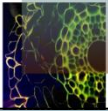


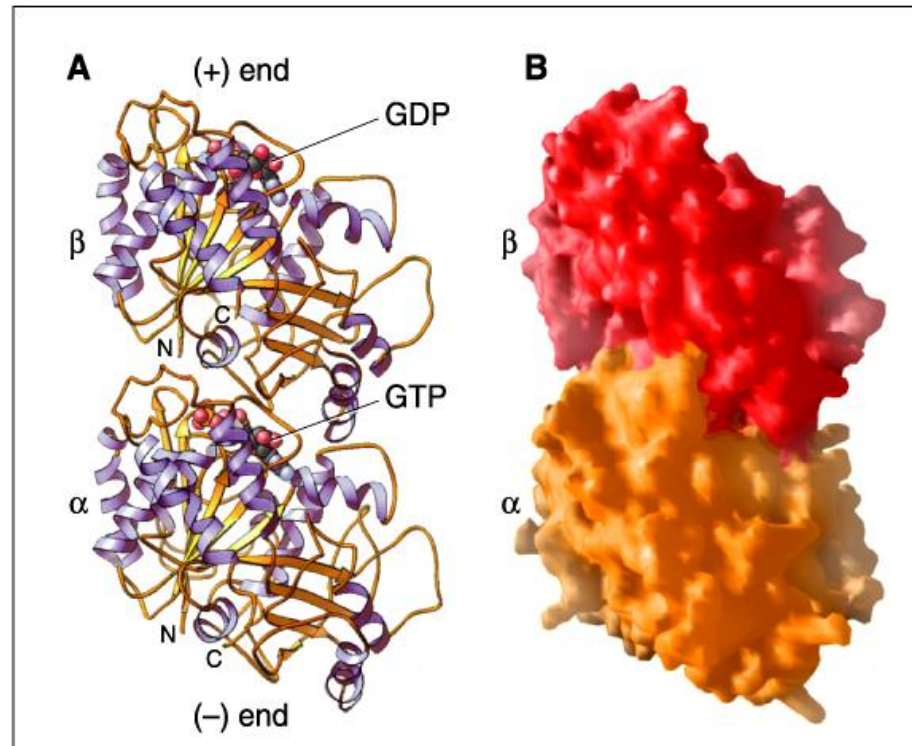
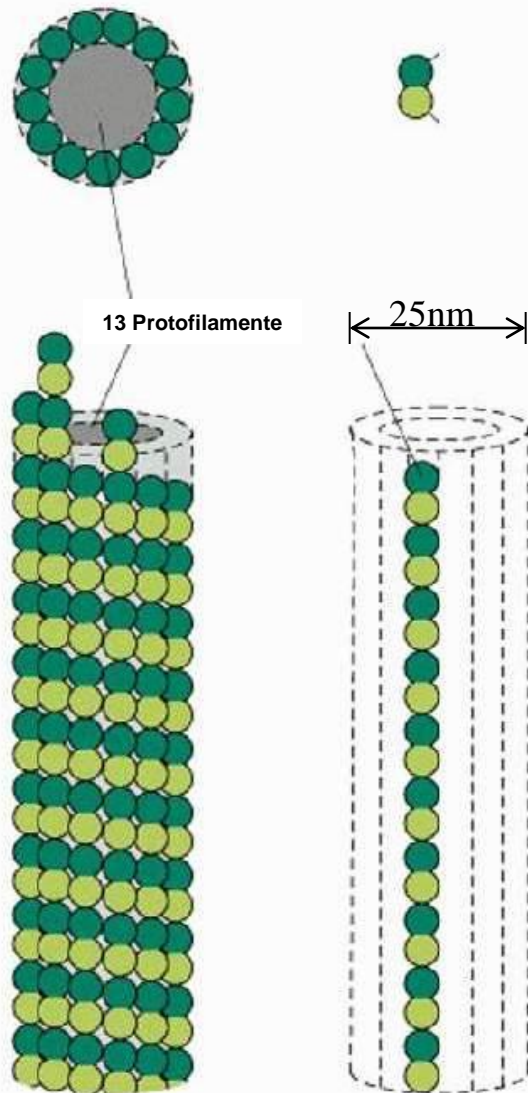
# Plant Cell, Development & Ultrastructure

**Plant Cell Biology Labs**

Download at:  
<http://goo.gl/111Tha>

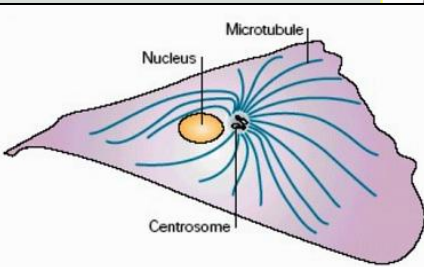
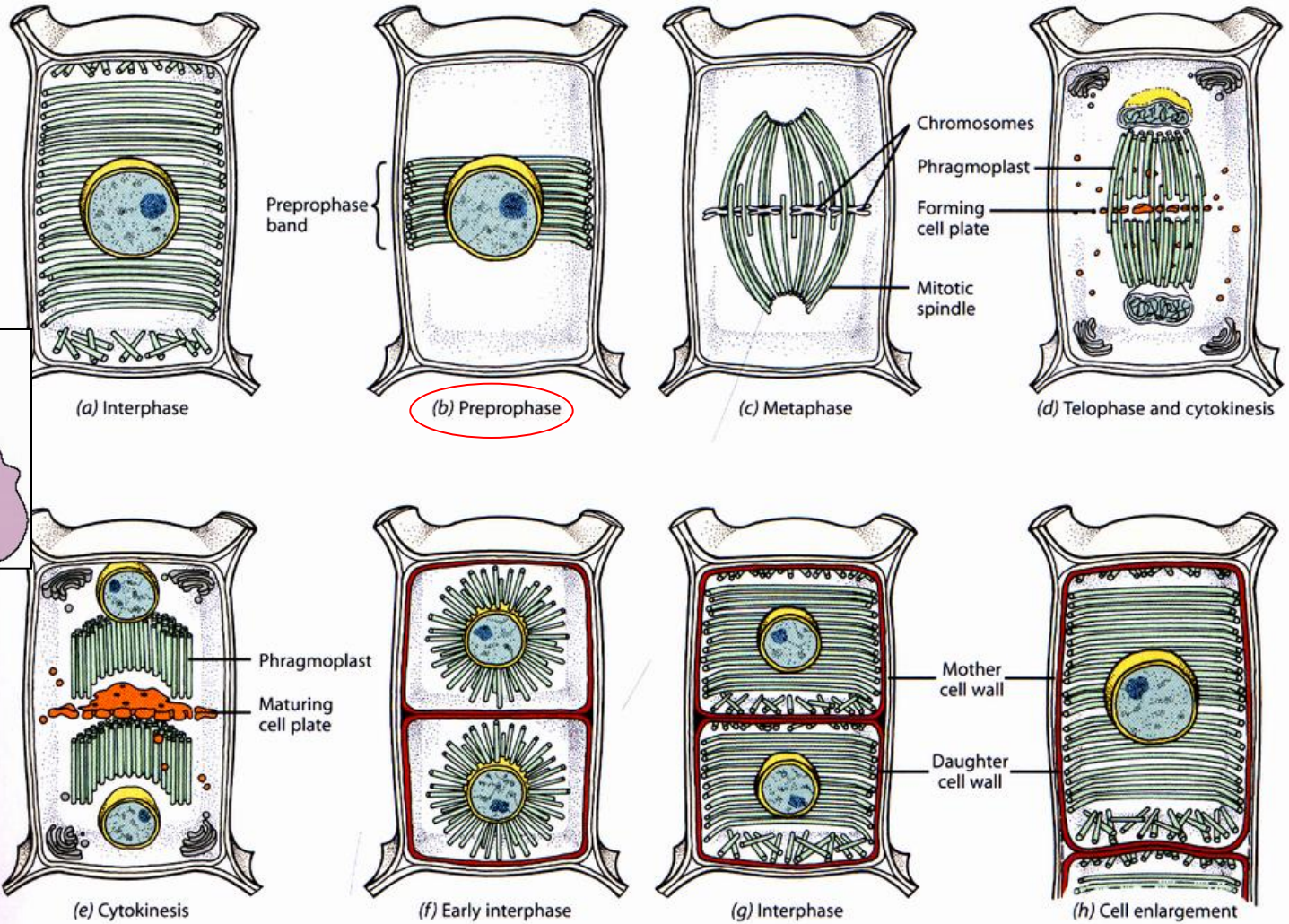


# Microtubules



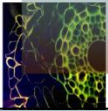
Elsevier Science (USA) items and derived items copyright © 2002, Elsevier Science (USA). All rights reserved.

# Plant MT Cytoskeleton

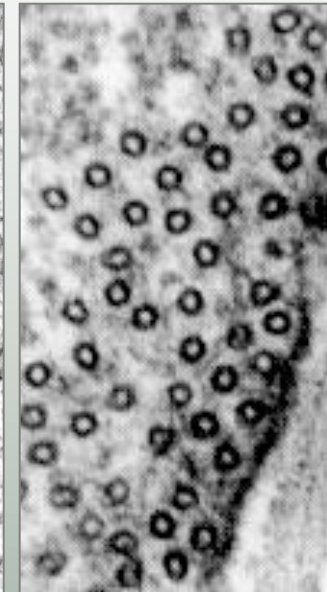
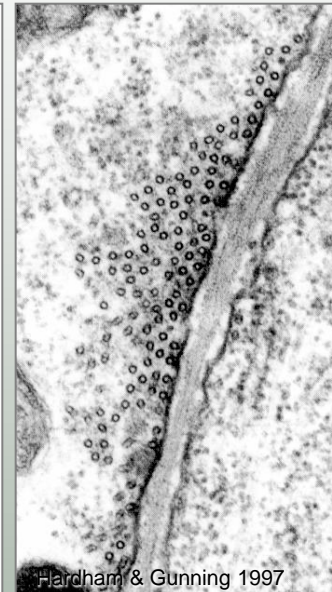
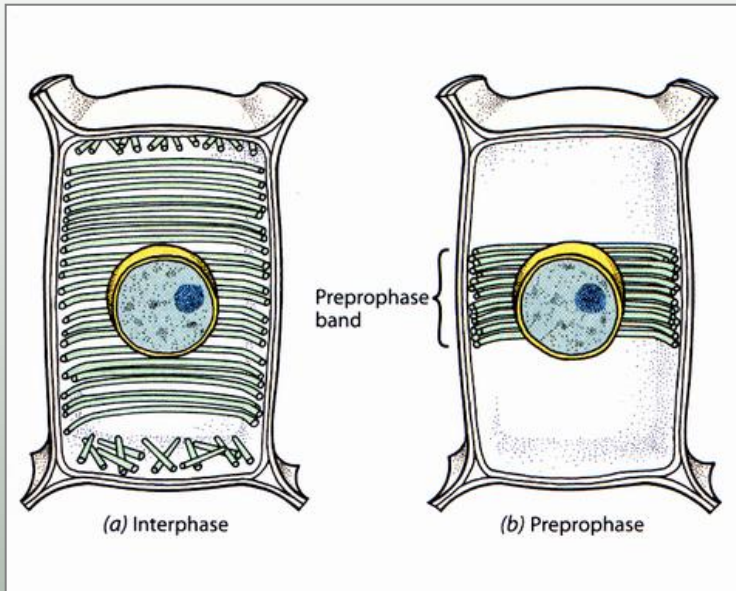


