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Schneider, R., Hanak, T., Persson, S. et al. (1 more author) (2016) Cellulose and callose synthesis and organization in focus, what's new? Current Opinion in Plant Biology, 34. C. pp. 9-16. ISSN 1369-5266

https://doi.org/10.1016/j.pbi.2016.07.007

Title:
Cellulose and callose synthesis and organization in focus, what's new?

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Abstract
Plant growth and development are supported by plastic but strong cell walls. These walls consist largely of polysaccharides that vary in content and structure. Most of the polysaccharides are produced in the Golgi apparatus and are then secreted to the apoplast and built into the growing walls. However, the two glucan polymers cellulose and callose are synthesized at the plasma membrane by cellulose or callose synthase complexes, respectively. Cellulose is the most common cell wall polymer in land plants and provides strength to the walls to support directed cell expansion. In contrast, callose is integral to specialized cell walls, such as the cell plate that separates dividing cells and growing pollen tube walls, and maintains important functions during abiotic and biotic stress responses. The last years have seen a dramatic increase in our understanding of how cellulose and callose are manufactured, and new factors that regulate the synthases have been identified. Much of this knowledge has been amassed via various microscopy-based techniques, including various confocal techniques and super-resolution imaging. Here, we summarize and synthesize recent findings in the fields of cellulose and callose synthesis in plant biology.

The Plant Cell Wall

All plant cells are encased by cell walls that provide protection of the plant against environmental conditions, dictate cell expansion needed to support plant morphology, and allow for solute transport between cells and from a plant's root to its shoot [1]. Hence, cell walls are essential for plants to grow and develop.

While cell wall composition and architecture change during development and in response to environmental stress, a range of polysaccharides provide the basic cell wall structure. The polysaccharides may be divided into two major classes based on their physio-chemical characteristics; i.e. the homopolysaccharides cellulose and callose, and the heteropolysaccharides pectins and hemicelluloses [2,3]. The latter two are referred to as matrix polysaccharides and are typically produced in the Golgi apparatus and subsequently secreted to the apoplast where they may be modified and incorporated into the growing cell wall [2,3]. In contrast, the β-1,4-glucan cellulose and the β-1,3-glucan callose are made at the plasma membrane by large glucan synthase complexes that are referred to as cellulose synthases (CesAs) and callose synthases (CalS or GSL for GLUCAN SYNTHASE-LIKE), respectively [4,5]. While cellulose is found in virtually all cell walls in land plants, callose is typically made in specialized walls that support cell division, tip growth, pollen and pollen tube development, and plasmodesmata function, as well as during responses to environmental stress.

Cellulose Synthesis

Cellulose is synthesized by massive CesA protein complexes that are 40-60 nm in diameter [6] and that typically consist of a heterotrimeric CesA core, and associated proteins, at the plasma membrane [4] (Fig. 1).

Cellulose Synthase Assembly and Secretion: The CesA complex is likely assembled in the Endoplasmic Reticulum (ER) or Golgi [4]. However, our understanding of what is regulating the assembly and the subsequent trafficking of the complex to the plasma membrane is very limited. Nevertheless, the recently identified STELLO (STL) proteins that interact with the CesAs in the Golgi have at least provided a first ingress on the assembly. Impaired STL function led to a redistribution of the CesAs in the Golgi, i.e. from peripheral to central Golgi localization, and to a reduction in CesA secretion and thus reduction in cellulose production [7*]. Interestingly, less CesA subunits were incorporated into CesA complexes in stl mutants, indicating that the STLs work as assembly factors for the CesA complex [7*]. Based on point mutations it appears likely that the STLs are glycosyltransferases, though the substrate of the STLs remains elusive.

Once the CesAs are assembled, they may be trafficked to the plasma membrane, possibly via the trans-Golgi network (TGN) and small vesicle compartments named Microtubule-Associated Cellulose Synthase Compartments (MASCs; [8])/Small CesA Compartments (SmaCCs; [9]; Fig. 1), where they typically are inserted in close vicinity to cortical microtubules [9]. Apart from the STLs, several factors influence the rate of CesA secretion, including the actin cytoskeleton [10], the kinesin FRA1 [11] and the acidification of the TGN [12]. Nevertheless, regulatory components that specifically impact CesA trafficking remain ill defined.

Cellulose Synthase at the Plasma Membrane: As alluded to above, the CesAs are typically delivered to the plasma membrane in close proximity of cortical microtubules [9]. Here, the CesAs are presumably activated by a currently unknown mechanism, and begin to track along the microtubules. It is plausible that phosphorylation of the CesAs could play a role in the activation. Indeed, mutations of several phosphorylation sites of different CesAs affect the tracking behaviour and the speed of the complexes [13-15]. The CesAs use UDP-glucose as substrate, and the catalytic activity of them is likely the driving force behind the motility of the complexes [16**]. Here, individual glucan chains crystallize into cellulose fibrils that are entangled by other cell wall polymers. Hence, further synthesis will push the CesA complexes forward through the membrane. The speed of the complexes would therefore be largely determined by the rate of synthesis, but also depend on other circumstances, such as temperature and the stiffness of the membrane and cellulose fibrils [17]. Using functional fluorescently-tagged primary wall CesAs, the speeds have been estimated to around 200-350 nm/min [18 to 21], which is in excellent agreement with theoretical estimates [17]. In contrast, measurements of CesA speeds around the columella in seed coats suggest that the CesA complexes here move with speeds below 100 nm/min [22]. In addition, fluorescently tagged secondary wall CesAs, which produce cellulose to support water-transporting xylem elements, migrated faster than the typical primary wall CesAs [23**]. In this outstanding study, the authors used a secondary wall inducible line in Arabidopsis thaliana (Arabidopsis) seedlings to outline the behaviour of fluorescently-labelled secondary wall CesAs during different phases of secondary wall production.

Another factor that can influence CesA velocity is its association with cortical microtubules [21]. Cortical microtubules guide the CesA complex via the protein Cellulose Synthase Interacting (CSI)1/POM2 [21,24,25]. Interestingly, the CSI1/POM2 may also contribute to the trafficking of the CesAs as the protein seems to be important for the re-establishment of CesAs at the plasma membrane after stress [26]. The recently reported Cellulose synthase-Microtubule Uncoupling (CMU)1 to 3 appear to stabilize the cortical microtubules, which prevent lateral displacement of them by the motile CesA complexes [27*]. Another protein family that contributes to the relationship between the CesAs and microtubules is the recently identified Companion of Cellulose Synthase (CC)1 to 4 [28**]. The C-terminal part of these proteins may interact with the CesAs while the N-terminal parts can bind to microtubules. Interestingly, the N-terminal part protects the cellulose producing capacity of the CesAs during adverse conditions, including salt stress, by promoting microtubule re-establishment during salt exposure [28**]. This study therefore provided a new member of the CesA complex that act as a protecting device for the plant to maintain cellulose synthesis during abiotic stress conditions. Apart from the CSI1/POM2 and the CC proteins, several other proteins affect cellulose synthesis, including CTL1, COBRA, TED6 and 7, and KORRIGAN [29-32]. Recently, KORRIGAN, which is an endo-1,4-glucanse, was shown to be part of the CesA complex [33-36]. These studies added further clues to how KORRIGAN might work in cellulose synthesis.

