Challenges in imaging cell walls

Alex Paredez, Dave Erhardt, Staffan Persson, Seth DeBolt & Chris Somerville

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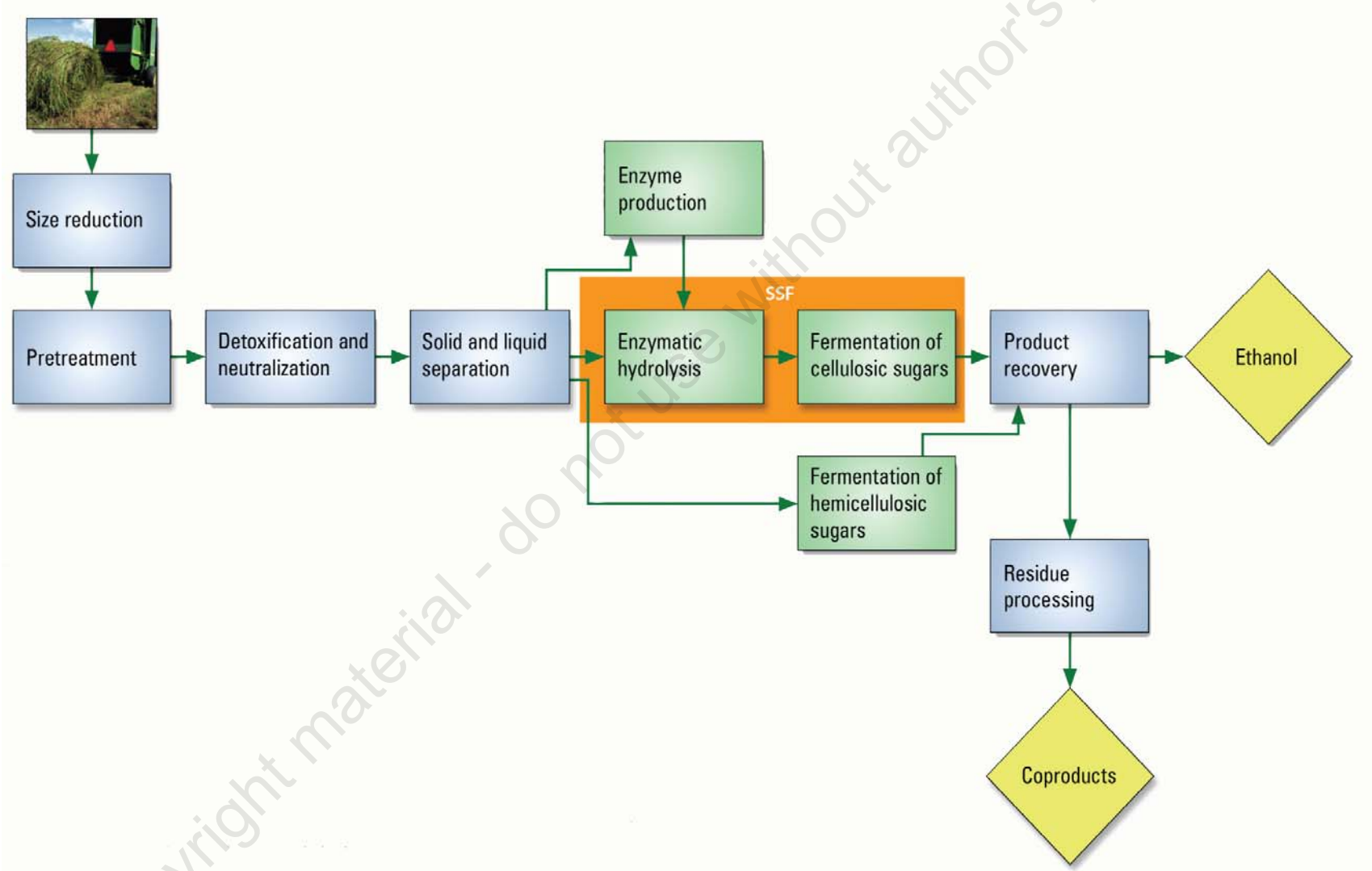
Steps in cellulosic ethanol production



From: Breaking the Biological Barriers to Cellulosic Ethanol

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Some imaging questions

- What is the structure of the polysaccharides?
- What is the fine structure of the wall?
- How does it assemble?
- How does the structure change during hydrolysis (or in mutants)?
- How is lignin associated?
- What is the structure of cellulose synthase?
- How does structure of the complex control the properties of cellulose?

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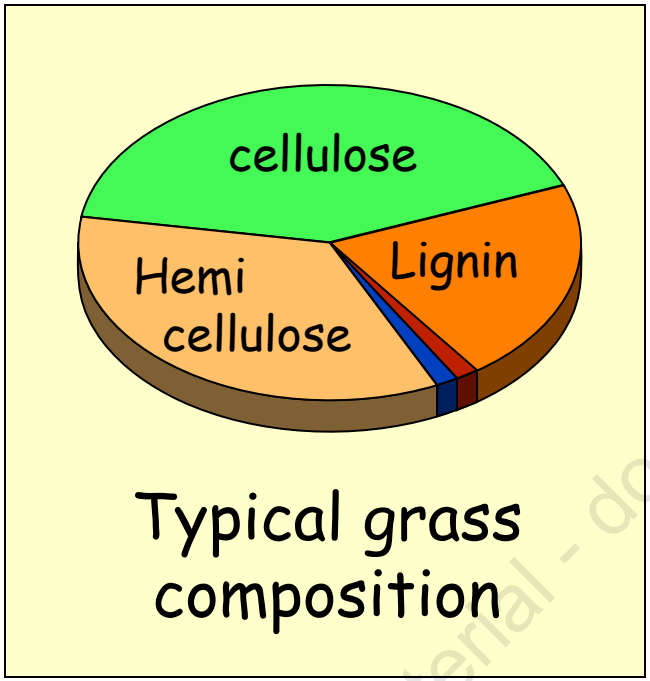
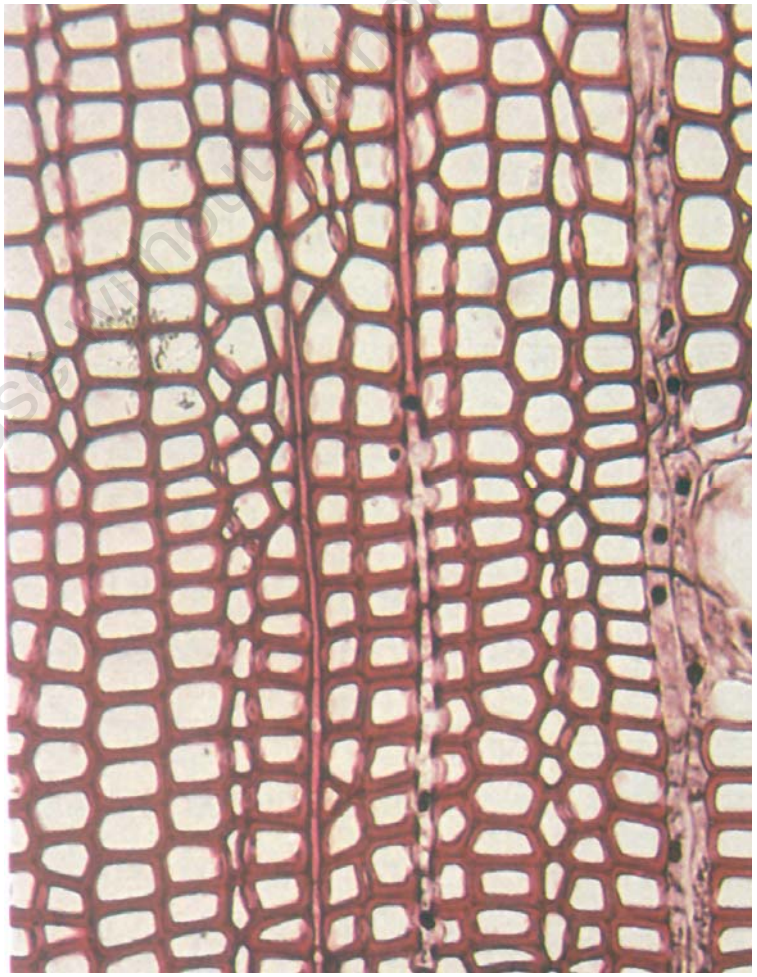
Plants are composed of polysaccharides and lignin



Typical grass composition



Section of a pine board

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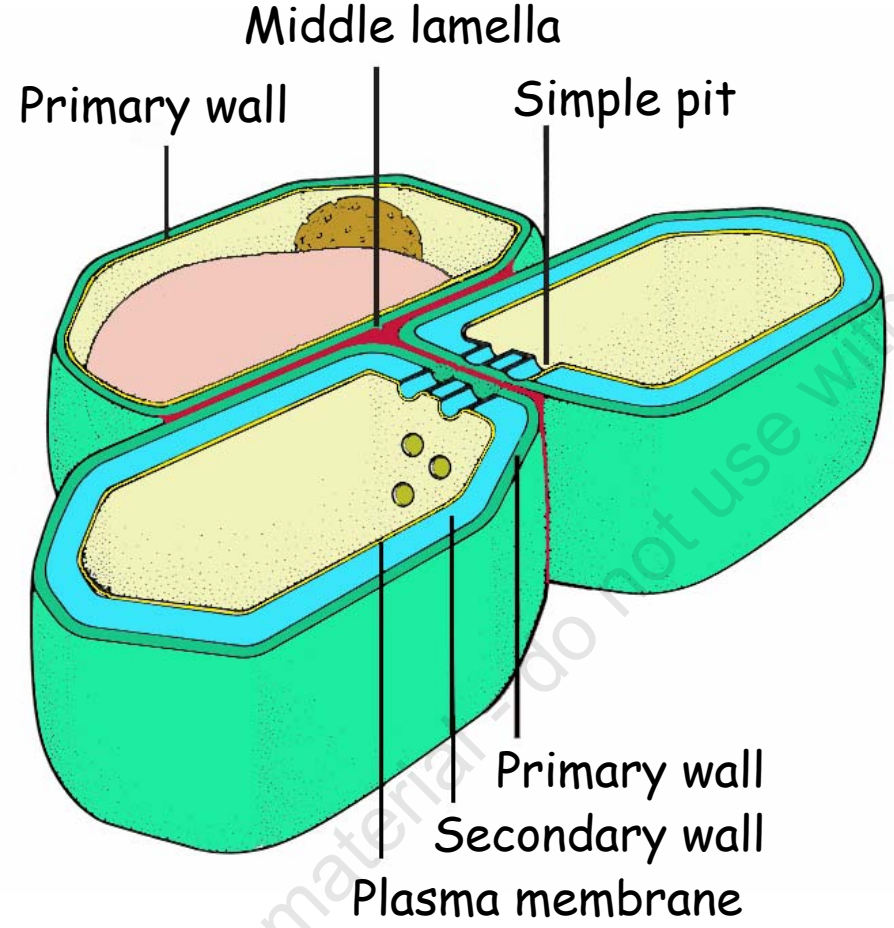
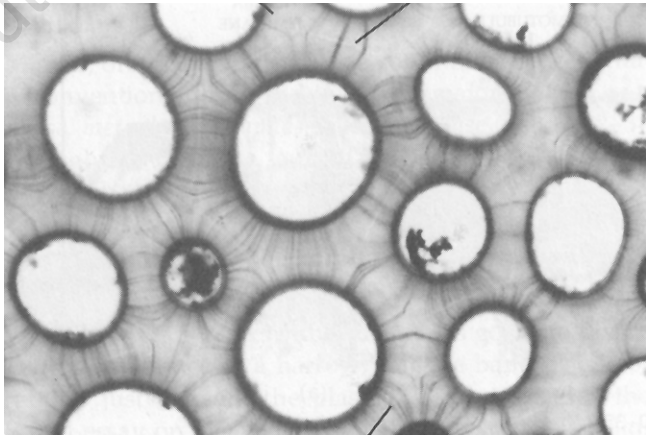
The properties of cell walls determine many aspects of plant life



From Taiz & Zeiger
Plant Physiology 1991



Cells in persimmon endosperm

From Raven, Evert & Eichhorn
Biology of Plants 1986

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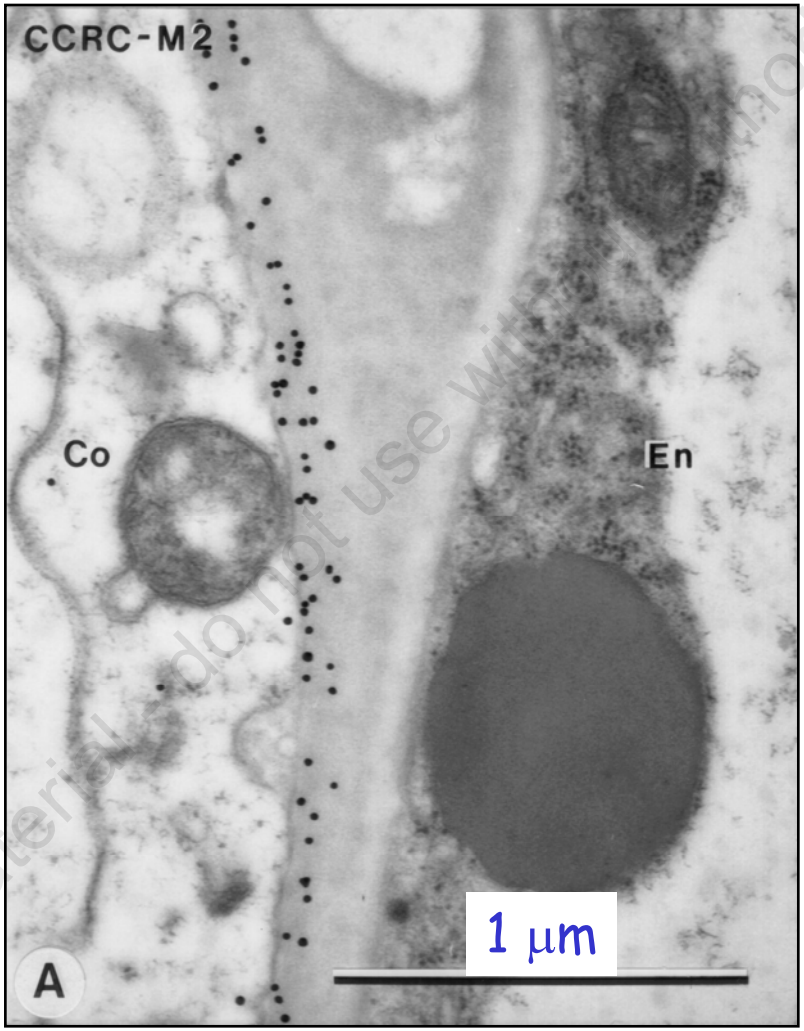
Cell-type specific localization of the rhamno-galacturonan I epitope recognized by CCRC-M2



Freshour *et al.* (1996) Plant Physiol. **110:** 1413-1429

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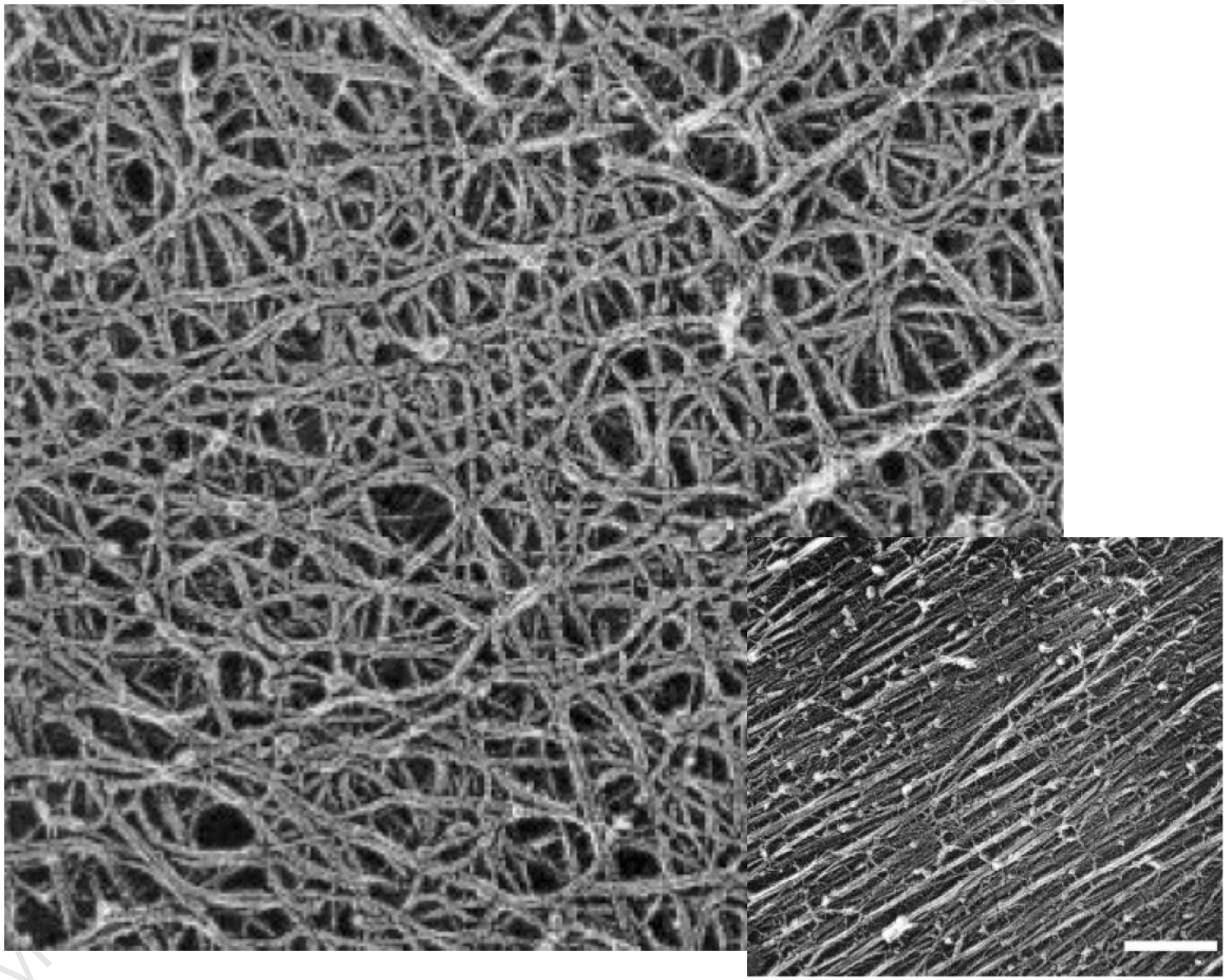
EM of onion cell wall surface



