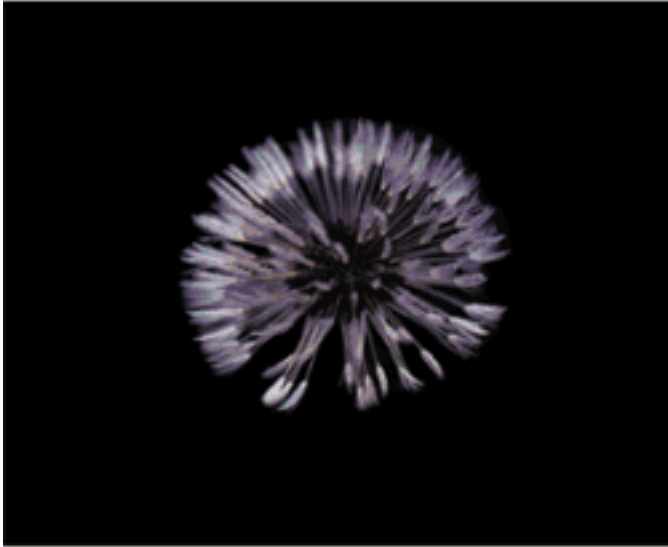
(A)



(B)



(C)



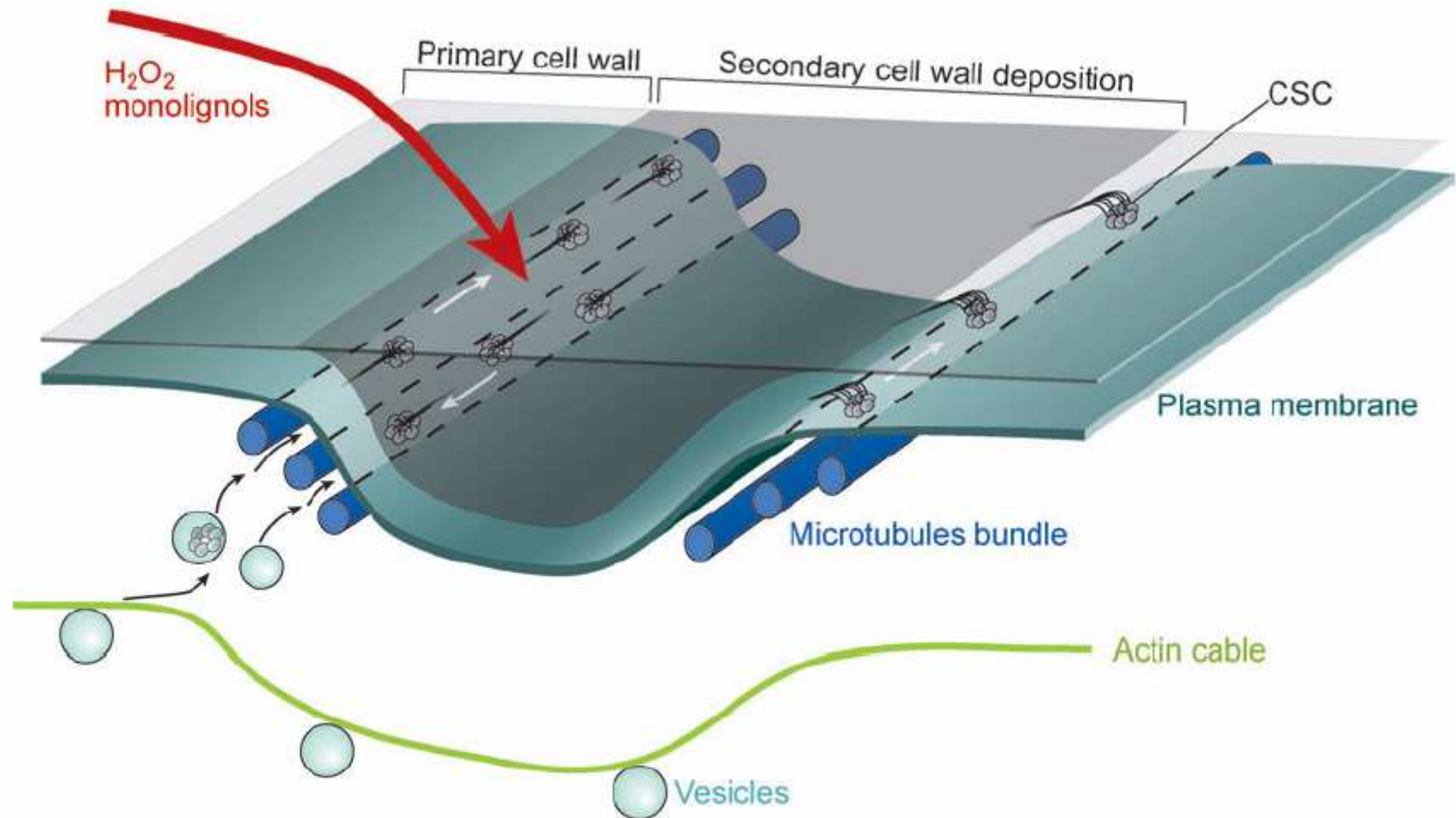


Figure 5

A model for secondary cell wall deposition. The actin cytoskeleton directs the delivery of vesicles containing hemicellulose and the cellulose synthase complex to the plasma membrane. Microtubule (MT) bundles mark the sites of vesicle insertion and maintain plasma membrane partitions, ensuring that the cellulose synthase complex moves parallel to the MTs, coaligning the MTs and the cellulose microfibrils. Adjacent cells deliver components that contribute to the synthesis of lignin in the secondary cell wall thickenings.