Much effort is undertaken to better understand structural features and the stoichiometry of the CesAs in the CSC in plants. Expression of the cytoplasmic domain, which holds the putative catalytic site, of CesAs has yielded structural estimates of this part of

the proteins [37,38]. However, the conclusions of these studies differ, and it is certainly plausible that the lack of the transmembrane domains surrounding the cytoplasmic domain influences the structures. Hence, robust structural estimates, similar to those for the bacterial catalytic subunit of cellulose synthase [16**], remain wanting. The CesA complex has traditionally been depicted as containing 36 CesA subunits [4]. This model has been challenged through several studies that have estimated that cellulose microfibrils typically contain about 18 to 24 cellulose chains (e.g. [39*,40]). Hence, the CesA complex may hold 18 to 24 CesA subunits, assuming that all CesAs actively make cellulose chains (Fig. 1). These data are in agreement with recent freeze fracture transmission electron microscopy analyses that argue for a six-fold trimeric CesA assembly [41]. Nevertheless, it will be important to verify these estimates by directly observing the number of CesA subunits in the complex. A recent elegant study used variable-angle epifluorescence microscopy (VAEM) in combination with quantitative photo-bleaching to enable optical assessment of the CesA3 stoichiometry in CesA complexes in epidermal hypocotyl cells of Arabidopsis [42]. By using a step-detection algorithm this study estimated the number of GFP-CesA3 molecules to be at least 10 in one CesA complex [42, 4]. Still, these estimates are likely low because the imaged plants were only in a partial-loss-of-function mutant (i.e. not a null mutant) and thus a portion of each complex might be comprised of non-fluorescent CesA3 molecules. Given that the CesA complexes appear to contain equimolar heterotrimeric CesAs, i.e. 1:1:1 of CesA1, 3 and 6-like CesAs and of CesA4, 7 and 8 in primary and secondary wall CesA complexes, respectively [43,44] the VAEM photo-bleach study [42] supports 36 CesA subunits per complex. However, it remains to be seen how these data will compare against EM-based work, if all CesA subunits in the complex are active, and if there are cell, tissue and species dependent differences of CesA complex size (Fig. 1).

Cellulose Synthase Internalization: Once the CesA complex has produced a cellulose microfibril it will need to be removed from the plasma membrane to allow for new CesAs to commence synthesis. This would entail stalling the complexes, severing of the cellulose chains from the CesAs, and internalization of the complex. Very little is known about these processes; however, it appears that the complexes can be internalized via clathrin mediated endocytosis (CME). Subunits of the canonical adaptor protein complex AP2 can interact with the CesAs in vitro and in yeast [45], and mutations in AP2µ may cause an over-accumulation of CesAs at the plasma membrane. In addition, components of the newly identified TPLATE complex [46] may work with the AP2 complex to facilitate CesA internalization [47]. While these data support an internalization of the CesAs via CME, direct evidences, e.g. via visualization of co-internalization of the CesAs and CME components, have not been reported.

Cellulose Microfibrils in the Wall: Traditionally, the cell wall was modelled as a ‘tethered network’ where individual cellulose fibrils were separated but also mechanically linked by pectins and hemicelluloses into a quasi-homogenous load bearing mesh [1,48]. Various conflicting results have provided a revised model including cellulose-cellulose junctions termed ‘biochemical hotspots’ [49]. Using atomic force microscopy (AFM) on cell walls of onion scale epidermis; these hotspots were made up of cellulose fibrils that merge into each other and that form a reticulated network. Randomly distributed on the µm-scale, these hotspots render cell wall stiffness and adhesiveness to be inhomogenously distributed on the nm-scale [50*]. Thus, the cell wall may not be accurately modelled using purely molecular models but instead need to incorporate meso-scale characteristics such as the packing, bundling, and orientation of cellulose fibrils, as well as the interaction between sequentially stacked lamellae. Super-resolution imaging of cellulose fibrils using total internal reflection fluorescence (TIRF) microscopy combined with direct stochastic optical reconstruction microscopy (dSTORM, [51]), a fast evolving localization microscopy technique, allowed visualization of cellulose fibrils in onion bulb epidermal cells [52] by the

use of the cellulose-specific dye Pontamine Fast Scarlet 4B (S4B, also called Direct Red 23, [53]). Here, the authors were able to visualize individual and branching cellulose fibrils with a lateral resolution below 100 nm on the outer epidermal cell wall, which indicated that microfibrils can aggregate during cell wall restructuring. While these efforts are clear improvements on confocal imaging of cell wall components, they remain well above the limit to resolve cellulose nanofibrils estimated by AFM to be in the range of three to four nm wide [50*].

This is also true for the β-1,3-glucan callose, the only other plant cell wall polymer that is directly synthesized at the plasma membrane. This may suggest similar mechanisms of biosynthesis; however, in contrast to cellulose biosynthesis, the knowledge of callose biosynthesis is still very limited.

Callose Synthesis

Unlike cellulose, callose is relatively sparsely produced in plants, ranging from about 0.3% (of total cell wall content) in Arabidopsis to about 5% in the energy crop Miscanthus x giganteus [54*]. Indeed, callose is produced mainly in specialized cell walls, which might explain the expansion and cell- or tissue-type specific expression of the GSL gene family members, comprising 12 members in Arabidopsis [55]. The GSLs fall into two major groups [55]: i) the ones contributing to fertility and cell division (GSL1, GSL2, GSL6, GSL8, GSL10) and ii) those that provide structural cell wall reinforcement (GSL5, GSL7, GSL12). The function of the remaining four GSLs has not been clarified. Similar to the CesAs, GSLs are integrated into the plasma membrane, with a putative preference for detergent-resistant membrane regions [56], and are predicted to contain 14 to 16 transmembrane domains [57].