From: McCann, Wells & Roberts J Cell Sci 96,323

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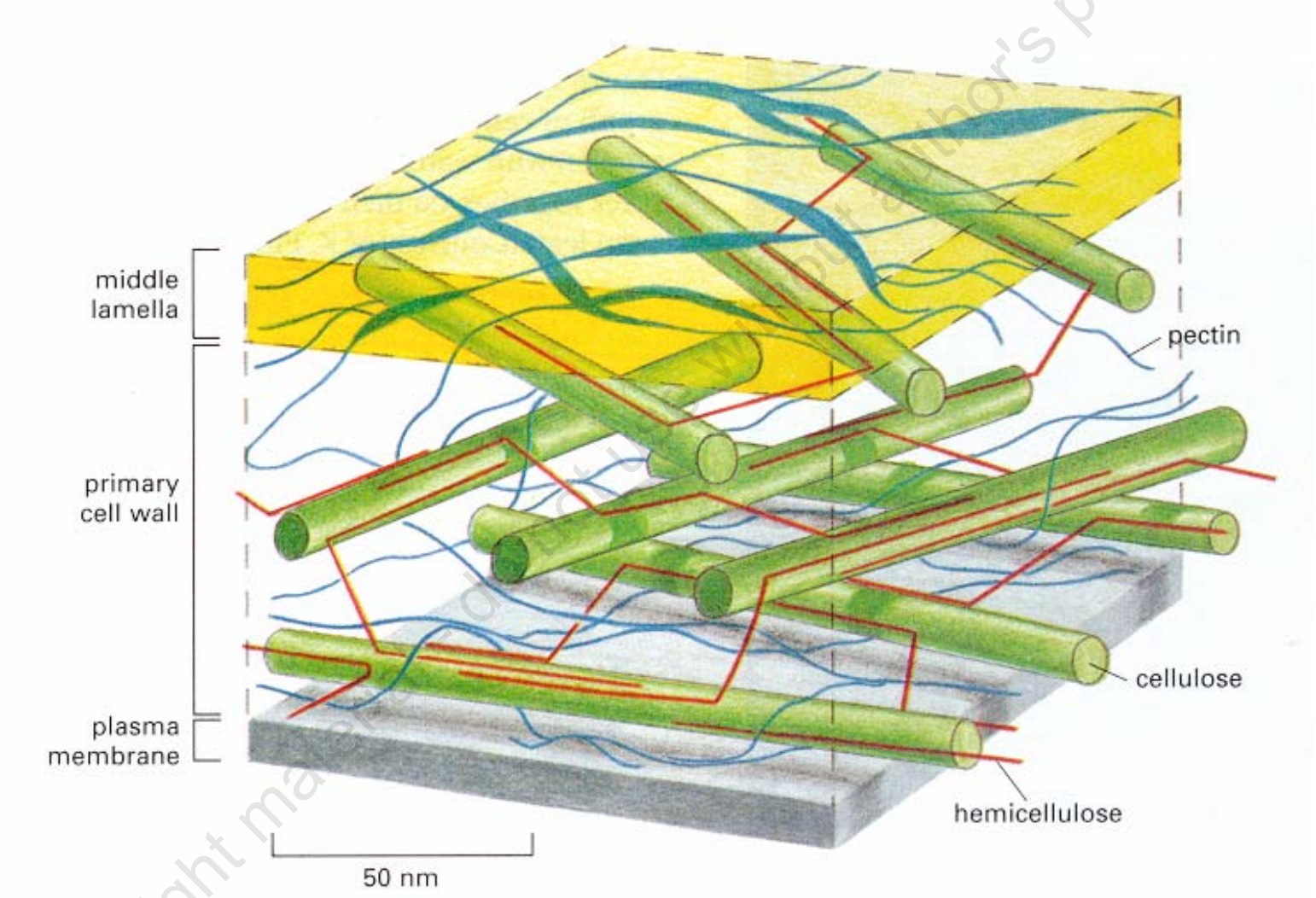
Model of the cellulose/hemicellulose and pectic cell wall networks



McCann & Roberts (1991) *The Cytoskeletal Basis of Plant Growth and Form*, p. 126

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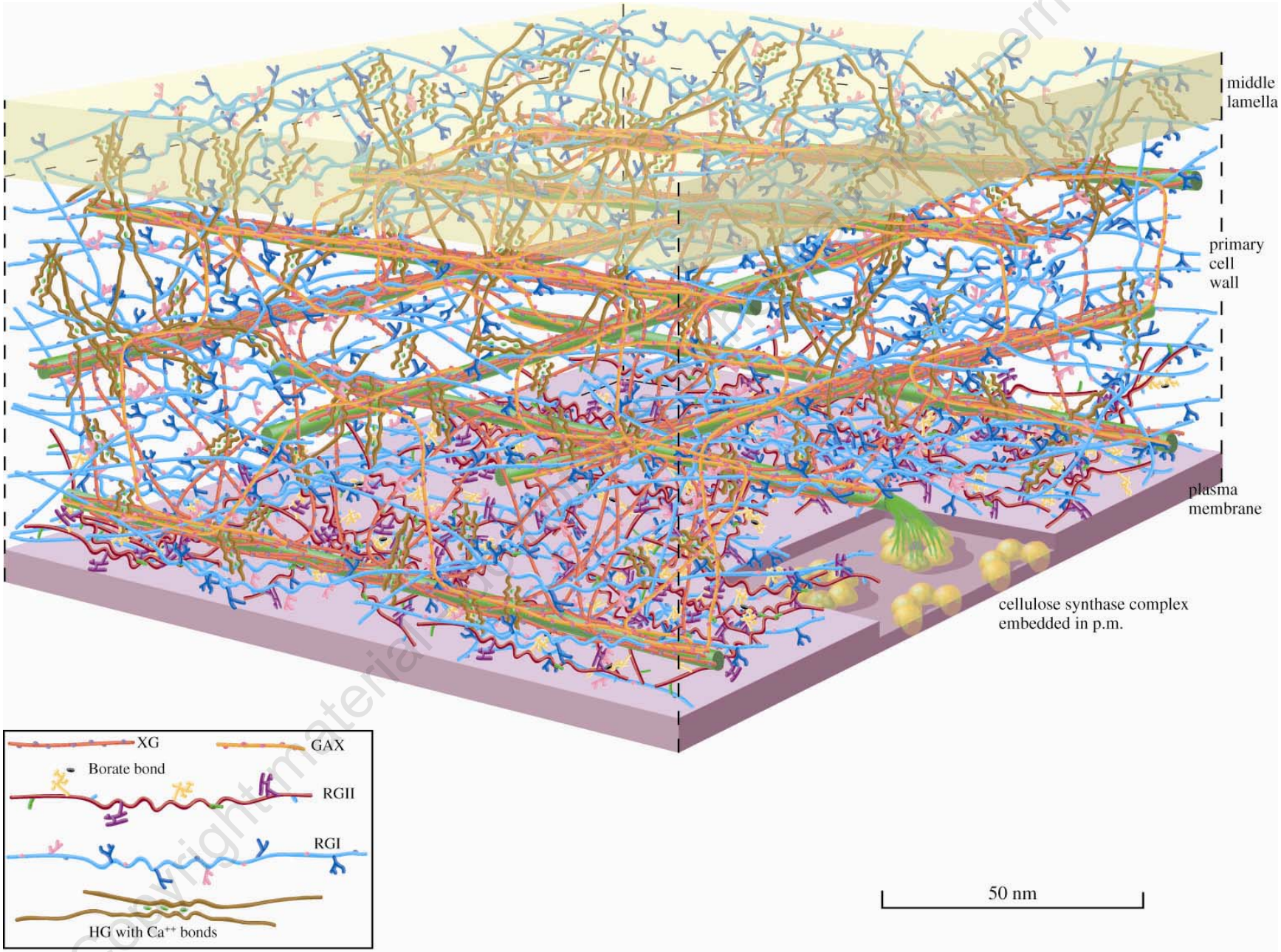
Scale model of a cell wall

Somerville et al. Science 2004



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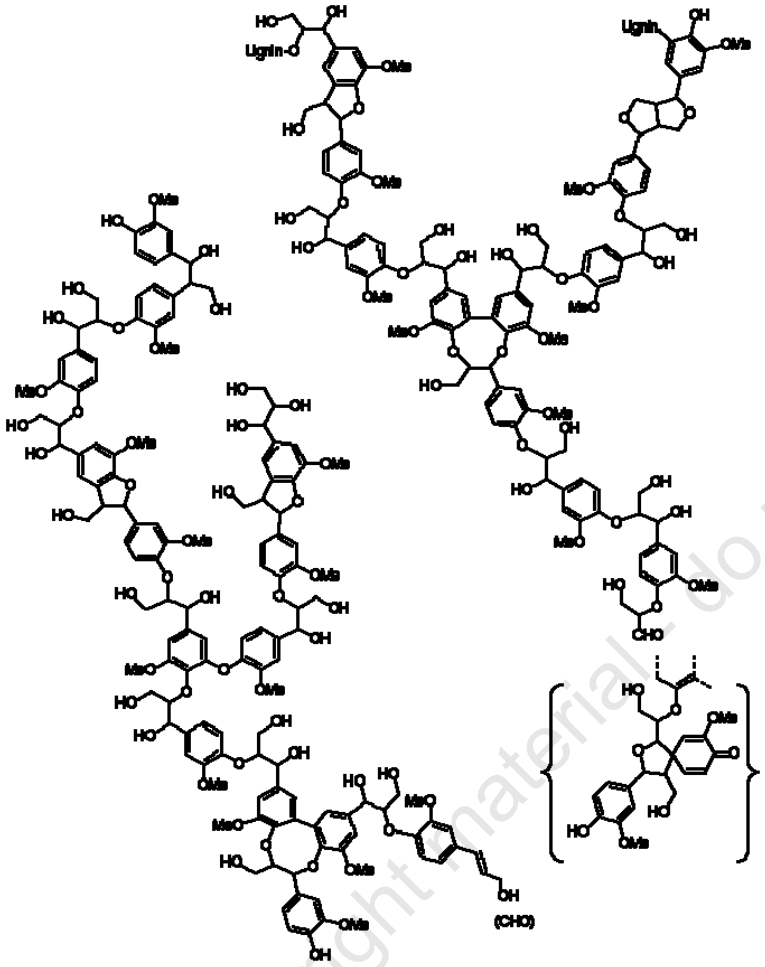
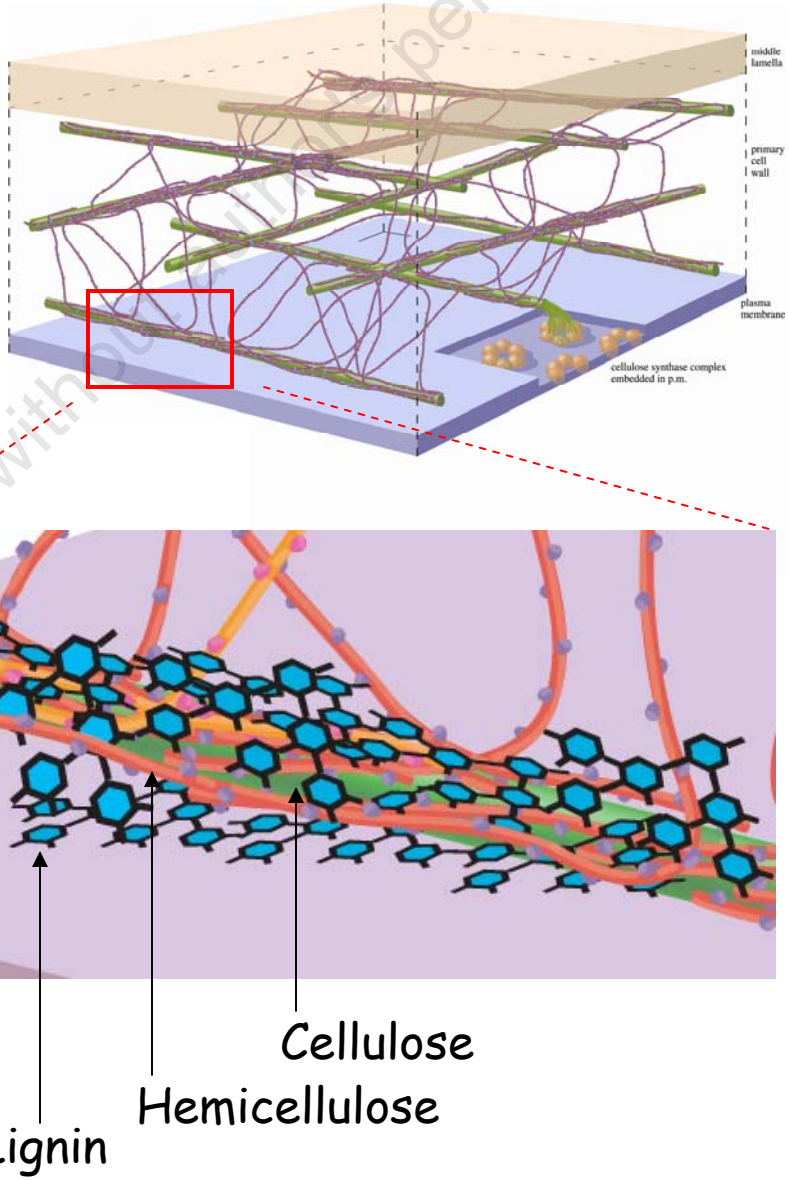
Lignin occludes polysaccharides





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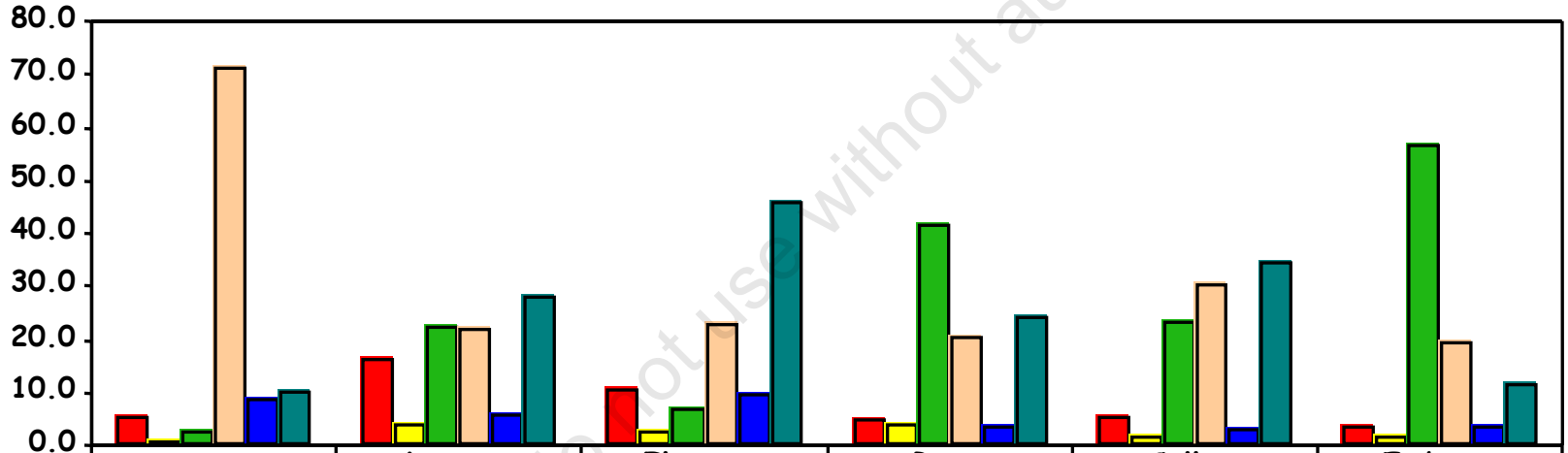
Cell wall Composition Varies in Different Tissues



	Stems	Leaves	Flowers	Root	Callus	Embryo
Rhamnose	5.5	16.7	10.7	5.0	5.6	4.0
Fucose	1.1	4.3	2.9	4.3	2.0	2.0
Arabinose	2.8	22.4	7.2	42.1	23.6	57.0
Xylose	71.4	22.0	23.2	20.6	30.7	20.0
Mannose	9.0	6.3	10.1	3.7	3.2	4.0
Galactose	10.3	28.3	46.0	24.4	34.8	12.0

Richmond and Somerville Plant Mol. Biol. 47,131

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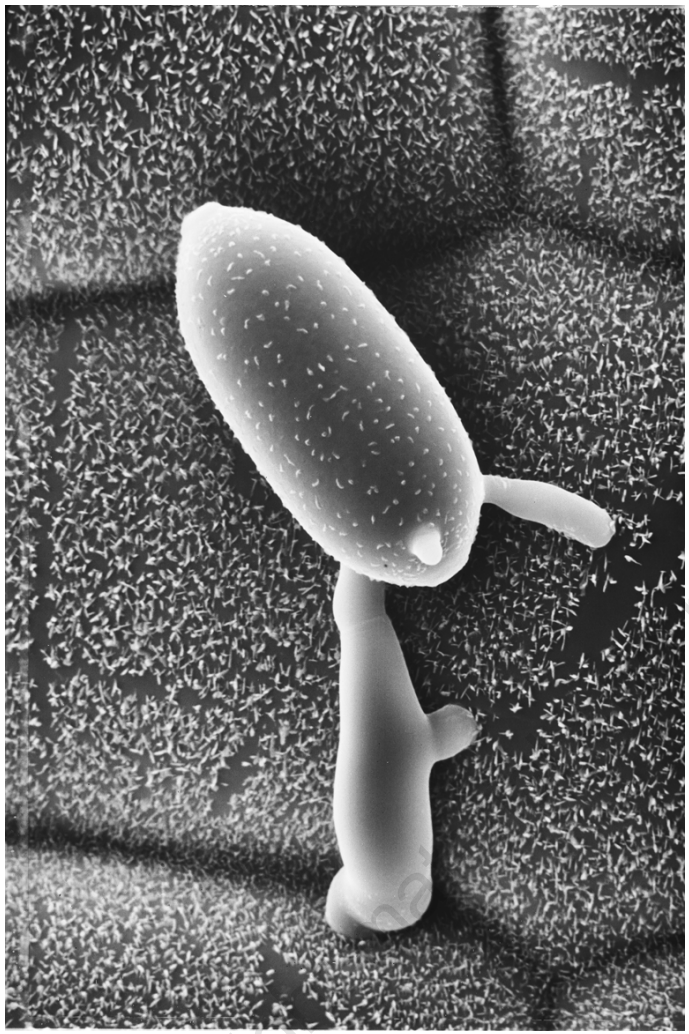
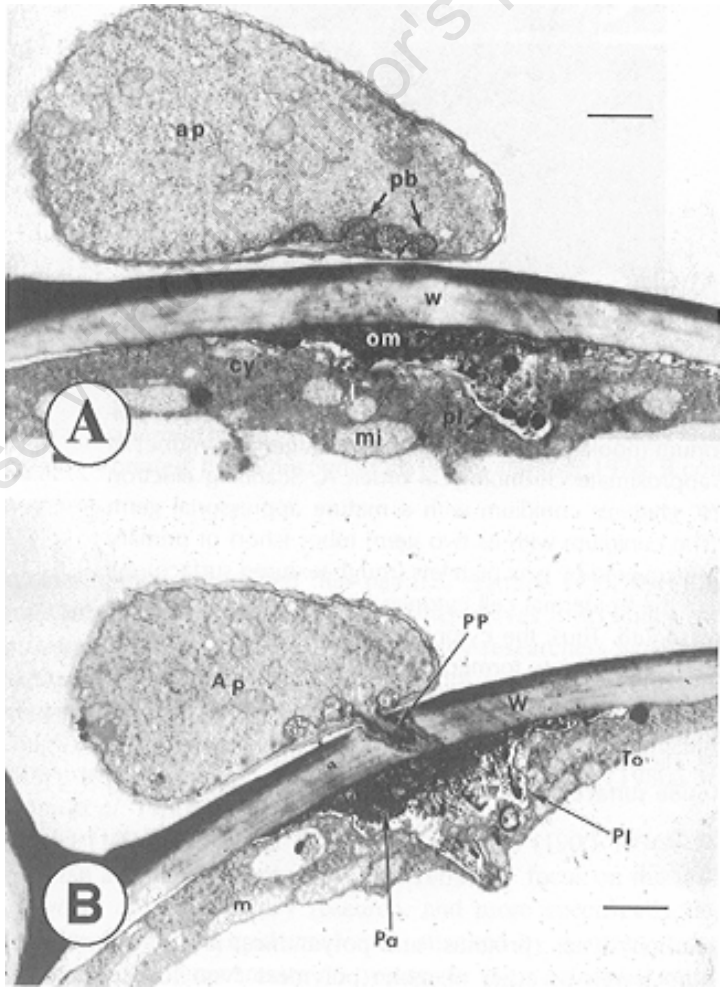
Fungal infection requires localized cell wall hydrolysis





Zeyen et al. 2002. In *The Powdery Mildews*

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Growth of *Erysiphe cichoracearum* on various mutants

