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Update

Callose synthesis at the center point of plant development—An evolutionary insight

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Abstract

Polar callose deposition into the extracellular matrix is tightly controlled in time and space. Its presence in the cell wall modifies the properties of the surrounding area, which is fundamental for the correct execution of numerous processes such as cell division, male gametophyte development, intercellular transport, or responses to biotic and abiotic stresses. Previous studies have been invaluable in characterizing specific callose synthases (CalSs) during individual cellular processes. However, the complex view of the relationships between a particular CalS and a specific process is still lacking. Here we review the recent proceedings on the role of callose and individual CalSs in cell wall remodelling from an evolutionary perspective and with a particular focus on cytokinesis. We provide a robust phylogenetic analysis of CalS across the plant kingdom, which implies a 3-subfamily distribution of CalS. We also discuss the possible linkage between the evolution of CalSs and their function in specific cell types and processes.

Callose as a polar cell wall component

The primary cell wall is a unifying characteristic of all plants and is composed of a variety of polysaccharides with distinct functions such as cellulose (conferring the cell wall mechanical stability while allowing its extensibility), hemicelluloses (affecting self-assembly of cellulose microfibrils and determining cell wall extensibility), and pectins (supporting cell wall integrity and controlling cell wall thickness) (Cosgrove 2022; Colin et al. 2023). This composition varies during plant ontogeny, with a controlled polar rearrangement of the cell wall facilitating distinct developmental processes and environmental responses in the inherently immobile plant cells (De Lorenzo et al. 2018; Gigli-Bisceglia et al. 2020; Zhang et al. 2021a). Unlike the 3 "canonical" cell wall components, β-1,3-glucan polysaccharide callose is a temporal part of the cell wall whose presence in the extracellular space, resulting from its polar synthesis, alters the physical and mechanical properties around the site of the deposition. Composed of β-1,3–linked glucose subunits that form the disaccharide laminaribiose and occasional β-1,6-glucosyl side chains, the final polysaccharide has an amorphous structure, in contrast to microcrystalline β-1,4 glucan cellulose (Stone 2009; Piršelová and Matušíková 2013; Zhang et al. 2021b). Even though the structural role of callose in the cell wall is still a subject of ongoing studies, reduced permeability to compounds (Heslop-Harrison and Mackenzie 1967; Yim and Bradford 1998; Gensler 2019) and enhanced rigidity at maintained flexibility (Parre and Geitmann 2005; Abou-Saleh et al. 2018; Kapoor and Geitmann 2023) are 2 primary consequences stemming from the callose synthesis. Moreover, the composition of callose distinct from other cell wall components enables its controlled degradation when it is no longer necessary, further punctuating its significance as a polar compound in both space and time.

The changes in cell wall properties driven by callose deposition are essential for many tissue and cell types. For example, during male gametophyte development (Fig. 1), the inherent

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ADVANCES BOX

- The recent explosion of quality omics data from different species across the plant kingdom enables the studies of the evolution of distinct cellular processes such as callose synthesis with unprecedented detail.
- Callose is polarly synthesized at the plasma membrane by the callose synthase (CalS).
- Polar synthesis of callose is essential for a portfolio of cellular processes, each controlled by a specific paralog from the CalS family.
- A comprehensive phylogenetic analysis revealed that CalS is highly conserved in the plant kingdom, and 3 ancestral subfamilies form the CalS family.

impermeability of callose protects the developing microsporocyte tetrads from the outer environment, preventing water uptake and subsequent microsporocyte rupture (Dong et al. 2005). Simultaneously, it acts as a mould for the deposited exine of the future pollen grain (Dong et al. 2005; Nishikawa et al. 2005). After the microspores reach full maturity, callose is selectively degraded, releasing the individual microspores from the tetrad (Stieglitz 1977). In the growing pollen tube, callose is the predominant polysaccharide in the subapical cell wall, conferring simultaneous rigidity and flexibility of the tissue, possibly in cooperation with cellulose (Parre and Geitmann 2005; Vogler et al. 2013; Abou-Saleh et al. 2018). The mechanical properties of the pollen tube cell wall enable the pollen tube to efficiently navigate through the transmitting tract of the style, improving but not substantiating the fertilization efficiency (Nishikawa et al. 2005).

Another process that utilizes the amorphous gel-like properties of callose is the regulation of intercellular connectivity (Fig. 1). As such, callose is synthesized in a controlled manner at the neck region of the plasmodesma, constricting its aperture and thus lowering the size exclusion limit (Fitzgibbon et al. 2010; Maule et al. 2012). By this simple mechanism, plants can control the transport of nutrients, proteins, and signaling compounds or prevent the spread of viruses (Vatén et al. 2011; Li et al. 2012b; Zavaliev et al. 2013; Ross-Elliott et al. 2017). Similarly, during the development of the phloem, callose deposition to the cell–cell junctions between the future sieve elements results in an increased sieve plate pore diameter, eventually affecting the rootward transport of nutrients (Xie et al. 2011). However, callose is also an essential regulator of transport in the fully developed phloem; callose impregnation of the sieve plates has been observed in response to a variety of stress treatments, including heat (McNairn and Currier 1968), cutting (Ehlers et al. 2000), leaf tip burning (Furch et al. 2010), and aphid feeding, obstructing the basipetal flow. Likewise, plants have evolved a 2-component mechanism activated upon phloem damage composed of a quick P-protein plugging of sieve plates, followed by their gradual impregnation by callose. This helps to prevent infection from spreading and restricts the amount of the escaped sap (Knoblauch and van Bel 1998; Ehlers et al. 2000; Furch et al. 2010).

One of the best models for studies of the polar secretion of callose is papilla formation during pathogen response (Fig. 1). The papilla is a dome-shaped callose-rich structure deposited at the location of the pathogen infection, acting as a reinforcement layer (Aist 1976), as well as preventing the diffusion and activity of the cell wall hydrolyzing enzymes (Eggert et al. 2015). Colocalization analysis of immunolabeled polysaccharides induced upon powdery mildew *Blumeria graminis* f. sp. *hordei* infection of barley revealed that papilla callose is accompanied by arabinoxylans, known cellulose cross-linkers (Chowdhury et al. 2014). Because callose-cellulose mixtures have been observed to possess improved resistance to orthogonal pressure (Abou-Saleh et al. 2018), arabinoxylan cross-linking leads to additional strengthening of the surrounding cell wall (Chowdhury et al. 2014). Even if the papilla fails, the callose creates a collar around the neck of the protruding hyphae, the constriction presumably restricting nutrient transport within the pathogen (Donofrio and Delaney 2001; Micali et al. 2011).

In all the aforementioned cases, callose is deposited as a secondary compound into the existing cell wall. However, this is not true throughout the plant body. In plants, the ultimate step of cell division, cytokinesis, depends on the formation of the cell plate, a membranous structure whose centrifugal growth controls the separation of daughter nuclei (Fig. 1). The cell plate is initialized by the fusion of Golgi-derived vesicles at the dividing cell equator, subsequently transforming into a tubulo-vesicular network. In contrast to cellulose deposited from the tubulo-vesicular network stage, callose is the first and fundamental polysaccharide of this nascent cell plate, present from the vesicle fusion stage for the duration of cytokinesis (Samuels et al. 1995; Miart et al. 2014). On the subcellular level, callose forms a uniform coat on the extracytoplasmic side of the plasma membrane, which is believed to enable the further spreading of cell plate tubules, eventually transforming into a smooth perforated sheet (Samuels et al. 1995; Jawaid et al. 2022). The sheet is then stabilized by cellulose microfibrils formed during the later stages of its development at a simultaneous degradation of callose (Samuels et al. 1995). Ultimately, callose also facilitates the perpendicular connection of the mature cell plate with the parental cell wall (Thiele et al. 2009).