Callose Synthase Assembly and Trafficking: The extent to which GSLs may form oligomers is not known; however, it is hypothesized that a CalS complex might contain a monomeric GTPase, UDP-glucose transferase (UGT), annexin, and sucrose synthase that may interact with the hydrophilic, intracellular loop of the GSLs [58]. These data are in part based on experiments on green algae [58] and Arabidopsis in which GSL6 was partially purified with the cell-wall associated proteins, phragmoplastin, and UGT1 [59]. More recently, a direct interaction of the stress-induced GSL5, also known as POWDERY MILDEW RESISTANT (PMR) 4, with the GTPase RabA4c was confirmed by in planta FRET (Förster/Fluorescence resonance energy transfer) and pull-down experiments [60]. It is plausible that the CalS complex is assembled in the ER, as indicated in tobacco pollen tubes [61*], and trafficked via the TGN to the plasma membrane [61*,62]. Vesicle trafficking of the CalS complex may also sustain translocation of plasma membrane-localized GSLs to sites of attempted pathogen penetrations where callose is deposited in so-called papillae for cell wall reinforcement [63]. In Arabidopsis, plasma membrane-based GSL5 was re-located to sites of papillae formation via vesicle-like bodies [64**]. Whether trafficking of CalS complexes is mediated by multi-vesicular bodies, as indicated from studies with barley [65*], and involves trafficking guided by actin filaments, as indicated in pollen tubes [61*], will, however, require further corroborations. The Arabidopsis GSL10 [66], GSL12 [67], and GSL5 [68-73] may be phosphorylated in response to various abiotic and biotic stresses. Results from yeast suggest that CalS activity might depend on its phosphorylation status [74-76], but trafficking could also represent a target for phosphorylation-dependent regulation, supported by the interactions between GSL5 and RabA4c in Arabidopsis [60].

Callose Microfibrils in the Wall: A common model of callose synthesis favoured a deposition of this glucan in the paramural space between the pre-existing cellulosic cell wall and the plasma membrane (Fig. 2). This model was mainly supported by analysis of callose deposition by electron microscopy at forming papillae where callosic papillae expanded to the cytosol [77]. Recent application of dSTORM prompted re-consideration of this model. Combined use of the dye S4B [53] and the callose-specific dye aniline blue fluorochrome

(ABF) [64**,78] facilitated three-dimensional, nanoscale cell wall images that revealed a direct interaction of cellulose and callose microfibrils within the area of forming callosic papillae, induced by pathogenic powdery mildew in Arabidopsis leaves [79**]. These results suggest a permeation of callose fibrils through internal cell wall nanopores, which would require a gel-like condition of callose that may be pH dependent [80]. Overexpression of GSL5 enhanced callose deposition at these interaction sites [64**] and expanded the cellulose/callose network, and led to formation of a callose layer on top of the pre-existing cellulosic cell wall structure [79**,80]. Interestingly, the formation of cellulose/callose networks, and layered cellulose/callose cell wall architecture, is not restricted to interaction sites with invading pathogens, but is also found in unchallenged leaf tissue of maize (Zea mays) and M. giganteus where it might represent an evolutionary adaption to environmental conditions. Indeed, dSTORM analysis revealed a clear, layered cellulose/callose architecture in cell walls of epidermal leaf cells, which are directly exposed to the environment [54*].

Outlook

The complexity of the cell wall has already received considerable attention from various sources of confocal and super-resolution microscopists. Nevertheless, firm understanding of the architecture of the walls and how the synthesis occurs will require studies on length scales below the diffraction limit. However, many of the super-resolution imaging areas are still in a phase of proofing applicability of techniques, and are typically not routinely used. Whereas dSTROM was used to visualize and reconstruct glucan polymers, live cell imaging of corresponding protein complexes in planta has not been achieved. Here, it is imperative that this microscopy improves speed of image acquisition, which has hampered plant cell biology applications. It is encouraging that localization microscopy is compatible with conventional fluorophores [81-83], and it should be possible to use existing lines with labelled CesAs and CalS. Here, it will be interesting to investigate whether also the CalS moves during callose deposition similar to what has been observed for the CesAs. Likewise, AFM might prove suitable to also detect callose architecture and to put such estimates in context to the recently reported cellulose networks. This may lead to the identification of other sites where cellulose and callose fibrils coincide to form glucan networks apart from those that have already been reported.

Although being an established method among mammalian researchers, TIRF microscopy has had limited success in the plant field. This is mainly due to the cell wall being relatively thick (>250 nm) and comprised of layers with variable refractive indices. Recent advances in live-cell imaging revealed that adaptive optics may be used to partly compensate for those aberrations [84], which should open up new and affordable ways of looking at the cell wall in detail. Together, these approaches offer interesting ways to approach cell wall biology and will certainly provide more mechanistic insights into cellulose and callose synthesis.

Acknowledgements

S.P. was supported by a R@MAP Professor grant at University of Melbourne, and via an ARC Discovery grant (DP150103495) and a DFG grant PE1642/6-1.

References

1. Somerville C, Bauer S, Brininstool G, Facette M, Hamann T, Milne J, Osborne E, Paredez A, Persson S, Raab T, Vorwerk S, Youngs H: **Toward a systems approach to understanding plant cell walls.** Science 2004, **306**:2206-11.
2. Scheller HV, Ulvskov P: **Hemicelluloses.** Annu Rev Plant Biol. 2010, **61**:263-89.
3. Mohnen D: **Pectin structure and biosynthesis.** Curr Opin Plant Biol. 2008, **11**:266-77.