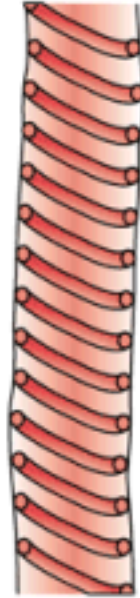
IMPREGNACE -

lignifikace a suberinizace

(A) Annular



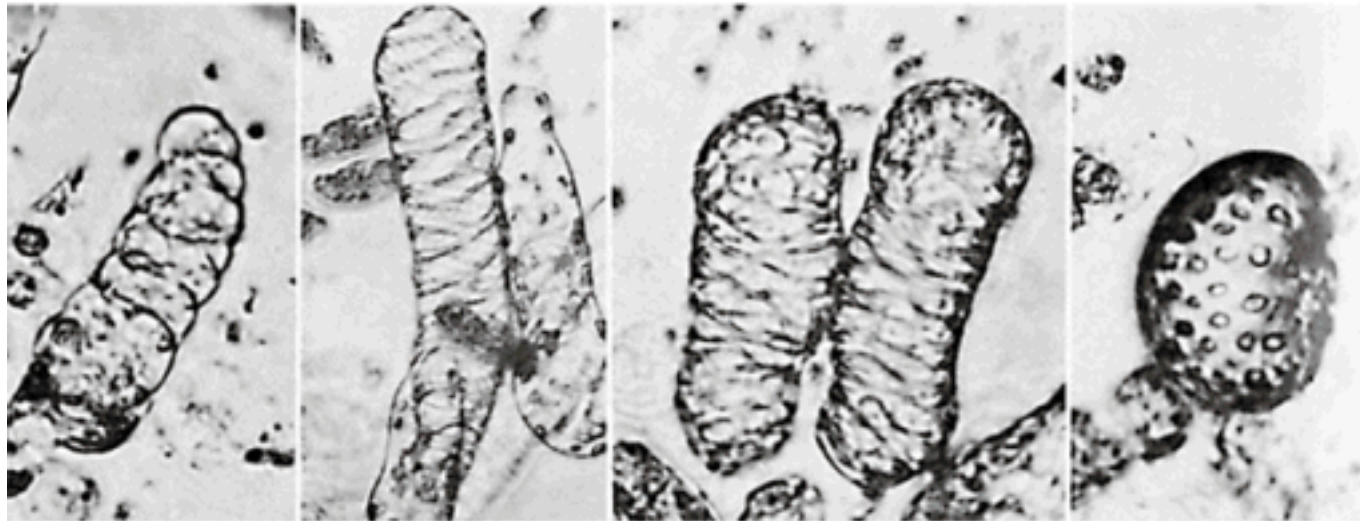
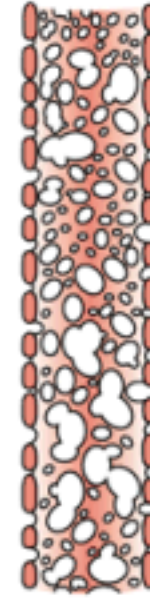
(B) Spiral



(C) Reticulate

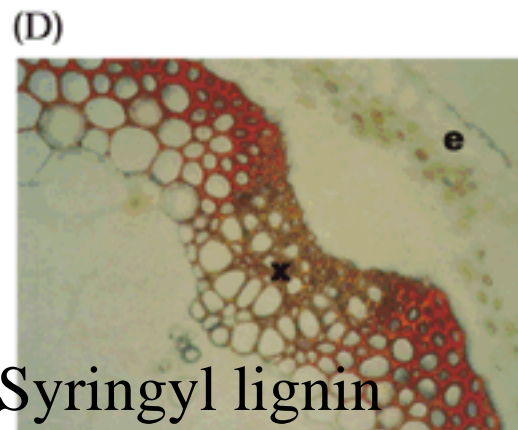
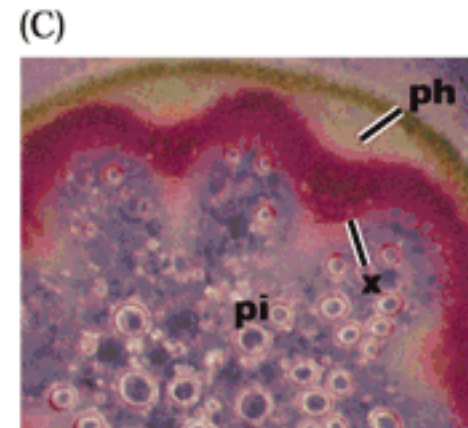
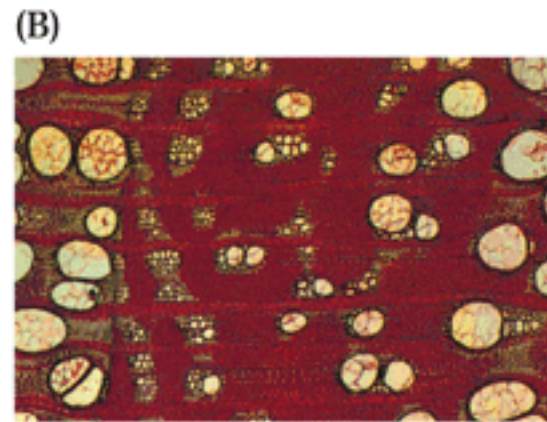
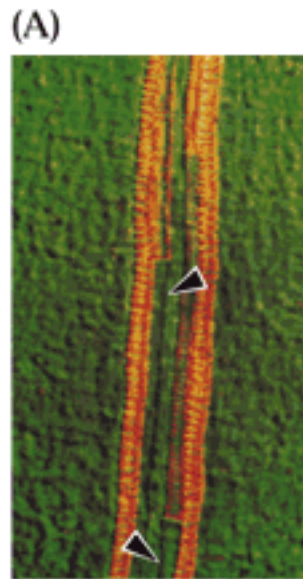


(D) Pitted

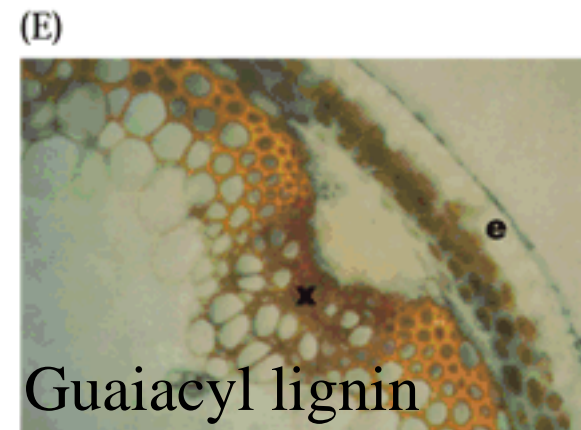


Model diferenciace xylemu in vitro u r. *Zinnia*

Barvení kyselým fuchsinem(A),
phloroglucinolem(B,C) a Mäuleovým
činidlem (D,E) na lignin.