Enzymatic control of polar callose deposition

The extracellular deposition of callose in plants depends on callose synthase (CalS), a large 200-kDa integral membrane protein. CalS is in Arabidopsis (*Arabidopsis thaliana*) encoded by a family of 12 genes, *CalS*/*Glucan synthase-like* 1 to 12. Its structure encompasses 3 functional regions—

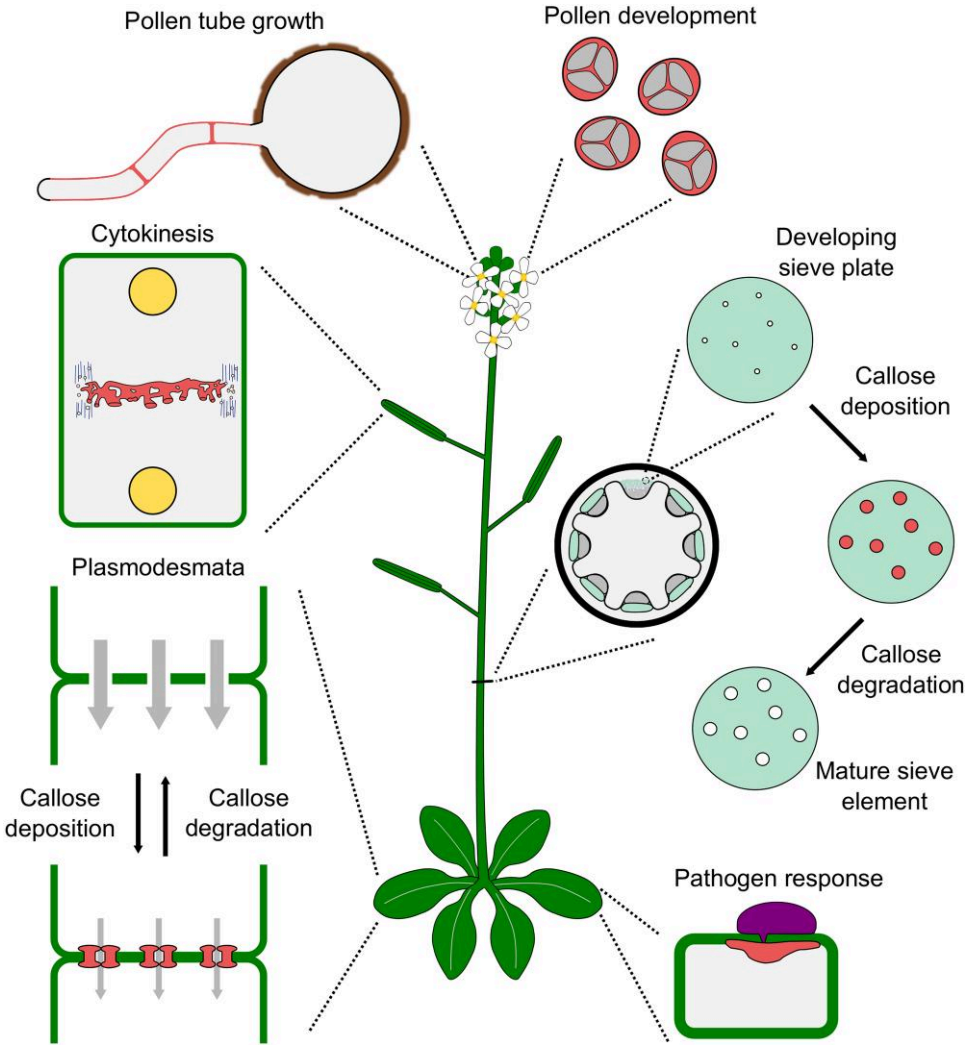

Figure 1. Polar deposition of callose in the individual cell types. Callose (light red) is synthesized during male gametophyte development on the outer side and between the tetrad microsporocytes. After pollen germination, callose is deposited in the subapical region of the protruding pollen tube and makes up callose plugs, which restrict the location of sperm cells at the tip. Controlled callose deposition at plasmodesmata also regulates symplastic transport, a special case employed in the developing phloem, where callose is synthesized at the future sieve element junctions and enlarges the sieve plate pore. During cytokinesis, callose is the main component of the nascent cell plate, which mediates the separation of the daughter nuclei. Callose is also essential in preventing pathogen entry during infection by forming a special thickened structure called the papilla.

Vta1 domain, FKS1 domain, and glucan synthase domain—as well as 16 transmembrane helices and a central cytoplasmic loop that confers the β-1,3-glucan synthesis activity (Záveská Drábková and Honys 2017). Data from protein–protein interaction assays suggest that CalS is part of a bigger structure called CalS complex (CALSC) (Verma and Hong 2001). In this complex, CalS interacts with phragmoplastin (with unknown function in CALSC), Rho of plants 1 small GTPase, and annexin (both possibly controlling CalS activity) (Hong et al. 2001; Verma and Hong 2001). The same studies also described UDP-glucose transferase as an interacting partner of CalS (presumably providing the UDP-glucose precursors for laminaribiose synthesis). However, a major UDP-glucose transferase isoform in Arabidopsis, UGT71C5, was shown to play an important role in abscisic acid homeostasis by glucosylating abscisic acid to an abscisic acid-glucose ester (Liu et al. 2015) and thus brings into question the role of this UDP-glucose transferase in the CALSC. It is therefore essential to address which UDP-glucose transferase isoform is part of CALSC and provide functional evidence of the interaction. Recently, additional partners of Arabidopsis CalS10, PLASMODESMATAL-RELATED PROTEIN 5 and β-1,3-glucanase, were identified; the same study also revealed the interaction of AtCalS10 with AtCalS9, providing the first evidence of CalS oligomerization (Saatian et al. 2018).

Polar callose synthesis assumes the specific regulation of CalS. This regulation can be mediated directly at the membrane by post-translational modifications of the functional protein. Indeed, several putative phosphorylation and glycosylation sites have been identified in the N-terminus of CalS

(Verma and Hong 2001). Moreover, several studies described the different CalS phosphorylation statuses after stress treatment, although the specific kinases and phosphatases remain unknown to this date (Ellinger and Voigt 2014). Alternatively to post-translational modifications, polar callose deposition might also be controlled by CalS trafficking. Indeed, inactive AtCalS5 and AtCalS12 have been observed to localize to Golgi and endomembrane system components, correlated with a lack of aniline blue staining of the callose signal (Xie et al. 2011; Ellinger et al. 2013). Similarly, the endomembrane fractions of the *Nicotiana alata* pollen tubes contained only trace amounts of active CalS compared with the plasma membrane fractions (Brownfield et al. 2008). Several distinct Arabidopsis CalSs have also been identified in exocytic vesicles, although the authors did not focus on CalS activity measurements (Drakakaki et al. 2012). These data altogether show that there are multiple levels of control of polar callose deposition.

Studies of other enzymes synthesizing polysaccharides at the plasma membrane, such as plant cellulose synthase (CESA) or fungal chitin synthase (ChS), can provide valuable insight into spatiotemporal regulation of the CalS dynamics, serving as a blueprint for future CALSC research. Recent proceedings have shown that CESA is transported to the plasma membrane via an endomembrane system-dependent manner. Inside the Golgi apparatus, CESA presumably undergoes post-translational modifications such as glycosylation (Nibbering et al. 2022) and assembles into a multimeric structure called the CESA complex, adopting a rosette-shaped architecture (Zhang et al. 2016; Purushotham et al. 2020). The newly formed complex is trafficked through the trans-Golgi network (TGN) toward the plasma membrane. Clathrin-mediated endocytosis further regulates its turnover at the plasma membrane, thus affecting the cellulose synthesis rate (Lei et al. 2015; McFarlane et al. 2021; Vellosillo et al. 2021). Alternatively, the extracellular deposition of cellulose is also modified by a portfolio of CESA interactors, which affect the dynamics of microfibril deposition in space and time (Li et al. 2012a; Vain et al. 2014; Liu et al. 2016; Schneider et al. 2022). A large body of data is available regarding trafficking and regulation of ChS. In brief, ChS is synthesized and packaged in the endoplasmic reticulum (Sánchez and Roncero 2022). Subsequently, COPII coatomer mediates ChS transport to Golgi (Jakobsen et al. 2013; Sacristan et al. 2013), where it is subject to further sorting; the incorrectly folded molecules return to endoplasmic reticulum via COPI-dependent retrograde transport, and Exomer promotes the anterograde transport through TGN toward the plasma membrane (Starr et al. 2012; Anton-Plagaro et al. 2021). During its trafficking by the endomembrane system, ChS forms an oligomeric structure consisting of multiple Chs subunits and a set of interacting factors, which together mediate correct ChS delivery while controlling its enzymatic activity at the distinct subcellular compartments (DeMarini et al. 1997; Reyes et al. 2007; Gohlke et al. 2017; Dharwada et al. 2018; Ren et al. 2022). Similarly to CESA, chitin deposition is terminated by ChS internalization (Reyes et al. 2007). Ultimately, the inactive ChS is either recycled back to the plasma membrane or marked by ubiquitin and sorted by the endosomal sorting complex required for transport complex for vacuolar degradation (Chuang and Schekman 1996; McMurray et al. 2012; Arcones et al. 2016; Knafler et al. 2019). Because CalS, CESA, and ChS are all large (>100 kDa) integral membrane proteins that mediate polysaccharide synthesis in the cell wall, similar mechanisms could perhaps underlie the tight control of callose synthesis, as well.