4. McFarlane HE, Döring A, Persson S: **The cell biology of cellulose synthesis.** Annu Rev Plant Biol. 2014, **65**:69-94.
5. Ellinger D, Voigt CA: **Callose biosynthesis in Arabidopsis with a focus on pathogen response: what we have learned within the last decade**. Ann Bot 2014, **114**:1349-1358.
6. Bowling A, Brown Jr RM: **A new view of cellulose synthase.** http://www.botany.utexas.edu/facstaff/facpages/mbrown/bowling/default2.html.
*7. Zhang Y, Nikolovski N, Sorieul M, Vellosillo T, McFarlane HE, Dupree R, Kesten C, Schneider R, Driemeier C, Lathe R, Lampugnani E, Yu X, Ivakov A, Doblin MS, Mortimer JC, Brown SP, Persson S, Dupree P: **Golgi-localized STELLO proteins regulate the assembly and trafficking of cellulose synthase complexes in Arabidopsis.** Nat Comm. 2016, **7**:11656.
*This paper outlines assembly factors of the cellulose synthase complex and how they contribute to a plant's ability to synthesise cellulose.
8. Crowell EF, Bischoff V, Desprez T, Rolland A, Stierhof YD, Schumacher K, Gonneau M, Höfte H, Vernhettes S: **Pausing of Golgi bodies on microtubules regulates secretion of cellulose synthase complexes in Arabidopsis.** Plant Cell. 2009, **21**:1141-54.
9. Gutierrez R, Lindeboom JJ, Paredez AR, Emons AM, Ehrhardt DW: (2009) **Arabidopsis cortical microtubules position cellulose synthase delivery to the plasma membrane and interact with cellulose synthase trafficking compartments.** Nat Cell Biol. 2009, 11:797-806.
10. Sampathkumar A, Gutierrez R, McFarlane HE, Bringmann M, Lindeboom J, Emons AM, Samuels L, Ketelaar T, Ehrhardt DW, Persson S: **Patterning and lifetime of plasma membrane-localized cellulose synthase is dependent on actin organization in Arabidopsis interphase cells.** Plant Physiol. 2013, **162**:675-88.
11. Zhu C, Ganguly A, Baskin TI, McClosky DD, Anderson CT, Foster C, Meunier KA, Okamoto R, Berg H, Dixit R: **The fragile fiber1 kinesin contributes to cortical microtubule-mediated trafficking of cell wall components.** Plant Physiol. 2015 167:780-92.
12. Luo Y, Scholl S, Doering A, Zhang Y, Irani NG, Di Rubbo S, Neumetzler L, Krishnamoorthy P, Van Houtte I, Mylle E, Bischoff V, Vernhettes S, Winne J, Friml J, Stierhof Y-D, Schumacher K, Persson S, Russinova E: **V-ATPase activity in the TGN/EE is required for exocytosis and recycling in Arabidopsis.** Nat Plants **1(7)**: doi:10.1038/nplants.2015.94.
13. Bischoff V, Desprez T, Mouille G, Vernhettes S, Gonneau M, Höfte H: **Phytochrome regulation of cellulose synthesis in Arabidopsis.** Curr Biol. 2011, **21**:1822-7.
14. Chen S, Ehrhardt DW, Somerville CR: **Mutations of cellulose synthase (CESA1) phosphorylation sites modulate anisotropic cell expansion and bidirectional mobility of cellulose synthase.** Proc Natl Acad Sci U S A. 2010, **107**:17188-93.
15. Chen S, Jia H, Zhao H, Liu D, Liu Y, Liu B, Bauer S, Somerville C: **Regulation of anisotropic cell expansion and bidirectional mobility of cellulose synthase by phosphorylation of S211 and T212 of CESA3.** Plant Physiol. 2016 Mar 11. pii: pp.01874.2015.
16. Morgan JL, Strumillo J, Zimmer J: **Crystallographic snapshot of cellulose synthesis and membrane translocation. Nature 2013, **493**:181-6.
**This study revealed the structure of the catalytically active (BcsA) and membrane-anchored (BcsB) parts of the cellulose synthase in Rhodobacter.
17. Diotallevi F, Mulder B: **The cellulose synthase complex: a polymerization driven supramolecular motor.** Biophys J. 2007, **92**:2666-73.

18. Paredez AR, Somerville CR, Ehrhardt DW: **Visualization of cellulose synthase demonstrates functional association with microtubules.** Science 2006, **312**:1491-5.
19. Persson S, Paredez A, Carroll A, Palsdottir H, Doblin M, Poindexter P, Khitrov N, Auer M, Somerville CR: **Genetic evidence for three unique components in primary cell-wall cellulose synthase complexes in Arabidopsis.** Proc Natl Acad Sci U S A. 2007, **104**:15566-71.
20. Desprez T, Juraniec M, Crowell EF, Jouy H, Pochylova Z, Parcy F, Höfte H, Gonneau M, Vernhettes S: **Organization of cellulose synthase complexes involved in primary cell wall synthesis in Arabidopsis thaliana.** Proc Natl Acad Sci U S A. 2007, **104**:15572-7.
21. Gu Y, Kaplinsky N, Bringmann M, Cobb A, Carroll A, Sampathkumar A, Baskin TI, Persson S, Somerville CR: **Identification of a cellulose synthase-associated protein required for cellulose biosynthesis.** Proc Natl Acad Sci U S A. 2010, **107**:12866-71.
22. Griffiths JS, Šola K, Kushwaha R, Lam P, Tateno M, Young R, Voiniciuc C, Dean G, Mansfield SD, DeBolt S, Haughn GW: **Unidirectional movement of cellulose synthase complexes in Arabidopsis seed coat epidermal cells deposit cellulose involved in mucilage extrusion, adherence, and ray formation.** Plant Physiol. 2015, **168**:502-20.
23. Watanabe Y, Meents MJ, McDonnell LM, Barkwill S, Sampathkumar A, Cartwright HN, Demura T, Ehrhardt DW, Samuels AL, Mansfield SD: **Visualization of cellulose synthases in Arabidopsis secondary cell walls. Science. 2015, **350**:198-203.
**In this outstanding study the authors describe how the CesAs move during the formation of secondary cell walls in Arabidopsis.
24. Bringmann M, Li E, Sampathkumar A, Kocabek T, Hauser MT, Persson S: **POM-POM2/cellulose synthase interacting1 is essential for the functional association of cellulose synthase and microtubules in Arabidopsis.** Plant Cell. 2012, **24**:163-77.
25. Li S, Lei L, Somerville CR, Gu Y: **Cellulose synthase interactive protein 1 (CSI1) links microtubules and cellulose synthase complexes.** Proc Natl Acad Sci U S A. 2012, **109**:185-90.
26. Lei L, Singh A, Bashline L, Li S, Yingling YG, Gu Y: **CELLULOSE SYNTHASE INTERACTIVE1 is required for fast recycling of cellulose synthase complexes to the plasma membrane in Arabidopsis.** Plant Cell. 2015, **27**:2926-40.
*27. Liu Z, Schneider R, Kesten C, Zhang Y, Somssich M, Zhang Y, Fernie AR, Persson S: **Cellulose-Microtubule Uncoupling proteins prevent lateral displacement of microtubules during cellulose synthesis in Arabidopsis.** Dev Cell. 2016 in press.
*In this paper, Liu et al report on a microtubule-associated protein family that prevents lateral microtubule displacement by the motile cellulose synthase complexes.
28. Endler A, Kesten C, Schneider R, Zhang Y, Ivakov A, Froehlich A, Funke N, Persson S: **A mechanism for sustained cellulose synthesis during salt stress. Cell. 2015 **162**:1353-64.
**This study identified a protein family that aid cellulose synthesis during adverse conditions by the re-establishment of the cortical microtubule array.
29. Sánchez-Rodríguez C, Bauer S, Hématy K, Saxe F, Ibáñez AB, Vodermaier V, Konlechner C, Sampathkumar A, Rüggeberg M, Aichinger E, Neumetzler L, Burgert I, Somerville C, Hauser MT, Persson S: **Chitinase-like1/pom-pom1 and its homolog CTL2 are glucan-interacting proteins important for cellulose biosynthesis in Arabidopsis.** Plant Cell. 2012, **24**:589-607.
30. Liu L, Shang-Guan K, Zhang B, Liu X, Yan M, Zhang L, Shi Y, Zhang M, Qian Q, Li J, Zhou Y: **Brittle Culm1, a COBRA-like protein, functions in cellulose assembly through binding cellulose microfibrils.** PLoS Genet. 2013, **9**:e1003704.