Col (wt) *pmr1* *pmr2* *pmr3* *pmr4* *pmr5* *pmr6*

Vogel et al., Plant J.,

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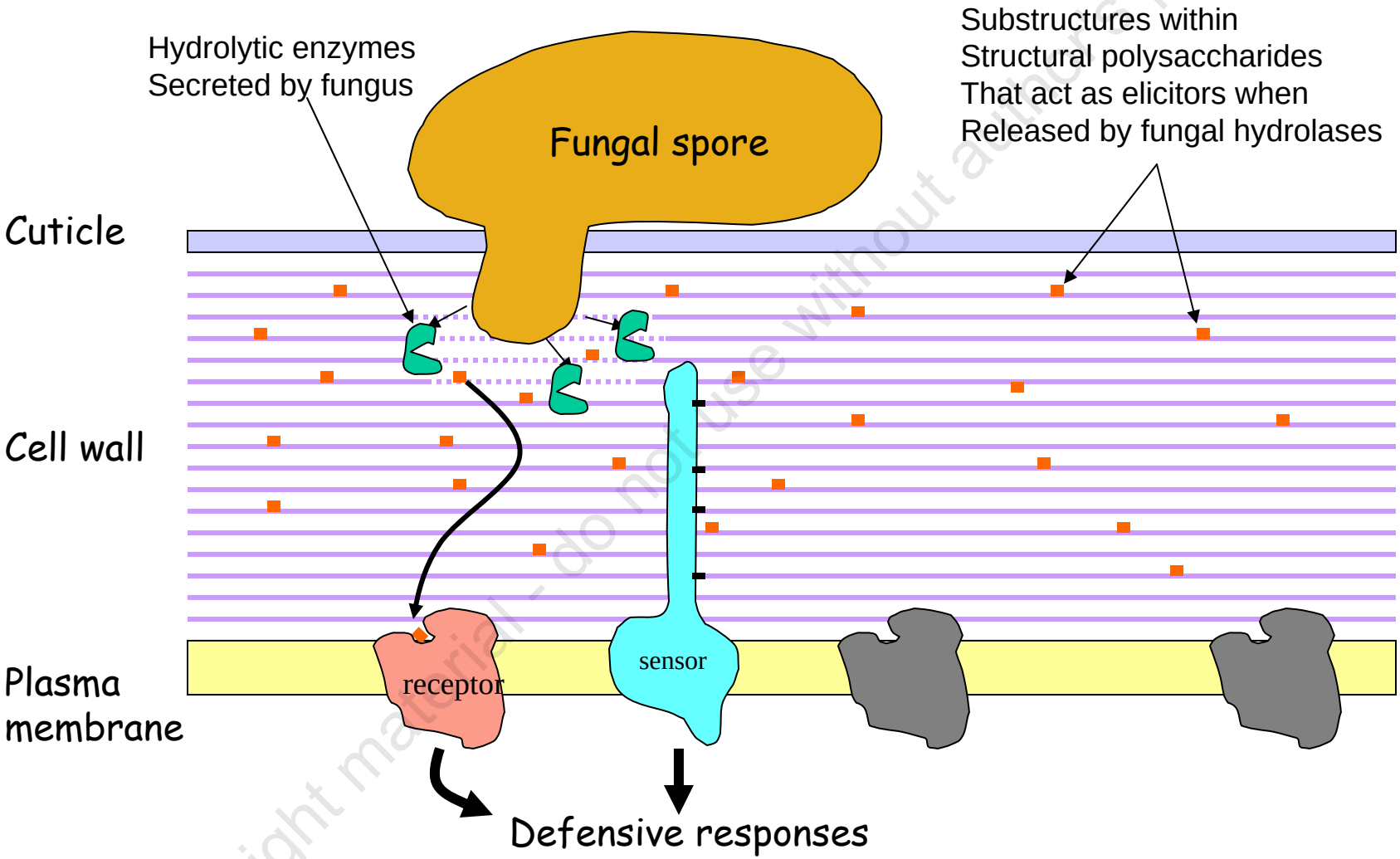
Albersheim hypothesis



Vorwerk et al Trends Plant Sci. 9, 203

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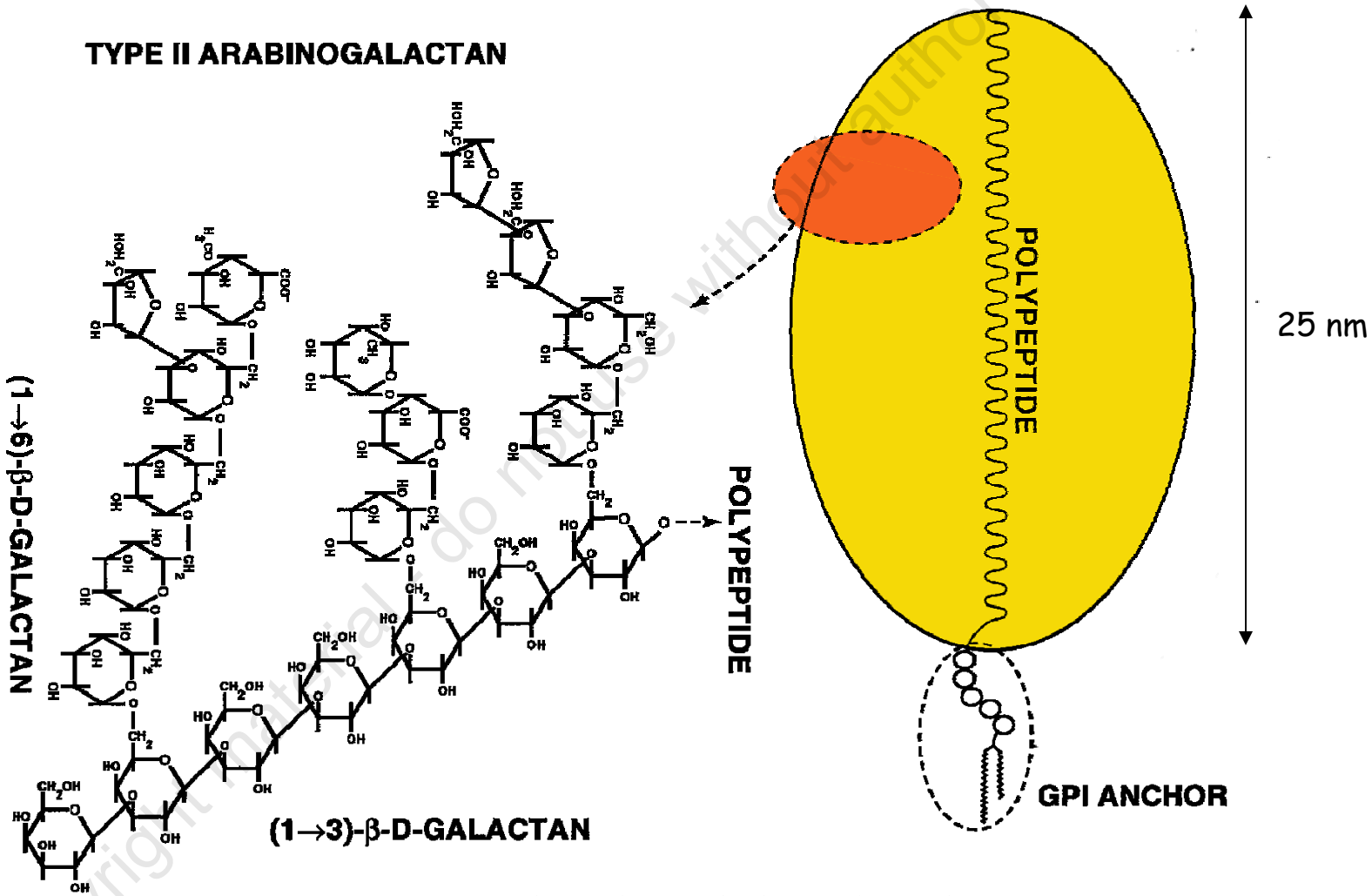
Plant walls contain about 100 proteoglycans



Majewska-Sawka and Nothnagel Plant Physiol. 122, 3

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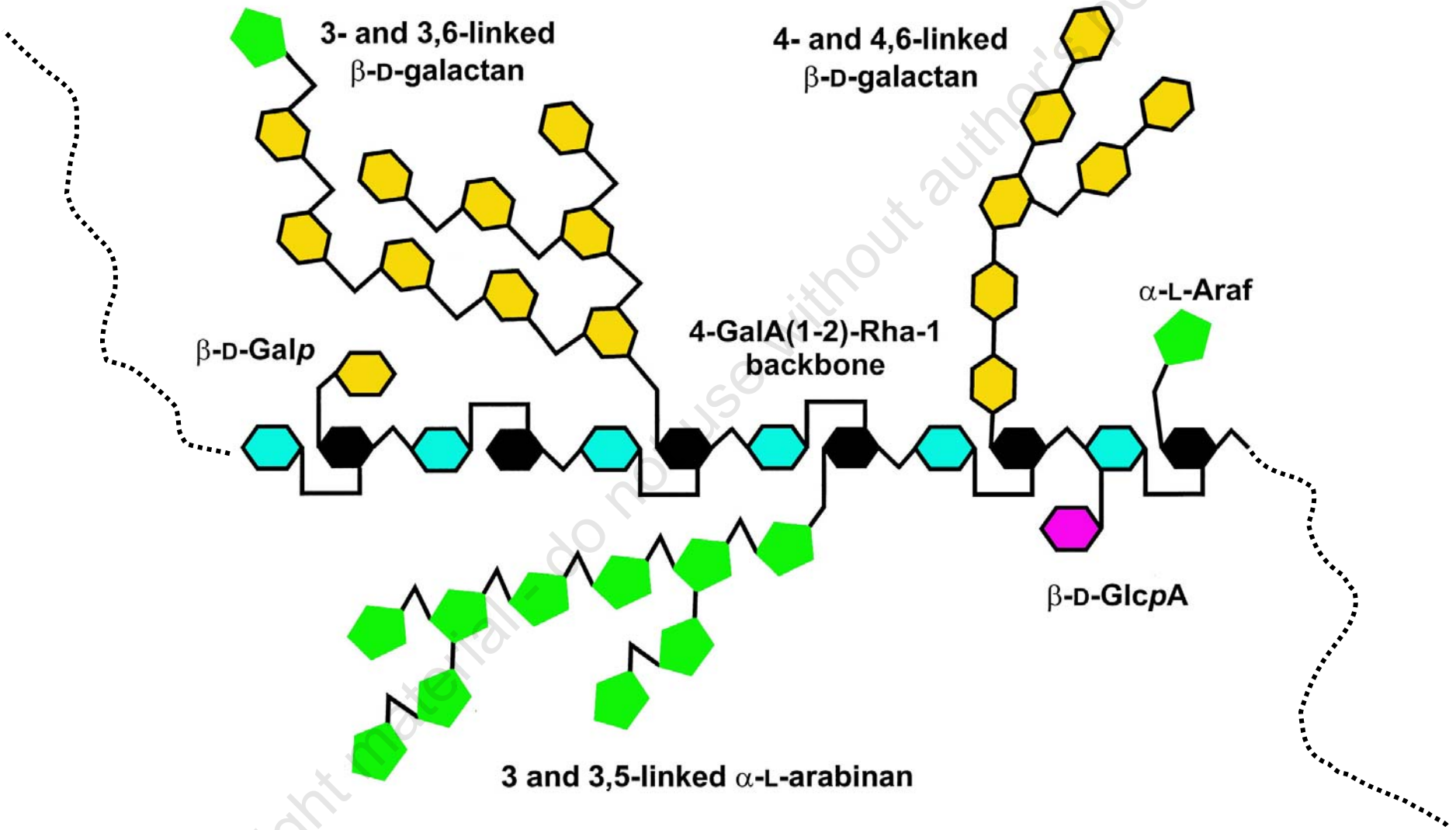
Illustrative structure of RGI



Ridley, O'Neill, Mohnen Phytochemistry 57, 929

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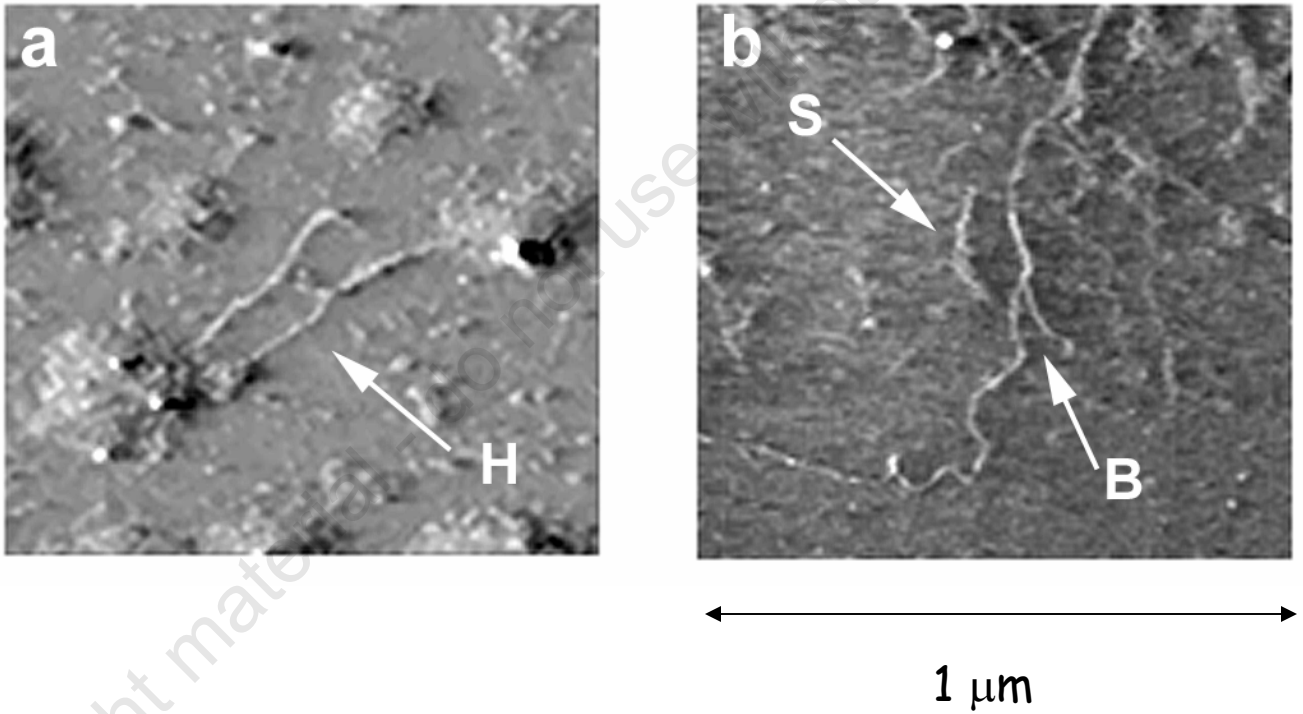
AFM image of xylan



Adams et al., Carb. Res. 338,771

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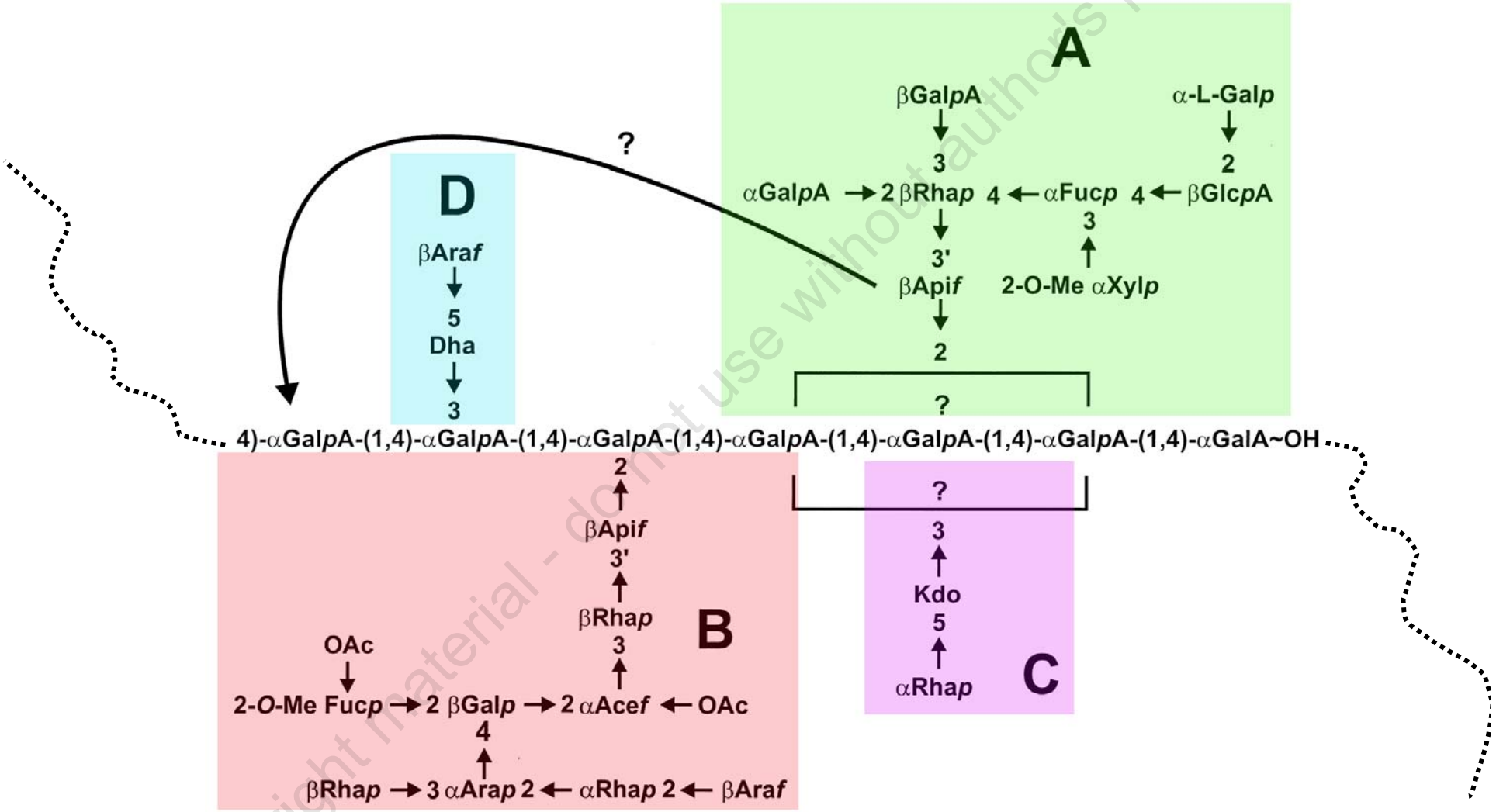
Structure of RGII



Ridley, O'Neill, Mohnen Phytochemistry 57, 929

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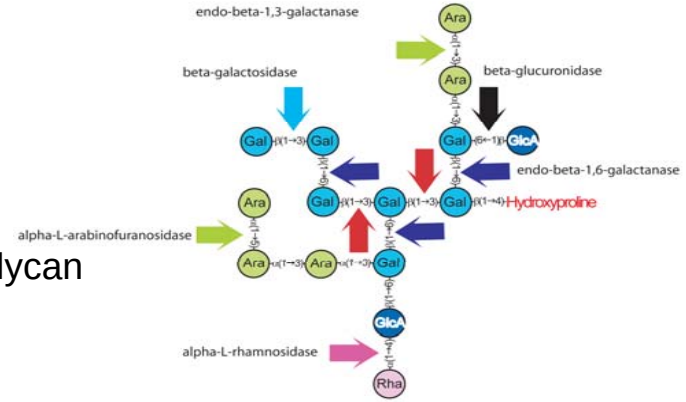
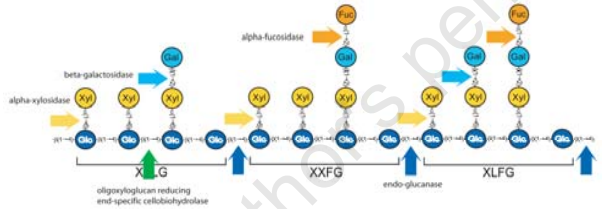
Cell wall toolkit