Besides its synthesis, the spatial and temporal polarity of callose is also controlled by its selective degradation. This degradation is facilitated by β-1,3-glucanases, a large set of hydrolytic enzymes. β-1,3-Glucanases contain several functional domains: a targeting sequence at the N terminus (for extracellular targeting), a glycosyl hydrolase domain (active core), a CBM43 domain (binding to callose), and a C-terminal sequence (Xu et al. 2016). As revealed from the analysis of their phylogenetic clustering, domain composition, and expression pattern, β-1,3-glucanases cluster into 13 functional groups (Doxey et al. 2007; Xu et al. 2016). On the subcellular level, the activity of β-1,3-glucanases is most notable in cell–cell junctions, where their activity loosens the callose-dependent constriction of plasmodesmata, thus effectively regulating the size exclusion limit. β-1,3-Glucanases are also essential for pollen, where they release the callose-bound microsporocytes from tetrads (Perrot et al. 2022). During infection, the β-1,3-glucanase activity has also been confirmed to digest the pathogen cell wall, often abundant in β-1,3-glucans (Ruiz-Herrera and Ortiz-Castellanos 2019; Wang et al. 2021; Liu et al. 2022b), which is probably a result of plant coevolution with biotic factors because the β-1,3-glucan hydrolyzing activity during cell division or cell wall remodeling is presumed to be the ancestral role of β-1,3-glucanases (Doxey et al. 2007).

Cytokinetic callose across the plant kingdom

The role of callose in cell plate formation is conserved throughout the Embryophyta clade. Naturally, a question arises: If all embryophytes divide by a callose-rich cell plate, what was the situation in their ancestors? In the Chlorophyta lineage of Viridiplantae, the cytokinesis depends on a centripetal cleavage furrow (Fig. 2A) (Cross and Umen 2015; Katsaros et al. 2017; von der Heyde and Hallmann 2022). In contrast, the high-resolution microscopy data determined a more complex situation in Streptophyta, the sister clade to Chlorophyta. As such, the Mesostigmatophyceae, Chlorokybophyceae, and Klebsormidiophyceae classes of Streptophyta all divide by a centripetal constriction (Fig. 2A) (Manton and Ettl 1965; Floyd et al. 1972; Lokhorst et al. 1988), whereas Charophyceae, Coleochaetophyceae, and Embryophyta strictly utilize a centrifugal mechanism of cytokinesis (Fig. 2A) (Gambardella and Alfano 1990; Cleary et al. 1992; Cook et al. 1998; Cook 2004; Smertenko et al. 2017; Kanazawa et al. 2020). These studies led to the proposition of centrifugally

dividing Phragmoplastophyta clade, a sister clade to Klebsormidiophyceae (Adl et al. 2012; Nishiyama et al. 2018), and also concurring that the last common ancestor of plants possessed Opisthokont-like cytokinesis, which was replaced with a cell plate-dependent mechanism in the rest of the lineage (Buschmann and Zachgo 2016). However, the evolutionary progression from the cleavage furrow to the cell plate has long been a mystery. The discovery of Zygnematophyceae as a sister clade to land plants provided a novel insight into the stages of cytokinesis evolution because some Zygnematophyceae, such as *Spirogyra* or *Penium margaritaceum*, divide by combined cytokinesis mediated by cleavage and cell plate simultaneously (Fig. 2A) (McIntosh and Pickett-Heaps 1995; Davis et al. 2020). These results also suggested possible cooperation between the 2 mechanisms. However, dual cytokinesis is not ubiquitous in Zygnematophyceae, with many representatives possibly exhibiting only the cleavage furrow (Hall et al. 2008; Hess et al. 2022). The latest data suggest that in the Zygnematophyceae, the cleavage furrow has reappeared secondarily, along with the reduction of their centrifugal division apparatus. Therefore, the emergence of centrifugal machinery within plants is more likely connected to the most recent common ancestor (MRCA) of Phragmoplastophyta (Buschmann and Zachgo 2016; Hess et al. 2022).

The distinct division between Klebsormidiophyceae and Phragmoplastophyta would hint at the vastly different processes underlying their cytokinesis mechanisms. However, the presence of callose during nuclei separation has also been observed in plants dividing by furrowing, most notably *Klebsormidium flaccidum* or *Fritschiella tuberosa* (Scherp et al. 2001), thus implying that the recruitment of callose in cross wall formation was a much earlier event, possibly linked to the emergence of multicellularity (Scherp et al. 2001). Because of this, several studies have tried to address the evolution of cytokinetic callose deposition by focusing on the borderline between the centripetally and centrifugally dividing streptophyte classes: bryophytes *sensu lato* (a sister group to vascular plants; Puttick et al. 2018; Harris et al. 2020) and streptophyte green algae. In Marchantiophyta representative *Riella helicophylla*, the callose content was present in the whole cell plate, although slightly increased in the contact sites between the cell plate and parental walls (Scherp et al. 2002). A similar pattern as in *Riella* was observed in furrowing *Zygnema* and *Klebsormidium crenulatum*, with callose detected during the centripetal progression of the cross wall. In contrast, callose was restricted to the central part of the cross wall in *K. nitens* (Herburger and Holzinger 2015). In both of these studies, the callose signal was not quantified, thus not allowing their direct comparison. In Zygnematophyceae representative *P. margaritaceum*, the cross wall is started by a constricting septum, which is then complemented by an expanding central punctum (a cell plate–like structure), both rich in callose (Fig. 2A) (Ochs et al. 2014; Davis et al. 2020). This mechanism is presumed to be reduced in a successor of *Spirogyra* cytokinesis, which is more closely related to canonical Embryophyta cytokinesis. Interestingly, in both species, callose synthesis inhibition by the Endosidin 7 treatment led to incomplete division and fused cells that exhibited a complete absence of the central punctum, whose function could not be supplemented by a functional cleavage apparatus. The Endosidin 7 treatment abolished the cell plate in Arabidopsis, showing that callose might not play a significant role during furrowing but does so in the cell plate formation (Park et al. 2014; Davis et al. 2020; Jawaid et al. 2022).

CalSs form a 3-subfamily system across the Phragmoplastophyta lineage

What could be the evolutionary driving force behind the mechanism of plant cytokinesis? The protein machinery facilitating nuclei separation is composed of several components, including microtubular phragmoplast, Phragmoplastin (also called Dynamin-related protein 1), SH3P and SNARE proteins, as well as their related partners, whose evolutionary contribution has been previously addressed in various insightful works (Buschmann and Zachgo 2016; Arakaki et al. 2017; Park et al. 2018; Buschmann and Müller 2019; Forero and Cvrčková 2019). Other essential players encompass actin filaments (especially during the cleavage furrow progression), motor proteins (POK2 and Myosin VIII), the TPLATE complex, or phospholipids (see Müller and Jürgens 2016; Smertenko et al. 2017; Caillaud 2019; Livanos and Müller 2019; Sinclair et al. 2022) (Fig. 2B). Another key component is CalS, which is responsible for callose deposition into the nascent cell plate. Previous analyses of CalS phylogeny, although mostly focused on a limited number of studied species and phylogenetic groups, identified many genes homologous to CalS across the plant kingdom (Yamaguchi et al. 2006; Schuette et al. 2009; Abercrombie et al. 2011; Piršelová and Matušíková 2013; Yu et al. 2016; Záveská Drábková and Honys 2017; Liu et al. 2018; Saatian et al. 2018; Granato et al. 2019; Davis et al. 2020; Feng et al. 2021; Cao et al. 2022; Wang et al. 2022).

The highly conserved presence of CalS in the plant lineage, combined with the pattern of callose deposition during cytokinesis, makes CalS a perfect subject for the studies of cell division evolution. To obtain a comprehensive view of CalS evolution, we constructed a phylogenetic tree from a diverse set of the CalS family protein sequences. To limit any bias in the analysis, we prepared a balanced dataset with the number of assayed species roughly equal between the main plant clades. Notably, concerning Klebsormidiophyceae and all Phragmoplastophyta, the CalS family forms 3 distinct monophyletic subfamilies, which we title CalSA, B, and C, respectively (Fig. 3 and Supplemental data). A similar 3-subfamily distribution was previously mentioned in several works (Schuette et al. 2009; Abercrombie et al. 2011; Piršelová and Matušíková 2013; Yu et al. 2016; Liu et al. 2018; Wang et al. 2022). However, not all groups within this proposed distribution contain paralogs in each subfamily. For example, Klebsormidiophyceae (*K. nitens*, *K. subtile*, and *Interfilum*