31. Endo S, Pesquet E, Yamaguchi M, Tashiro G, Sato M, Toyooka K, Nishikubo N, Udagawa-Motose M, Kubo M, Fukuda H, Demura T: **Identifying new components participating in the secondary cell wall formation of vessel elements in zinnia and Arabidopsis.** Plant Cell. 2009, **21**:1155-65.
32. Rejab NA, Nakano Y, Yoneda A, Ohtani M, Demura T: **Possible contribution of TED6 and TED7, secondary cell wall-related membrane proteins, to evolution of tracheary element in angiosperm lineage.** Plant Biotechnol. 2015, **4**:343-347
33. Nicol F, His I, Jauneau A, Vernhettes S, Canut H, Höfte H: **A plasma membrane-bound putative endo-1,4-beta-D-glucanase is required for normal wall assembly and cell elongation in Arabidopsis.** EMBO J. 1998, **17**:5563-76.
34. Vain T, Crowell EF, Timpano H, Biot E, Desprez T, Mansoori N, Trindade LM, Pagant S, Robert S, Höfte H, Gonneau M, Vernhettes S: **The cellulase KORRIGAN is part of the cellulose synthase complex.** Plant Physiol. 2014, **165**:1521-1532.
35. Lei L, Zhang T, Strasser R, Lee CM, Gonneau M, Mach L, Vernhettes S, Kim SH, J Cosgrove D, Li S, Gu Y: **The jiaoyao1 mutant is an allele of korrigan1 that abolishes endoglucanase activity and affects the organization of both cellulose microfibrils and microtubules in Arabidopsis.** Plant Cell. 2014, **26**:2601-2616.
36. Mansoori N, Timmers J, Desprez T, Alvim-Kamei CL, Dees DC, Vincken JP, Visser RG, Höfte H, Vernhettes S, Trindade LM: **KORRIGAN1 interacts specifically with integral components of the cellulose synthase machinery.** PLoS One. 2014, **9**:e112387.
37. Olek AT, Rayon C, Makowski L, Kim HR, Ciesielski P, Badger J, Paul LN, Ghosh S, Kihara D, Crowley M, Himmel ME, Bolin JT, Carpita NC: **The structure of the catalytic domain of a plant cellulose synthase and its assembly into dimers.** Plant Cell. 2014, **26**:2996-3009.
38. Vandavasi VG, Putnam DK, Zhang Q, Petridis L, Heller WT, Nixon BT, Haigler CH, Kalluri U, Coates L, Langan P, Smith JC, Meiler J, O'Neill H: **A structural study of CESA1 catalytic domain of Arabidopsis cellulose synthesis complex: Evidence for CESA trimers.** Plant Physiol. 2016, **170**:123-35.
*39. Fernandes AN, Thomas LH, Altaner CM, Callow P, Forsyth VT, Apperley DC, Kennedy CJ, Jarvis MC: **Nanostructure of cellulose microfibrils in spruce wood.** Proc Natl Acad Sci U S A. 2011, **108**:E1195-203.
*In this study, application of wide-angle X-ray scattering, small-angle neutron scattering, and deuteration-FTIR revealed microfibril dimension and organisation with unprecedented accuracy. The authors suggest that microfibrils are twisted and have about 24 chains.
40. Newman RH, Hill SJ, Harris PJ: **Wide-angle x-ray scattering and solid-state nuclear magnetic resonance data combined to test models for cellulose microfibrils in mung bean cell walls.** Plant Physiol. 2013, **163**:1558-67.
41. Nixon BT, Mansouri K, Singh A, Du J, Davis JK, Lee JG, Slabaugh E, Vandavasi VG, O'Neill H, Roberts EM, Roberts AW, Yingling YG, Haigler CH: **Comparative structural and computational analysis supports eighteen cellulose synthases in the plant cellulose synthesis complex.** Sci Rep. 2016, **6**:28696.
42. Chen Y, Deffenbaugh NC, Anderson CT, Hancock WO: **Molecular counting by photobleaching in protein complexes with many subunits: best practices and application to the cellulose synthesis complex.** Mol Biol Cell. 2014, **25**:3630-42.
43. Gonneau M, Desprez T, Guillot A, Vernhettes S, Höfte H: **Catalytic subunit stoichiometry within the cellulose synthase complex.** Plant Physiol. 2014, **166**:1709-12.
44. Hill JL Jr, Hammudi MB, Tien M: **The Arabidopsis cellulose synthase complex: a proposed hexamer of CESA trimers in an equimolar stoichiometry.** Plant Cell. 2014, 26:4834-42.