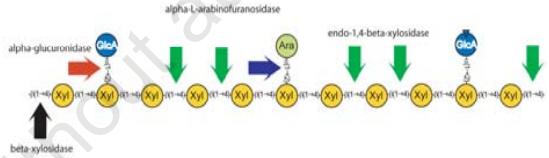
AGP glycan



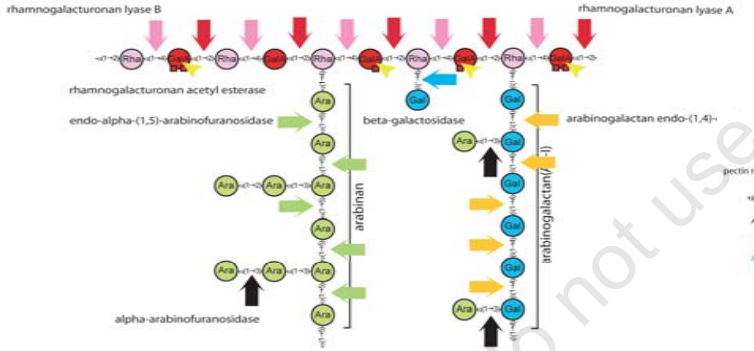
XG



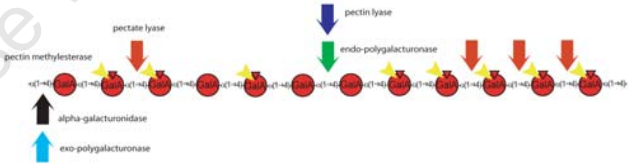
GAX



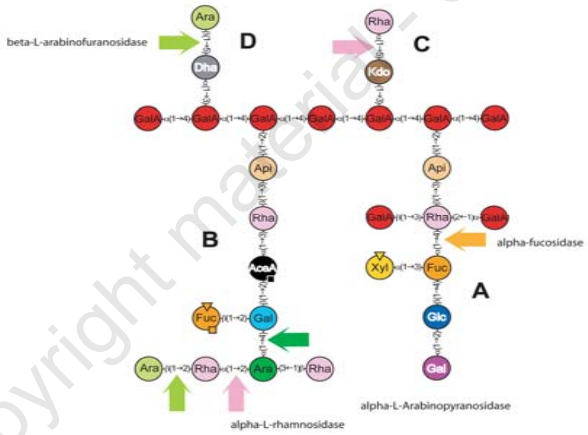
RGI



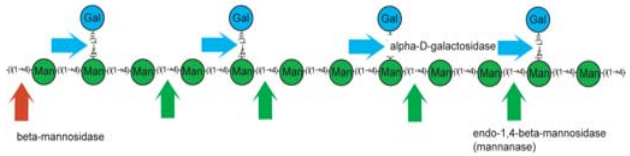
HG



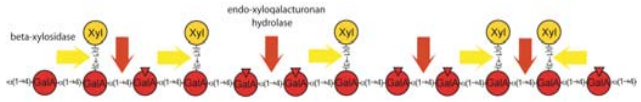
RGII



Galactomannan



Xylogalacturonan



Bauer et al., PNAS 2005

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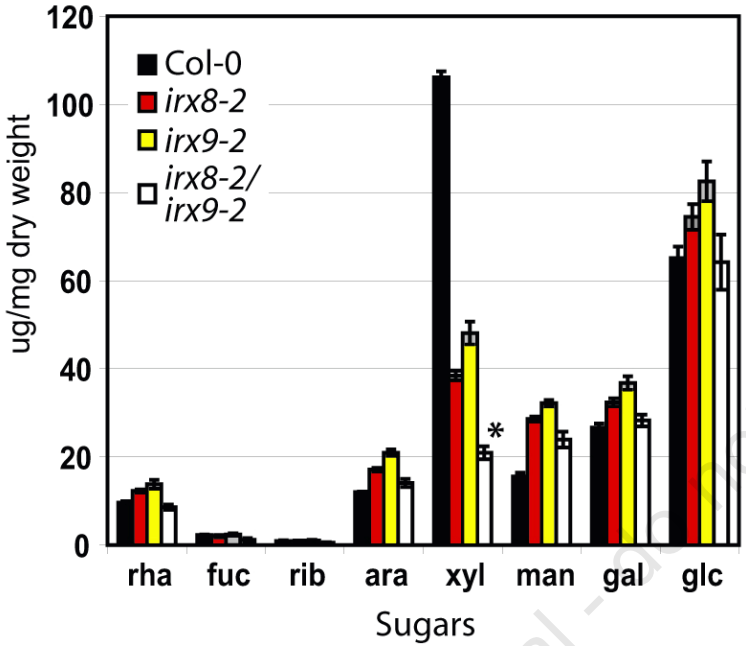
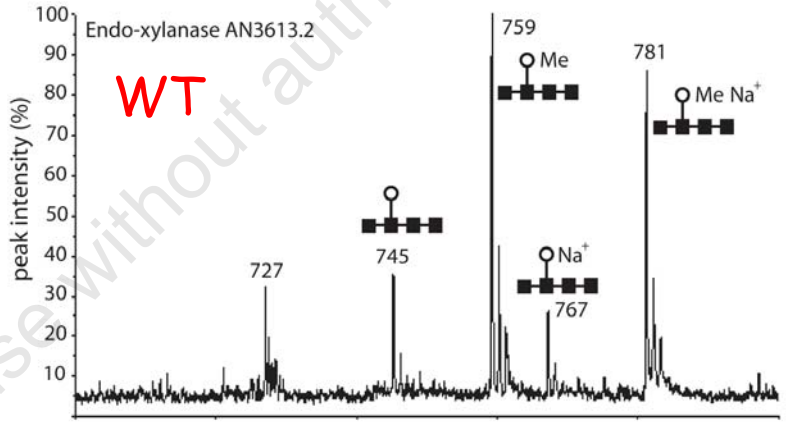
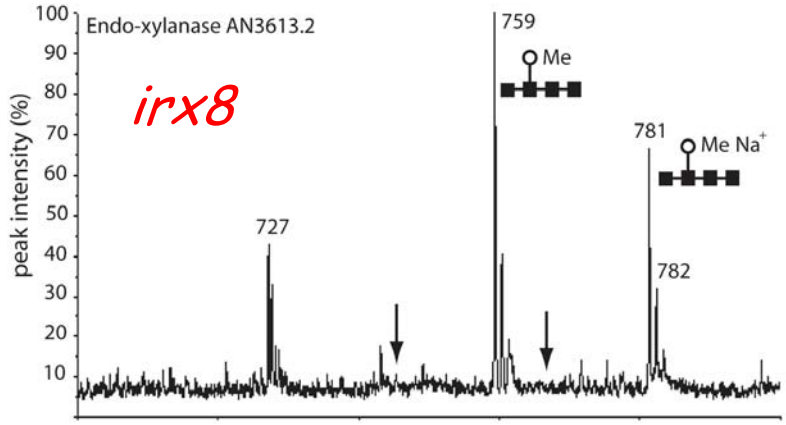
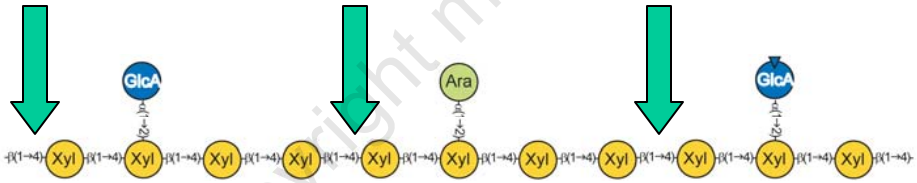
Irx8 & 9 mutants are deficient in xylan







M/z



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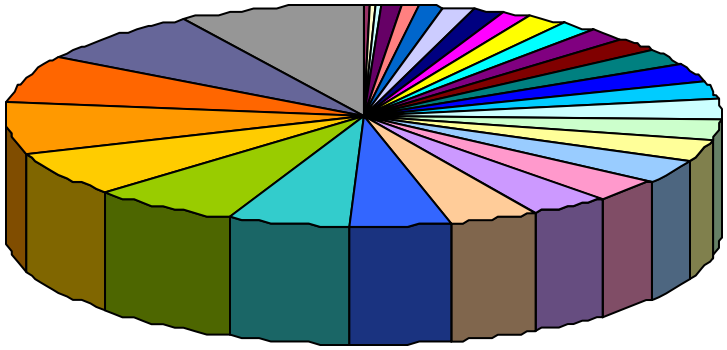
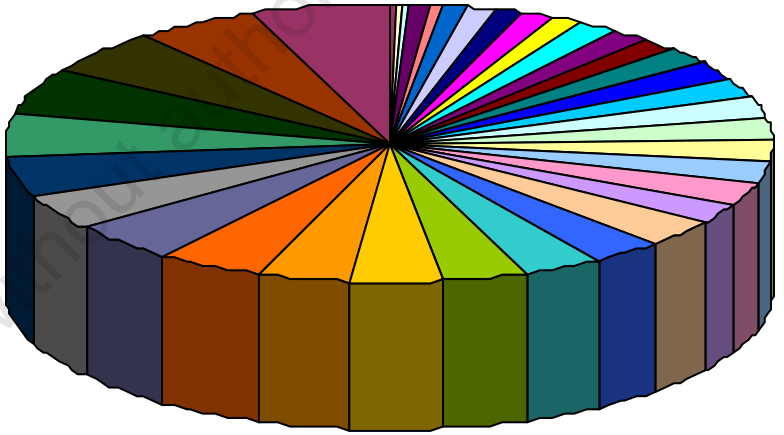
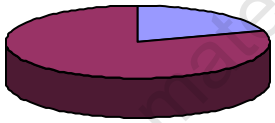
At least 1000 genes are implicated in polysaccharide synthesis and modification



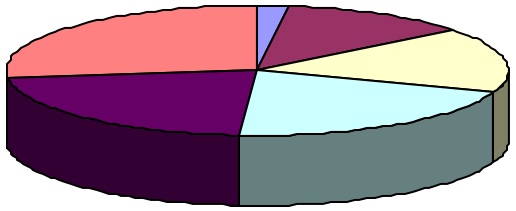
379 glycosidases & transglycosidases (32 families)



425 glycosyltransferases (38 families)



34 polysacharide lyases



110 esterases

http://afmb.cnrs-mrs.fr/CAZY/

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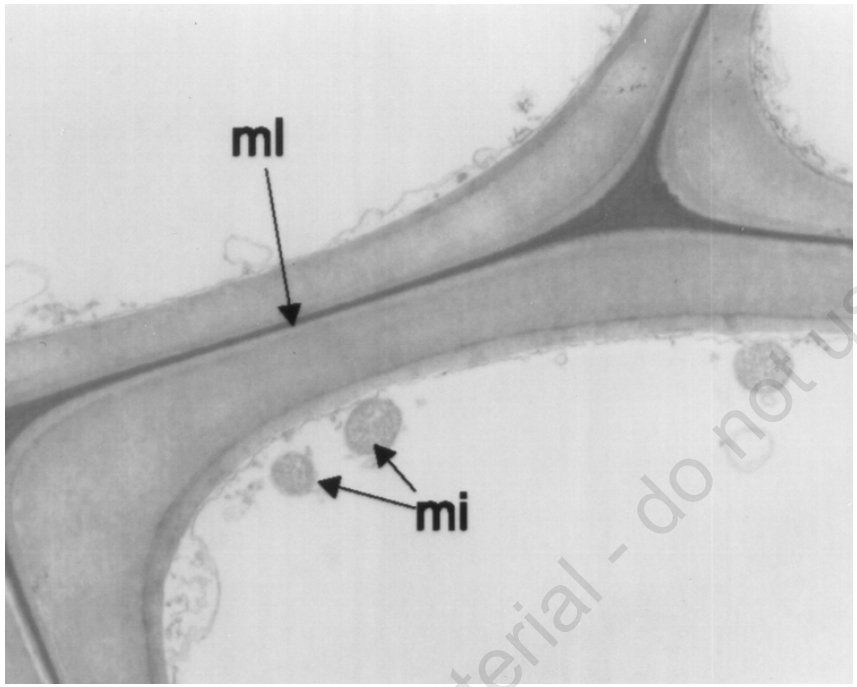
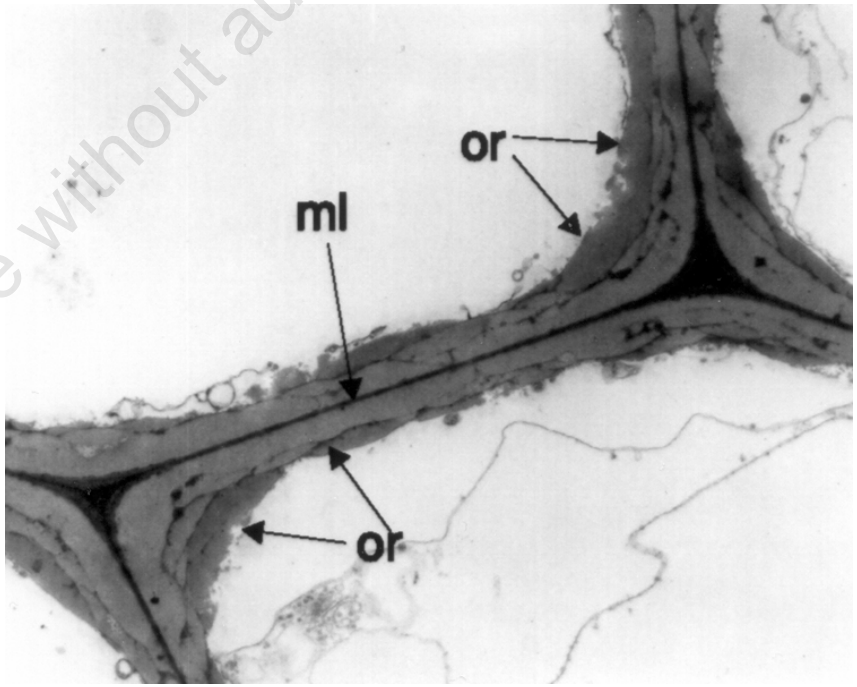
Cell walls are organized on cellulose



Wild type



Cellulose-deficient mutant

Turner and Somerville Plant Cell 9,689

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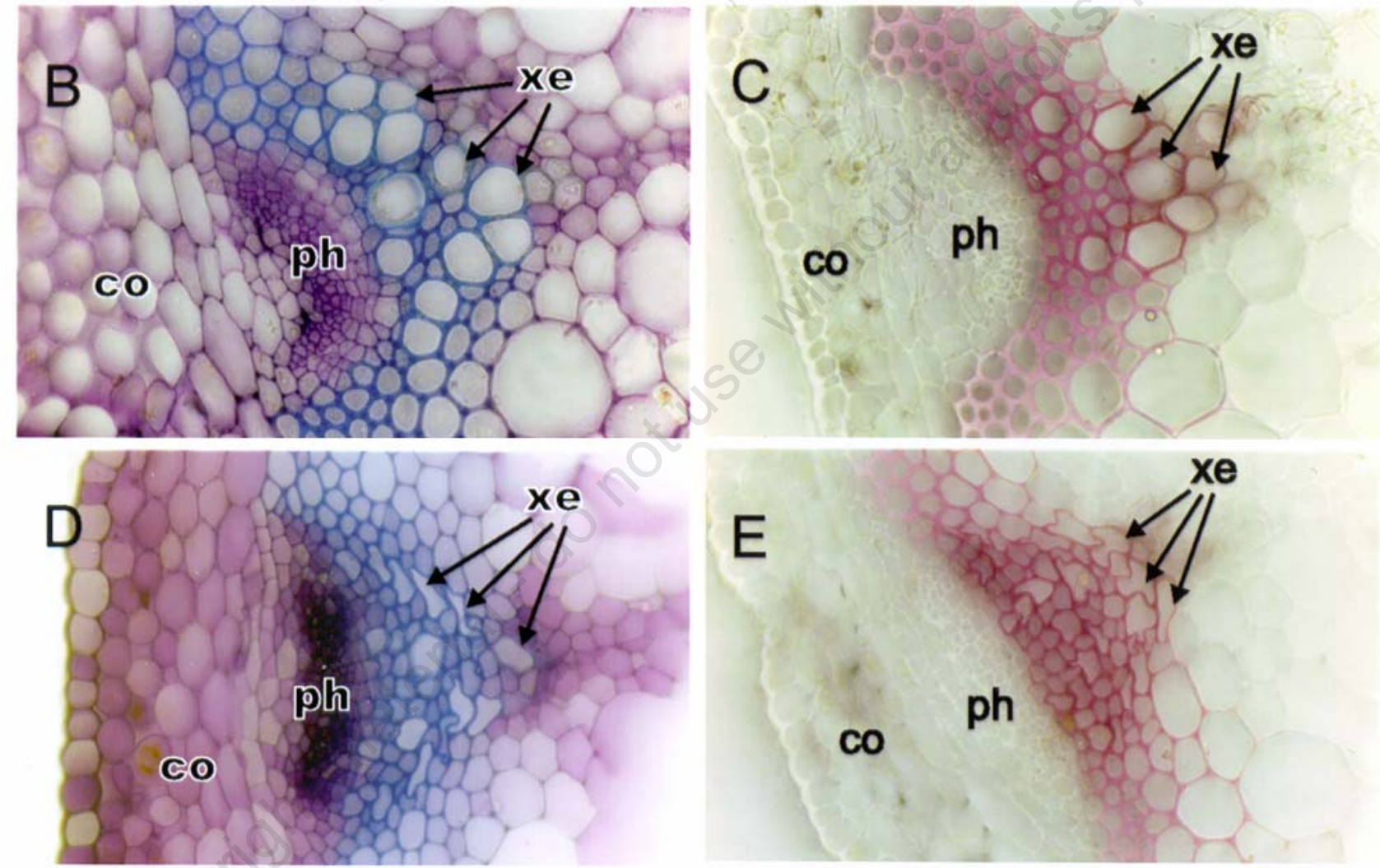
Irregular xylem (*irx*) mutants



Turner and Somerville Plant Cell 9,689

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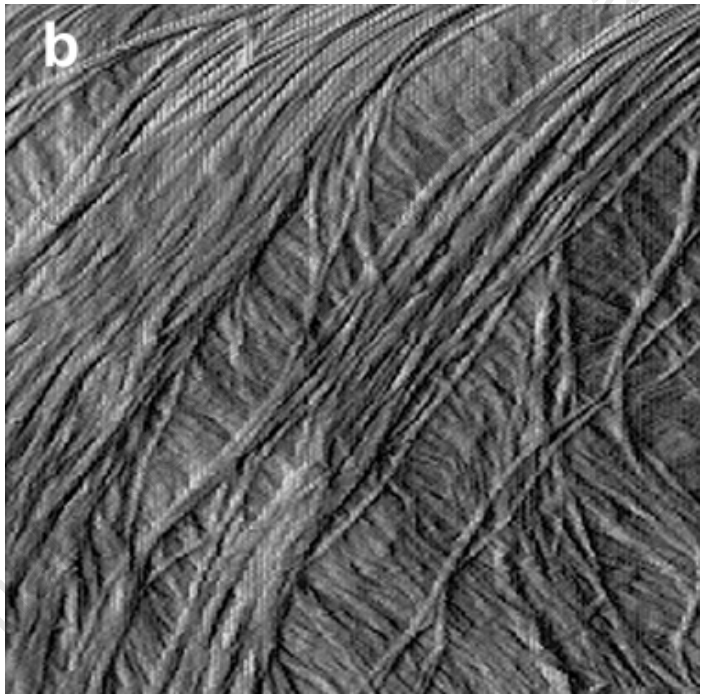
AFM image of cellulose