Syringyl lignin



Guaiacyl lignin

Lignin

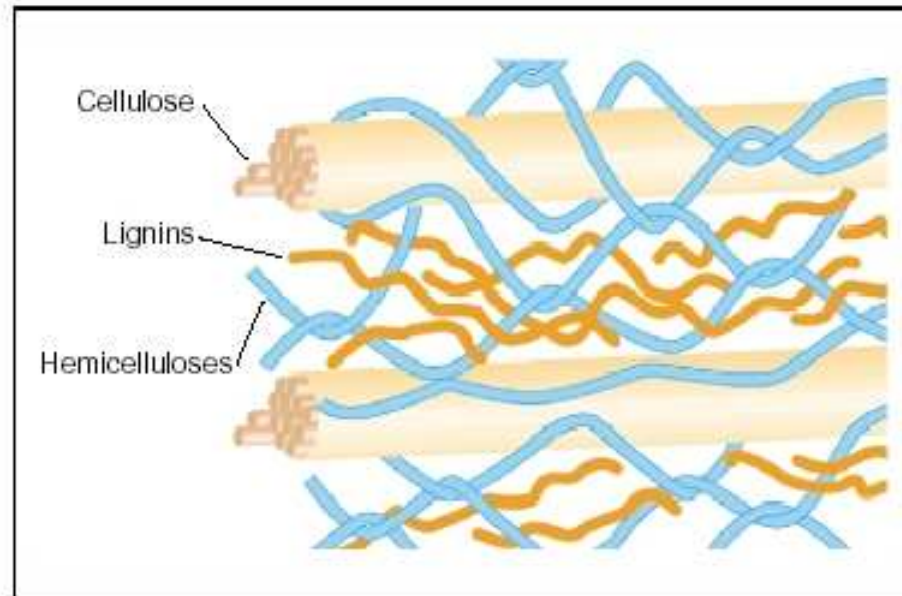
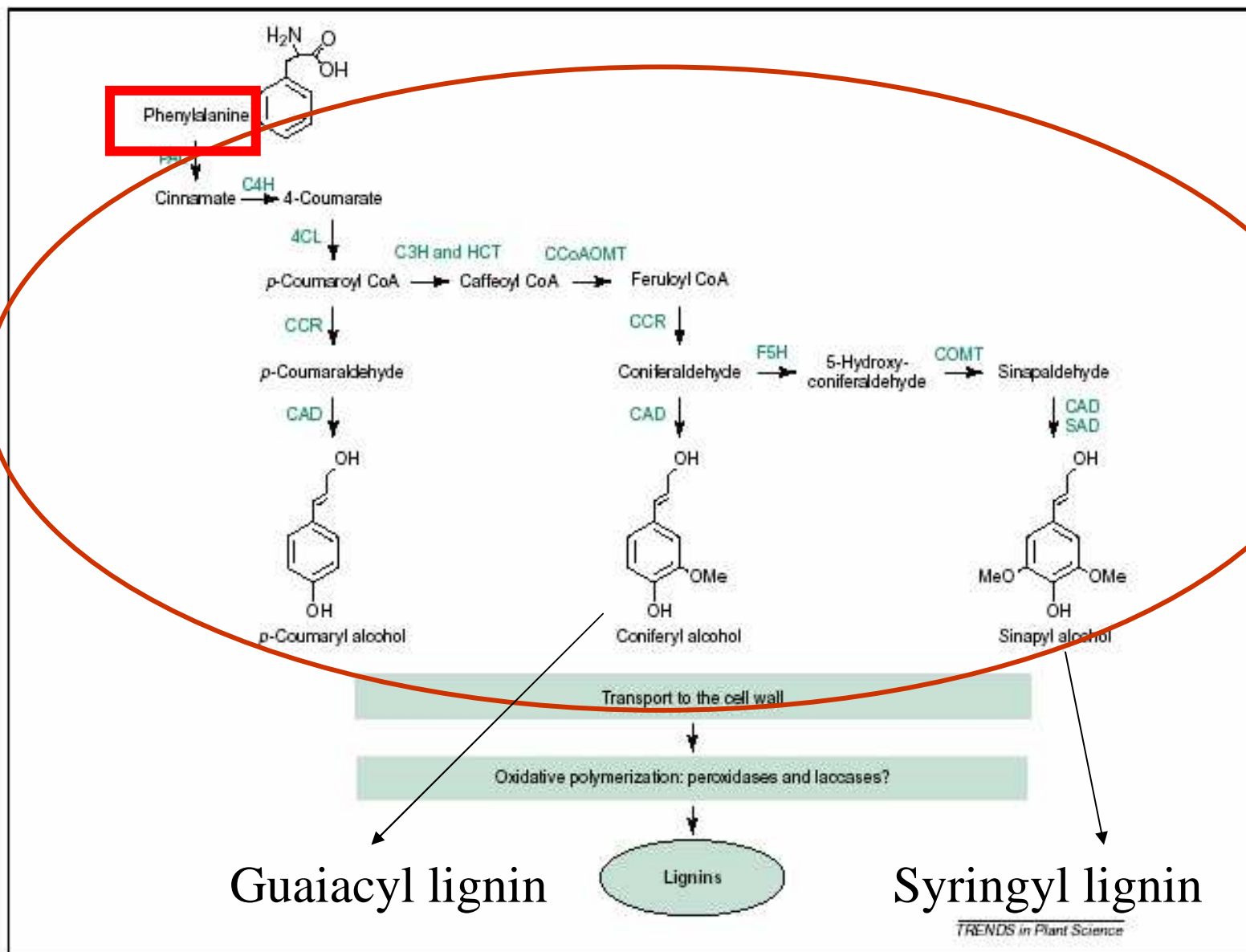
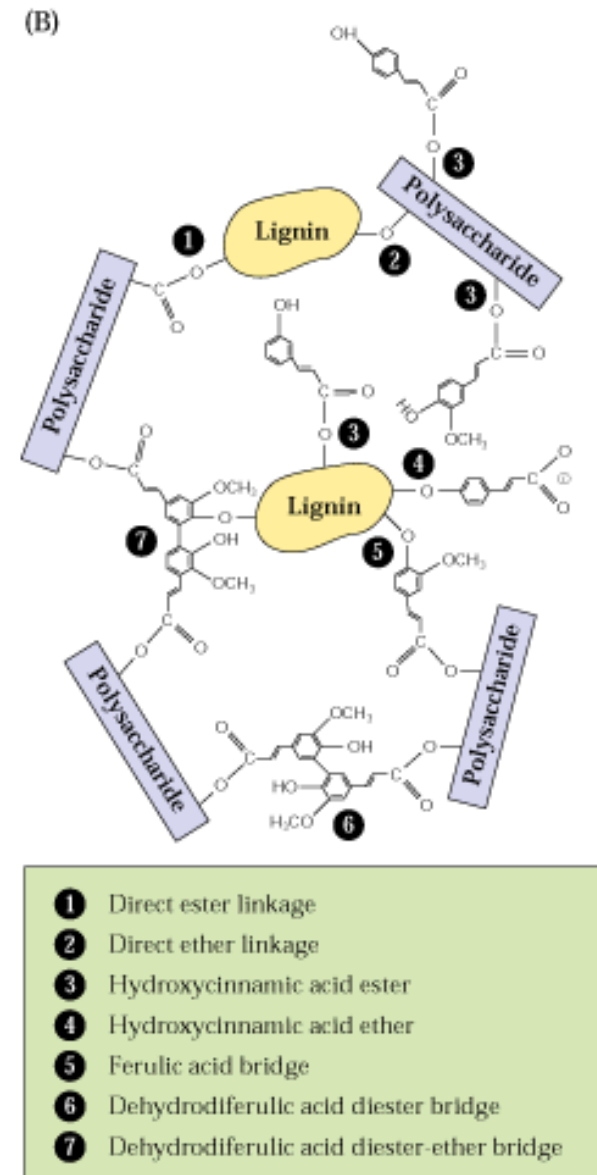
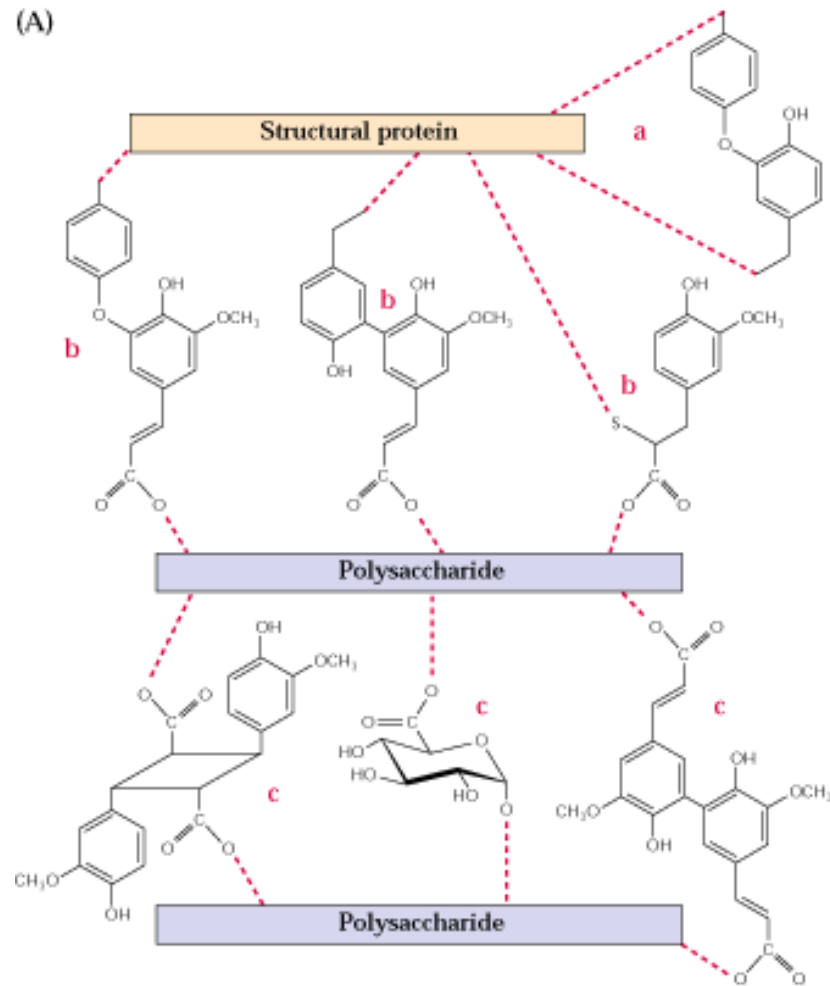