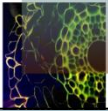
Animal MT cytoskeleton





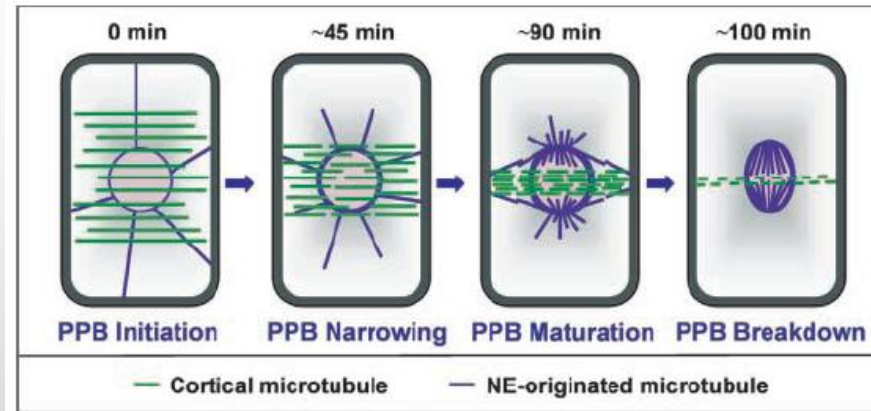
# Preprophase Band of MTs





# Preprophase Band Formation

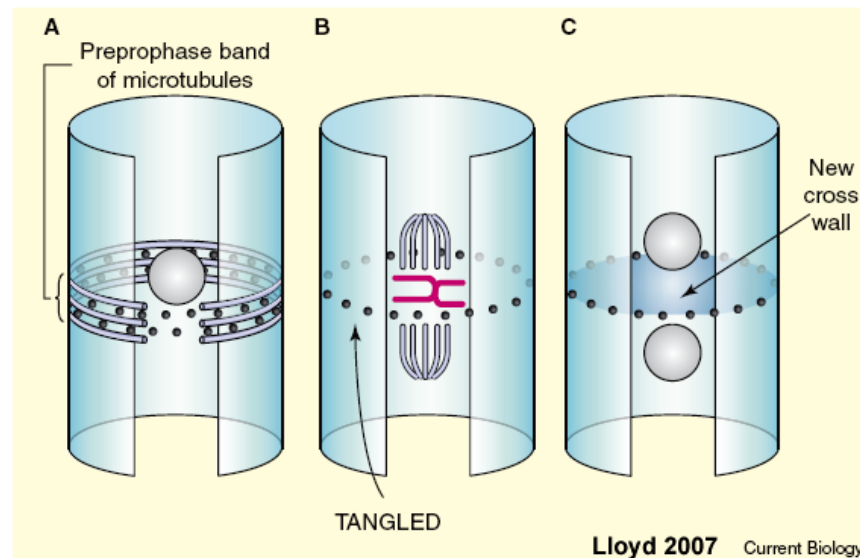
## Time course of PPB-formation



Dhonukshe & Gadella 2003

**Proposed function of PPB is to determine the division plane by inserting specific markers in the plasmamembrane**

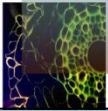
**One of the best candidates for such a marker is the tan-Protein**



**TAN-mutants in maize**

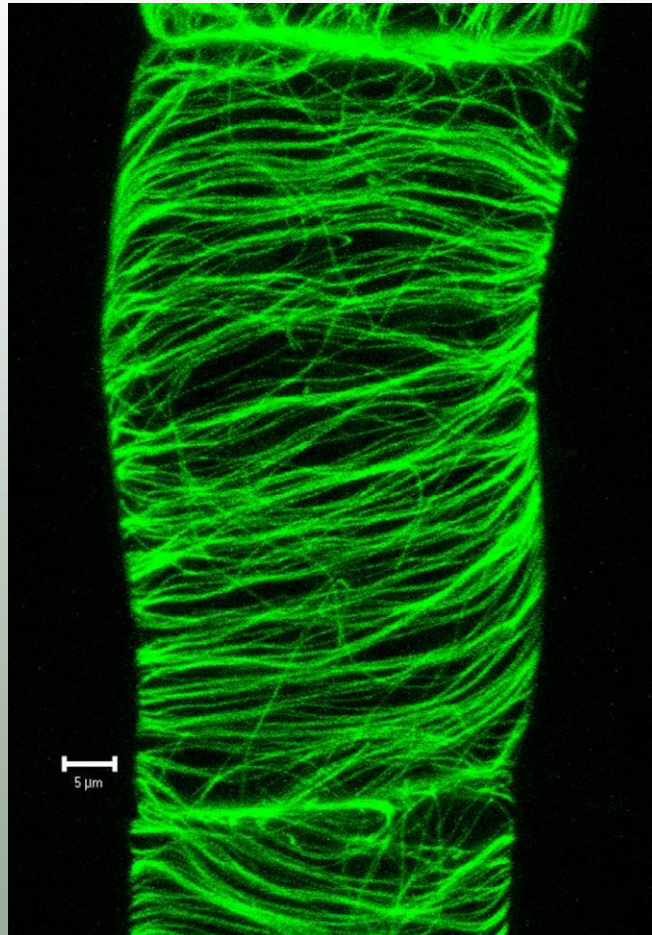
**Phenotype:**  
**Cell plate misorientation**