45. Bashline L, Li S, Anderson CT, Lei L, Gu Y: **The endocytosis of cellulose synthase in Arabidopsis is dependent on μ2, a clathrin-mediated endocytosis adaptin.** Plant Physiol. 2013, **163**:150-60.
46. Gadeyne A, Sánchez-Rodríguez C, Vanneste S, Di Rubbo S, Zauber H, Vanneste K, Van Leene J, De Winne N, Eeckhout D, Persiau G, Van De Slijke E, Cannoot B, Vercruysse L, Mayers JR, Adamowski M, Kania U, Ehrlich M, Schweighofer A, Ketelaar T, Maere S, Bednarek SY, Friml J, Gevaert K, Witters E, Russinova E, Persson S, De Jaeger G, Van Damme D: **The TPLATE adaptor complex drives clathrin-mediated endocytosis in plants.** Cell. 2014, **156**:691-704.
47. Bashline L, Li S, Zhu X, Gu Y: **The TWD40-2 protein and the AP2 complex cooperate in the clathrin-mediated endocytosis of cellulose synthase to regulate cellulose biosynthesis.** Proc Natl Acad Sci U S A. 2015, **112**:12870-5.
48. McCann MC, Roberts K: **Architecture of the primary cell wall.** The cytoskeletal basis of plant growth and form. 1991, 109-129.
49. Park YB, Cosgrove DJ: **A revised architecture of primary cell walls based on biomechanical changes induced by substrate-specific endoglucanases.** Plant Physiol. 2012, **158**:1933-43.
*50. Zhang T, Zheng Y, Cosgrove DJ: **Spatial organization of cellulose microfibrils and matrix polysaccharides in primary plant cell walls as imaged by multichannel atomic force microscopy.** Plant J. 2016, **85**:179-92.
*This study used AFM to probe microfibril organization and cell wall architecture in epidermal onion cells, which revealed a reticulated network of microfibrils and how these interact with different cell wall domains.
51. van de Linde S, Löschberger A, Klein T, Heidbreder M, Wolter S, Heilemann M, Sauer M: **Direct stochastic optical reconstruction microscopy with standard fluorescent probes.** Nat Protoc. 2011, **6**:991-1009.
52. Liesche J, Ziomkiewicz I, Schulz A: **Super-resolution imaging with Pontamine Fast Scarlet 4BS enables direct visualization of cellulose orientation and cell connection architecture in onion epidermis cells.** BMC Plant Biol. 2013, **13**:226.
53. Anderson CT, Carroll A, Akhmetova L, Somerville C: **Real-time imaging of cellulose reorientation during cell wall expansion in Arabidopsis roots.** Plant Physiol. 2010 **152**:787-96.
*54. Falter C, Zwikowics C, Eggert D, Blumke A, Naumann M, Wolff K, Ellinger D, Reimer R, Voigt CA: **Glucanocellulosic ethanol: the undiscovered biofuel potential in energy crops and marine biomass**. Sci Rep 2015, **5**:13722.
*In this study, application of super-resoluition microscopy revealed cellulose/callose network formation in unchallenged plant tissue.
55. Verma DP, Hong Z: **Plant callose synthase complexes**. Plant Mol Biol 2001, **47**:693-701.
56. Srivastava V, Malm E, Sundqvist G, Bulone V: **Quantitative proteomics reveals that plasma membrane microdomains from poplar cell suspension cultures are enriched in markers of signal transduction, molecular transport and callose biosynthesis**. Mol Cell Proteomics 2013.
57. Ostergaard L, Petersen M, Mattsson O, Mundy J: **An Arabidopsis callose synthase**. Plant Mol Biol 2002, **49**:559-566.
58. Stone B: **Callose and Related Glucans**. In eLS. Edited by: John Wiley & Sons, Ltd; 2006.
59. Hong Z, Zhang Z, Olson JM, Verma DP: **A novel UDP-glucose transferase is part of the callose synthase complex and interacts with phragmoplastin at the forming cell plate**. Plant Cell 2001, **13**:769-779.

60. Ellinger D, Glockner A, Koch J, Naumann M, Sturtz V, Schutt K, Manisseri C, Somerville SC, Voigt CA: **Interaction of the Arabidopsis GTPase RabA4c with its effector PMR4 results in complete penetration resistance to powdery mildew**. Plant Cell 2014, **26**:3185-3200.

*61. Cai G, Faleri C, Del Casino C, Emons AM, Cresti M: **Distribution of callose synthase, cellulose synthase, and sucrose synthase in tobacco pollen tube is controlled in dissimilar ways by actin filaments and microtubules**. Plant Physiol 2011, **155**:1169-1190.

*This study described the importance of the cytoskeleton for cellulose and callose synthases.

62. Drakakaki G, van de Ven W, Pan S, Miao Y, Wang J, Keinath NF, Weatherly B, Jiang L, Schumacher K, Hicks G, et al.: **Isolation and proteomic analysis of the SYP61 compartment reveal its role in exocytic trafficking in Arabidopsis**. Cell Res 2012, **22**:413-424.

63. Nielsen ME, Feechan A, Bohlenius H, Ueda T, Thordal-Christensen H: **Arabidopsis ARF-GTP exchange factor, GNOM, mediates transport required for innate immunity and focal accumulation of syntaxin PEN1**. Proc Natl Acad Sci U S A 2012, **109**:11443-11448.

64. Ellinger D, Naumann M, Falter C, Zwikowics C, Jamrow T, Manisseri C, Somerville SC, Voigt CA: **Elevated early callose deposition results in complete penetration resistance to powdery mildew in Arabidopsis. Plant Physiol 2013, **161**:1433-1444.

**This study proved that extra callose deposition supports plant resistance to pathogens.

*65. Bohlenius H, Morch SM, Godfrey D, Nielsen ME, Thordal-Christensen H: **The multivesicular body-localized GTPase ARFA1b/1c is important for callose deposition and ROR2 syntaxin-dependent preinvasive basal defense in barley**. Plant Cell 2010, **22**:3831-3844.

*In this sudy, the importance of spatial and temporal coordination of callose synthase transport by vesicles was shown.

66. Benschop JJ, Mohammed S, O'Flaherty M, Heck AJ, Slijper M, Menke FL: **Quantitative phosphoproteomics of early elicitor signaling in Arabidopsis**. Mol Cell Proteomics 2007, **6**:1198-1214.

67. Nuhse TS, Stensballe A, Jensen ON, Peck SC: **Large-scale analysis of in vivo phosphorylated membrane proteins by immobilized metal ion affinity chromatography and mass spectrometry**. Mol Cell Proteomics 2003, **2**:1234-1243.

68. Kline KG, Barrett-Wilt GA, Sussman MR: **In planta changes in protein phosphorylation induced by the plant hormone abscisic acid**. Proc Natl Acad Sci U S A 2010, **107**:15986-15991.

69. Nakagami H, Sugiyama N, Mochida K, Daudi A, Yoshida Y, Toyoda T, Tomita M, Ishihama Y, Shirasu K: **Large-scale comparative phosphoproteomics identifies conserved phosphorylation sites in plants**. Plant Physiol 2010, **153**:1161-1174.

70. Nuhse TS, Bottrill AR, Jones AM, Peck SC: **Quantitative phosphoproteomic analysis of plasma membrane proteins reveals regulatory mechanisms of plant innate immune responses**. Plant J 2007, **51**:931-940.

71. Reiland S, Finazzi G, Endler A, Willig A, Baerenfaller K, Grossmann J, Gerrits B, Rutishauser D, Gruissem W, Rochaix JD, et al.: **Comparative phosphoproteome profiling reveals a function of the STN8 kinase in fine-tuning of cyclic electron flow (CEF)**. Proc Natl Acad Sci U S A 2011, **108**:12955-12960.