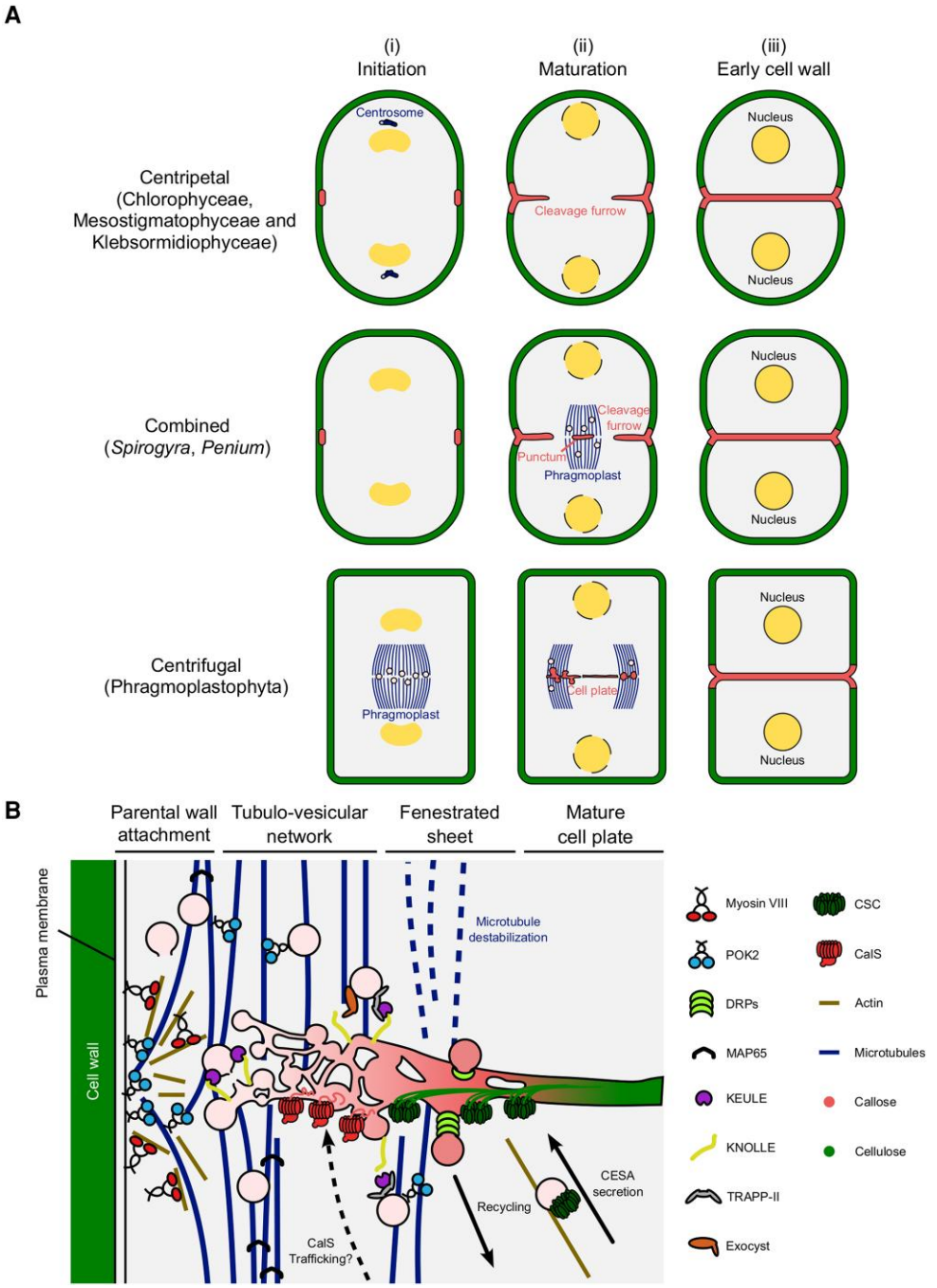

Figure 2. Plant cytokinesis in space and time. **A)** Cytokinetic mechanism of the specific plant groups. The algae from *Chlorophyta*, *Mesostigmatophyceae*, and *Klebsormidiophyceae* lineages divide by centripetal constriction, mediated by the cleavage furrow. This is presumably the basal mechanism of plant cytokinesis. Selected *Zygnematophyceae* species undergo a combined form of cytokinesis, characterized by the complementary action of the inward-growing cleavage furrow and the outward building of the cell plate–like structure punctum. Due to the distinct evolution of *Zygnematophyceae*, this is considered to be a result of the secondary reduction of land plant cytokinesis because the rest of the plants from the *Phragmoplastophyta* lineage exhibit a "canonical" centrifugal mode of cytokinesis. This depends on the cell plate, a membranous structure created by the phragmoplast-mediated delivery of vesicles fusing at the division plane and its progressive enlargement towards the cell edge. In all cases, the nascent cross wall is enriched by the polysaccharide callose. **B)** Overview of selected molecular players involved in centrifugal cytokinesis. The vesicles containing the necessary membrane material are delivered as a cargo of Kinesin motor proteins (including POK2) along the phragmoplast microtubules, nucleated by MAP65. Their docking to the cell plate membrane is promoted by tethering complexes TRAPP-II and Exocyst, and the final fusion requires KEULE and the SNARE protein KNOLLE. The fused vesicles form a tubulo-vesicular network, which is further planarized by CalS-dependent callose deposition into a fenestrated sheet. The synthesis of cellulose microfibrils by the CSC stabilizes the cell plate and enables its maturation. During cell plate growth, the excess membrane material is recycled endocytotically with the help of Dynamin-related proteins (DRPs). The cytokinesis is finalized by the fusion of the cell plate with the parental cell wall, guided by POK2 and Myosin VIII.

paradoxum) possess 1 CalS clustering inside the subfamily A. In addition to this CalSA, *K. nitens* and *K. subtile* contain an additional isoform forming an individual clade sister to the rest of the *Streptophyta* lineage (Fig. 3). On the other hand, contrary to Klebsormidiophyceae, CalSA is absent in *Chara braunii* and *Nitella mirabilis* but also in Anthocerotophyta. As for the rest of the bryophyte clade, Marchantiophyta species have only a single paralog per subfamily, and mosses exhibit large gene multiplications, especially in the subfamilies B and C. This pattern differs from Euphyllophyta, whose CalSs have predominantly diverged in the subfamily A (especially because most assayed Embryophyta species have a comparable number of roughly 12 CalS paralogs), possibly reflecting the differences in selection pressures and developmental innovations during the bryophytes and Embryophyta evolution (Schuette et al. 2009).

Similarly to the rest of the plant lineages with the A-B-C divergence, in each of the Zygnematophyceae species, there are several CalS paralogs. Their distribution across the 3 subfamilies follows a similar pattern as in bryophytes, with the early diverging species exhibiting a CalSA loss and the later diverging species retaining all CalS paralogs. Paralogs from each subfamily are present in *P. margaritaceum* and *Mesotaenium kramstae* (Desmidiales and Zygnematales, respectively), whereas a subfamily A paralog is absent in *Mesotaenium endlicherianum* (Serritaeniales). However, it cannot be ruled out that the genome and transcriptome coverage was insufficient to identify all CalS paralogs within the assayed species (Zhou and von Schwartzenberg 2020; Hess et al. 2022). This also implies that the MRCA of Zygnematophyceae and Embryophyta already possessed a complete set of CalS, maintained from the Charophyceae MRCA. Although *Mesostigma viride*, *Chlorokybus atmophyticus*, and the Chlorophyceae algae *Chlamydomonas reinhardtii*, *Volvox carteri*, and *Chromochloris zoofingiensis* also contain several CalS paralogs, their divergence is most likely a more recent event, unrelated to the aforementioned A-B-C subfamilies. Instead, all CalS from Mesostigmatophyceae form a sister clade to the A, B, and C subfamily system. Likewise, the Chlorophyceae CalS cluster into an independent monophyletic clade.

Besides the general 3-subfamily distribution, the distribution of CalS genetic structure between the paralogs also appears to correlate, at least in Arabidopsis. All AtCalS paralogs contain approximately 40 introns, except for CalSC subfamily members, which have the least number of introns. In contrast, CalSB paralogs generally have longer introns, and CalSAs exhibit an intermediary composition (Enns et al. 2005). The evolution of each major subfamily tightly follows the generally accepted phylogeny of the plant kingdom (One Thousand Plant Transcriptomes Initiative 2019). As such, the CalSC subfamily is characterized by AtCalS11 and 12, which have most likely undergone a recent duplication, a similar case as in monocot *Brachypodium distachyon*. This pattern of recent duplications is primarily conserved in all Spermatophyta, whereas in Monilophyta (represented by *Ceratopteris richardii* and *Pteris vittata*), the CalSCs have undergone several rounds of duplication independent from the rest of the Euphyllophyta lineage. In subfamily B, an analogous multiplication within Monilophyta can be observed, while 2 independent lineages in Arabidopsis represented by AtCalS9 and AtCalS10 have evolved within Spermatophyta. Finally, the CalSA subfamily consists of 3 distinct branches. Of these, the CalS5-related clade is distinguishable already in Monilophyta, not only Spermatophyta as observed previously by (Abercrombie et al. 2011), forming a rather evolutionarily early diverging branch. This is in contrast with other CalSA members within Euphyllophyta, which form distinct branches starting from the level of Gymnospermae, encompassing AtCalS1-4 and AtCalS6-8, respectively, with a mutual sister clade containing Monilophyta CalSs.