Figure 1. Schematic representation of the lignified secondary wall. In addition to cellulose, lignins and hemicelluloses, other cell wall constituents of minor abundance, including proteins and low-molecular weight phenolics, are not indicated on the figure.

Phenylpropanoidní metabolismus vedoucí ke vzniku monolignolů – p-kumaryl alkoholu, koniferyl alkoholu, a sinapyl alkoholu.



Stěnový cross-linking



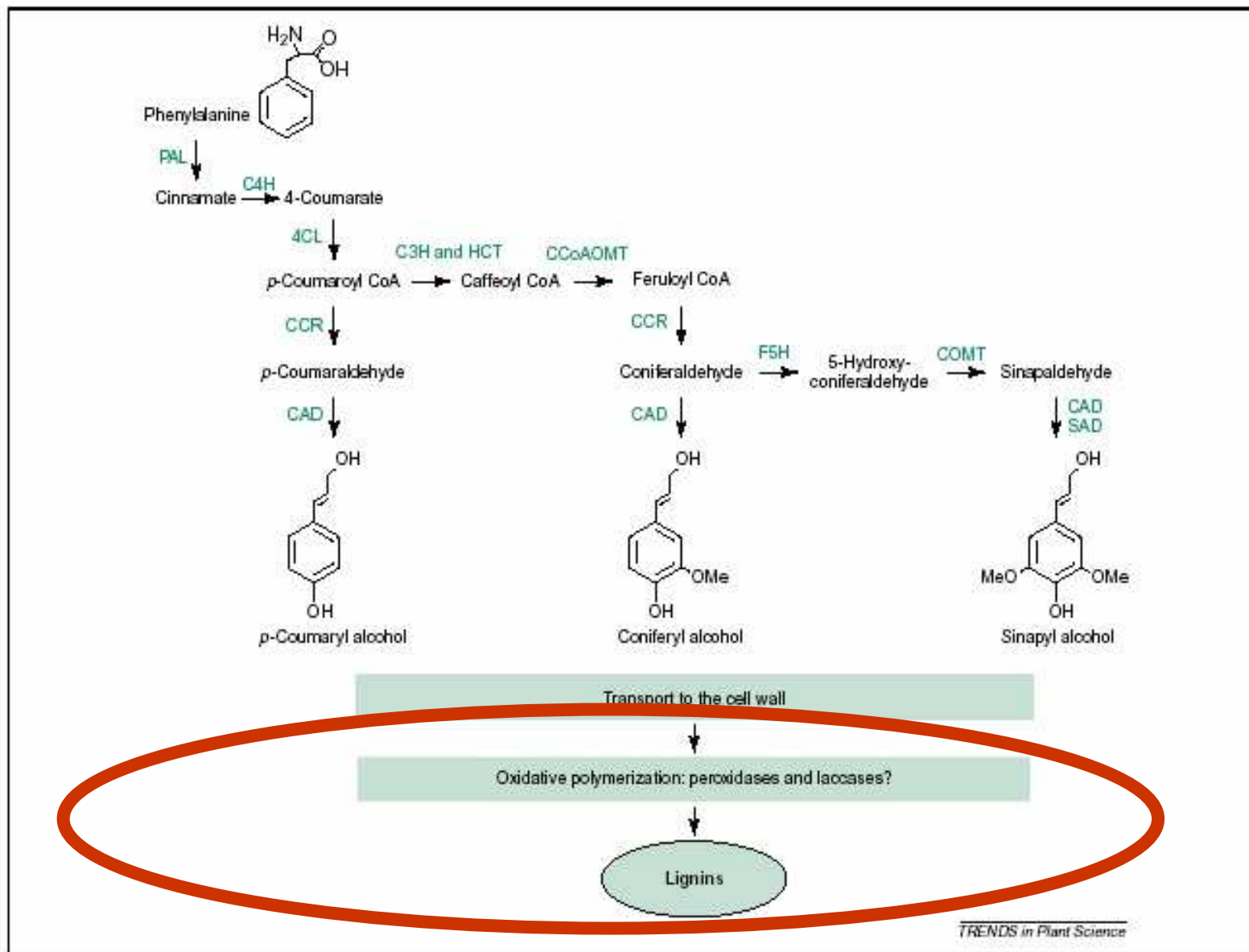
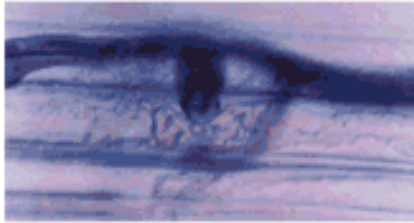