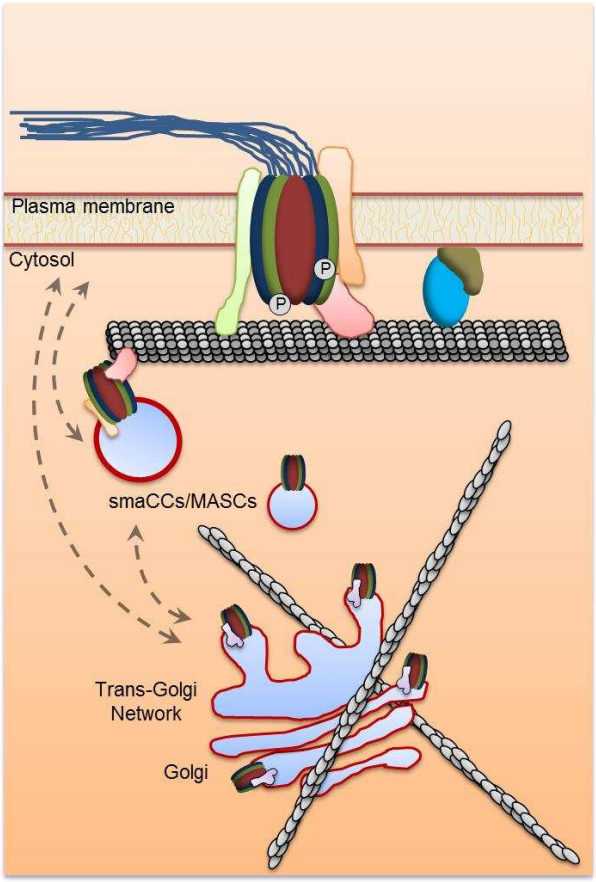
72. Reiland S, Messerli G, Baerenfaller K, Gerrits B, Endler A, Grossmann J, Gruissem W, Baginsky S: **Large-scale Arabidopsis phosphoproteome profiling reveals novel chloroplast kinase substrates and phosphorylation networks**. Plant Physiol 2009, **150**:889-903.

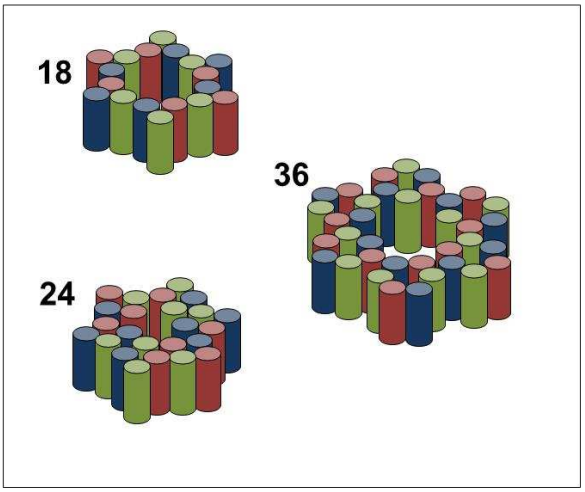
73. Sugiyama N, Nakagami H, Mochida K, Daudi A, Tomita M, Shirasu K, Ishihama Y: **Large-scale phosphorylation mapping reveals the extent of tyrosine phosphorylation in Arabidopsis**. Mol Syst Biol 2008, **4**:193.
74. Calonge TM, Arellano M, Coll PM, Perez P: **Rga5p is a specific Rho1p GTPase-activating protein that regulates cell integrity in Schizosaccharomyces pombe**. Mol Microbiol 2003, **47**:507-518.
75. Ishiguro J, Shibahara K, Ueda Y, Nakamura K: **Fission yeast TOR signaling is essential for the down-regulation of a hyperactivated stress-response MAP kinase under salt stress**. Mol Genet Genomics 2013, **288**:63-75.
76. Qadota H, Python CP, Inoue SB, Arisawa M, Anraku Y, Zheng Y, Watanabe T, Levin DE, Ohya Y: **Identification of yeast Rho1p GTPase as a regulatory subunit of 1,3-β-glucan synthase**. Science 1996, **272**:279-281.
77. Nishimura MT, Stein M, Hou BH, Vogel JP, Edwards H, Somerville SC: **Loss of a callose synthase results in salicylic acid-dependent disease resistance**. Science 2003, **301**:969-972.
78. Evans NA, Hoyne PA, Stone BA: **Characteristics and specificity of the interaction of a fluorochrome from aniline blue (sirofluor) with polysaccharides**. Carbohydrate Polymers 1984, **4**:215-230.
79. Eggert D, Naumann M, Reimer R, Voigt CA: **Nanoscale glucan polymer network causes pathogen resistance. Sci Rep 2014, **4**:4159.
**This study showed the appicability of super-resultion microscopy in generating nanoscale, 3D-images of different cell wall polymers.30.
80. Voigt CA: **Callose-mediated resistance to pathogenic intruders in plant defense-related papillae**. Front Plant Sci 2014, **5**:168.
81. Lemmer P, Gunkel M, Weiland Y, Muller P, Baddeley D, Kaufmann R, Urich A, Eipel H, Amberger R, Hausmann M, et al.: **Using conventional fluorescent markers for far-field fluorescence localization nanoscopy allows resolution in the 10-nm range**. J Microsc 2009, **235**:163-171.
82. Platonova E, Winterflood CM, Ewers H: **A simple method for GFP- and RFP-based dual color single-molecule localization microscopy**. ACS Chem Biol 2015, **10**:1411-1416.
83. Ries J, Kaplan C, Platonova E, Eghlidi H, Ewers H: **A simple, versatile method for GFP-based super-resolution microscopy via nanobodies**. Nat Methods 2012, **9**:582-584.
84. Betzig E: **Nobel Lecture: Single molecules, cells, and super-resolution optics.** Rev Mod Phys 2015, 87(4), 1153-1168.

Figure legends:

Figure 1. Cellulose synthase and synthesis. Left panel: Cellulose is produced by large cellulose synthase (CesA) complexes at the plasma membrane. The CesA complex is assembled in the Golgi, or possibly the Endoplasmic Reticulum, with the aid of the recently described STELLO proteins. The CesA complex is secreted/internalized to/from the plasma membrane via the Trans-Golgi Network and/or small CesA compartments that can interact with microtubules or the actin cytoskeleton (indicated by dashed lines). The CesA complex is inserted next to cortical microtubules and guided via CSI1/POM2 along the microtubules, which are stabilized by CMU proteins, during cellulose synthesis. The Companion of CesA (CC) proteins are part of the CesA complex and protect the cellulose producing capacity against environmental stress. The endo-glucanase KORRIGAN also partake in the CesA complex and may contribute to correct microfibril formation or in the severing of cellulose chains during or after synthesis. Several CesA subunits may be phosphorylated, which regulates the behaviour of the CesA complex. Right panel: The CesA complex has traditionally been anticipated to contain 36 CesA subunits; however, recent estimates of cellulose fibril width have suggested that the complexes may contain only 18 or possibly 24 CesA subunits. Alternatively, the CesA complex still holds 36 subunits, but not all of them are active.