**Function:**  
**TAN-protein interacts with kinesin**

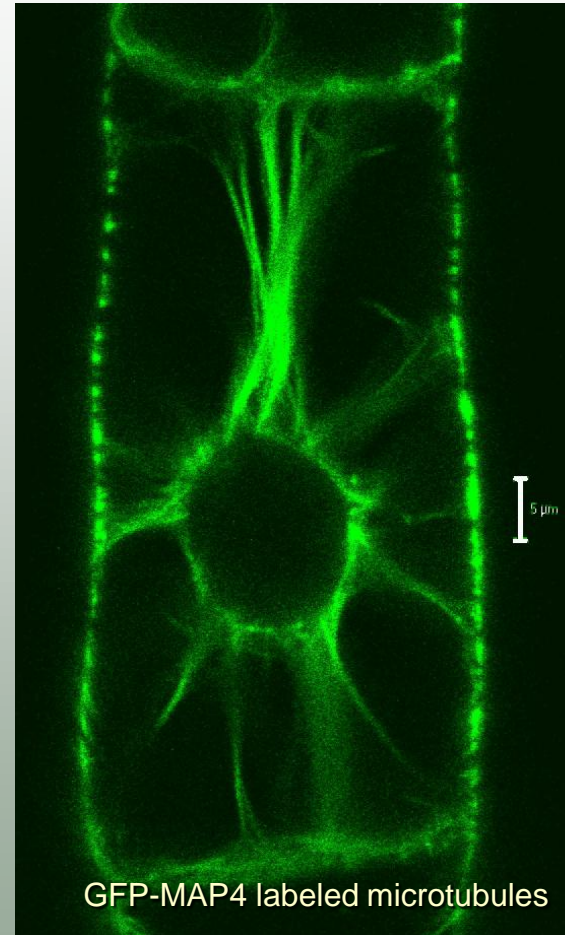


# Transition from Interphase to Pro-Metaphase

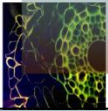
Cortical MT-System



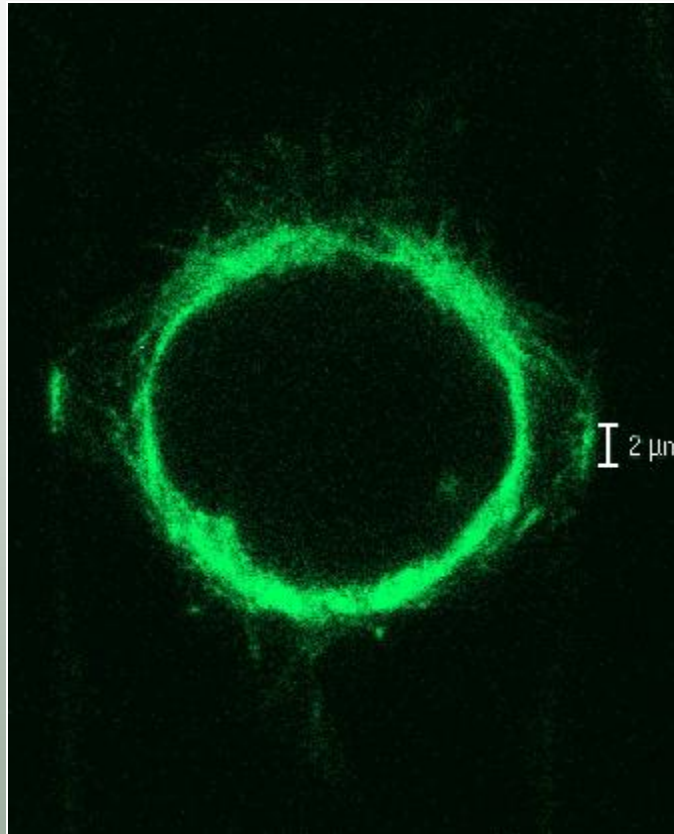
Perinuclear MT-System



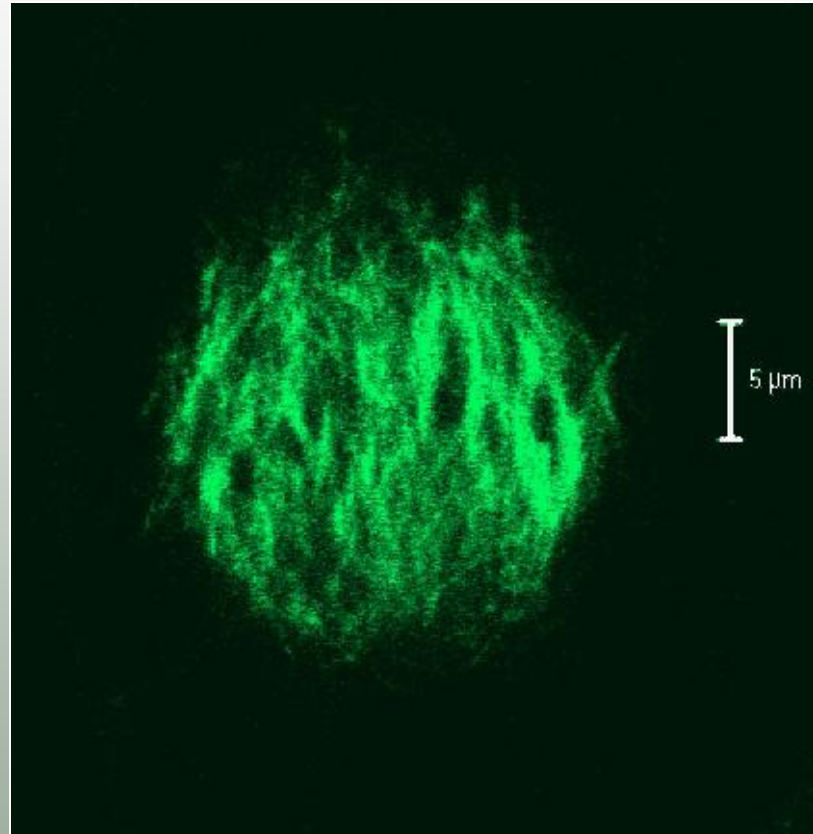




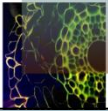
# Secondary Cell Wall Formation



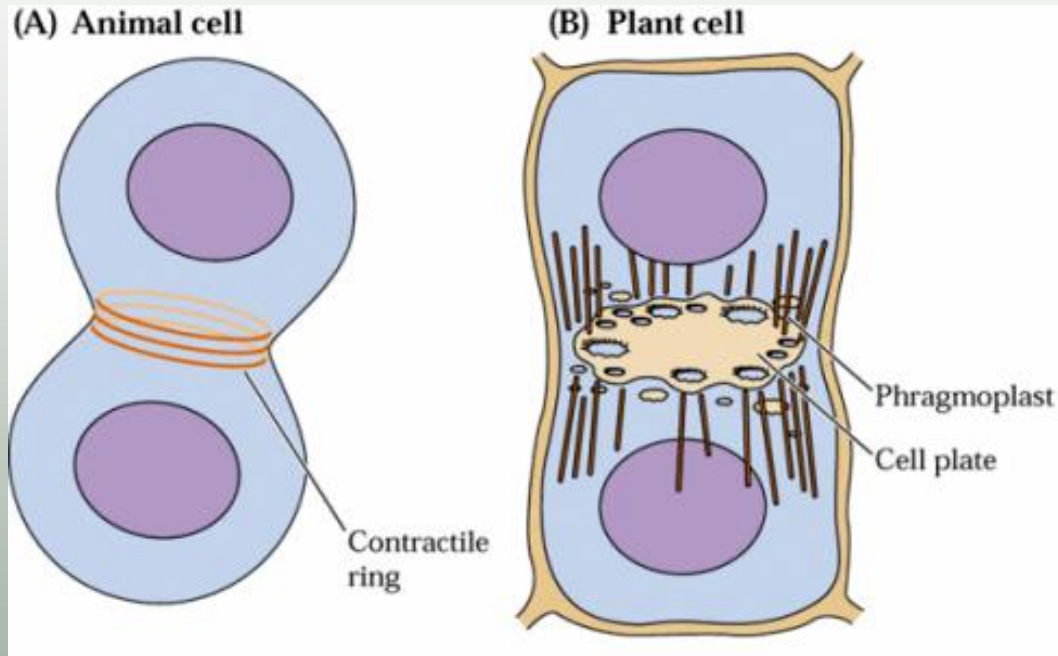
Gmycledetail1



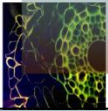
Gmycledetail2



# Cytokinesis



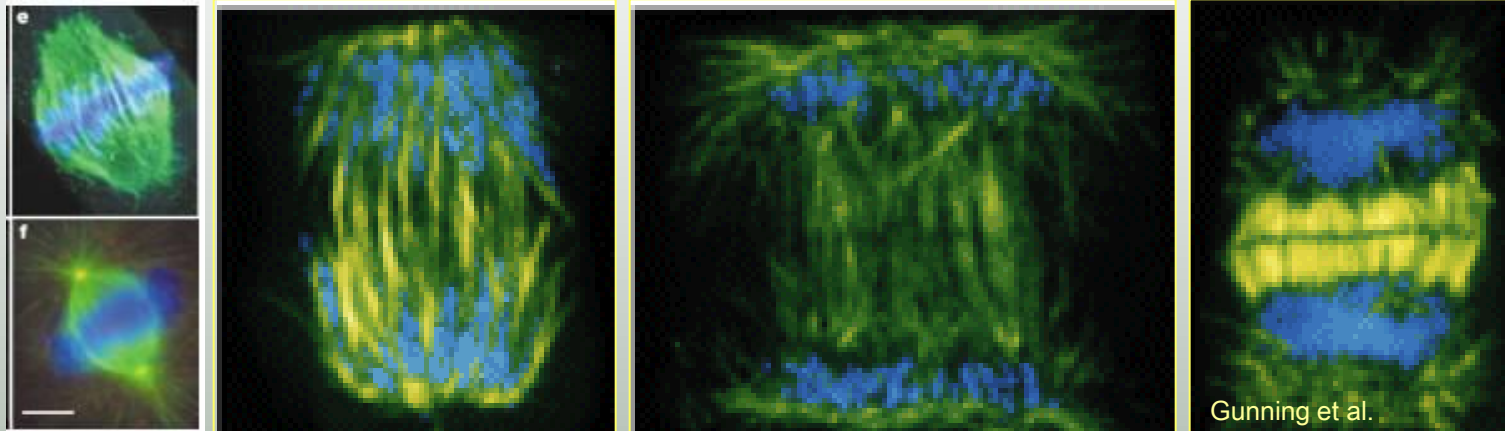




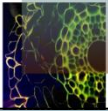
# Phragmoplast Formation



plant



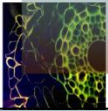
animal



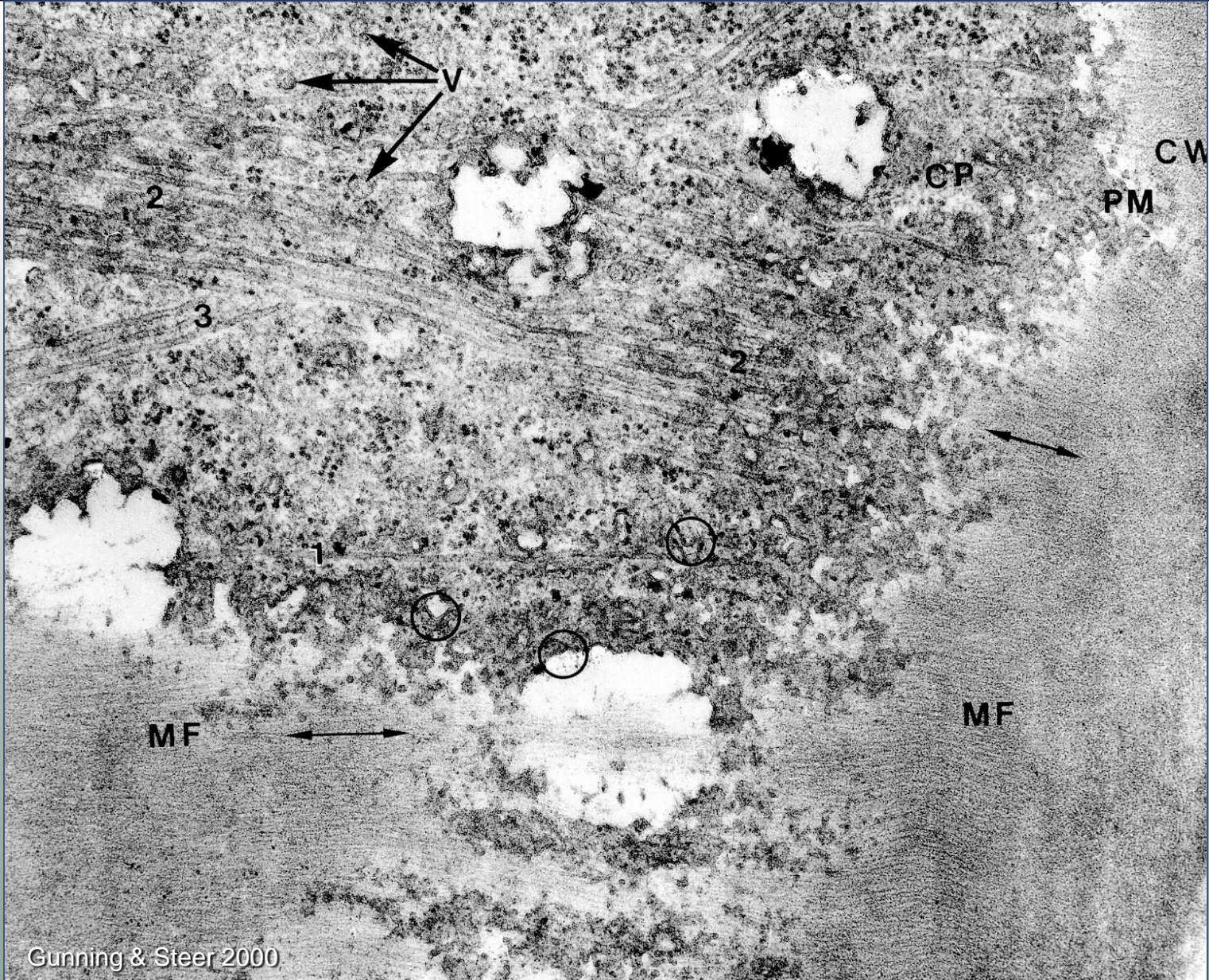
# Cortical MTs in Young Cells



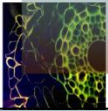




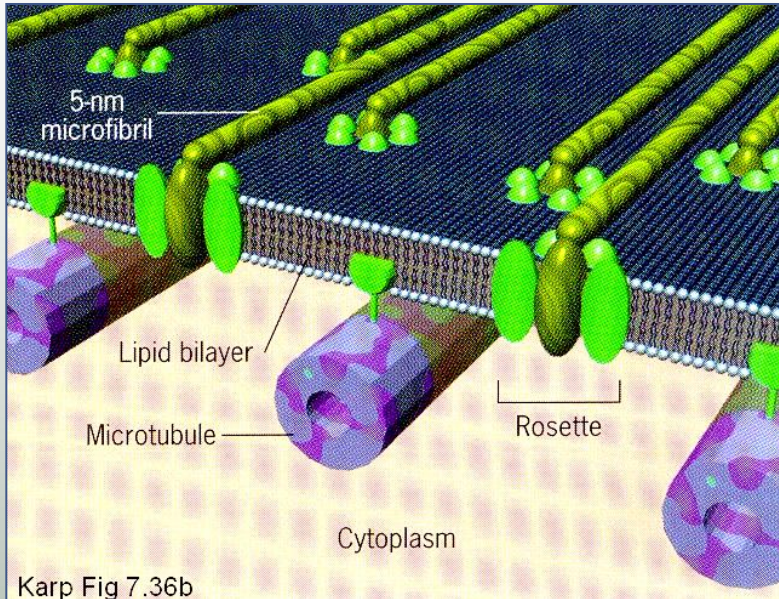
# MT-Microfibril Co-Alignment



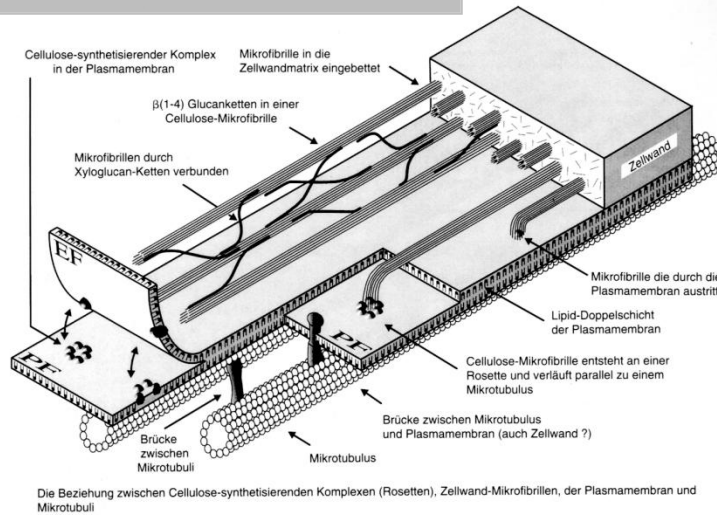
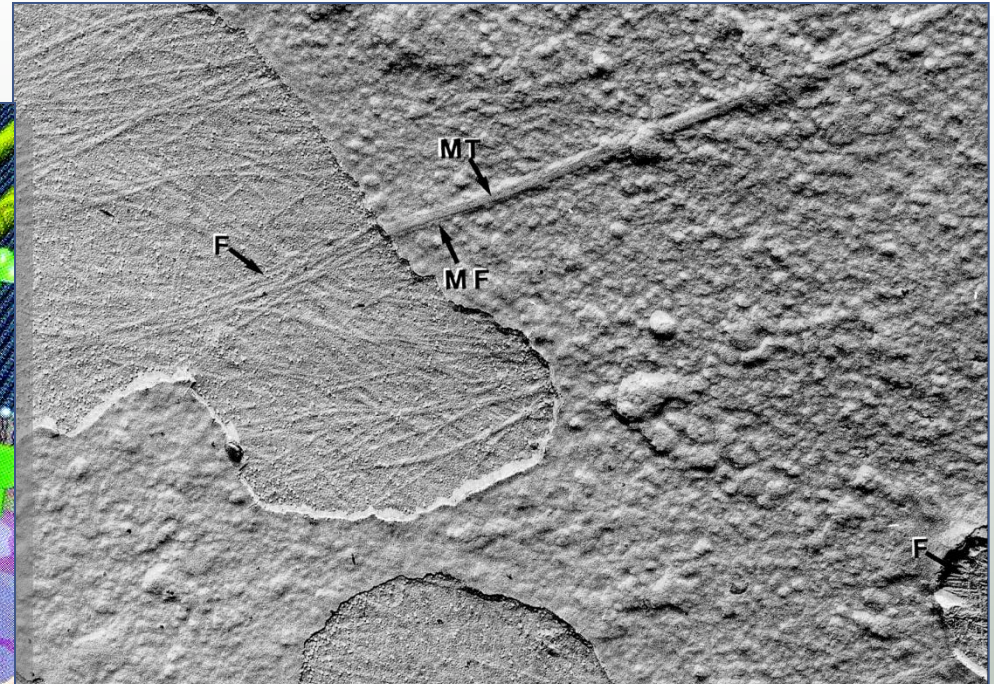