Courtesy of Vic Morris

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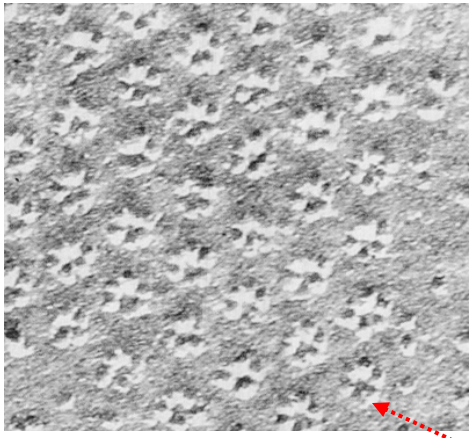
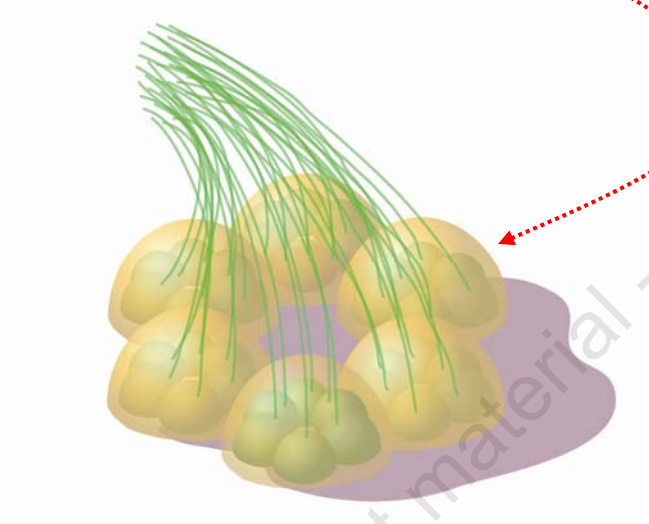
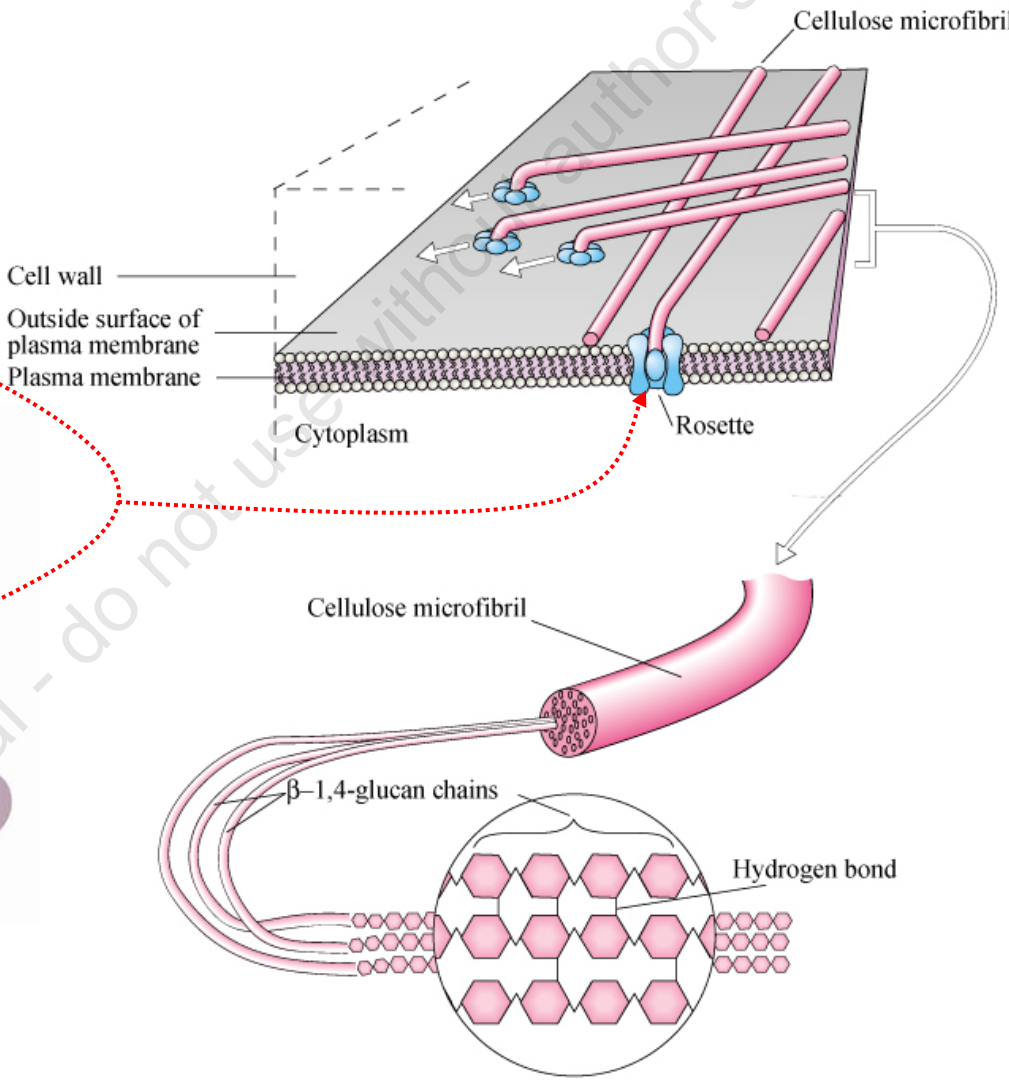
Cellulose is synthesized at the plasma membrane







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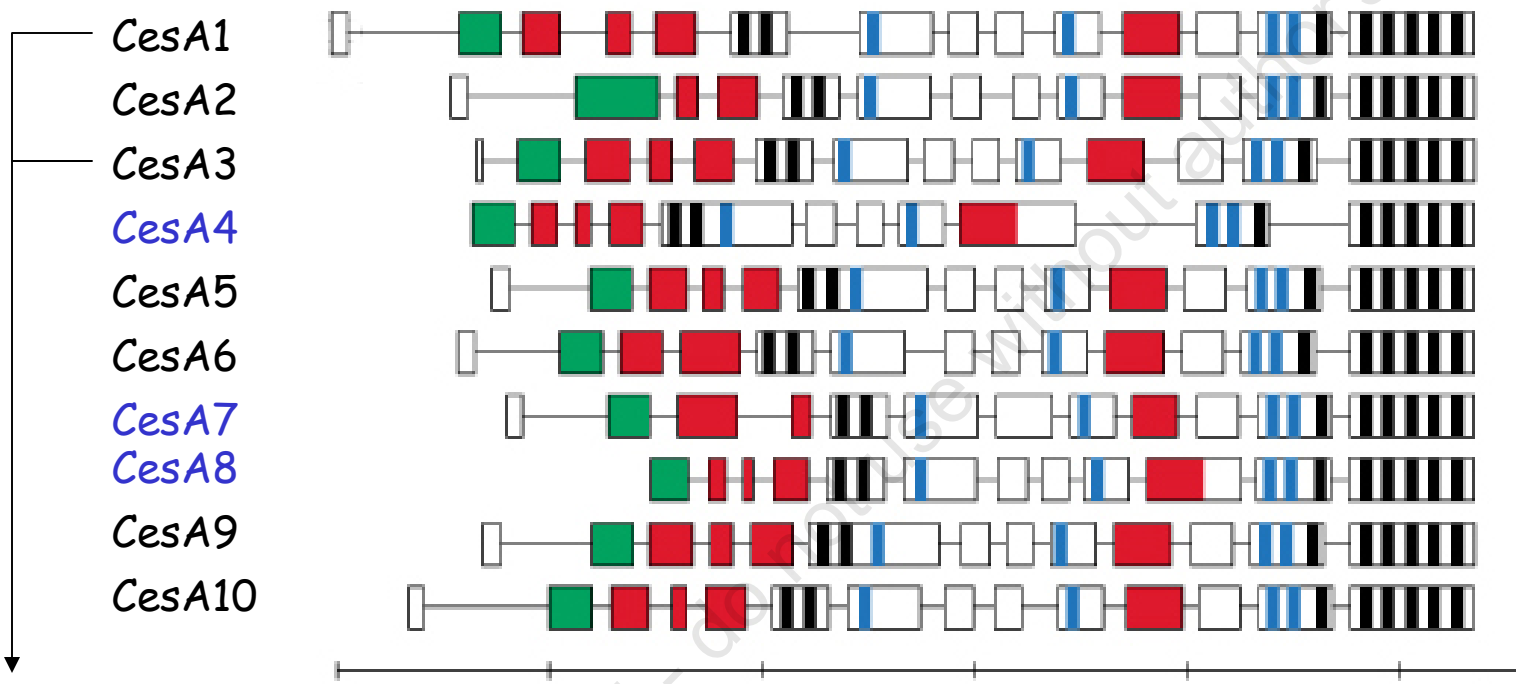
The Arabidopsis CesA gene family



Conserved residues in domains A and B
Zinc finger domain with CXXC motif
Variable regions
Transmembrane domain

Richmond, Genome Biology 1(4)3001.1

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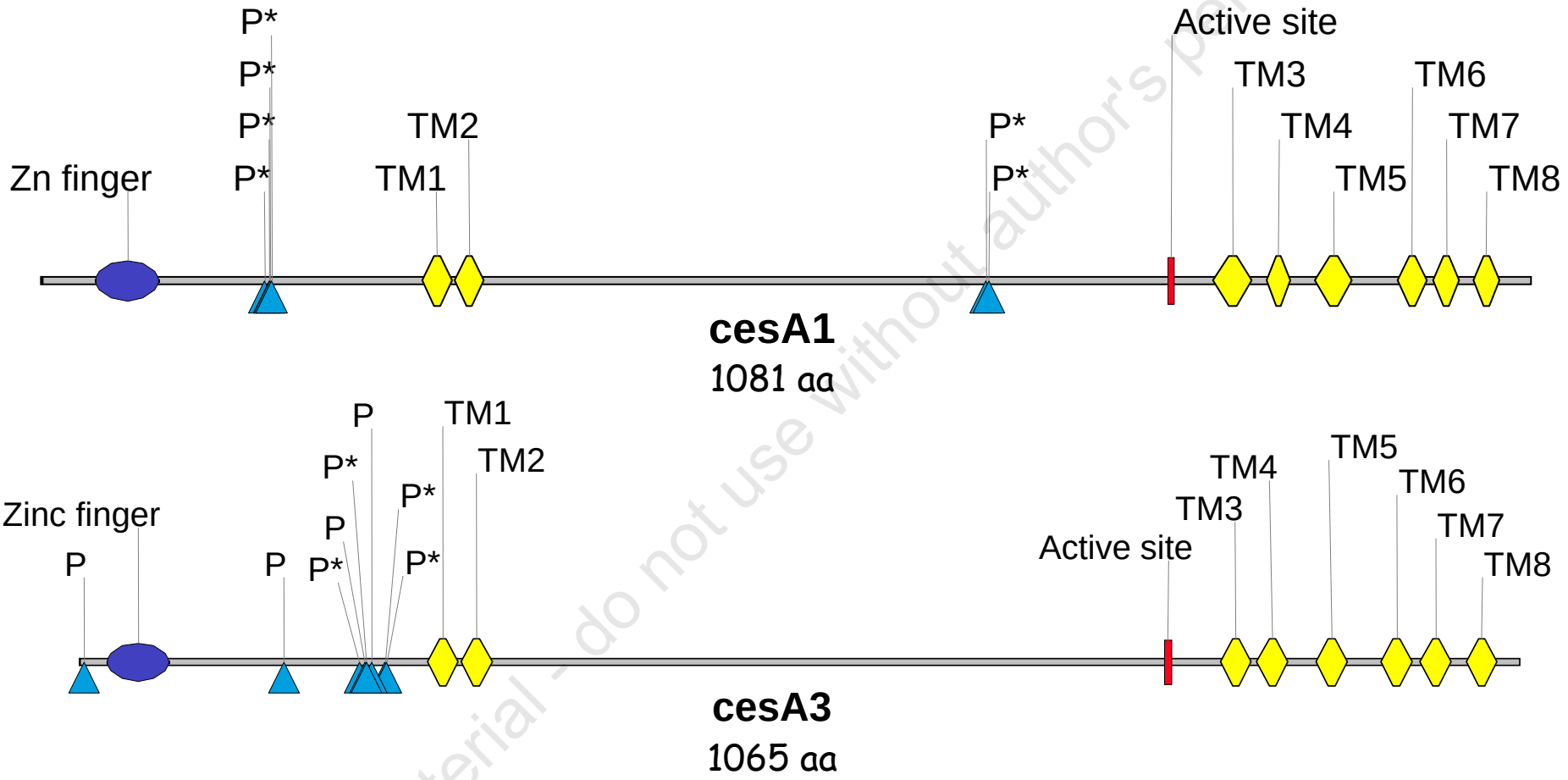
Structure of the CESA1 & CESA3 proteins



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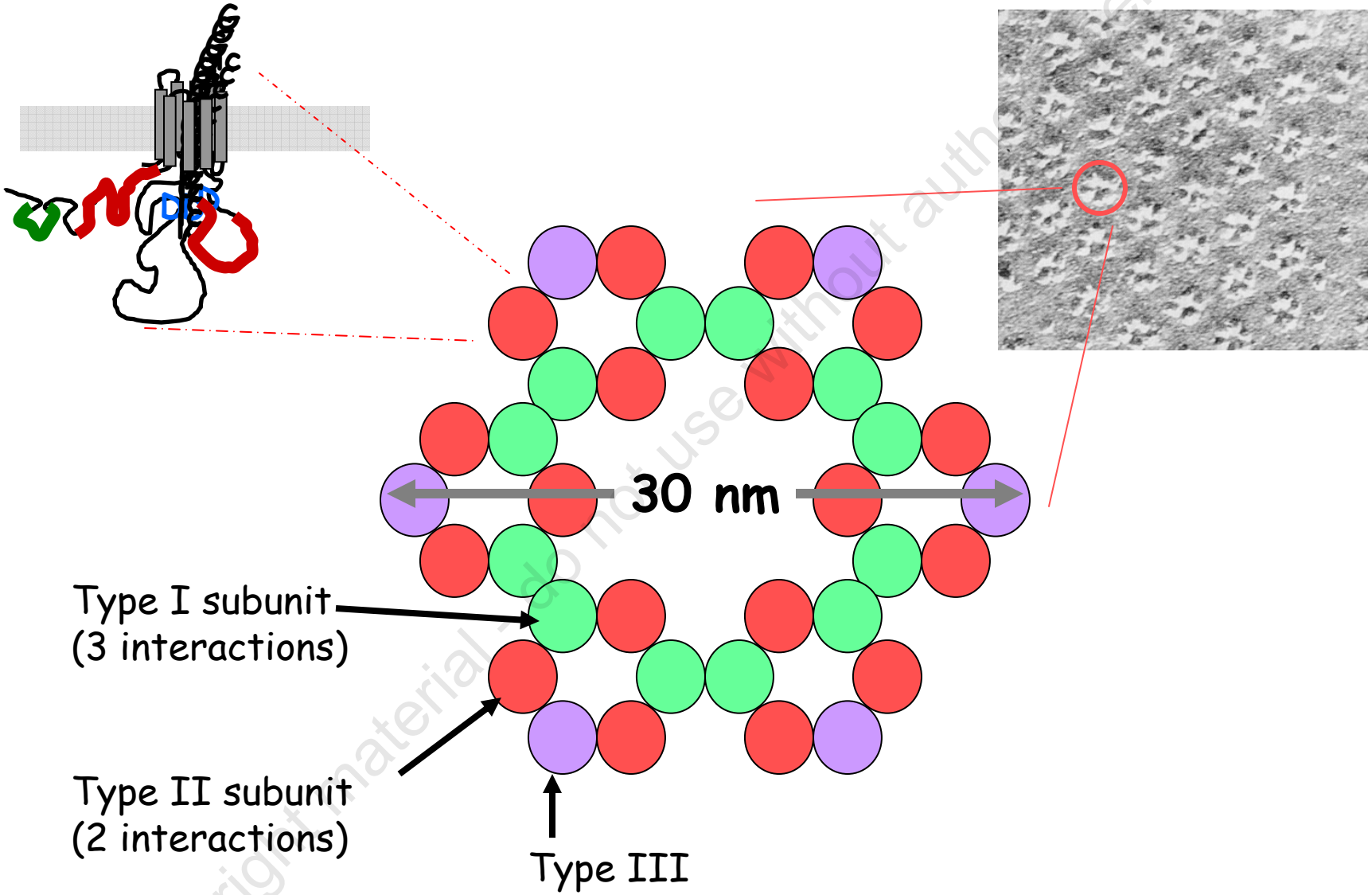
Heteromeric model for cellulose synthase



Scheible et al PNAS 98,10079

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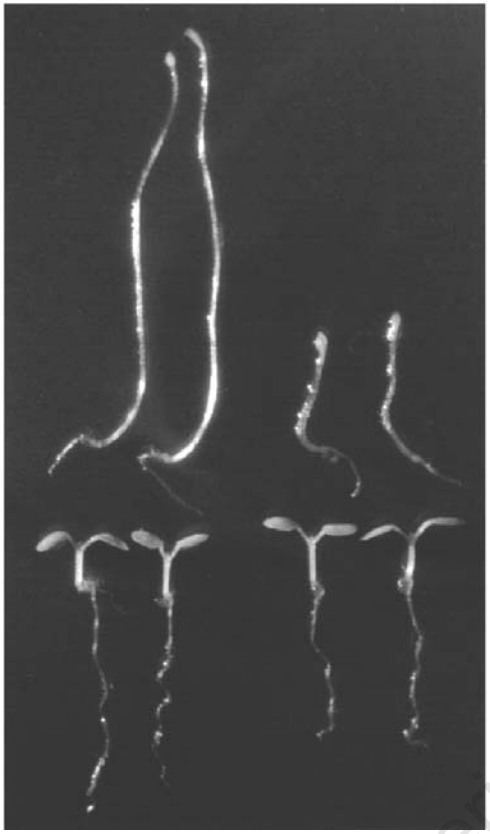
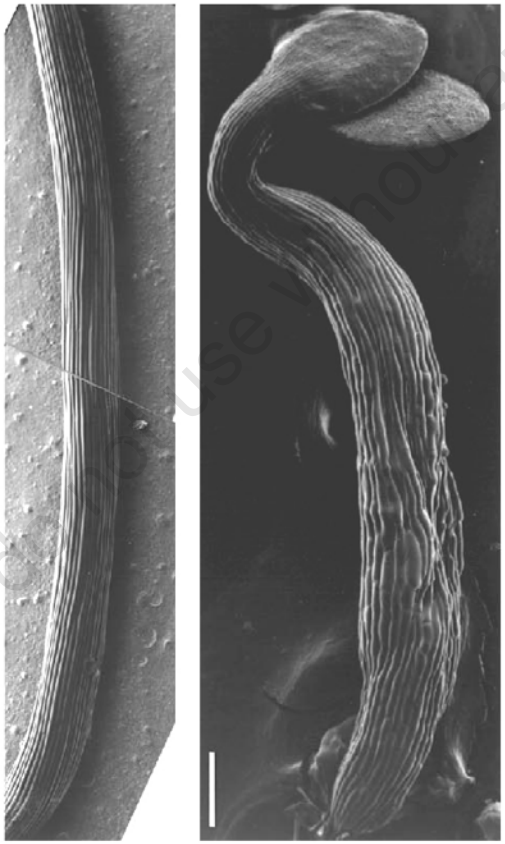
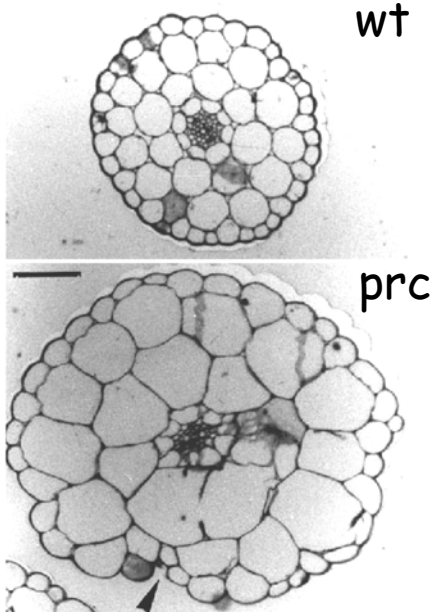
Procuste has defective cesA6