A major role for CalSs in cell division

The emergence of novel gene families and their further expansion is a staple feature of plant transition to land (One Thousand Plant Transcriptomes Initiative 2019; Bowles et al. 2020). Moreover, the functional specialization is driven by whole genome duplications, which occurred after plant territorialization, as well as the duplication of individual genes (One Thousand Plant Transcriptomes Initiative 2019; Stull et al. 2021). The colonization of the land and the subsequent burst of land plant species was further enabled by accompanying diversification and modification of specialized metabolic pathways (such as callose synthesis) (Rieseberg et al. 2023). Nevertheless, these do not single-handedly explain the divergence into 3 subfamilies observed in the CalS phylogeny because multiple paralogs that belong to the distinct subfamilies were presumably already present in the aquatic Phragmoplastophyta ancestor (Fig. 4). We also suggest the 3-subfamily system has already been present in some form in the MRCA of Klebsormidiophyceae and Phragmoplastophyta due to the Klebsormidiophyceae homolog cluster within the CalSA subfamily. Interestingly, the divergence of CalS into the 3 subfamilies strikingly coincides with the emergence of centrifugal cytokinesis mediated by the phragmoplast and cell plate (Buschmann and Zachgo 2016). Indeed, Charophyceae, which divide by a centrifugal growth, are the most evolutionarily distant Phragmoplastophyta group from Embryophyta that exhibit the 3-subfamily divergence (Nishiyama et al. 2018). The number and distribution of CalSs from Coleochaetophyceae, a paraphyletic, cell plate–dependent group between Charophyceae and Zygnematophyceae (Ruhfel et al. 2014; One Thousand Plant Transcriptomes Initiative 2019), remains unknown. A similar CalS inventory as in *Chara* is present in the cell plate–dependent Anthocerotophyta *Anthoceros agrestis* and *Leiosporoceros dusii*. Marchantiophyta dividing by centrifugal cytokinesis (Scherp et al. 2002) contain all 3 subfamily representatives. The same is true for the rest of the Embryophyta, in which a large CalS multiplication occurred across the gene family. Similarly, *P. margaritaceum*,

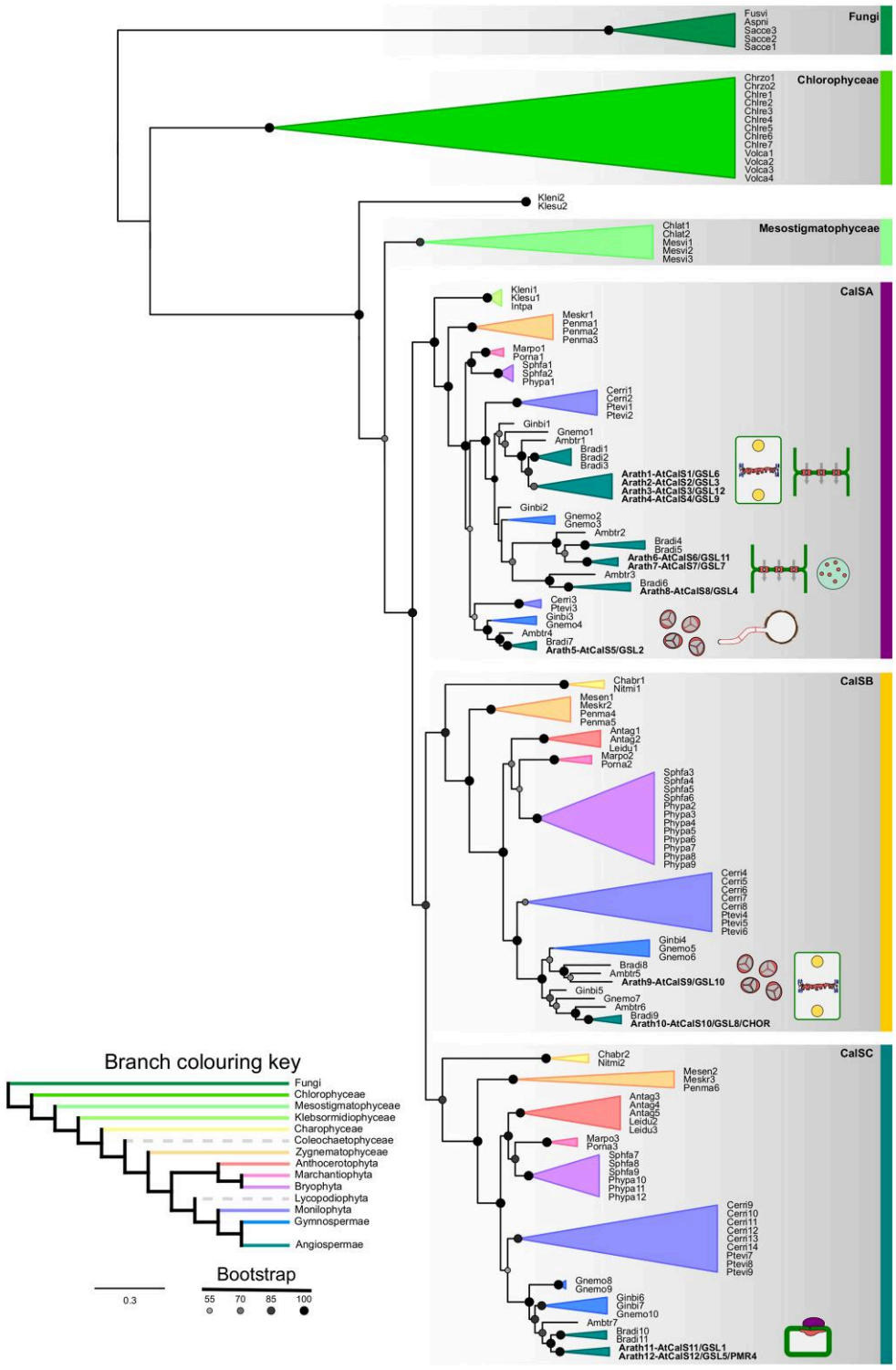

Figure 3. Maximum likelihood phylogenetic tree of the CalS family. Callose synthases from the land plants *Zygnematophyceae*, *Charophyceae*, and *Klebsormidiophyceae* cluster into 3 distinct subfamilies: CalSA, B, and C. The positions of individual Arabidopsis paralogs are highlighted. For the tree calculations, at least 2 species per big taxonomic group across the *Streptophyta* were employed. These are color-coded as shown in the branch coloring key, with *Coleochaetophyceae* and *Lycopodiophyta* greyed out and dashed to signify the omitted groups. The phylogenetic tree is rooted with the fungal homologs of CalS, β-1,3-glucan synthases present in *Saccharomyces cerevisiae*, *Fusarium virgulis*, and *Aspergillus niger*. The circle size and brightness represent bootstrap values >55%, as calculated from 500 replicates. The scale bar represents 0.3 expected substitutions per site. The full tree and the details can be found in Supplemental Fig. S1.

a Zygnematophyceae representative with combined cytokinesis, also contains homologs in each subfamily. We thus hypothesize that the CalS divergence was necessary to evolve centrifugal cell division machinery.

Although polar callose synthesis is crucial for the correct execution of cytokinesis, only 2 CalS paralogs in Arabidopsis have been described to localize to the nascent cell plate and synthesize callose there: AtCalS1 and AtCalS10 from the subfamily A and B, respectively (Hong et al. 2001; Töller et al. 2008; Thiele et al. 2009; Saatian et al. 2018). If we hypothesize that establishing the 3 subfamilies is related to the evolution of centrifugal cytokinesis, then either A or B subfamily should contain cytokinesis-specific CalS. Consistently, CalSA and CalSB representatives are present in the Zygnematophyceae species tested previously for the polar localization of callose during cell division. It is plausible these specific paralogs likewise control the maturation of the observed centrifugally expanding cell plate–like structure during *Penium* and *Spirogyra* cytokinesis (Davis et al. 2020). Even though AtCalS10 has also been described in a variety of unrelated processes, for example, the division of pollen generative cells or in plasmodesmata, the mutant *cals10* plants are seedling lethal because of the impaired callose synthesis during cytokinesis (Töller et al. 2008; Saatian et al. 2018). Interestingly, mutation of AtCalS1 causes no visible phenotype, possibly due to its sequence and expression similarity to AtCalS2, 3, and 4 (Dong et al. 2008). Except for AtCalS3, these all are functionally unknown, and their activity could play a redundant role to AtCalS1 during cytokinesis. Even though CalSA is perhaps the first diverging subfamily, its emergence does not necessarily sanction cell plate–dependent cell division (a case of Klebsormidiophyceae). Moreover, CalS phylogeny with the history of multiple independent CalSA subfamily losses in Charophyceae, Serritaeniales, and Anthocerotophyta shows that they were perhaps insignificant in regards to the centrifugal cytokinesis of Phragmoplastophyta.