# MT-Microfibril Co-Alignment



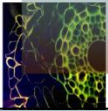
Karp Fig 7.36b



Die Beziehung zwischen Cellulose-synthetisierenden Komplexen (Rosetten), Zellwand-Mikrofibrillen, der Plasmamembran und Mikrotubuli

Gunning et al. 2000

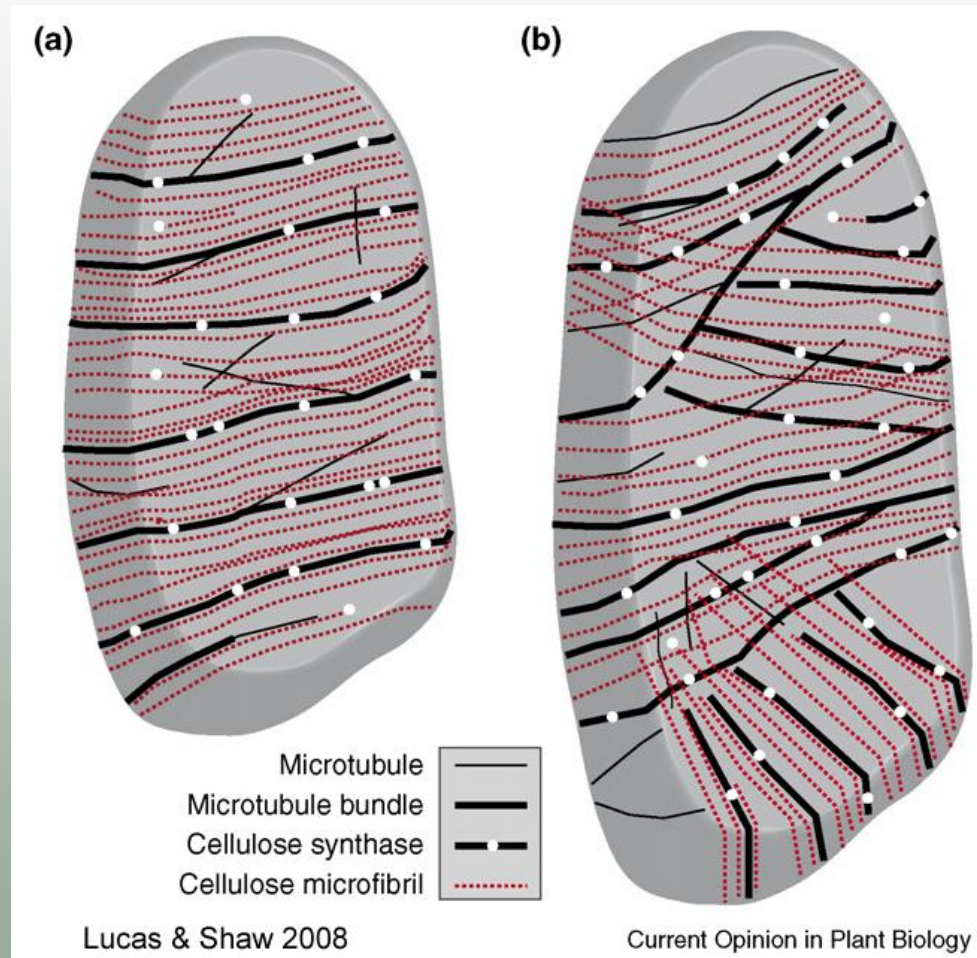


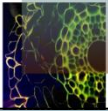


# MT-Microfibril Co-Alignment

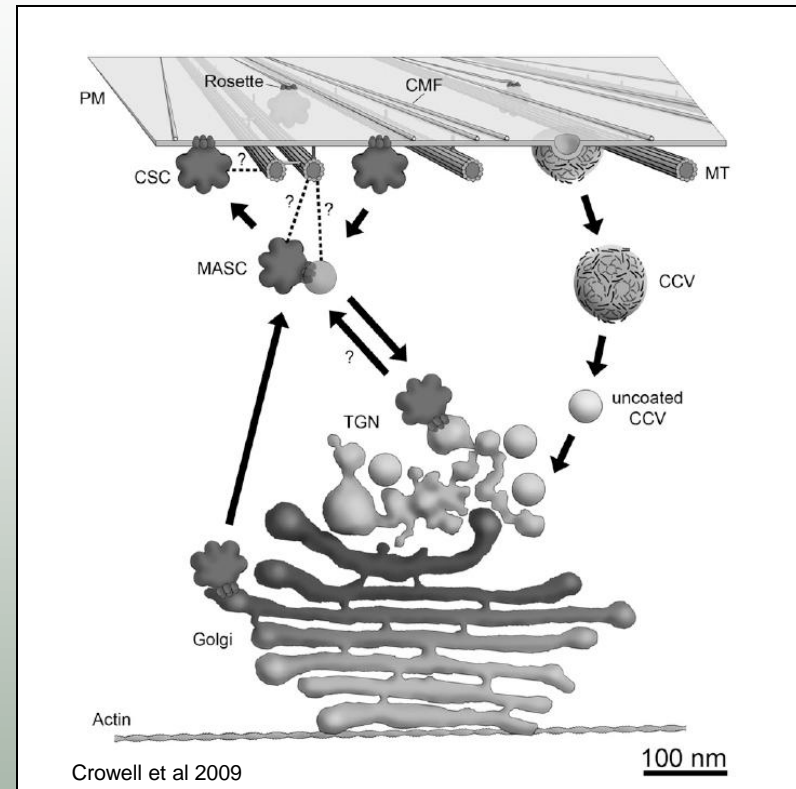
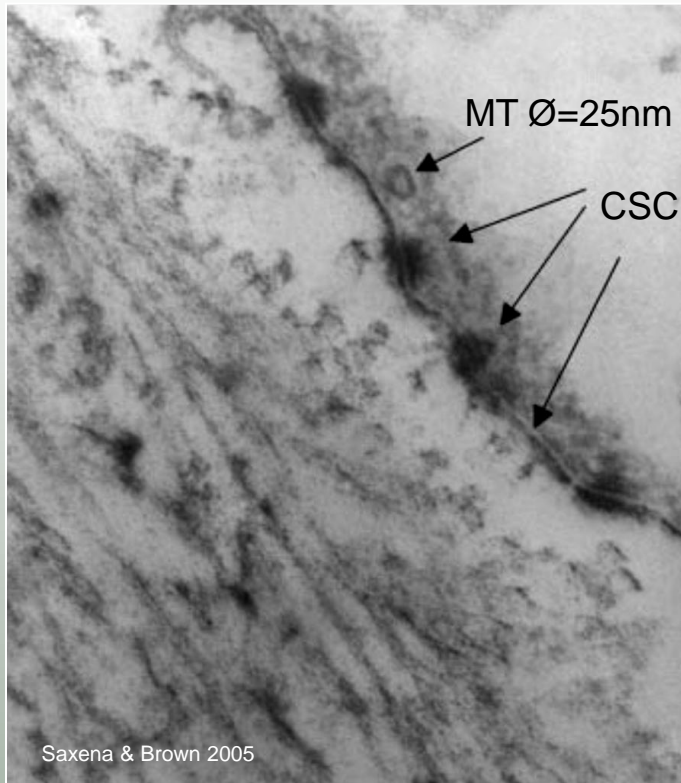


## Distribution of CesaA Complexes Alongside Microtubules



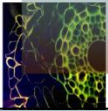


# Delivery of Terminal Complexes to PM

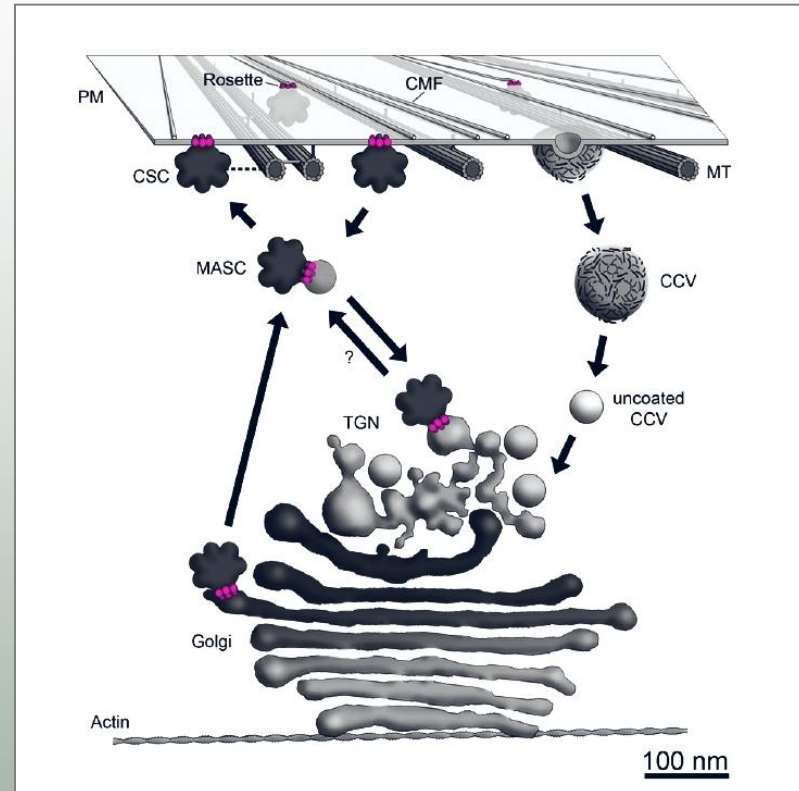
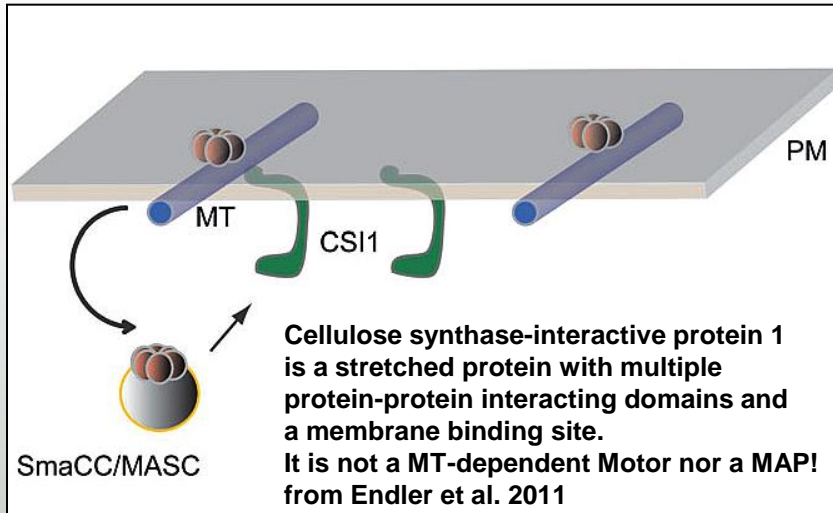


Cellulose synthase complexes (CSC) have a very large cytoplasmic domain ( $\varnothing$  ca. 30nm), they are delivered one by one from the TGN. In the process of delivery to and retrieval from the plasma membrane they interact with microtubules. CMF – cellulose microfibril, PM – plasma membrane, CSC – cellulose synthase complex, MASC - microtubule-associated cellulose synthase compartment, TGN – transgolgi network, CCV – clathrin coated vesicles.