Desnos et al., Development 122,683

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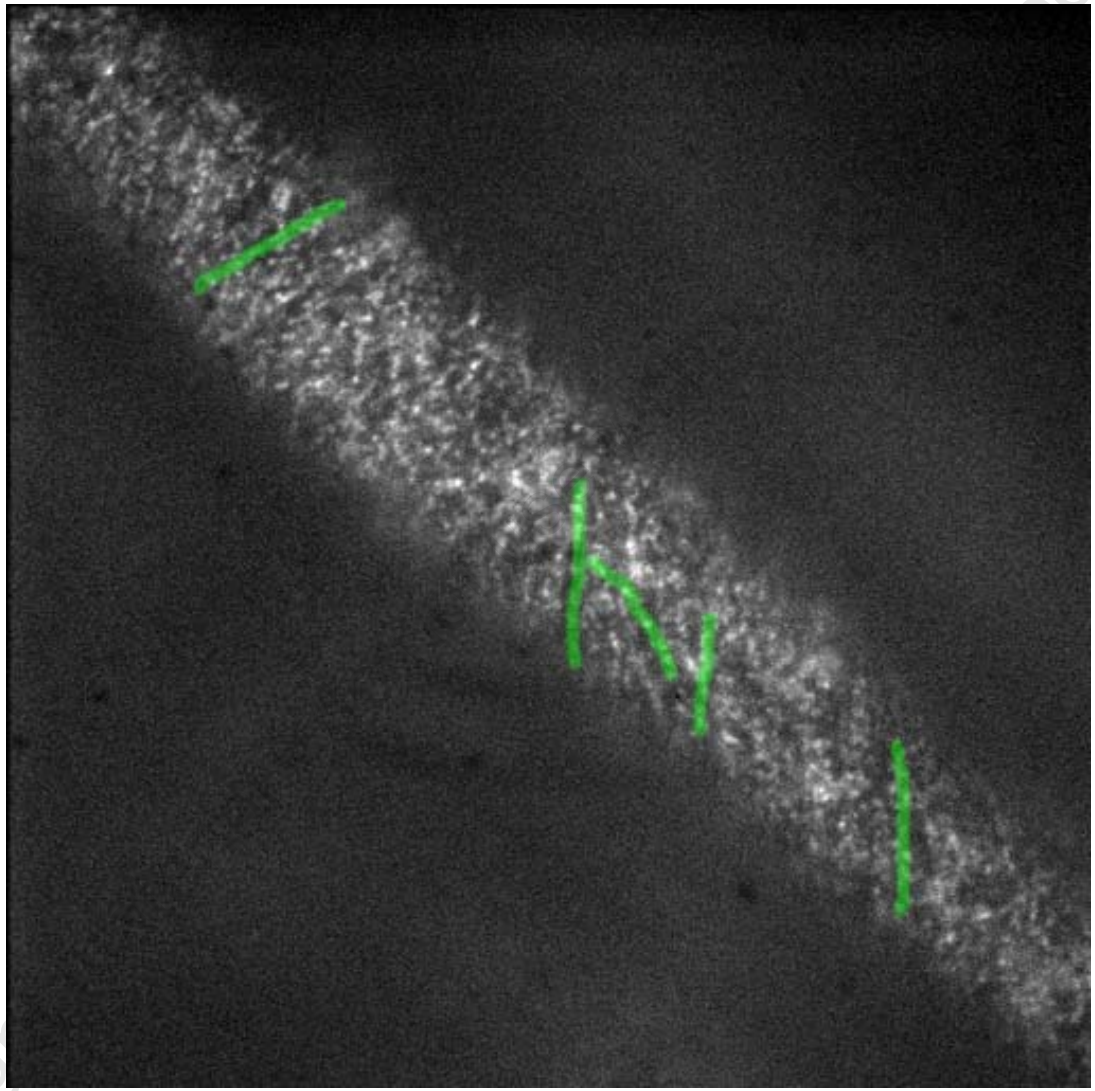
GFP:CESA6 in hypocotyl cells



Paredez, Ehrhardt & Somerville, Science 2006

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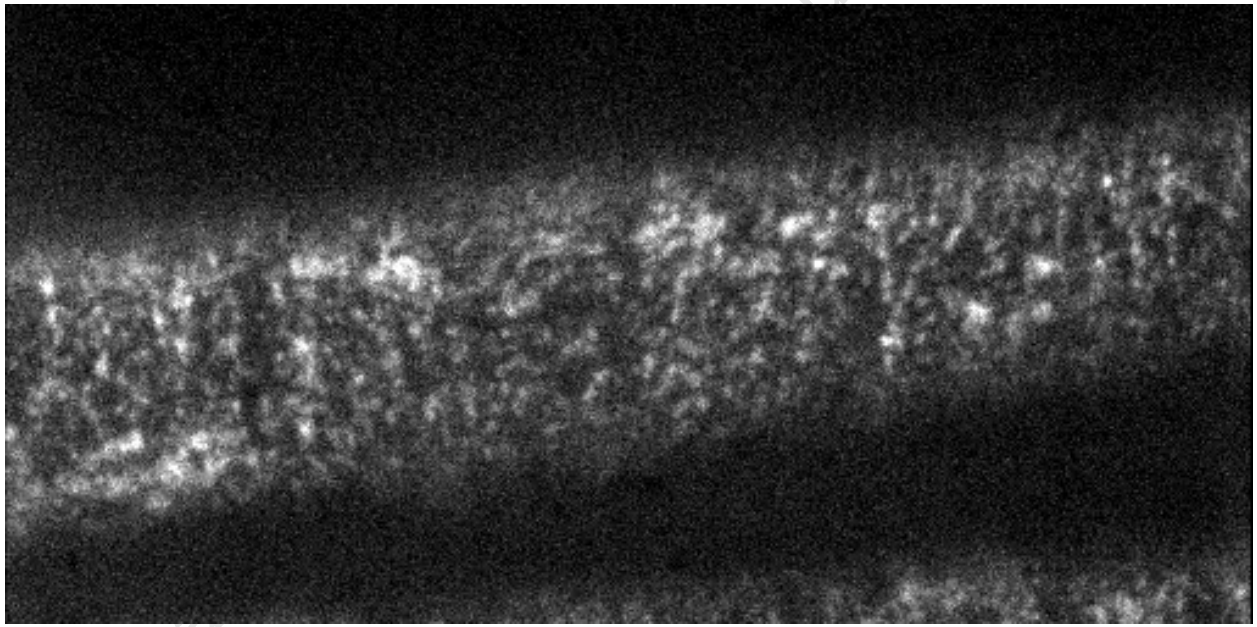
GFP:CESA6
One second image every 30 sec for 8 min



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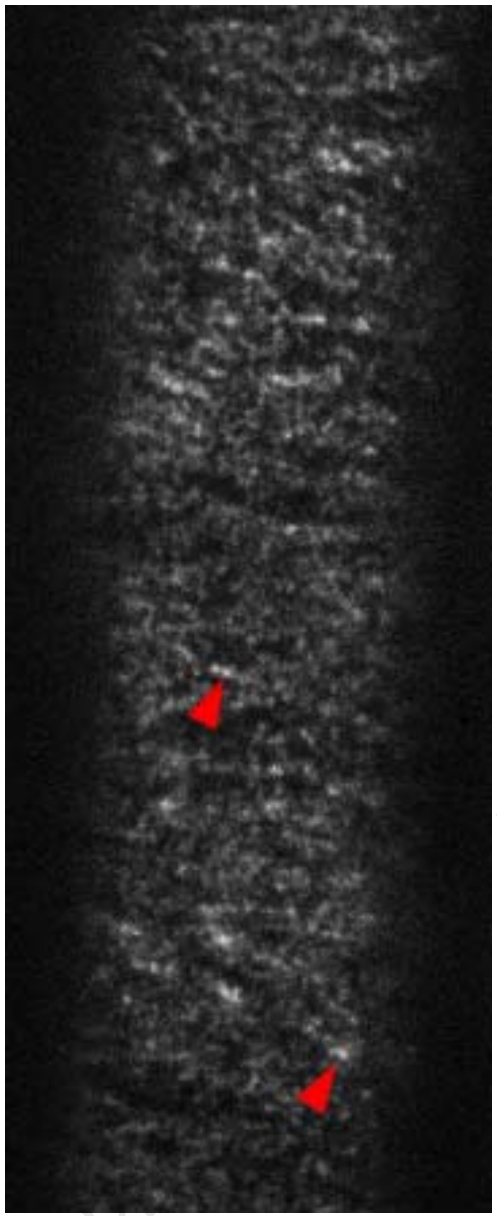
YFP:CESA6 Under Native Promoter



- Citrine YFP genomic fusion rescues *prc1-1*
- 10 minute movie frames acquired every 10 seconds
- Frames averaged in groups of 3
- Rapidly Growing tissue, original translated and required frame alignment for analysis

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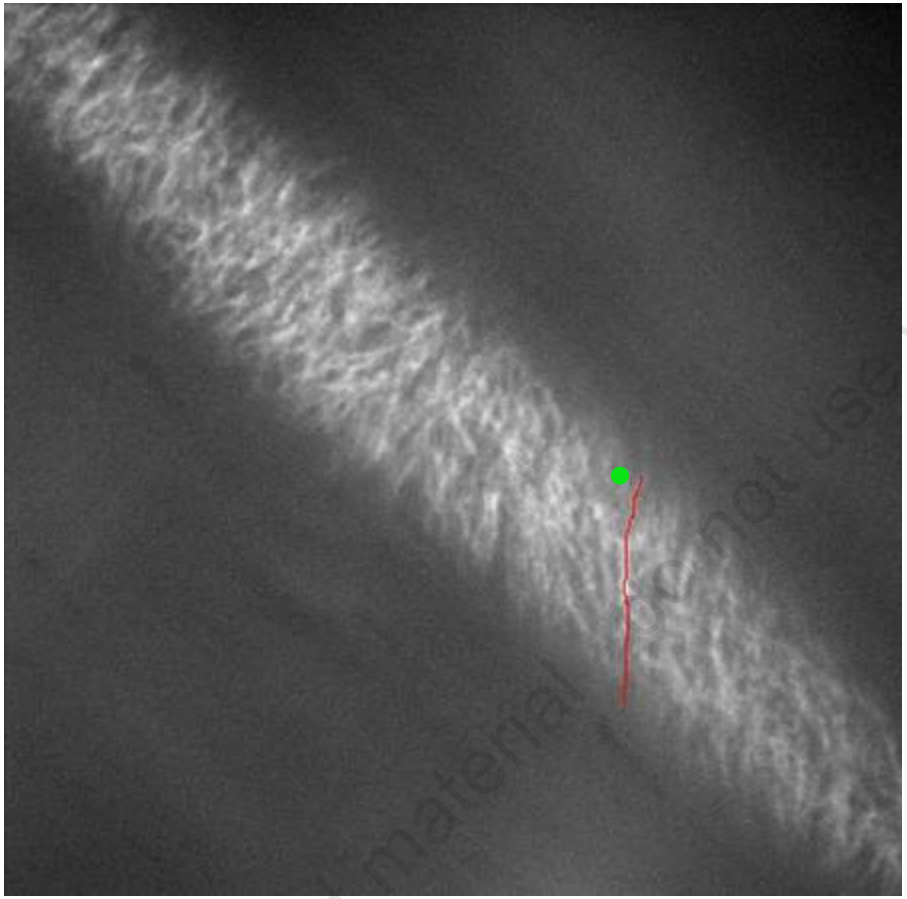
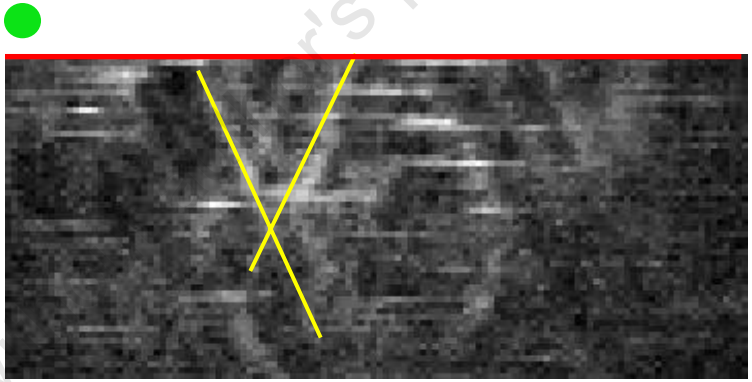
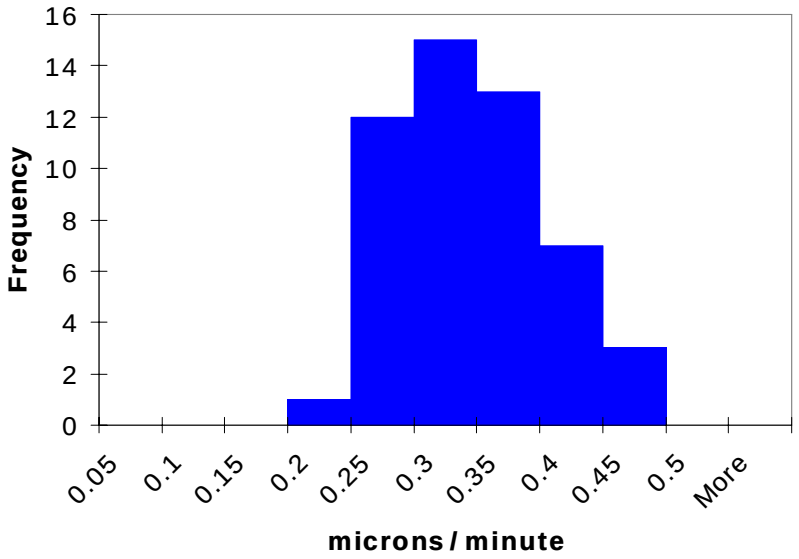
Particle velocities are steady and bi-directional along individual striations







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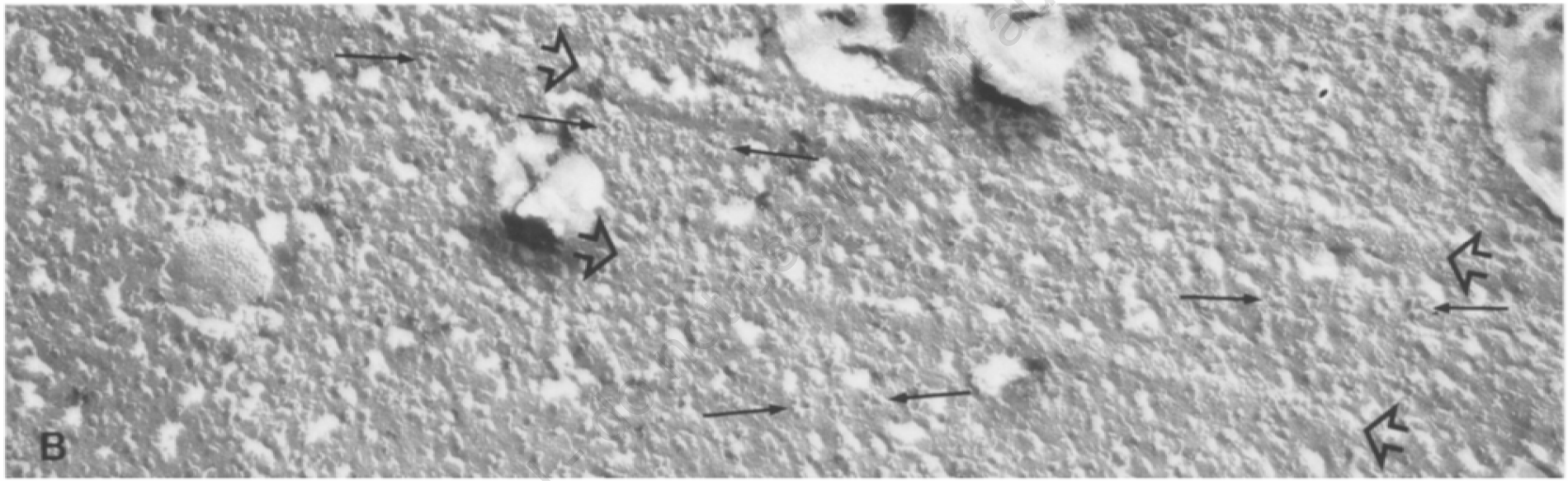
Rows of Rosettes



Giddings and Staehelin Planta 173 (1988)

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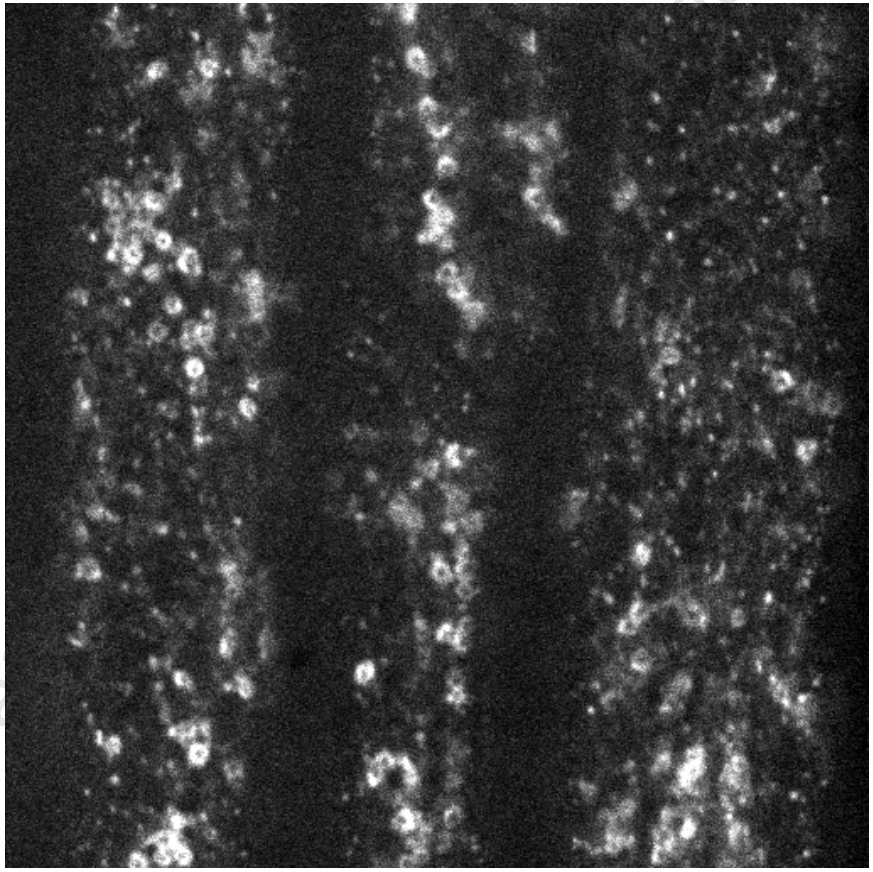
Cellulose synthase assembles in the Golgi



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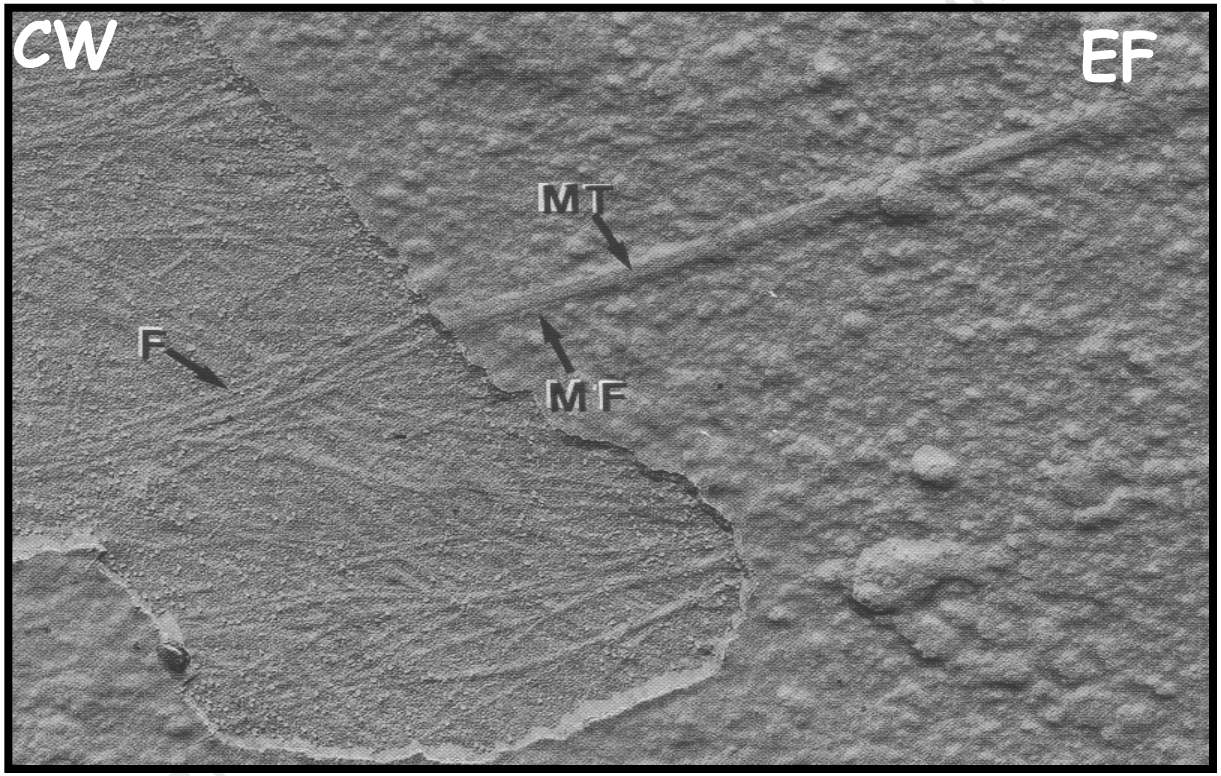
Cellulose deposition is aligned with cortical microtubules