Figure 2. Simplified view of the most favoured lignin biosynthetic pathway in angiosperms. The implication of laccases in the oxidation of monolignols remains to be elucidated. Abbreviations: CAD, cinnamyl alcohol dehydrogenase; CCoAOMT, caffeoyl-CoA O-methyltransferase; C3H, p-coumarate 3-hydroxylase; C4H, cinnamate 4-hydroxylase; CCR, hydroxycinnamoyl-CoA reductase; 4CL, 4-coumarate CoA ligase; COMT, caffeic acid/5-hydroxyferulic acid O-methyltransferase also known as AldOMT, 5-

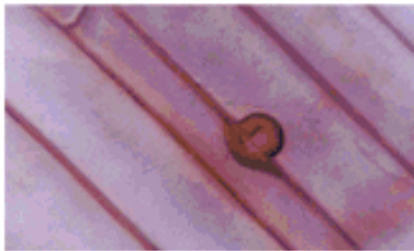
Lignifikace v obraně

Colletotrichum atakuje b. kukuřice - obranná papila akumul. kalosu a lignin.

(A)



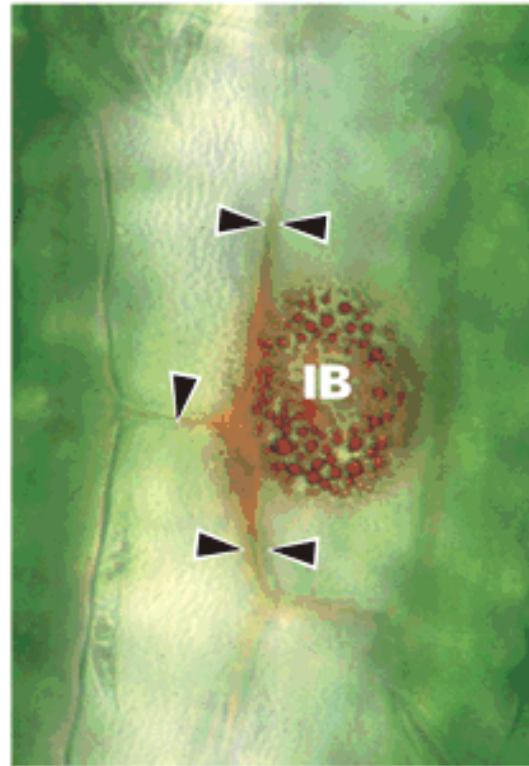
(B)



(C)



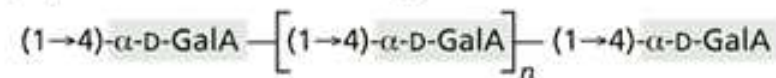
(D)



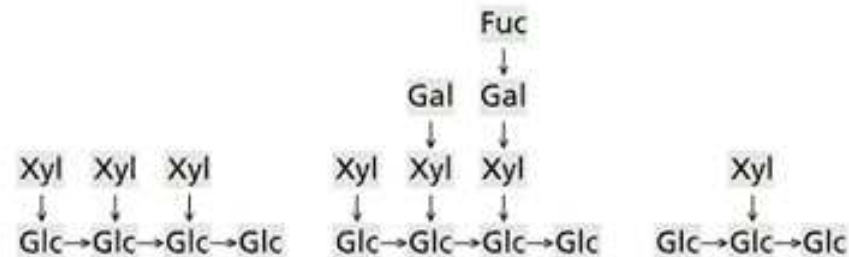
Důležitá
úloha
aktinu!

Biologicky aktivní oligosacharidy spouštějí obrannou reakci.