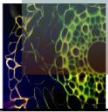


# Delivery of Terminal Complexes to PM

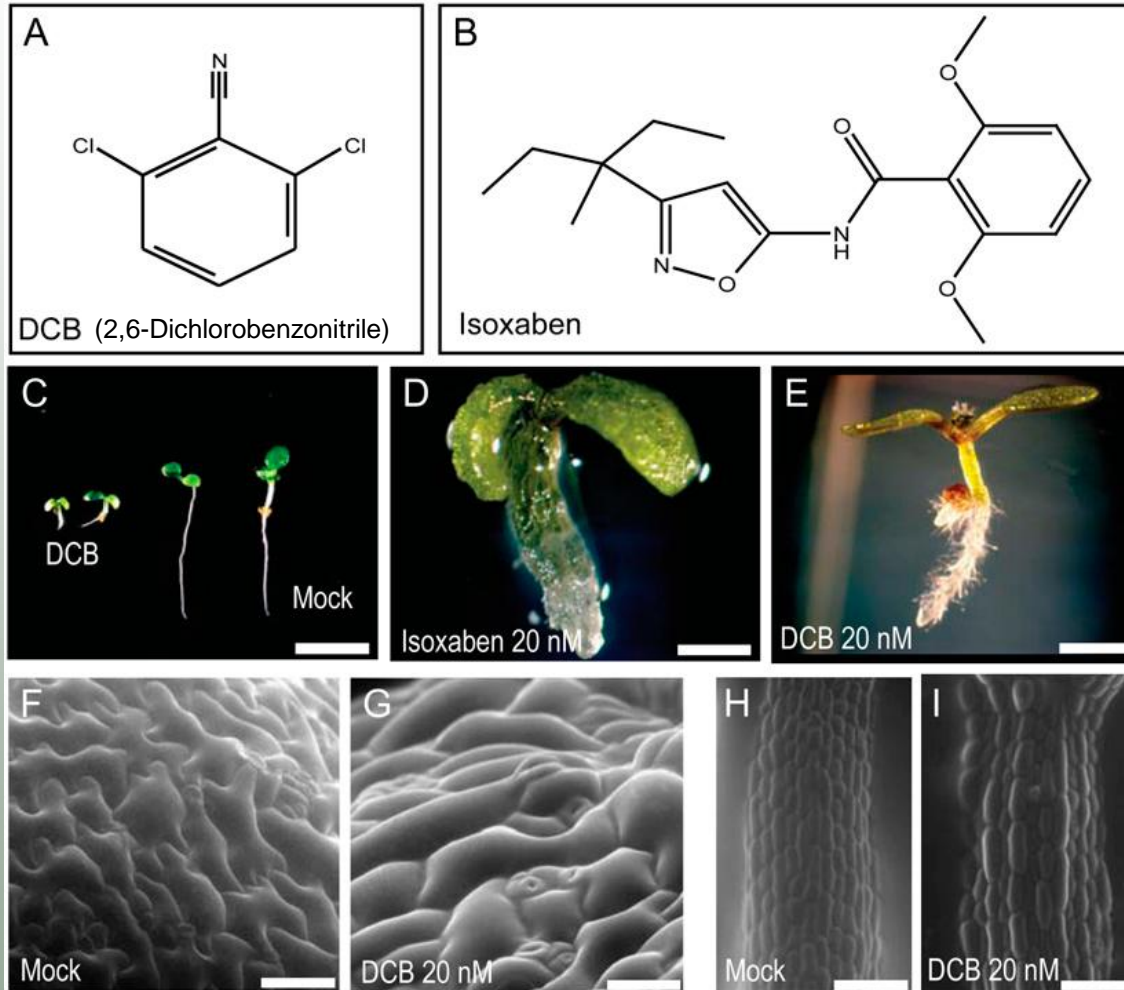


Cellulose synthase complexes (CSC) have a very large cytoplasmic domain ( $\emptyset$  ca. 30nm), they are delivered one by one from the TGN to the PM. In the process of delivery to and retrieval from the PM they interact with microtubules.

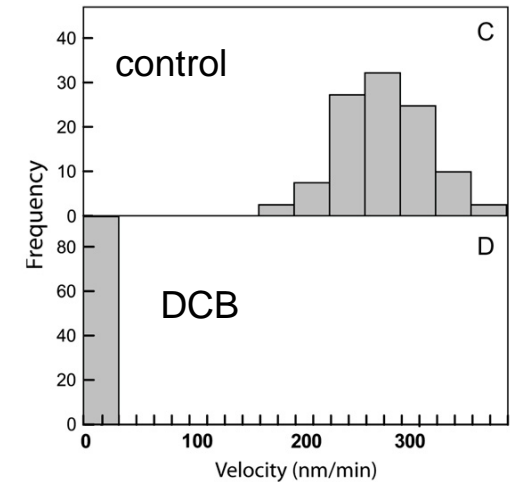
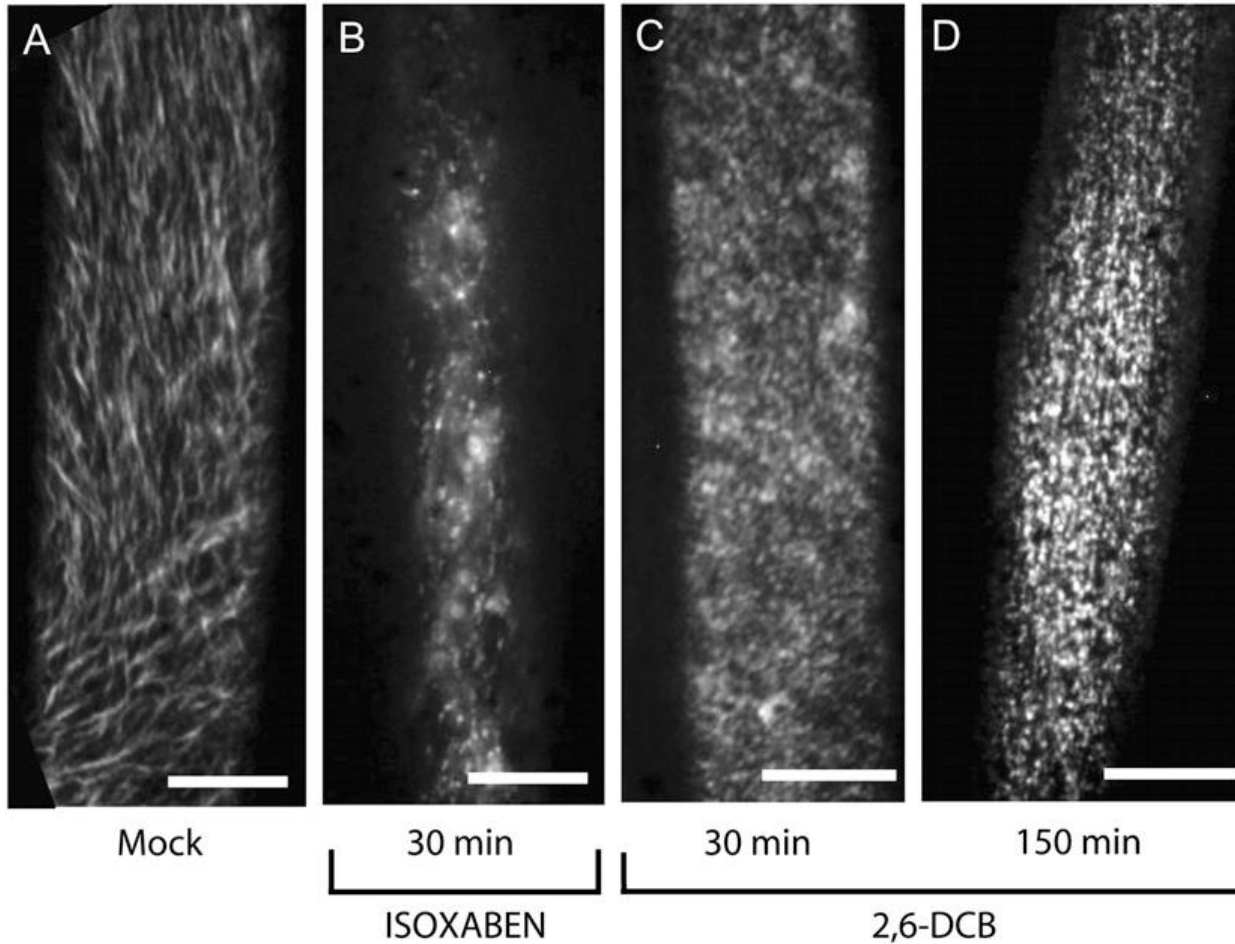
CMF – cellulose microfibril  
PM – plasma membrane  
CSC – cellulose synthase complex  
MASC - microtubule-associated cellulose synthase compartment  
TGN – transgolgi network  
CCV – clathrin coated vesicles



# Inhibition of Cellulose Synthesis



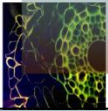
multiple frames overlay



Velocity profile

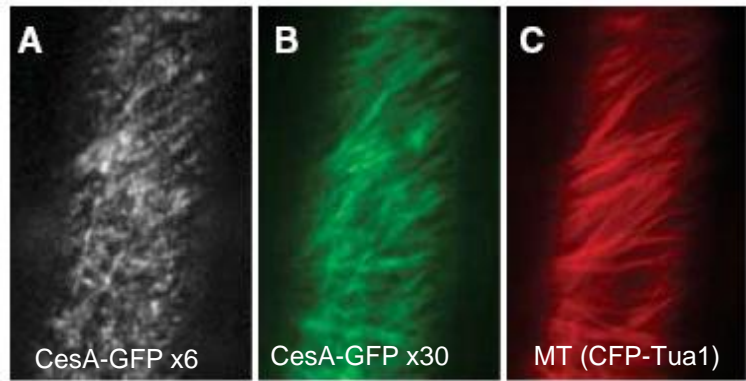
Live-cell imaging of YFP::CESA6 in untreated cells (A), and cells treated with isoxaben (B), or DCB (C and D). Each image represents a time-averaged projection of 60 frames spaced 5 s apart.