Gunning and Steer, 1996

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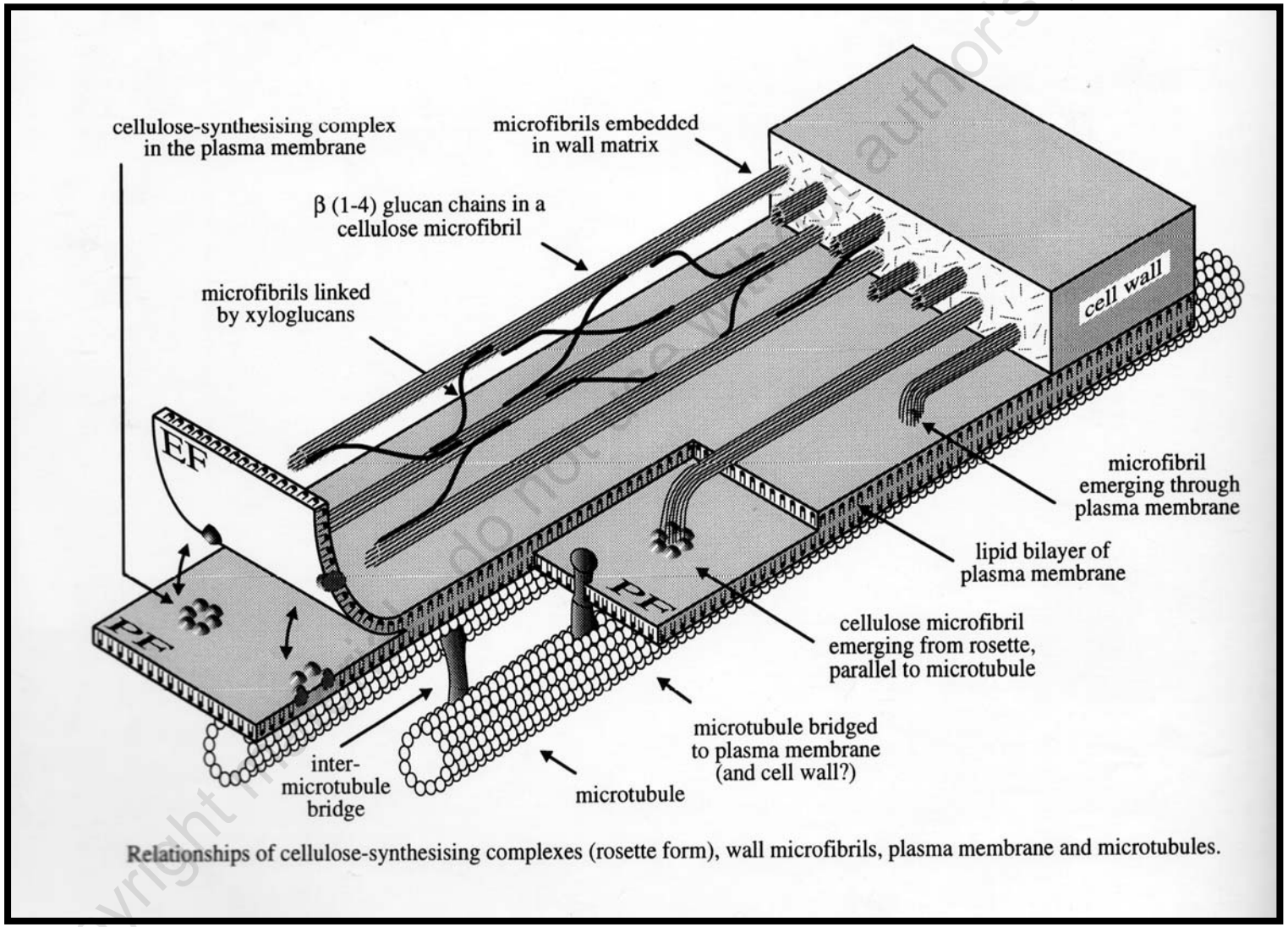
Cellulose synthase may be linked to the cytoskeleton



Relationships of cellulose-synthesising complexes (rosette form), wall microfibrils, plasma membrane and microtubules.

Gunning and Steer, 1996

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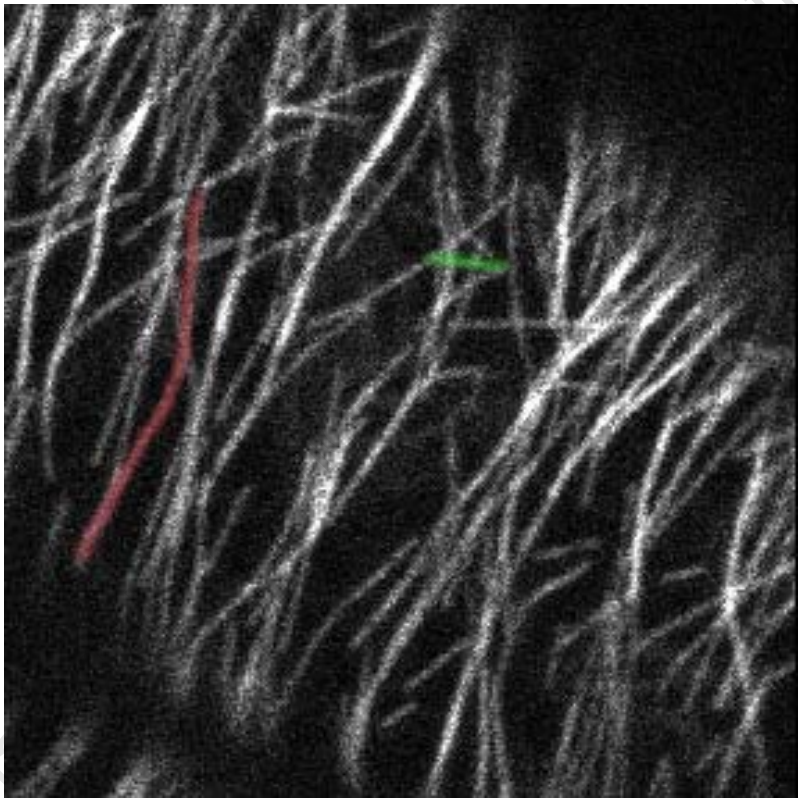
Cortical microtubules treadmill and bundle in an angle dependent manner



Dave Ehrhardt

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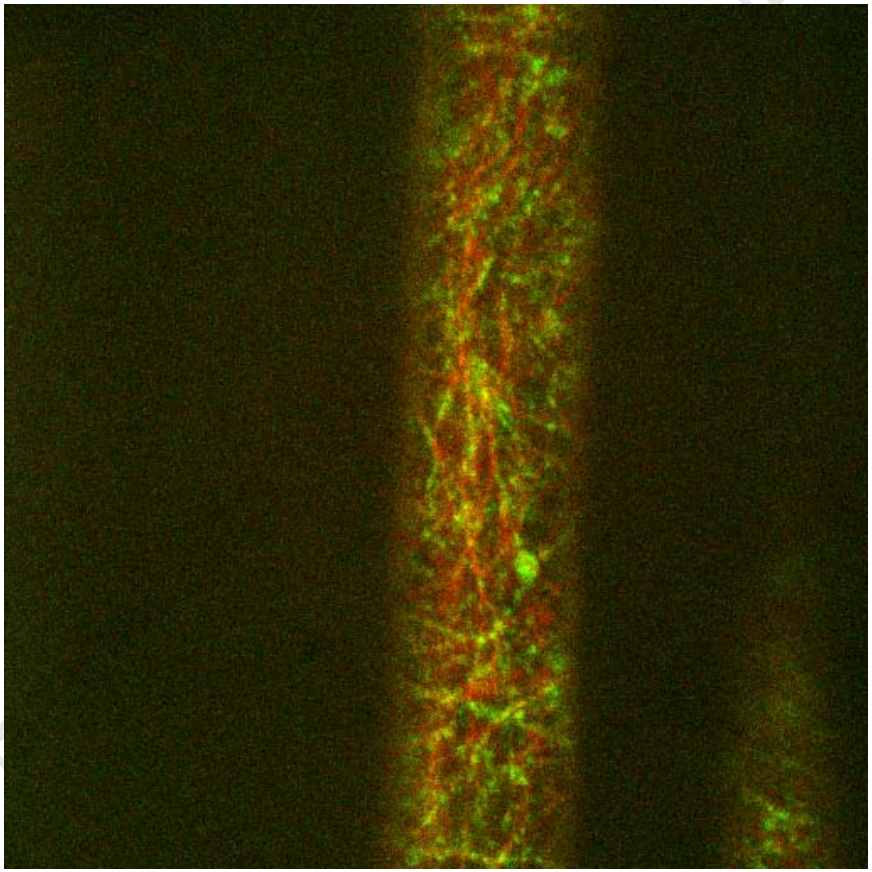
Cellulose synthase (green)
tubulin (red)



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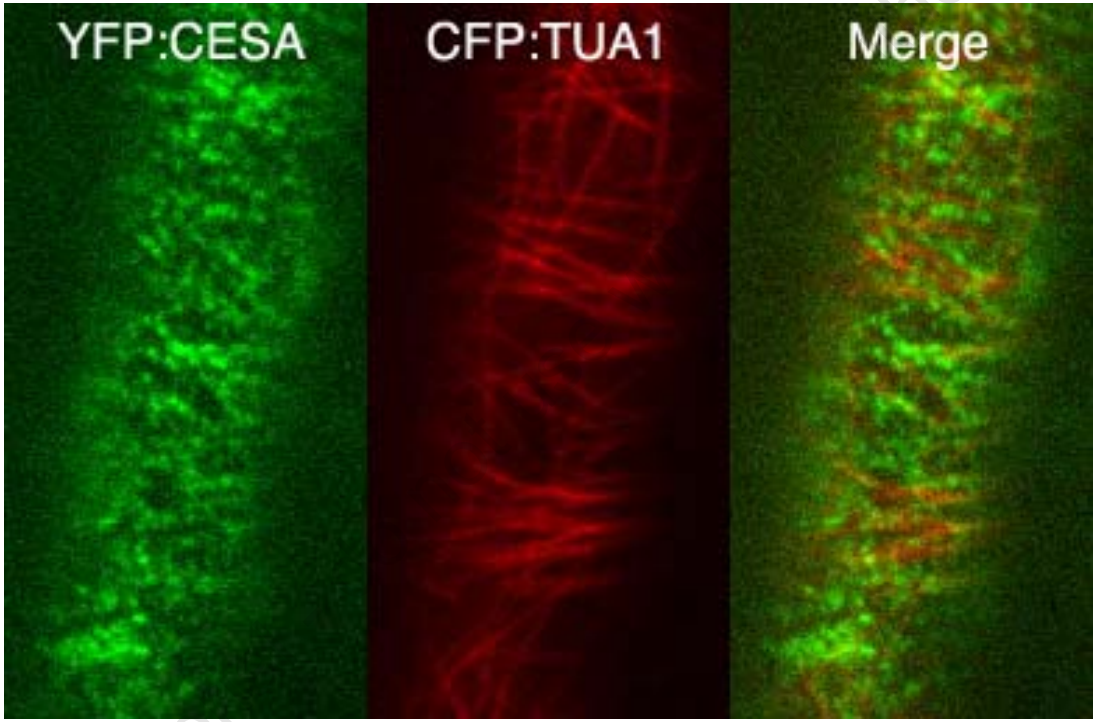
Light induced re-orientation is coordinated



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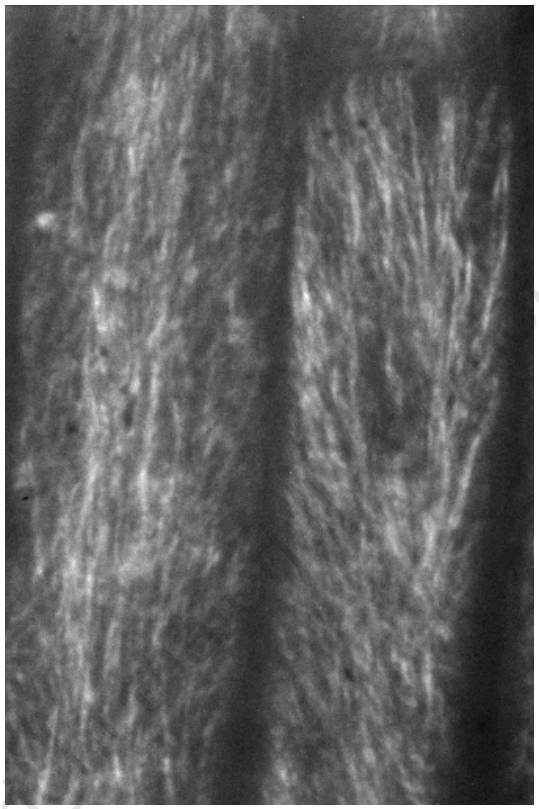
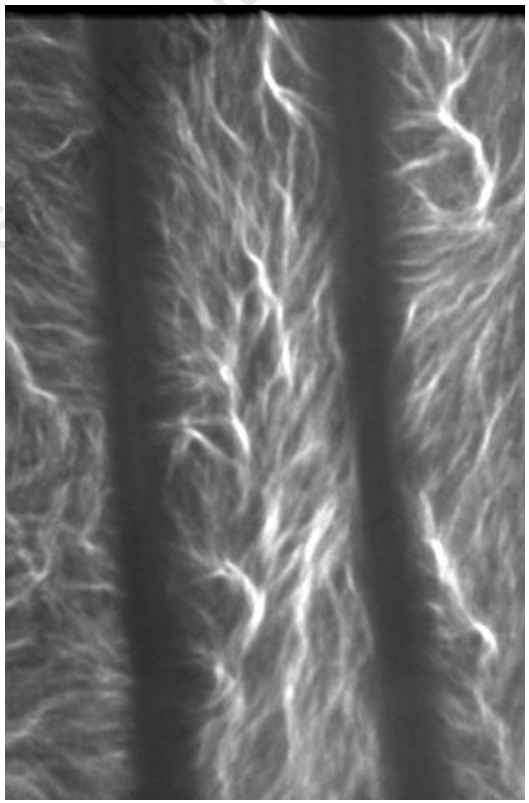
Microtubules Are Important for Maintaining Normal Patterns of CESA Movement

No oryzalin



10uM Oryzalin 45min



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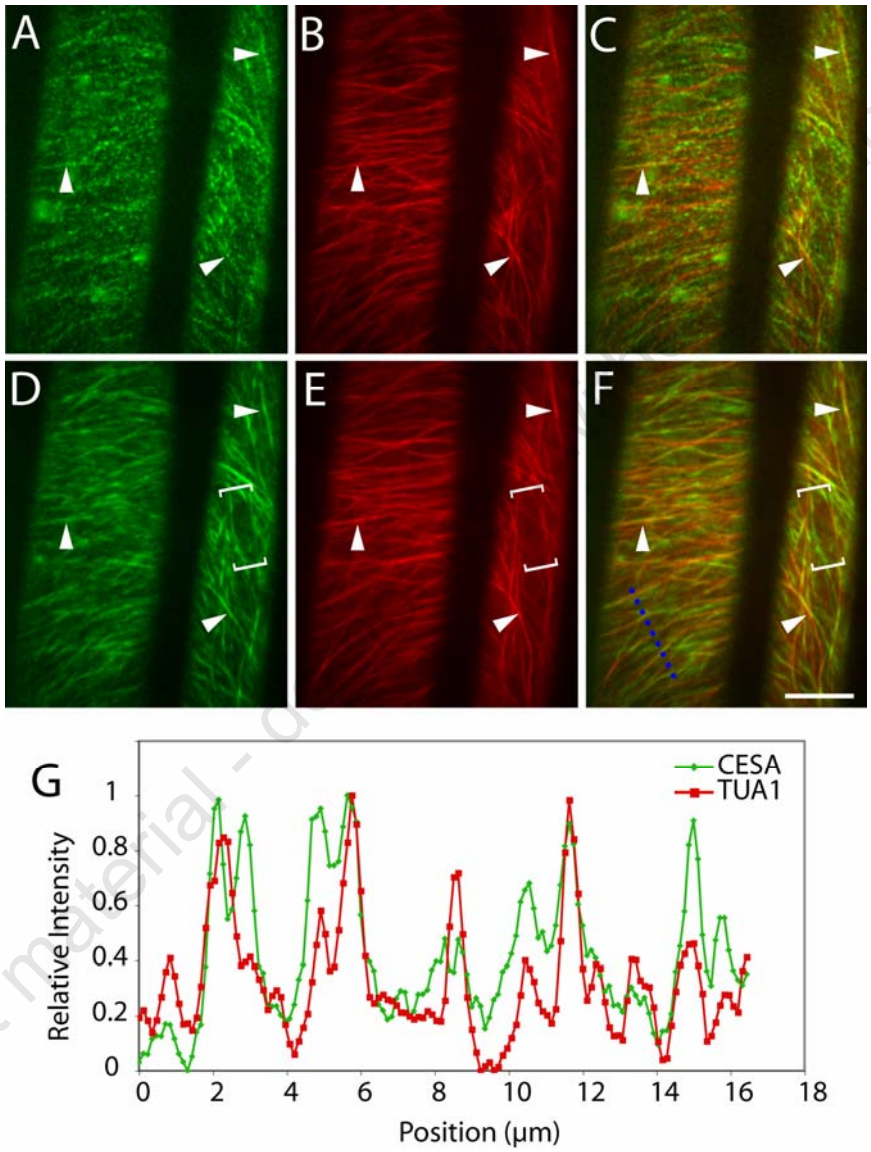
YFP-CESA6 and CFP-TUA1 Co-localize ~60%