Figure 2. Model of cellulose/callose polymer network formation. Cellulose/callose polymer network formation has been observed i) in unchallenged plant epidermal leaf cells of maize and Miscanthus where this layered network is exposed to the external environment; and ii) at infection sites of plant epidermal leaf cells of Arabidopsis where the stress-induced callose synthase GSL5 is responsible for callose synthesis and its deposition in defense-related papillae (e.g. induced by fungal pathogens through attempted penetration along infection hyphae). Super-resolution microscopy suggests a spatial and temporal coordination of cellulose and callose synthesis to form a layered glucan polymer network in unchallenged maize and Miscanthus leaf tissue. Here, cellulose/callose cell wall architecture seems to be a general cell wall adaption or modification, which is not stress-induced. In contrast, stress-induced cellulose/callose polymer network formation follows a sequential glucan synthesis where callose microfibrils would be deposited in the paramural space between the pre-existing cellulosic cell wall from where they migrate via nanopores through the cellulosic cell wall to form the callosic papilla. Ongoing cellulose synthesis outside the infection area is assumed.


Plasma membrane
Cytosol
P
P
smaCCs/MASCs
Trans-Golgi
Network
Golgi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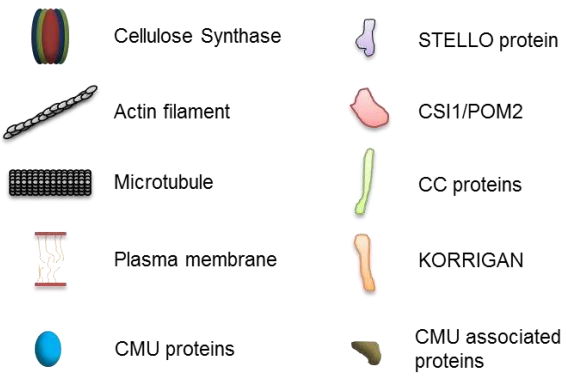
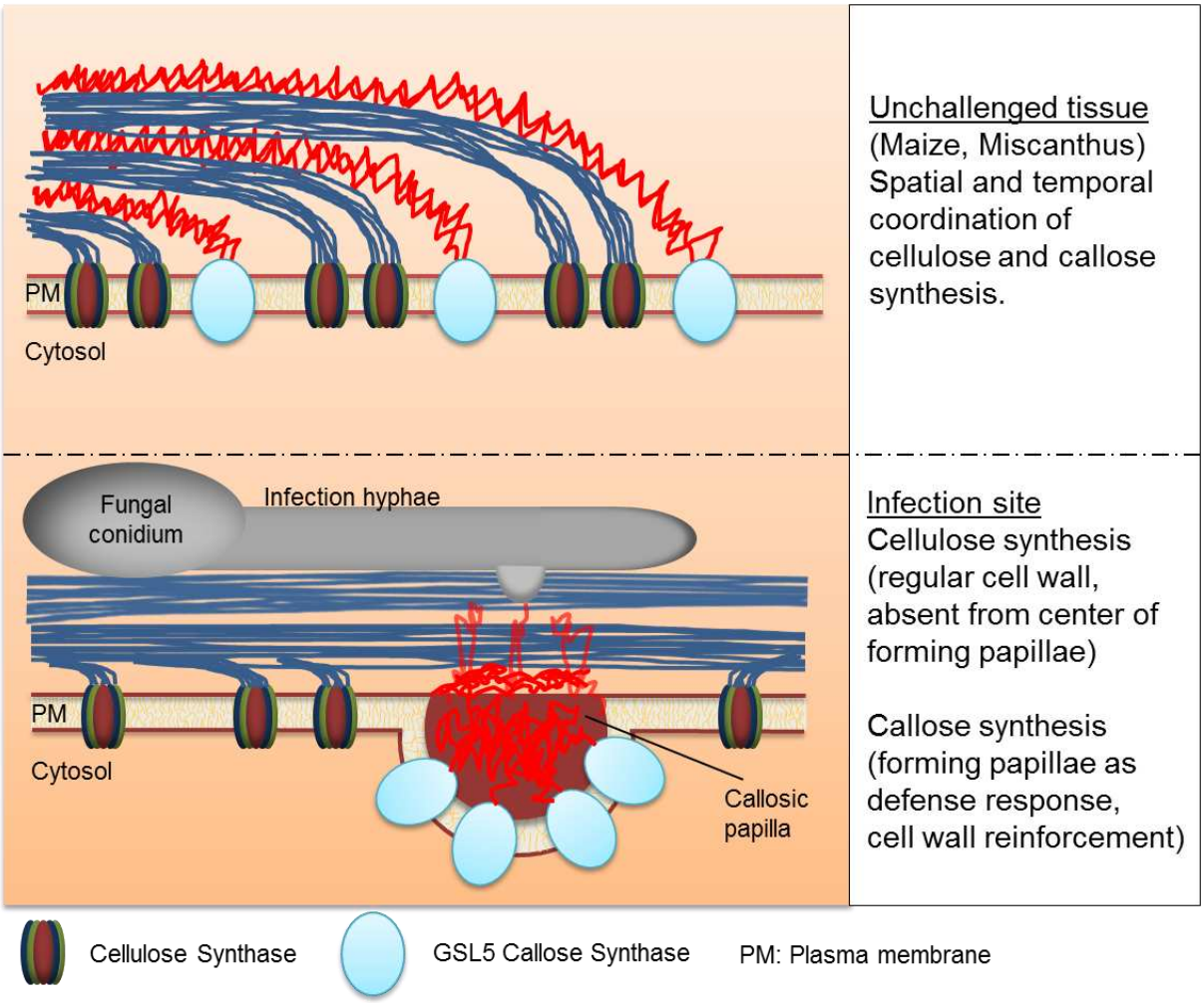

Cellulose Synthase
Actin filament
Microtubule
Plasma membrane
CMU proteins
STELLO protein
CSI1/POM2
CC proteins
KORRIGAN
CMU associated proteins


PM
Cytosol
Fungal conidium
Infection hyphae
PM
Cytosol
Callosic papilla
Unchallenged tissue
(Maize, Miscanthus)
Spatial and temporal coordination of cellulose and callose synthesis.
Infection site
Cellulose synthesis (regular cell wall, absent from center of forming papillae)
Callose synthesis (forming papillae as defense response, cell wall reinforcement)
Cellulose Synthase
GSL5 Callose Synthase
PM: Plasma membrane