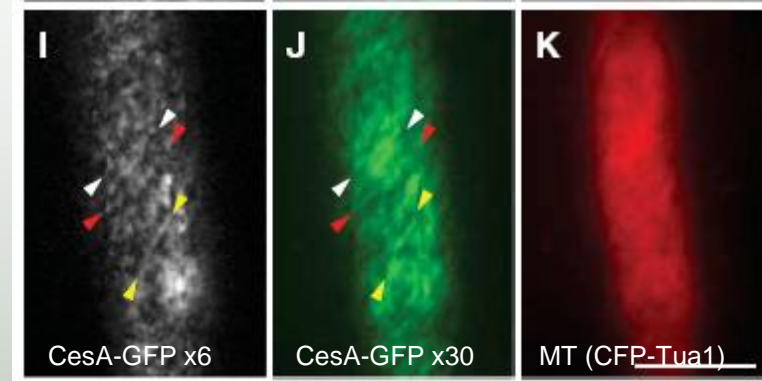


## Argument in Favour of MT-Independent Self-Organization of CES-Complexes

Control (multiple frames overlay)

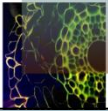


20  $\mu$ M Oryzalin (multiple frames overlay)



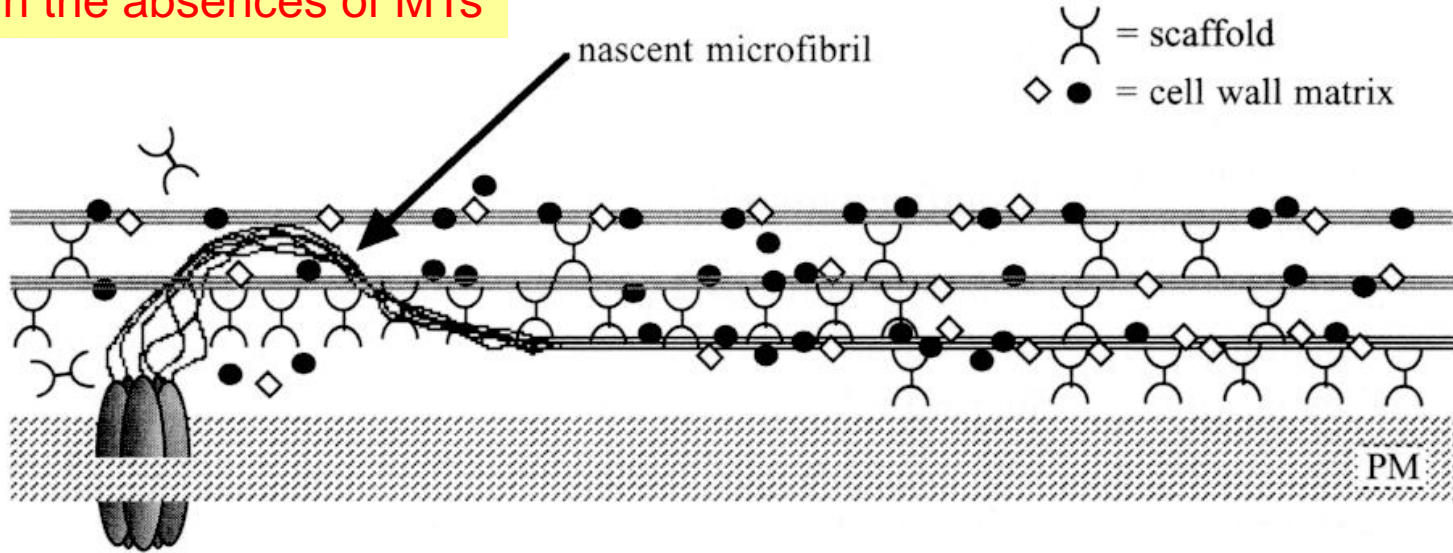
Peredez et al. 2009

- CesA can reorganize into linear tracks in oryzalin-treated cells (in the absence of cortical MTs). This suggests that MTs may play a shepherding role in organizing cellulose deposition rather than serving as a required partner.
- The velocity of the CesA-complexes in the plasma membrane does not change, when MTs are depolymerized with oryzalin.

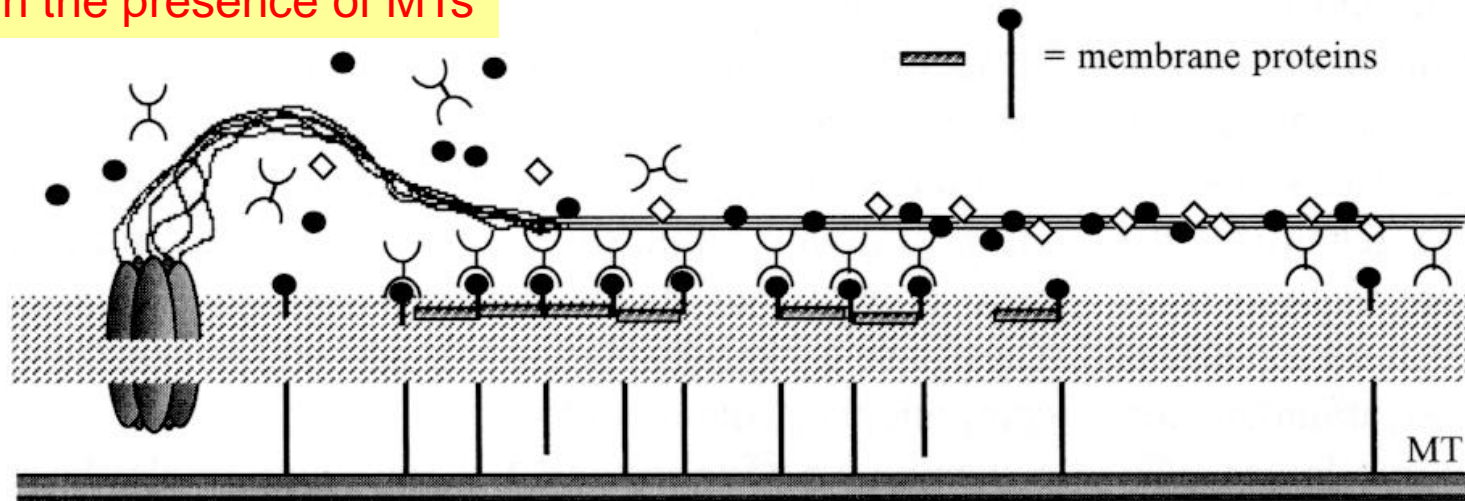


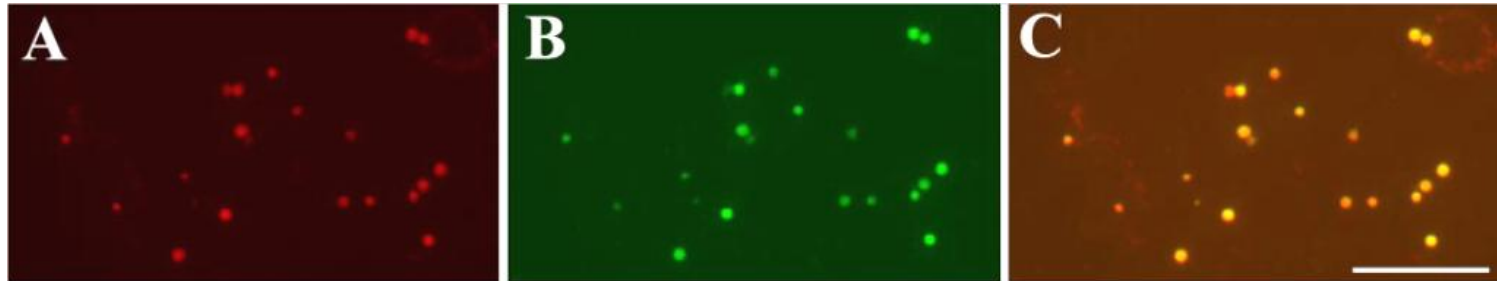
# MT-Microfibril Co-Alignment

## In the absence of MTs

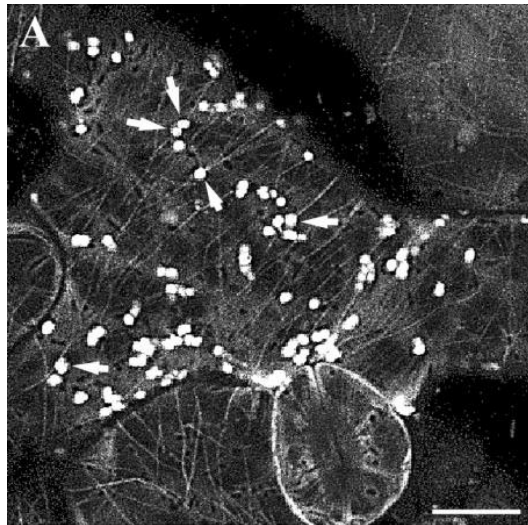


## In the presence of MTs

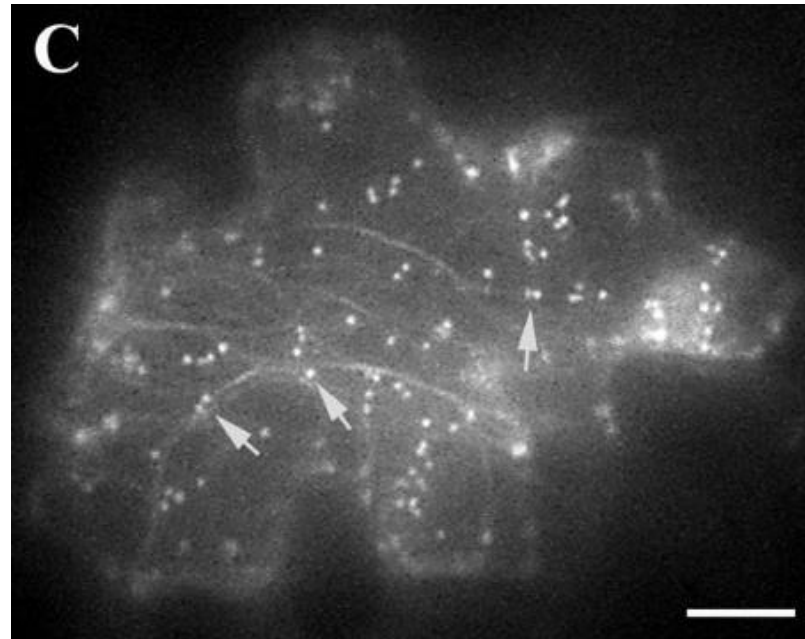




Colocalization of AtKinesin-13 (A) with the Golgi apparatus (B) in leaf mesophyll protoplasts isolated from a STtmd-GFP plant (Sialyl-transferase-transmembrane domain).

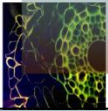


Association of Golgi stacks (N-acetylglucosaminyl transferase I -GFP label) with cortical microtubules (GFP-alpha-6 tubulin).