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Something else is involved

Oryzalin

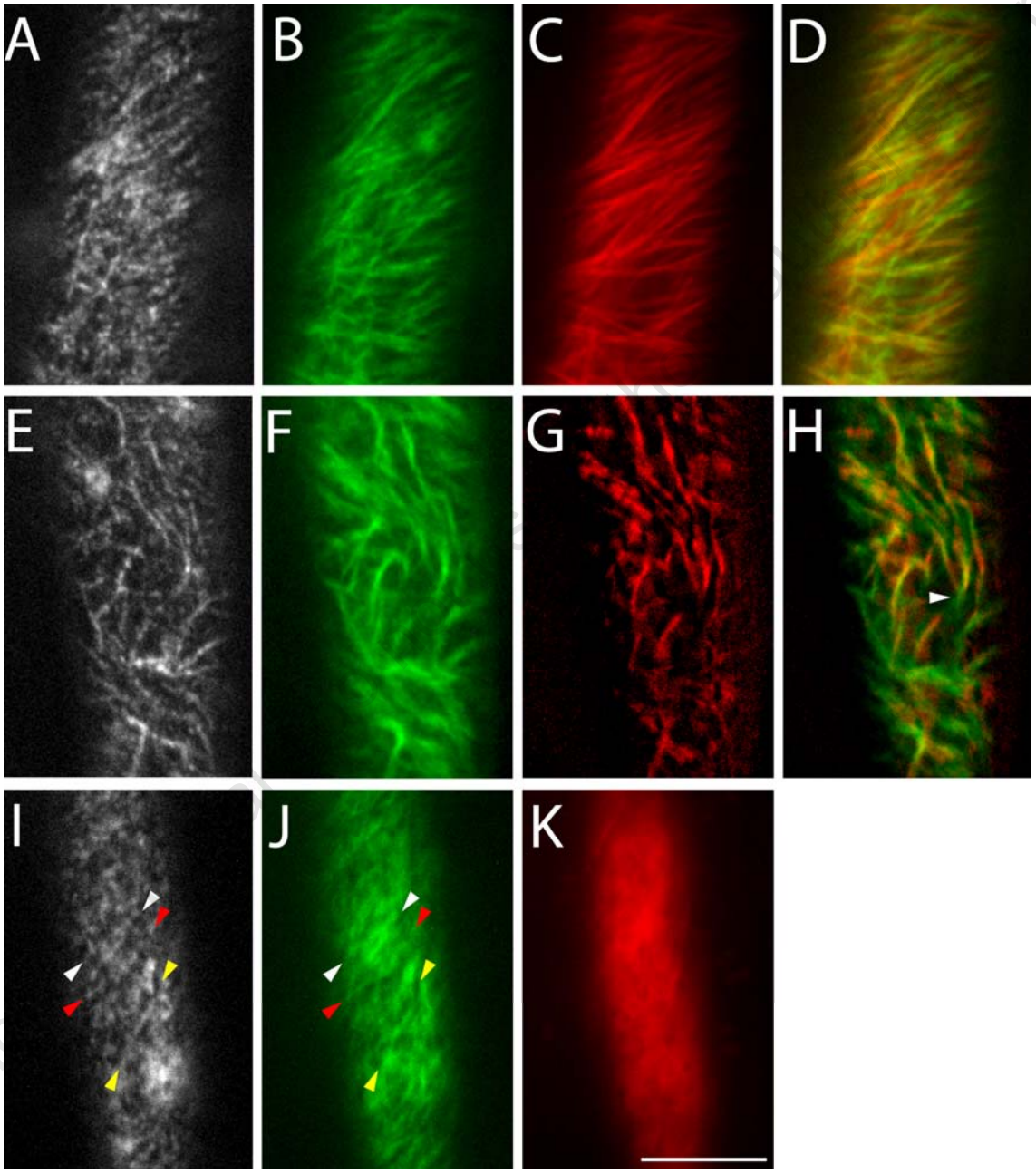
Control

10 uM

Time-3.6h

20 uM

Time-7h



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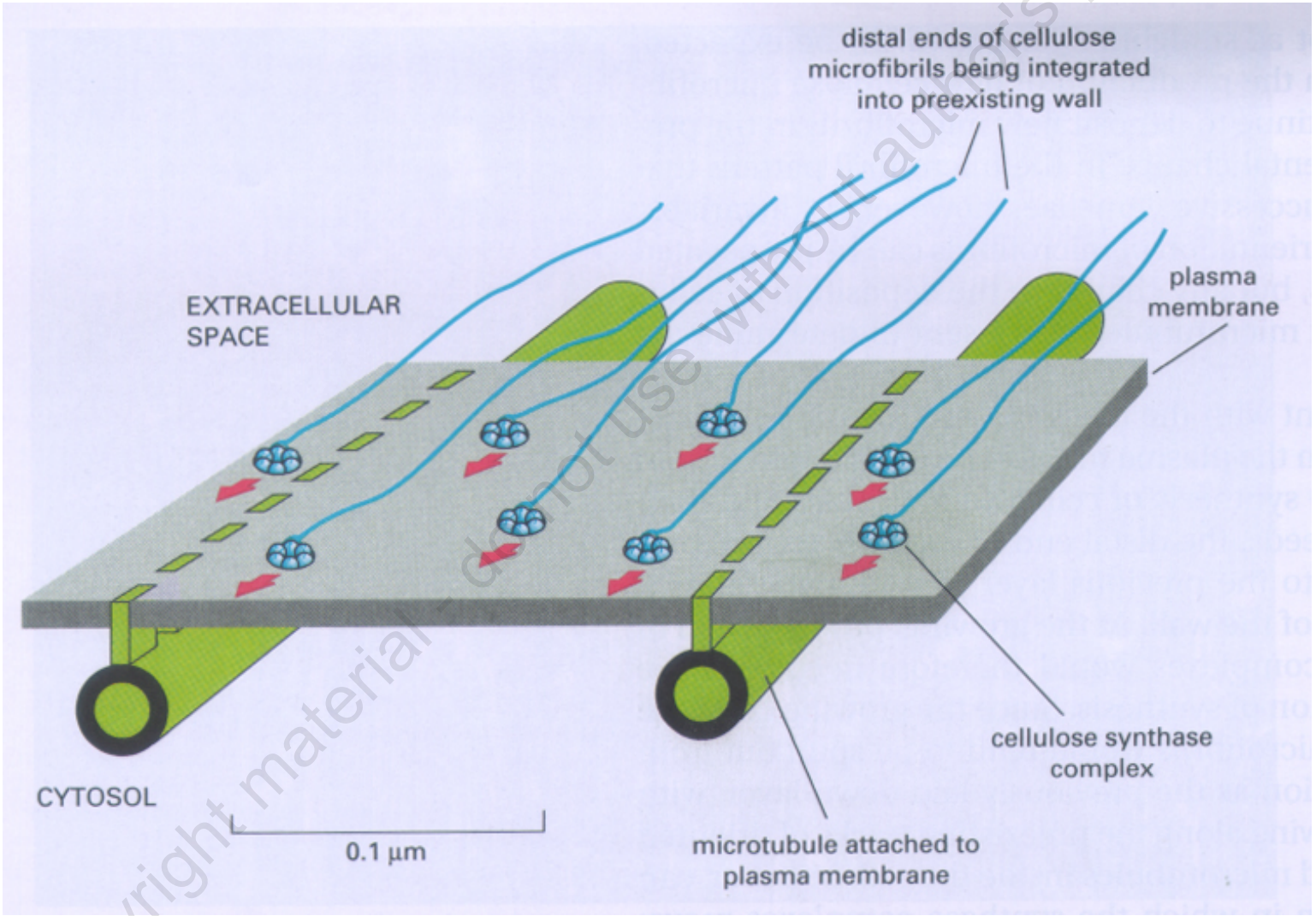
Bumper model of cellulose synthesis



Alberts, 2002

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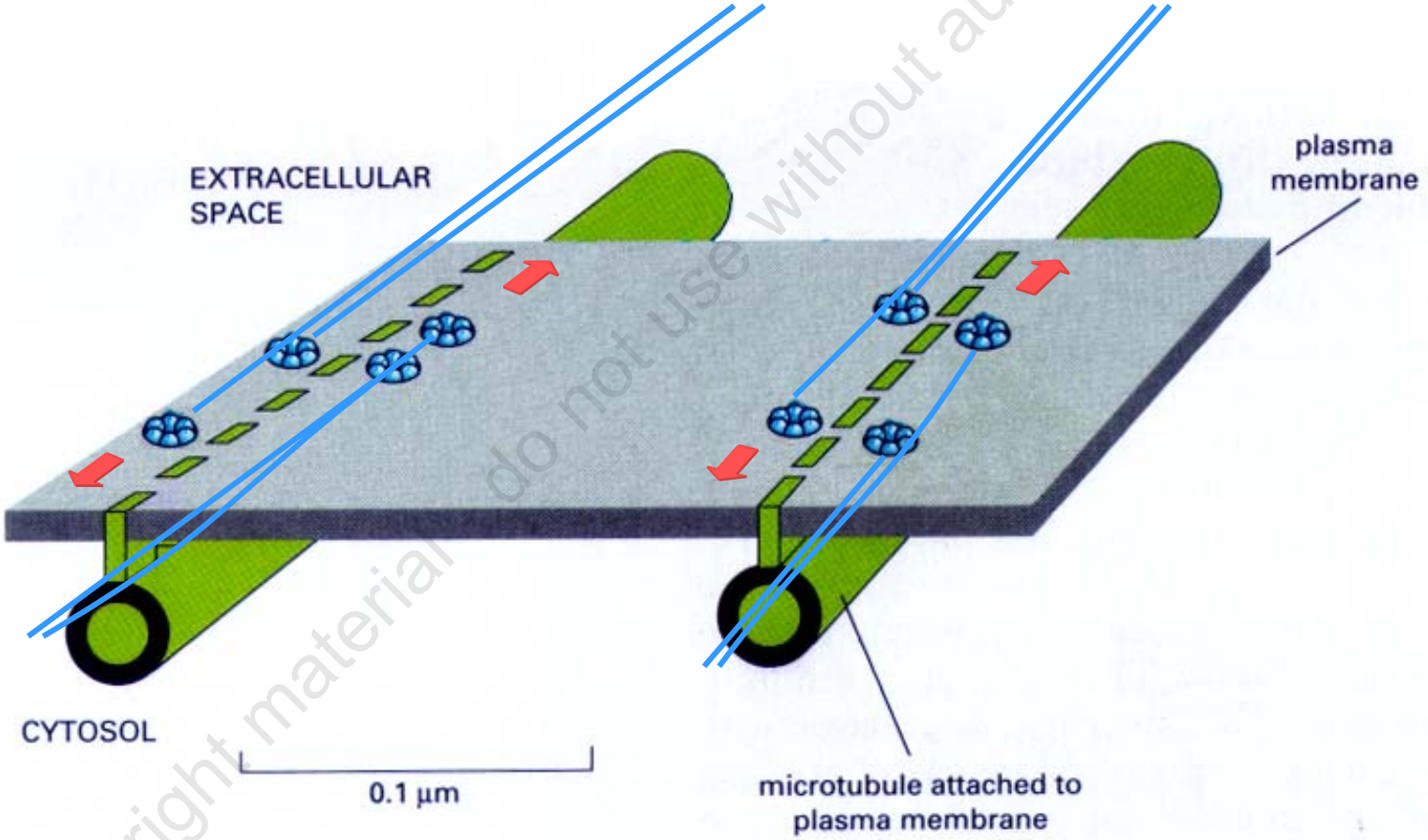
Revised bumper model of cellulose synthesis



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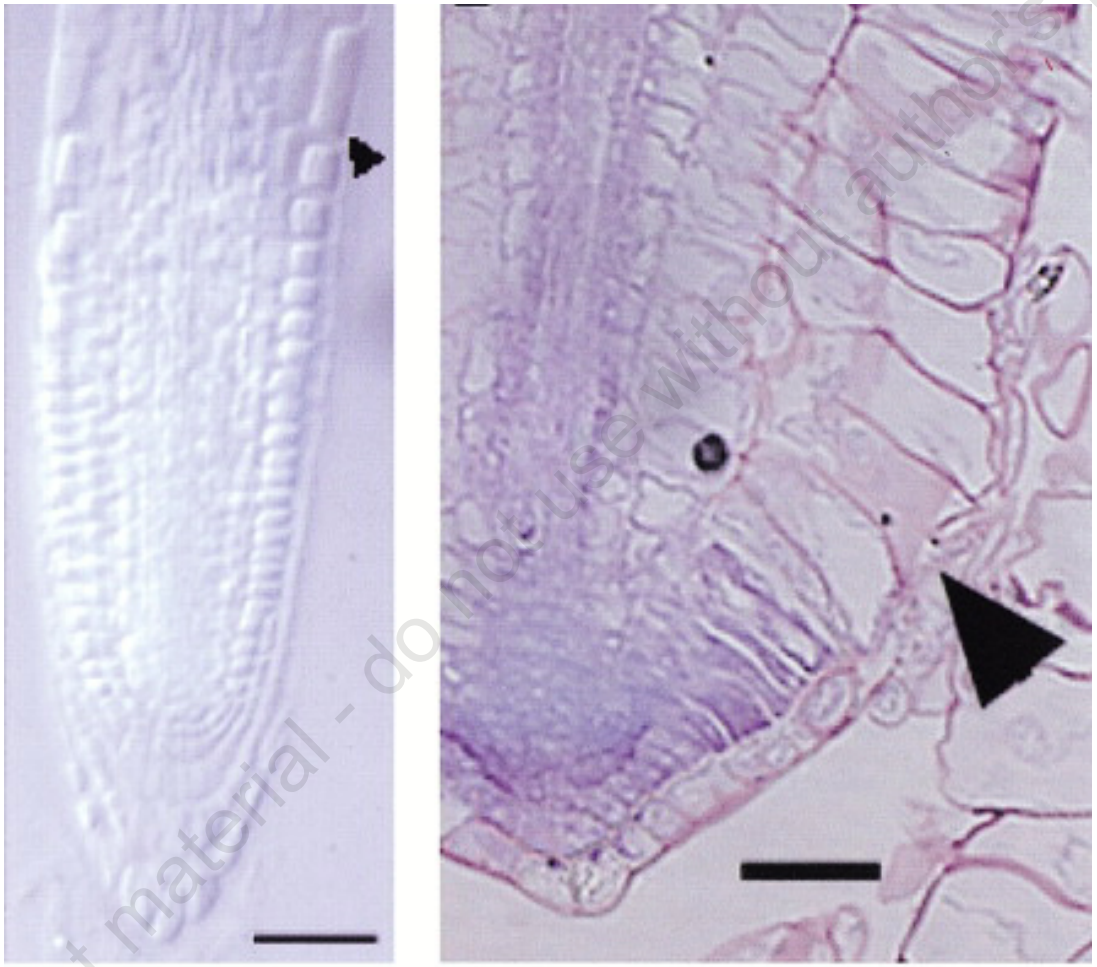
Cobra phenotype on high sucrose media



Wt

Mutant

Schindelman et al, *Genes & Development* 15,1115 (2001)

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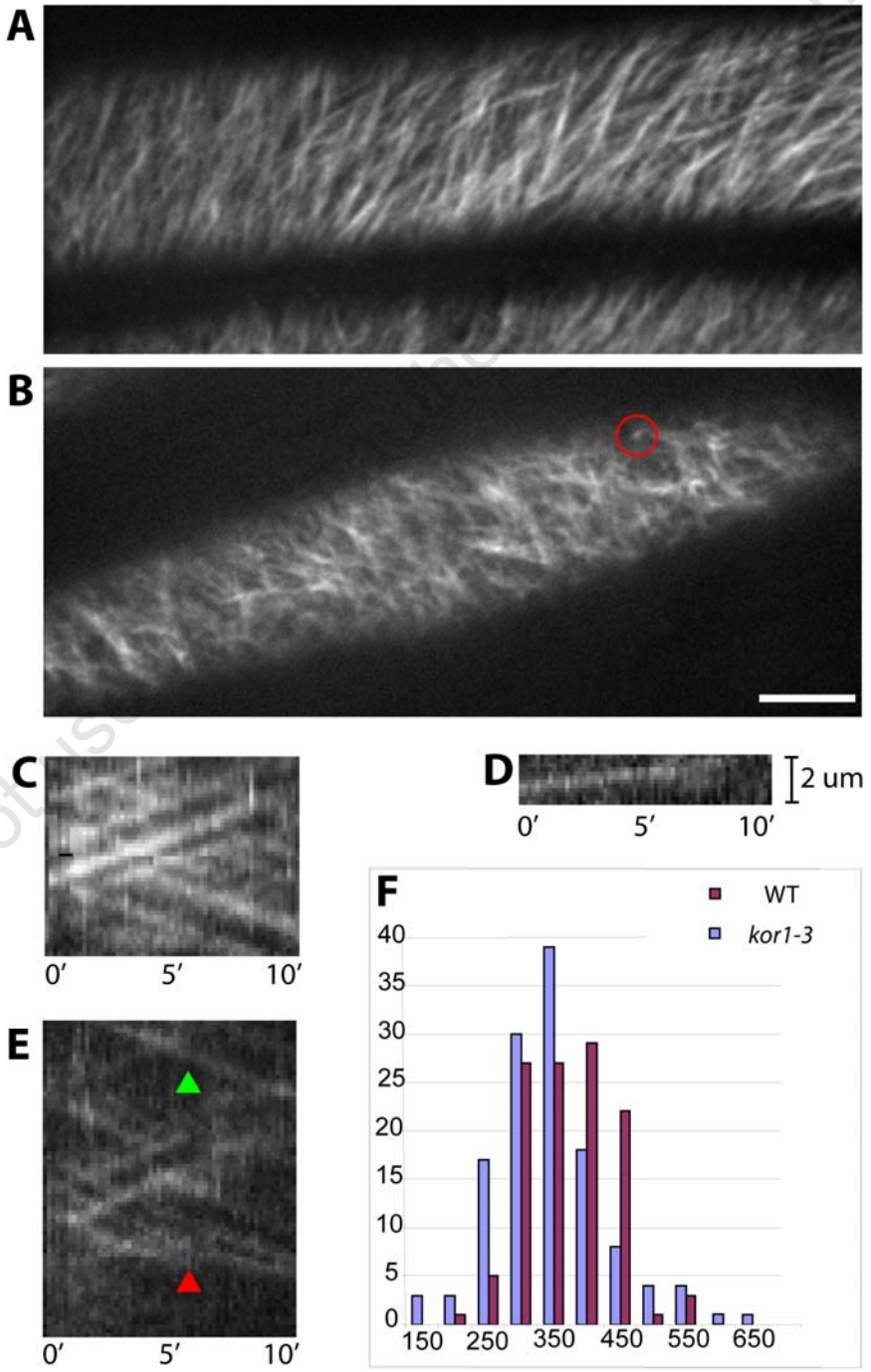
Kor alters CESA6 movement



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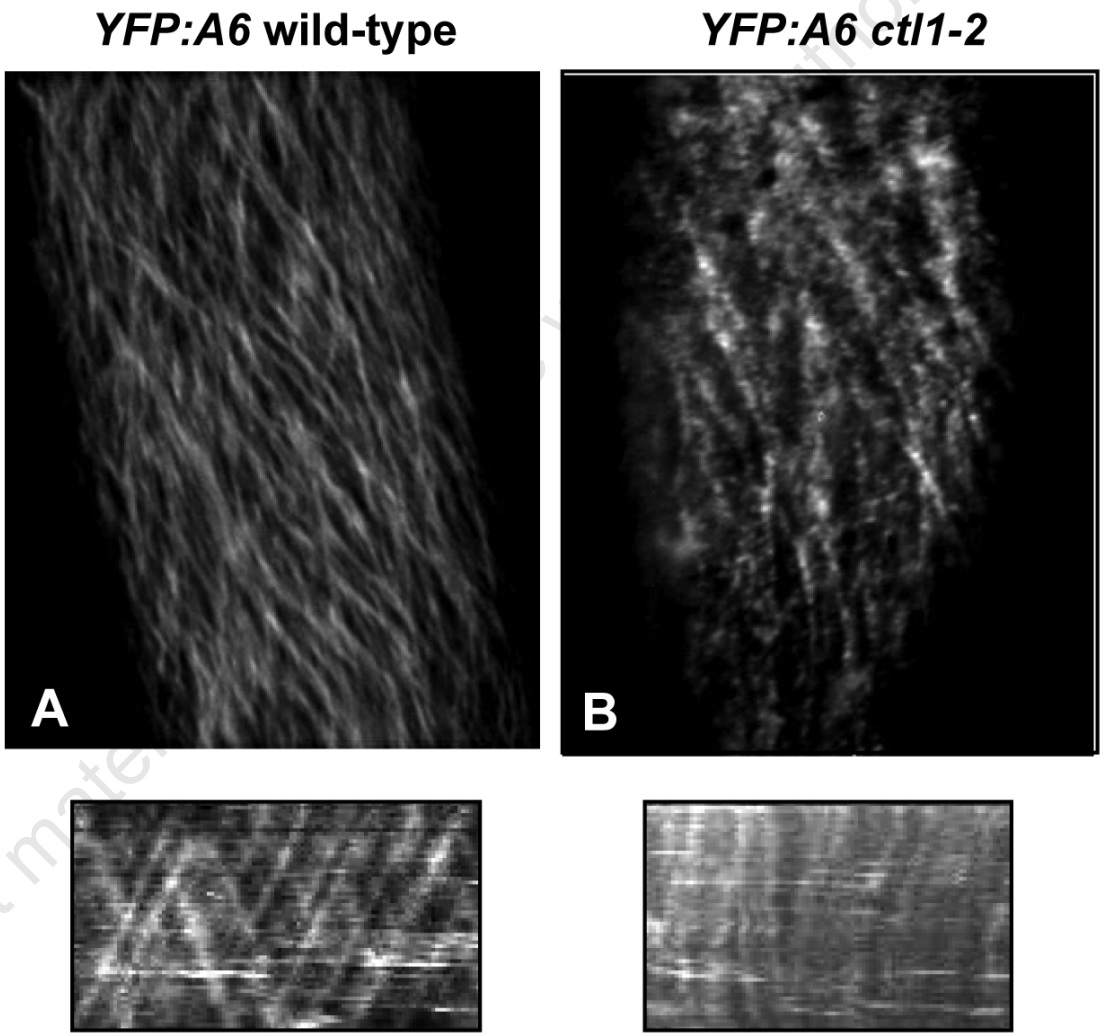
ctl1 alters the rate of movement of cellulose synthase



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In conclusion: Some imaging questions

- What is the structure of the polysaccharides?
- What is the fine structure of the wall?
- How does it assemble?
- How does the structure change during hydrolysis (or in mutants)?
- How is lignin associated?
- What is the structure of cellulose synthase?
- How does structure of the complex control the properties of cellulose?

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Acknowledgements

Dave Ehrhardt

Alex Paredez

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