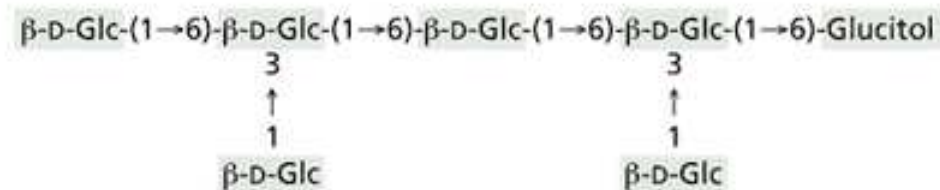
(A) Galacturonic acid oligomers



(B) Three oligosaccharins from xyloglucan



(C) Oligosaccharin from fungal cell wall

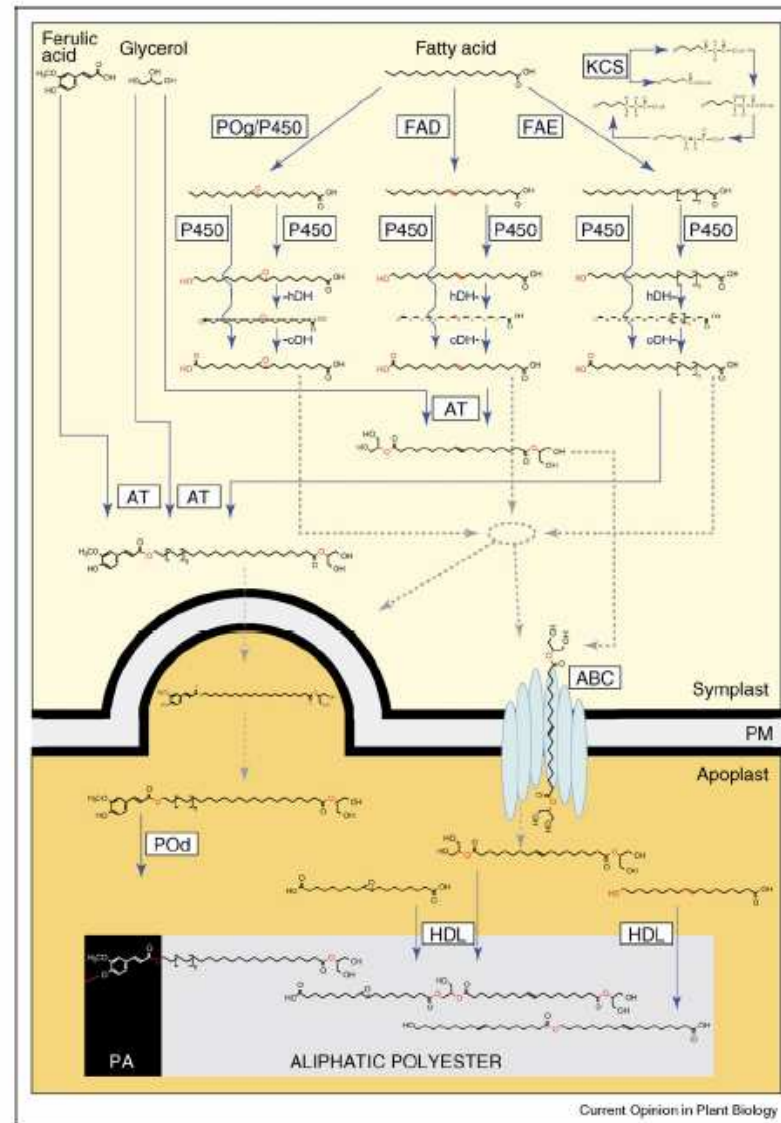


Web Figure 15.5.A Structure of biologically active oligosaccharins. (A) Oligomers of galacturonic acid are released during pectin degradation. The most active fragments are 10 to 15 residues long. (B) Three oligosaccharins derived from xyloglucan. (C) An oligosaccharin derived from the degradation of fungal (*Phytophthora*) cell walls. Such oligosaccharides stimulate defense responses. **(Click image to enlarge.)**

Figure 1

hlavní monomer
hydroxy-alifatické
kyseliny (C16-C30)

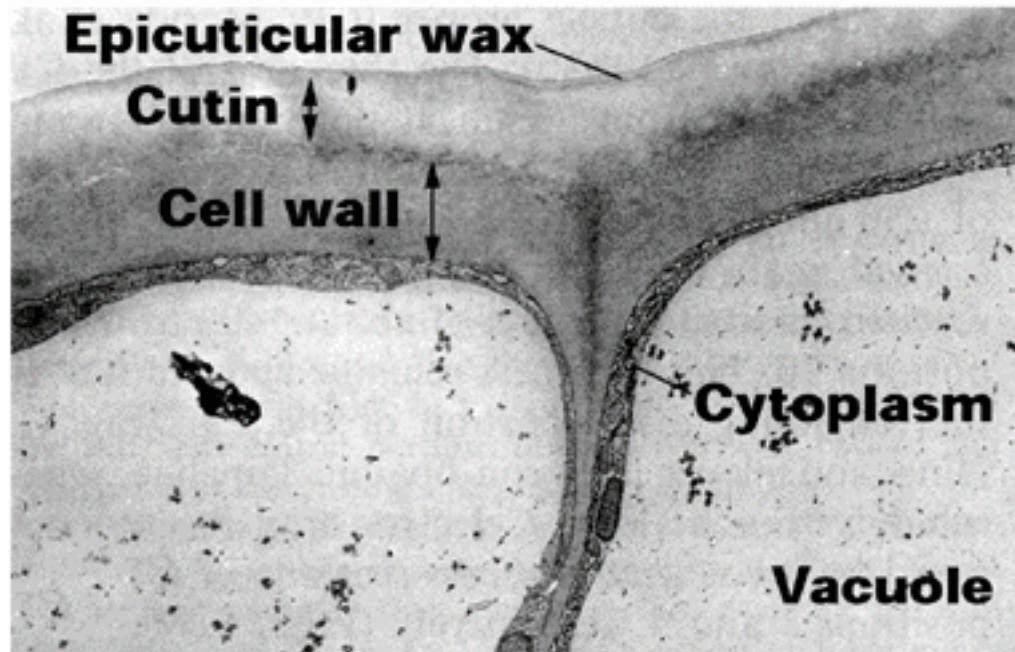
Pod=PEROXIDÁZY



Simplified model of the suberin biosynthetic pathway (adapted from [15^{***}]). The building blocks for suberin are glycerol and C₁₆ and C₁₈ fatty acids derived from primary metabolic activity, and ferulic acid, a product of the phenylpropanoid pathway. Depending on the species, fatty acid suberin precursors undergo mid-chain modifications, resulting in unsaturated or epoxy fatty acids. Saturated fatty acids can be elongated up to C₃₀ suberin precursor. The majority of the suberin precursors enter a ω -oxidation pathway, resulting in ω -hydroxyacids and α,ω -diacids. The ω -hydroxyacids and α,ω -diacids form esters by linkage to glycerol and/or ferulic acid or are interlinked. After export to the apoplast, the mono- and oligomeric building blocks will be polymerized into the suberin macromolecule. The deduced catalysts that are typically responsible for the diagrammed reactions are shown. ABC, ATP binding cassette transporter; AT, acyltransferase; FAD, fatty acid desaturase; FAE, fatty acid elongase complex; KCS, β -ketoacyl-CoA synthases; hDH, ω -hydroxyacid dehydrogenase; HDL, α,β -hydrolase; oDH, ω -oxoacid dehydrogenase; P450, cytochrome P450 monooxygenase; PA, polyaromatic domain; PM, plasma membrane; POd, peroxidase; POg, peroxygenase.

- KUTIKULA

(A)



(B)



Kutikula – lipidní polyester - **Lipofilní polyalifatický.**

Origin of Leaf Hydrophobicity

Cause: Waxy Outer Layer and Surface Roughness

- **Wax Crystals on Epidermal Cells :Crystal Density determines actual hydrophobicity.**
- **Long hair like structures (trichomes) and bumpy protrusions induce surface roughness.**

Effect: Nature's Self Cleaning Mechanism

- **Dirt particles (spores, disease fungi) adhere more strongly to water than the leaf and are consequently washed away.**
- **Lack of water on surface prevents disease organisms from germinating and growing as they cannot survive.**