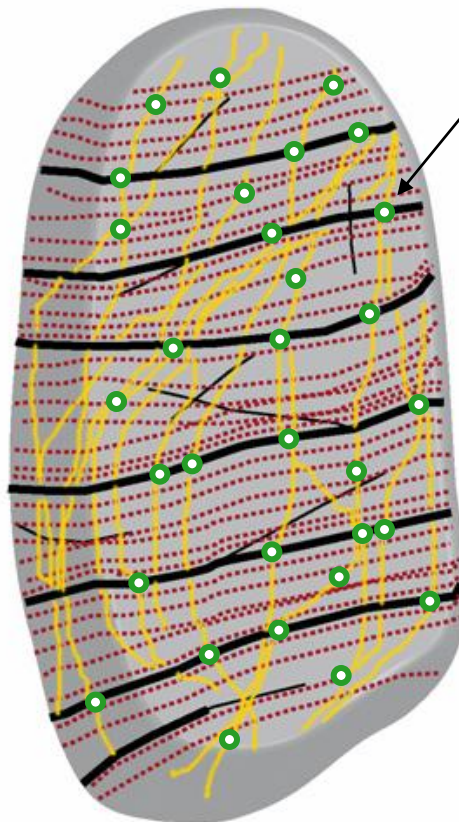
Association of Golgi (NAGT-GFP) with actin filaments (GFP-talin)



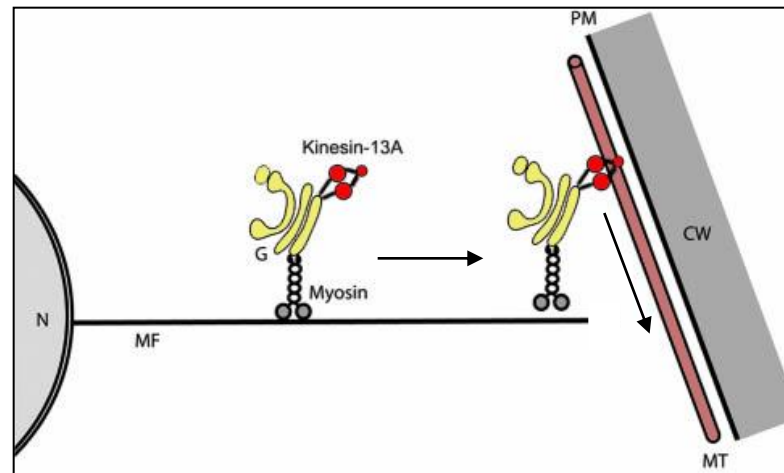


# MT-Microfibril Co-Alignment

Distribution of golgi bodies at cross over points between actin filaments and microtubules

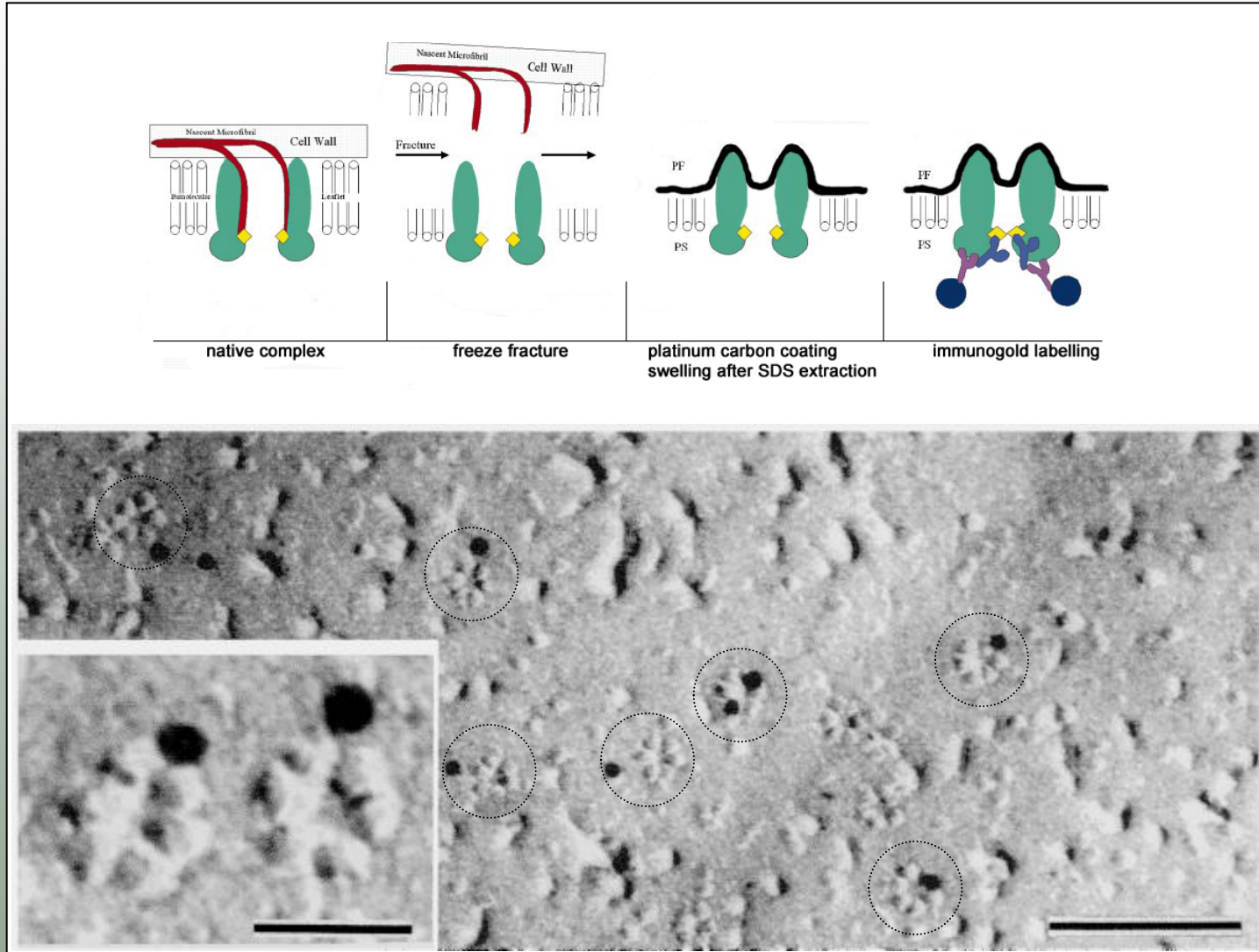


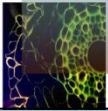
- ..... cellulose microfibrils
- single microtubules
- microtubule bundles
- actin filaments
- golgi stack



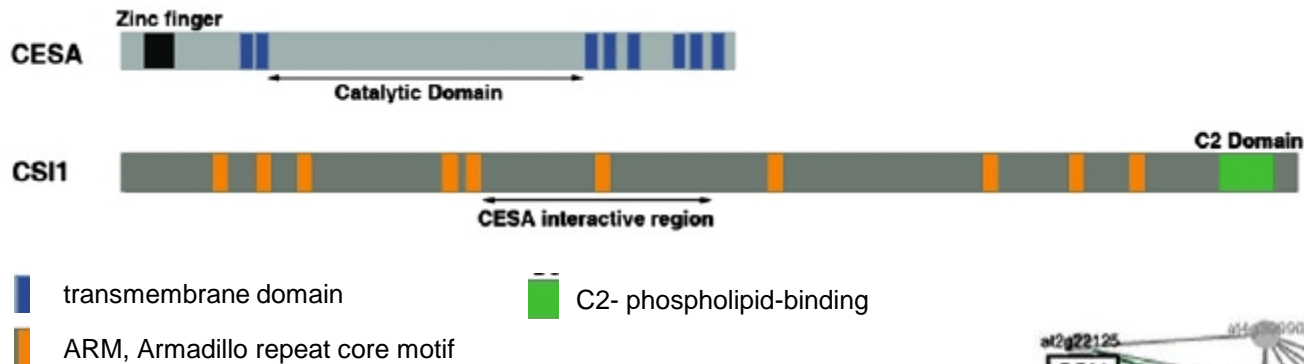
Proposed model for the function of kinesin 13A in plant cells

Golgies pause at cross over points between MTs and actin filaments, CesA containing vesicles are released, CesA complexes become inserted in the PM alongside MTs and start to produce cellulose microfibrils, which tend to co-align with microfibrils already laid down in the cell wall



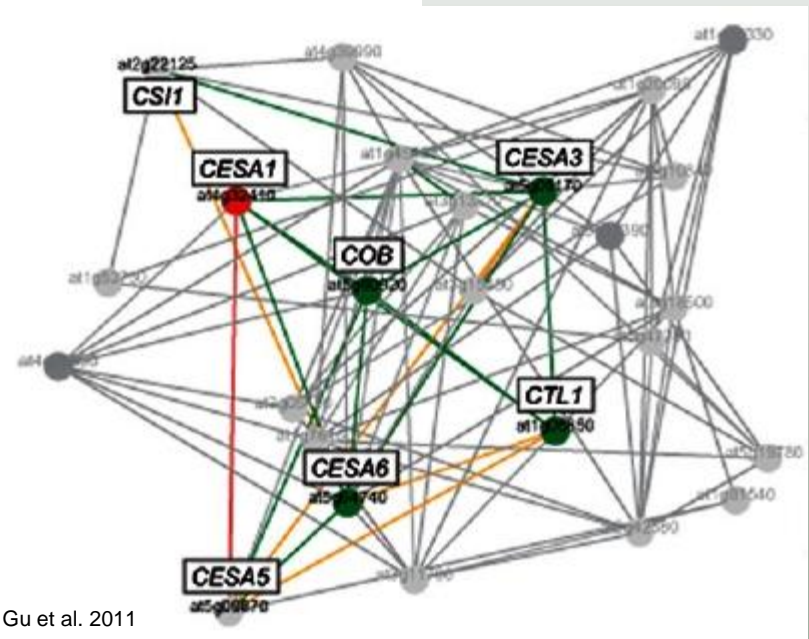


# MT-CSA Co-Alignment



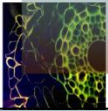
Cellulose synthase-interactive protein 1 is a stretched protein with multiple protein-protein interacting domains (ARM) and a phospholipid (membrane)- binding site. It is not a MT-dependent Motor nor a MAP!

from Endler et al. 2011



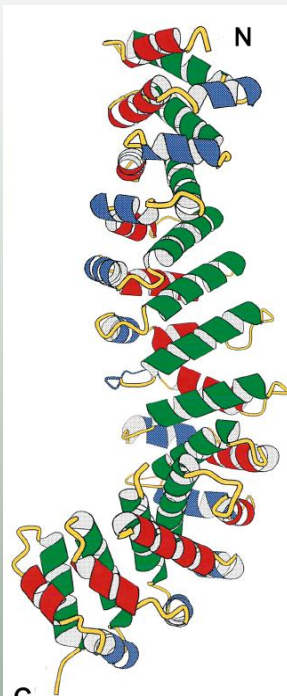
coexpression relationships between CSI1, CESA isoforms and cobra. red = very strong, orange = strong, green = medium strong, grey = weak  
 CTL1=chitinase-like 1