Another paralog from the subfamily B, AtCalS9, is essential for the entry of a microsporocyte into mitosis, and its mutants are gametophyte lethal (Töller et al. 2008; Huang et al. 2009). Although the rice homolog of AtCalS9 and 10 has been implicated in vascular tissue development, this has been mostly extrapolated from its promoter activity and not on the level of a functional protein (Song et al. 2016). Is it therefore possible that the AtCalS9- and 10-containing B subfamily's function in cell division is conserved within Phragmoplastophyta? Proteins that execute indispensable processes with a low margin of error (such as cytokinesis) have a generally cosmopolitan pattern of expression and are conserved during evolution (Lloyd et al. 2015). Indeed, AtCalS9 and 10, as well as their orthologs in *Pyrus brettschneiderii*, are expressed throughout plant ontogeny (Záveská Drábková and Honys 2017; Cao et al. 2022). Moreover, CalSB subfamily proteins are always present in all species exhibiting the centrifugal division, at least in one copy. Compared with Spermatophyta, both Bryophyta and Monilophyta contain many copies of CalS from subfamily B, which form 2 deep branches. These data suggest that for the correct execution of centrifugal cytokinesis, the presence of CalSB is strictly required.

From pollen development to plant defense—are the roles of individual CalSs conserved?

Although some CalSA subfamily representatives are active in cytokinesis, the function of CalSA paralogs is far from restricted to cell division. The phylogenetic analyses showed the early divergence of the AtCalS5-like branch conserved from Monilophyta to Angiospermae. The callose production during male gametophyte ontogeny and pollen tube growth was found to be highly dependent on AtCalS5, whose disruption is gametophyte lethal (Dong et al. 2005; Nishikawa et al. 2005; Xie et al. 2011; Mizuta and Higashiyama 2014). This is consistent with the localization of GFP-fused AtCalS5 into the developing Arabidopsis pollen and callose staining between the microsporocytes, as well as the gametophyte-specific expression of AtCalS5 (Dong et al. 2005; Záveská Drábková and Honys 2017). Similarly, in other Angiospermae and Gymnospermae, CalS5 mediates pollen tube growth as well (Brownfield et al. 2008; Abercrombie et al. 2011; Cao et al. 2022; Kapoor and Geitmann 2023). These results validate the previously proposed hypothesis that CalS5-controlled deposition of callose has been conserved from the common ancestor of seed plants. Our phylogeny also provides a new insight into the role of callose in the evolution of pollen tubes, which is manifested particularly by the changes in the subcellular localization of callose as well as callose plug emergence (compared with Gymnospermae), possibly facilitating its rapid growth as observed in Angiospermae (Williams 2008; Lora et al. 2016; Dehors et al. 2019). Interestingly, in *Pinus taeda*, pollen tube growth has been connected to its homolog, termed CalS13, which is only distantly related to AtCalS5, showing at least a partial functional redundancy between the individual CalS subfamilies in plant evolution (Abercrombie et al. 2011).

Continuing with other CalSA subfamily paralogs, the presence of AtCalS8 was linked to callose deposition in the plasmodesmata of the phloem pole pericycle, possibly affecting the phloem unloading (Ross-Elliott et al. 2017; Liu et al. 2022a). The analyses of *cals7* (close homolog of AtCalS8) mutant Arabidopsis lines revealed its function in the deposition of callose at the cell–cell junctions of the newly forming sieve plates. Such specificity was also supported by the phloem-specific expression pattern of the AtCalS7 promoter (Barratt et al. 2011; Xie et al. 2011), as well as the actual localization of fluorescently fused AtCalS7 (Kalmbach et al. 2023), implying the conservation of phloem activity within the CalS7/8 (and presumably CalS6-8) cluster of the subfamily A. However, even though the *cals7* lines had a virtually non-existent callose synthesis in the forming sieve plates, this could be mitigated by the increased expression of AtCalS3 (utilizing a gain-of-function

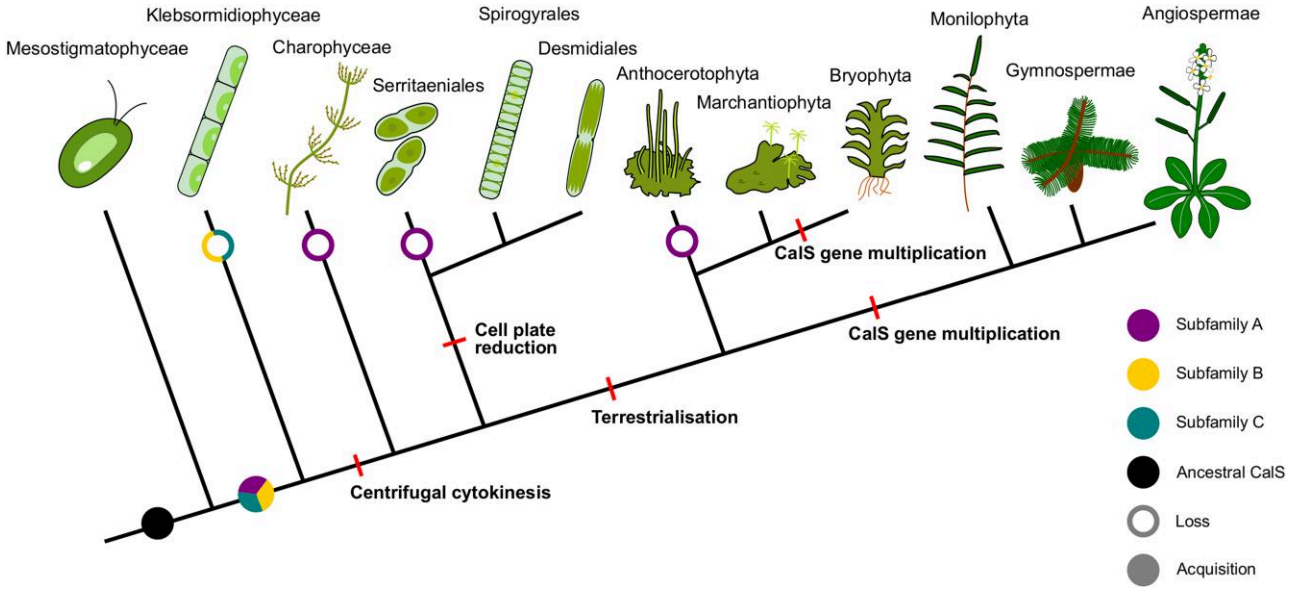

Figure 4. A summary of callose synthase evolution in the streptophyte lineage of plants. The divergence of CalSs into 3 independent subfamilies likely happened in the most recent common ancestor of *Klebsormidiophyceae* and *Phragmoplastophyta* (with CalSB and C or their predecessor lost in the *Klebsormidiophyceae*). The presence of the B and C subfamily correlates with the evolutionary emergence of the centrifugal cytokinesis machinery, phragmoplast and cell plate. The subfamily A has been lost independently multiple times in the *Charophyceae*, *Serritaeniales*, and *Anthocerotophyta*. After transitioning from water to land, the CalS family underwent several multiplications in true mosses, but especially in Euphyllophytes, which could drive or be driven by their functional specialization and/or whole genome duplications.

cals3 allele), hinting at the cooperation between AtCalS7 and AtCalS3 in phloem ontogeny (Vatén et al. 2011). AtCalS3 is a plasmodesmatal CalS, which, although belonging to the same subfamily A, clusters with CalS1, 2, and 4. Although this could still be explained by the developmental relatedness of sieve plates and plasmodesmata (Kalmbach and Helariutta 2019), other paralogs, including ones from different subfamilies (AtCalS1, AtCalS3 AtCalS8, AtCalS10, and AtCalS12) have been observed to regulate the permeability of plasmodesmata (Guseman et al. 2010; Cui and Lee 2016; Saatian et al. 2018, 2023). This is unlikely an experimental artefact because the same paralogs were identified in a poplar plasmodesmatal fraction proteomic screen with the addition of CalS12 and CalS9 (Leijon et al. 2018). Interestingly, AtCalS1–3 have all been implicated in cell-to-cell connectivity, increased upon the effector protein binding from *Phytophthora* (Tomczynska et al. 2020).