Agrochemical Delivery

Importance

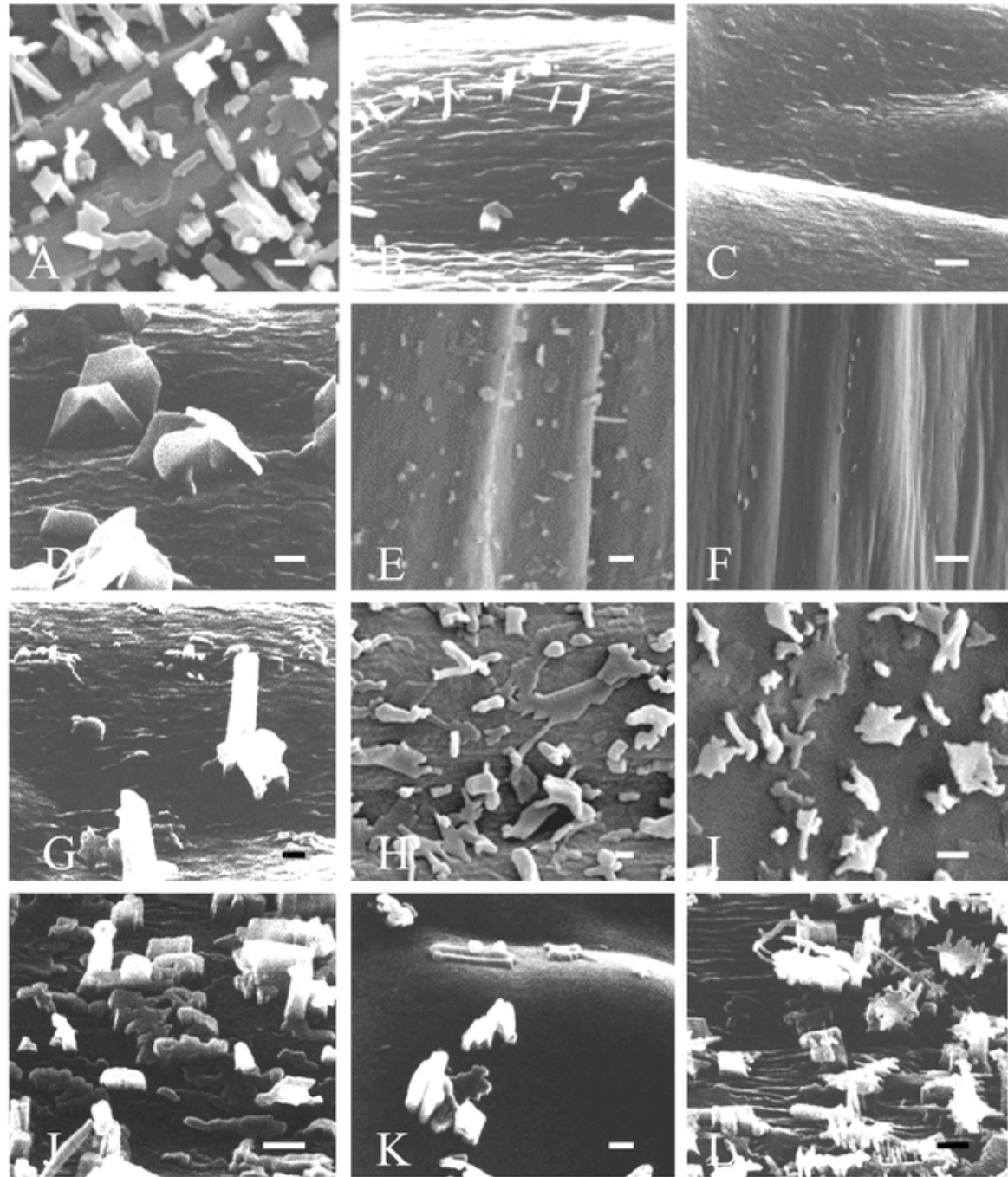
- Agrochemical spray on leaves

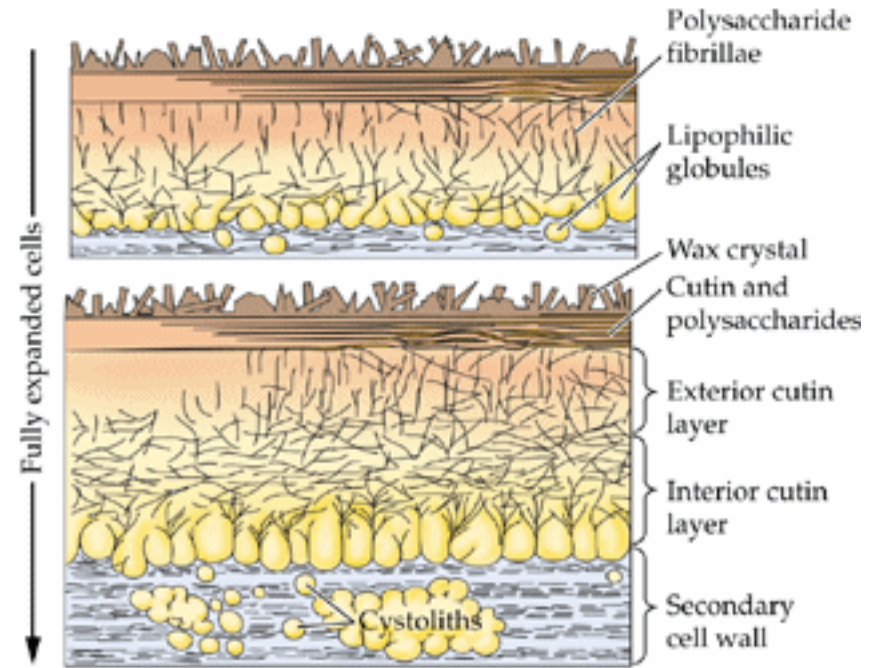
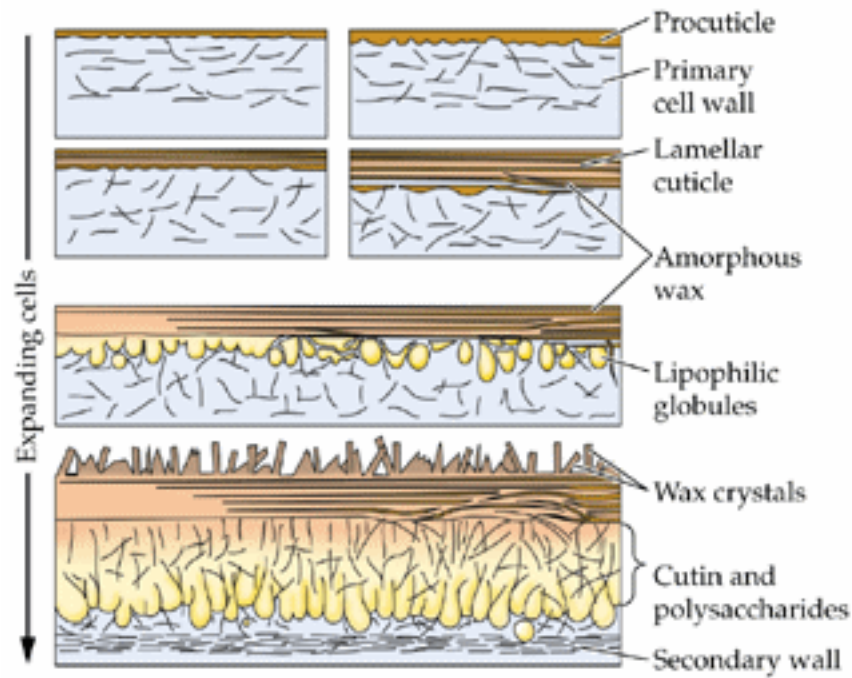


Ways to enhance spreading

- Temperature
- Coating the surface
- Addition of a surfactant
----> Surfactant
enhanced spreading

Alex Couzis, CCNY





Prekurzory kutikuly jsou na povrch epidermis „pumpovány“ ABC transportery.