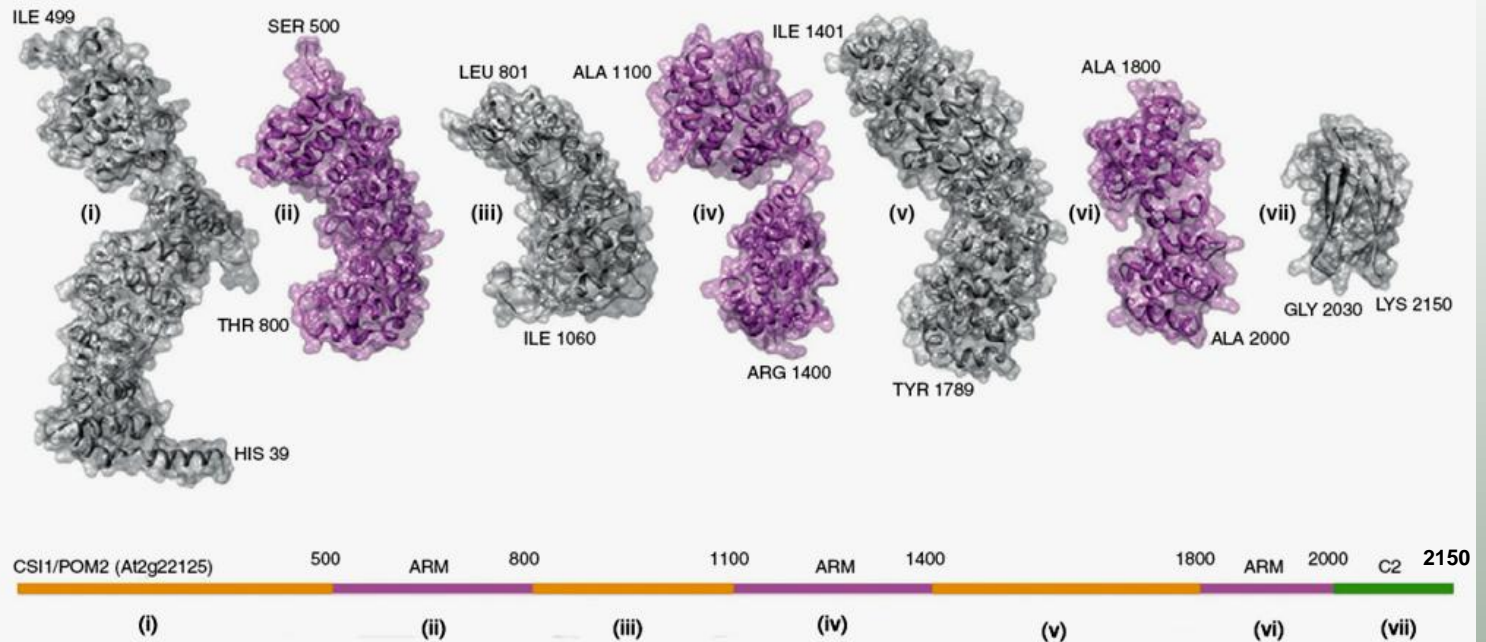
# MT-CSA Co-Alignment

## Domain structure of CSI1



C

Huber et al. 1997

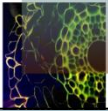


Bringmann et al. 2012

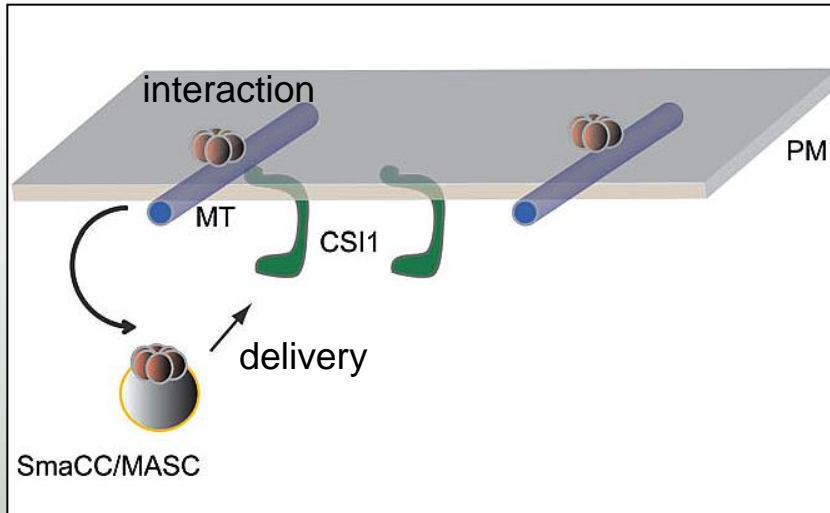
TRENDS In Plant Science

armadillo repeat motifs  
in animal  $\beta$ -catenin  
red-green-blue denote  
one repeat unit of ca.  
40 amino acids.

CSI1 is made of 6 armadillo-like sequences forming a long superhelical rod with a C2 motif at the C-terminal end

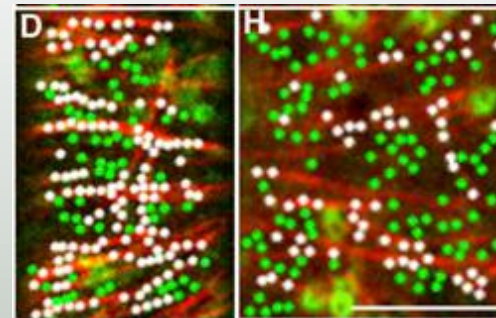


# MT-CSA Co-Alignment



Cellulose synthase-interactive protein 1 is a stretched protein with multiple protein-protein interacting domains and a membrane binding site. It is not a MT-dependent Motor nor a MAP! from Enderl et al. 2011

wild type      CSI1-3 mutant



Li et al. 2012

70.1%

40%

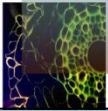


interaction with microtubules



no interaction with microtubules

co-alignment of YFP-CESA6 with RFP-TUA5



## Distribution of CesA/CSI-Complexes Alongside Microtubules

Time Average Over 61 Frames

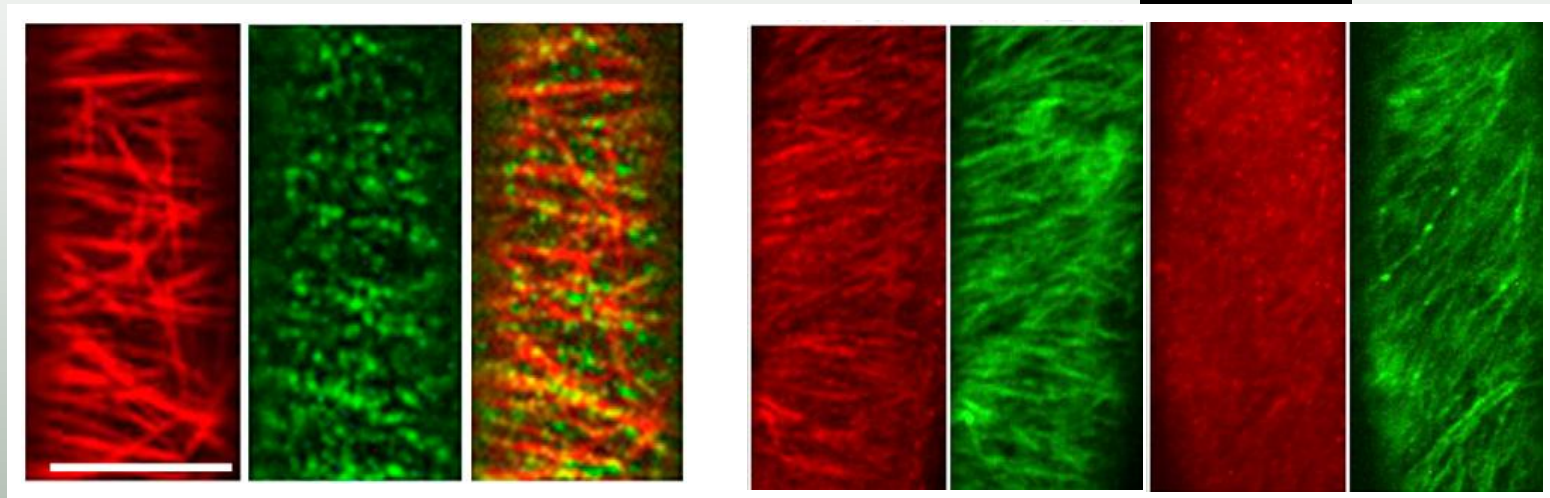
single frame

movement

movement

no movement

movement



RFP-TUA5

GFP-CSI

overlay

RFP-CSI1

GFP-CESA

RFP-CSI1

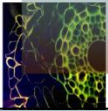
GFP-CESA

control

control

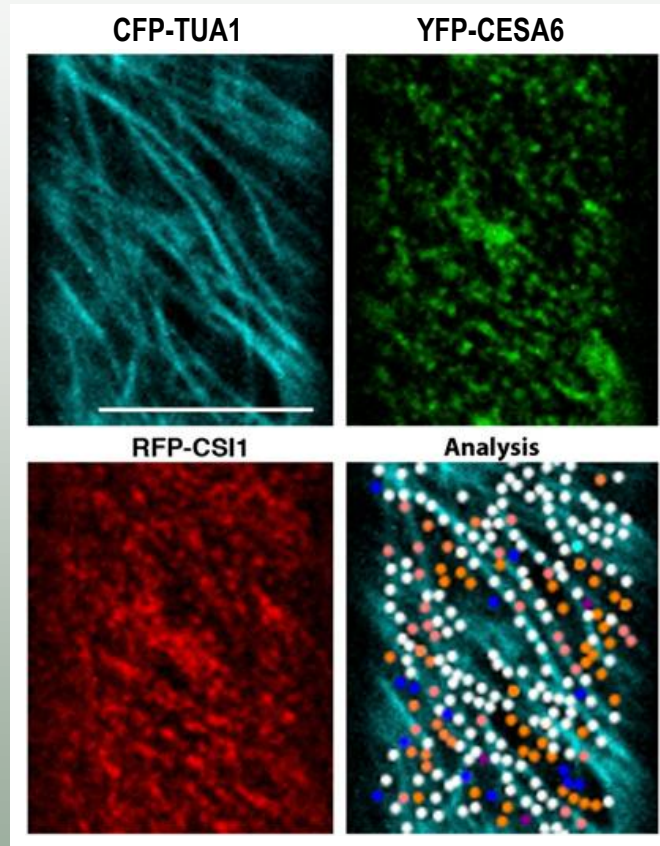
10hrs 20µM Oryzalin



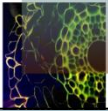


# MT-Microfibril Co-Alignment

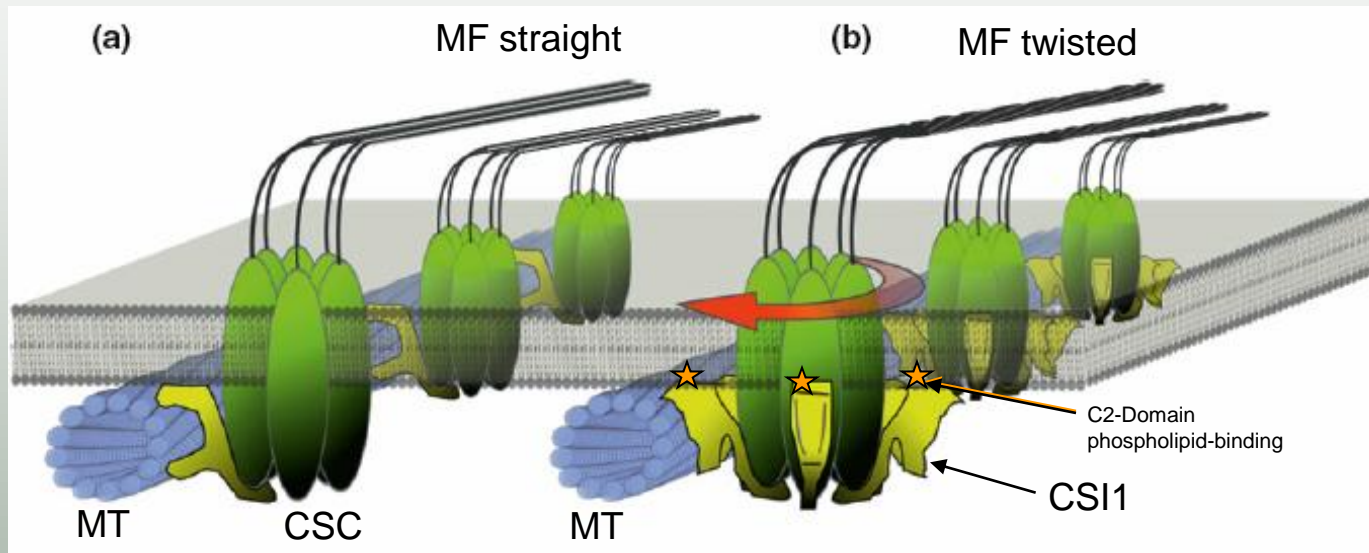
## Distribution of CesA/CSI complexes alongside microtubules



%	CFP-TUA1	YFP-CESA6	RFP-CSI1	
59,2	X	X	X	CESA associated with CSI & MT
21,5		X	X	CESA associated with CSI
11,2	X		X	MT associated with CSI
1,3			X	solitary CSI
0,5	X	X		MT associated with CESA
6,3		X		solitary CESA



# Model of MT-CSA Co-Alignment



Bringmann et al. 2012

- CSC – cellulose synthesizing complex
- CSI1 – cellulose synthase interacting protein 1
- MT – microtubules
- MF – cellulose microfibril