A member of the subfamily C, AtCalS12 has been identified as an essential player in papilla formation during powdery mildew infection (Jacobs et al. 2003; Wawrzynska et al. 2010; Ellinger et al. 2013). Contrary to expectations, the *cals12* mutants exhibited improved resistance to a pathogen after the plant infection (Jacobs et al. 2003; Dong et al. 2008; Wawrzynska et al. 2010). A recent study showed that CalS homologs in *Zygnema* (clustering within the CalSC and CalSB subfamilies) co-express with several components of the plant immune system (Feng et al. 2023), suggesting that the involvement of CalSC representatives in plant defense has been conserved in some form in the MRCA of Zygnematophyceae and land plants, although more insight into this is still required. Nevertheless, the function of CalSCs is not exclusive only to pathogen response because *cals11* and *cals12* exhibit defective phenotypes also in the generative tissues, ranging from reduced seed viability in single mutants and various combinations of null allele hemizygotes to pollen infertility in the double mutant (Enns et al. 2005). A similar situation was also observed in rice (Shi et al. 2015). AtCalS12 is indeed rather strongly expressed in the male gametophyte, which would validate its supporting role in pollen development (Záveská Drábková and Honys 2017).

Altogether, the discussed results suggest that even the distantly related CalS paralogs may be active in similar processes requiring polar callose synthesis. Perhaps the activity of distinct CalS paralogs should be viewed as partially universal, with its specificity in a given cell type and developmental process preferred but not restricted. This substantiates the evolution of CalS, which eventually resulted in the explosion of CalS paralogs in Embryophyta, their complex regulation and mutual interaction enabling the fine-tuning of callose deposition in a large portfolio of cell types and processes during plant development. Alternatively, the function of individual CalSs could be mediated by additional interactors that localize to such cell types and facilitate the correct execution of processes requiring polar callose synthesis.

Concluding remarks and perspectives

Although callose has been known to be deposited in different cells across the plant body for more than a century, it is rarely

considered a polar compound despite the fact that due to its high spatial and temporal specificity, callose synthesis is an archetypal polar process. Moreover, the role of callose synthesis in plant development has only begun to unravel in the last 2 decades. This was enabled by reverse genetic approaches or analyses of specific CalS expression, which have been mostly restricted for usage in Arabidopsis and a few closely related species. Alternatively, visualization of callose either by aniline blue staining or immunostaining with antibodies against callose has been insightful for determining the subcellular localization of callose deposition but requires tissue fixation, leading to the loss of temporal information and possible artefacts.

All living organisms rely on cytokinesis to correctly segregate the daughter nuclei. In most eukaryotic species, cytokinesis is carried out in a centripetal way. Therefore, it is fascinating that land plants have evolved a largely novel centrifugal mechanism facilitating nuclei separation during their evolution. Plant biologists have naturally tried to describe the underlying mechanisms, and, as such, the importance of polar callose deposition for cell plate formation was described. However, the aforementioned methodical drawbacks have, for a long time, hampered the efforts to study cytokinesis from an evolutionary perspective.

In recent years, the exponential increase of the available genomes has provided the basis for studies of plant phylogeny based on molecular data. Moreover, by enabling us to analyze the tissue-specific expression of the putative orthologs of the protein of interest, this has also improved our ability to track the evolution of gene and protein players driving the plant ontogeny across the plant kingdom. Indeed, several CalS paralogs with their function conserved during the evolution were proposed. This update review summarizes the recent proceedings on the role of individual CalSs in distinct cellular processes utilizing polar callose synthesis, which, combined with a complex phylogenetic analysis of CalS, implies the relationship between the evolution of callose-dependent processes (especially cell plate formation), the emergence of multiple CalS paralogs, and their recruitment in these processes. We also confirm the presence of CalS homologs in all plants, thus corroborating the previous observations of callose deposition across the plant kingdom (Scherp et al. 2001). Our analysis can serve as the basis for the experimental validation of the evolutionary conservation of CalS function in future studies.

Although identifying individual CalS activity in specific cell types can already provide valuable information into the polarity of callose synthesis, it offers little insight into the upstream regulatory processes controlling it (see outstanding questions box). The regulation of CalS activity has only begun to be studied, particularly the possible post-translational modification sites. However, any signaling components governing these modifications remain largely unknown. The number of available methods to analyze protein–protein interactions has surged in the past few years, including those compatible with large integral membrane proteins, for example, proximity biotinylation (Arora et al. 2020). In the near future, we expect an identification of proteins controlling the activity of specific CalS paralogs (kinases, phosphatases). Similarly, because CalS acts as part of a larger complex, we anticipate broadening the portfolio of known CALSC components, providing more insight into the mechanism of callose synthesis at the molecular level. These may also include players whose interaction with CalS is characteristic of callose deposition in the specific cell type. Therefore, their coevolution could drive the assumed CalS 3-subfamily divergence and functional specialization. Ultimately, by combining these approaches with the techniques for sensitive spatio-temporal analyses (e.g. live-cell microscopy of fluorescently tagged CalSs, cell fractionation of specific tissues), we can gain an unprecedented level of detail into the dynamics of subcellular CalS localization, players controlling the trafficking and degradation of individual CalSs, and eventually answer the question of what makes the callose synthesis polar in regards to both space and time (see outstanding questions box).

OUTSTANDING QUESTIONS BOX

- What is the subcellular localization of specific CalS homologs, and does it correlate with the callose synthesis in the distinct tissues?
- What controls the activity of individual CalSs in different cell types and processes?
- Is callose an essential component for centripetal septum formation?
- Are there evolutionarily conserved functions within the CalSA, B, and C subfamilies?
- Which CalS possessed the ancestral role in cytokinesis? Could the CalS duplications play a role in the cell plate establishment?

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Author contributions

R.P., D.U., and S.H. conceived and wrote the manuscript. S.H. and D.U. performed the phylogenetic analysis. D.U. generated the figures.

Supplemental data

The following materials are available in the online version of this article.

Supplemental Figure S1.

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Conflict of interest statement. All authors declare that they have no conflicts of interest.

References

Abercrombie JM, O'Meara BC, Moffatt AR, Williams JH. Developmental evolution of flowering plant pollen tube cell walls: Callose Synthase (CalS) gene expression patterns. EvoDevo. 2011:**2**(1):14. https://doi.org/10.1186/2041-9139-2-14

Abou-Saleh RH, Hernandez-Gomez MC, Amsbury S, Paniagua C, Bourdon M, Miyashima S, Helariutta Y, Fuller M, Budtova T, Connell SD, et al. Interactions between callose and cellulose revealed through the analysis of biopolymer mixtures. Nat Commun. 2018:**9**(1):4538. https://doi.org/10.1038/s41467-018-06820-y

Adl SM, Simpson AGB, Lane CE, Lukeš J, Bass D, Bowser SS, Brown MW, Burki F, Dunthorn M, Hampl V, et al. The revised classification of eukaryotes. J Eukaryot Microbiol. 2012:**59**(5):429–514. https://doi.org/10.1111/j.1550-7408.2012.00644.x

Aist JR. Papillae and related wound plugs of plant cells. Annu Rev Phytopathol. 1976:**14**(1):145–163. https://doi.org/10.1146/annurev.py.14.090176.001045

Anton-Plagaro C, Sanchez N, Valle R, Mulet JM, Duncan MC, Roncero C. Exomer complex regulates protein traffic at the TGN through differential interactions with cargos and clathrin adaptor complexes. FASEB J. 2021:**35**(6):e21615. https://doi.org/10.1096/fj.202002610R

Arakaki Y, Fujiwara T, Kawai-Toyooka H, Kawafune K, Featherston J, Durand PM, Miyagishima S, Nozaki H. Evolution of cytokinesis-related protein localization during the emergence of multicellularity in volvocine green algae. BMC Evol Biol. 2017:**17**(1):243. https://doi.org/10.1186/s12862-017-1091-z

Arcones I, Sacristán C, Roncero C. Maintaining protein homeostasis: early and late endosomal dual recycling for the maintenance of intracellular pools of the plasma membrane protein chs3. MBoC. 2016:**27**-(25):4021–4032. https://doi.org/10.1091/mbc.E16-04-0239

Arora D, Abel NB, Liu C, Van Damme P, Yperman K, Eeckhout D, Vu LD, Wang J, Tornkvist A, Impens F, et al. Establishment of proximity-dependent biotinylation approaches in different plant model systems. Plant Cell. 2020:**32**(11):3388–3407. https://doi.org/10.1105/tpc.20.00235

Bacic A, Fincher G, Stone BA. Chemistry of β-glucans. Chemistry, biochemistry, and biology of 1–3 Beta glucans and related polysaccharides. San Diego (CA): Elsevier; 2009. p. 5–46.