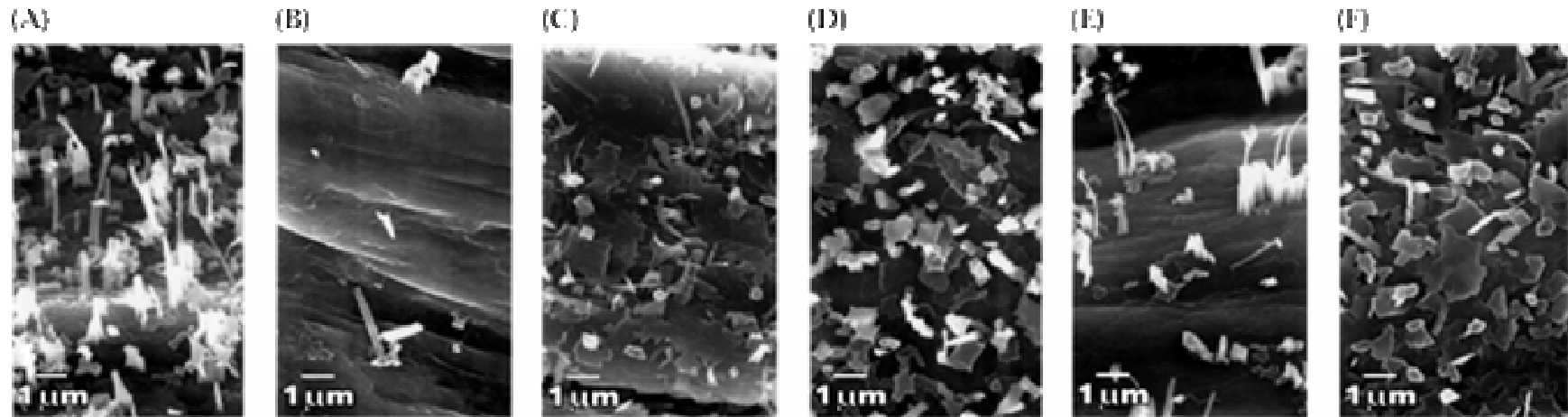
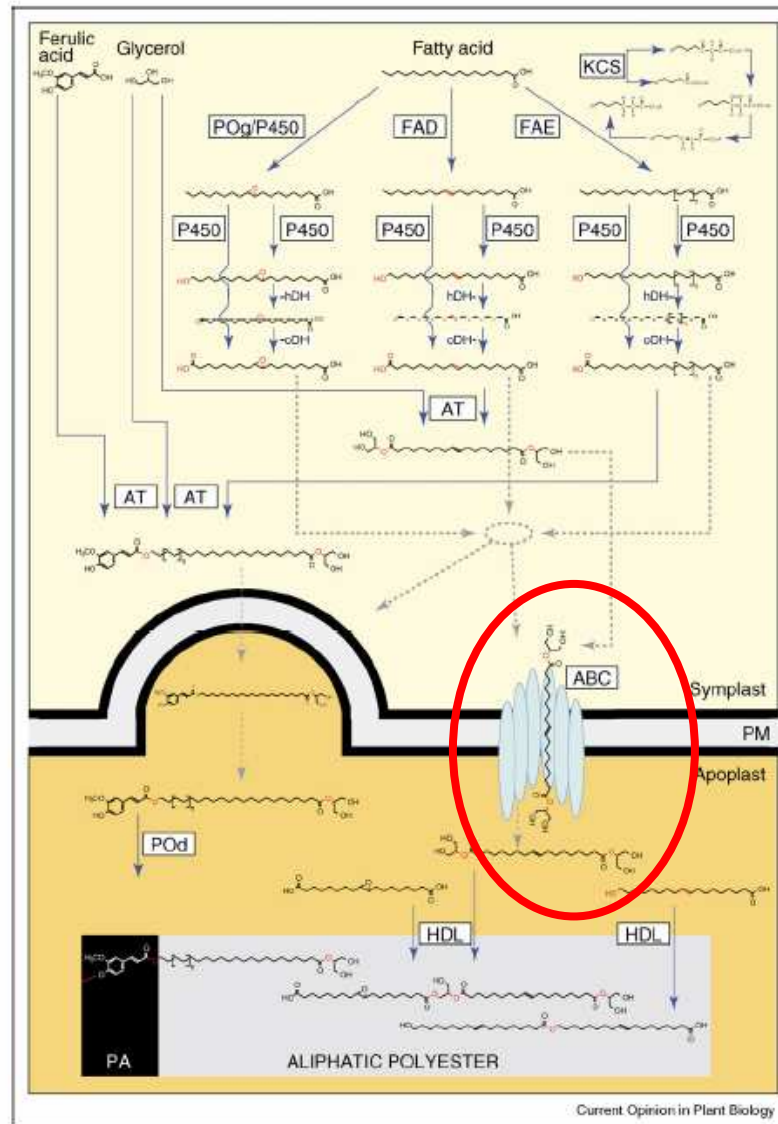


Figure 1



Podobně jako při ukládání suberinu.

Simplified model of the suberin biosynthetic pathway (adapted from [15th]). The building blocks for suberin are glycerol and C₁₆ and C₁₈ fatty acids derived from primary metabolic activity, and ferulic acid, a product of the phenylpropanoid pathway. Depending on the species, fatty acid suberin precursors undergo mid-chain modifications, resulting in unsaturated or epoxy fatty acids. Saturated fatty acids can be elongated up to C₃₀ suberin precursor. The majority of the suberin precursors enter a ω -oxidation pathway, resulting in ω -hydroxyacids and α,ω -diacids. The ω -hydroxyacids and α,ω -diacids form esters by linkage to glycerol and/or ferulic acid or are interlinked. After export to the apoplast, the mono- and oligomeric building blocks will be polymerized into the suberin macromolecule. The deduced catalysts that are typically responsible for the diagrammed reactions are shown. ABC, ATP binding cassette transporter; AT, acyltransferase; FAD, fatty acid desaturase; FAE, fatty acid elongase complex; KCS, β -ketoacyl-CoA synthases; hDH, ω -hydroxyacid dehydrogenase; HDL, α,β -hydrolase; oDH, ω -oxoacid dehydrogenase; P450, cytochrome P450 monooxygenase; PA, polyaromatic domain; PM, plasma membrane; POd, peroxidase; POg, peroxygenase.