Barratt DHP, Kölling K, Graf A, Pike M, Calder G, Findlay K, Zeeman SC, Smith AM. Callose synthase GSL7 is necessary for normal phloem transport and inflorescence growth in Arabidopsis. Plant Physiol. 2011:**155**(1):328–341. https://doi.org/10.1104/pp.110.166330

Bowles AMC, Bechtold U, Paps J. The origin of land plants is rooted in two bursts of genomic novelty. Curr Biol. 2020:**30**(3):530–536.e2. https://doi.org/10.1016/j.cub.2019.11.090

Brownfield L, Wilson S, Newbigin E, Bacic A, Read S. Molecular control of the glucan synthase-like protein NaGSL1 and callose synthesis during growth of *Nicotiana alata* pollen tubes. Biochem J. 2008:**414**(1):43–52. https://doi.org/10.1042/BJ20080693

Buschmann H, Müller S. Update on plant cytokinesis: rule and divide. Curr Opin Plant Biol. 2019:**52**(6):97–105. https://doi.org/10.1016/j.pbi.2019.07.003

Buschmann H, Zachgo S. The evolution of cell division: from streptophyte Algae to land plants. Trends Plant Sci. 2016:**21**(10):872–883. https://doi.org/10.1016/j.tplants.2016.07.004

Caillaud M-C. Anionic lipids: a pipeline connecting key players of plant cell division. Front Plant Sci. 2019:**10**(419). https://doi.org/10.3389/fpls.2019.00419

Cao P, Tang C, Wu X, Qian M, Lv S, Gao H, Qiao X, Chen G, Wang P, Zhang S, et al. Pbrcals5, a callose synthase protein, is involved in pollen tube growth in Pyrus bretschneideri. Planta. 2022:**256**(2):22. https://doi.org/10.1007/s00425-022-03931-1

Chowdhury J, Henderson M, Schweizer P, Burton RA, Fincher GB, Little A. Differential accumulation of callose, arabinoxylan and cellulose in nonpenetrated versus penetrated papillae on leaves of barley infected with *Blumeria graminis* f. sp. *hordei*. New Phytol. 2014:**204**(3):650–660. https://doi.org/10.1111/nph.12974

Chuang JS, Schekman RW. Differential trafficking and timed localization of two chitin synthase proteins, Chs2p and Chs3p. J Cell Biol. 1996:**135**(3):597–610. https://doi.org/10.1083/jcb.135.3.597

Cleary AL, Brown RC, Lemmon BE. Establishment of division plane and mitosis in monoplastidic guard mother cells of Selaginella. Cell Motil Cytoskeleton. 1992:**23**(2):89–101. https://doi.org/10.1002/cm.970230202

Colin L, Ruhnow F, Zhu J-K, Zhao C, Zhao Y, Persson S. The cell biology of primary cell walls during salt stress. Plant Cell. 2023:**35**(1):201–217. https://doi.org/10.1093/plcell/koac292

Cook ME. Cytokinesis in *Coleochaete orbicularis* (charophyceae): an ancestral mechanism inherited by plants. Am J Bot. 2004:**91**(3):313–320. https://doi.org/10.3732/ajb.91.3.313

Cook ME, Graham LE, Lavin CA. Cytokinesis and nodal anatomy in the charophycean green alga chara zeylanica. Protoplasma. 1998:**203**(1–2):65–74. https://doi.org/10.1007/BF01280588

Cosgrove DJ. Building an extensible cell wall. Plant Physiol. 2022:**189**(3):1246–1277. https://doi.org/10.1093/plphys/kiac184

Cross FR, Umen JG. The *Chlamydomonas* cell cycle. Plant J. 2015:**82**(3):370–392. https://doi.org/10.1111/tpj.12795

Cui W, Lee J-Y. Arabidopsis callose synthases CalS1/8 regulate plasmodesmal permeability during stress. Nat Plants. 2016:**2**(5):16034. https://doi.org/10.1038/nplants.2016.34

Davis DJ, Wang M, Sørensen I, Rose JKC, Domozych DS, Drakakaki G. Callose deposition is essential for the completion of cytokinesis in the unicellular alga, *Penium margaritaceum*. J Cell Sci. 2020:**133**(19):jcs249599. https://doi.org/10.1242/jcs.249599

De Lorenzo G, Ferrari S, Giovannoni M, Mattei B, Cervone F. Cell wall traits that influence plant development, immunity and bioconversion. Plant J. 2018:**97**(1):134–147.

Dehors J, Mareck A, Kiefer-Meyer M-C, Menu-Bouaouiche L, Lehner A, Mollet J-C. Evolution of cell wall polymers in tip-growing land plant gametophytes: composition, distribution, functional aspects and their remodeling. Front Plant Sci. 2019:**10**(441). https://doi.org/10.3389/fpls.2019.00441

DeMarini DJ, Adams AEM, Fares H, Virgilio CD, Valle G, Chuang JS, Pringle JR. A septin-based hierarchy of proteins required for localized deposition of chitin in the Saccharomyces cerevisiae cell wall. J Cell Biol. 1997:**139**(1):75–93. https://doi.org/10.1083/jcb.139.1.75

Dharwada ST, Dalton LE, Bean BDM, Padmanabhan N, Choi C, Schluter C, Davey M, Conibear E. The chaperone Chs7 forms a stable complex with Chs3 and promotes its activity at the cell surface. Traffic. 2018:**19**(4):285–295. https://doi.org/10.1111/tra.12553

Dong X, Hong Z, Chatterjee J, Kim S, Verma DPS. Expression of callose synthase genes and its connection with Npr1 signaling pathway during pathogen infection. Planta. 2008:**229**(1):87–98. https://doi.org/10.1007/s00425-008-0812-3

Dong X, Hong Z, Sivaramakrishnan M, Mahfouz M, Verma DPS. Callose synthase (CalS5) is required for exine formation during microgametogenesis and for pollen viability in Arabidopsis: callose is required for exine formation. Plant J. 2005:**42**(3):315–328. https://doi.org/10.1111/j.1365-313X.2005.02379.x

Donofrio NM, Delaney TP. Abnormal callose response phenotype and hypersusceptibility to *Peronospora parasitica* in defense-

compromised Arabidopsis *nim1–1* and salicylate hydroxylase-expressing plants. MPMI. 2001:**14**(4):439–450. https://doi.org/10.1094/MPMI.2001.14.4.439

Doxey AC, Yaish MWF, Moffatt BA, Griffith M, McConkey BJ. Functional divergence in the Arabidopsis -1,3-glucanase gene family inferred by phylogenetic reconstruction of expression states. Mol Biol Evol. 2007:**24**(4):1045–1055. https://doi.org/10.1093/molbev/msm024

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**(2):413–424. https://doi.org/10.1038/cr.2011.129

Eggert D, Naumann M, Reimer R, Voigt CA. Nanoscale glucan polymer network causes pathogen resistance. Sci Rep. 2015:**4**(1):4159. https://doi.org/10.1038/srep04159

Ehlers K, Knoblauch M, van Bel AJE. Ultrastructural features of well-preserved and injured sieve elements: minute clamps keep the phloem transport conduits free for mass flow. Protoplasma. 2000:**214**(1–2):80–92. https://doi.org/10.1007/BF02524265

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**(3):1433–1444. https://doi.org/10.1104/pp.112.211011

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**(6):1349–1358. https://doi.org/10.1093/aob/mcu120

Enns LC, Kanaoka MM, Torii KU, Comai L, Okada K, Cleland RE. Two callose synthases, GSL1 and GSL5, play an essential and redundant role in plant and pollen development and in fertility. Plant Mol Biol. 2005:**58**(3):333–349. https://doi.org/10.1007/s11103-005-4526-7

Feng J, Chen Y, Xiao X, Qu Y, Li P, Lu Q, Huang J. Genome-wide analysis of the *CalS* gene family in cotton reveals their potential roles in fiber development and responses to stress. PeerJ. 2021:**9**(e12557). https://doi.org/10.7717/peerj.12557

Feng X, Zheng J, Irisarri I, Yu H, Zheng B, Ali Z, de Vries S, Keller J, Fürst-Jansen JMR, Dadras A, et al. Chromosome-level genomes of multicellular algal sisters to land plants illuminate signaling network evolution. bioRxiv. 2023:**1**(2023.01.31.526407). https://doi.org/10.1101/2023.01.31.526407

Fitzgibbon J, Bell K, King E, Oparka K. Super-Resolution imaging of plasmodesmata using three-dimensional structured illumination microscopy. Plant Physiol. 2010:**153**(4):1453–1463. https://doi.org/10.1104/pp.110.157941

Floyd GL, Stewart KD, Mattox KR. Cellular organization, mitosis, and cytokinesis in the ulotrichalean alga, *Klebsormidium*. J Phycol. 1972:**8**:176–184. https://doi.org/10.1111/j.1529-8817.1972.tb04021.x

Forero AB, Cvrčková F. SH3Ps—evolution and diversity of a family of proteins engaged in plant cytokinesis. IJMS. 2019:**20**(22):5623. https://doi.org/10.3390/ijms20225623

Furch ACU, Zimmermann MR, Will T, Hafke JB, van Bel AJE. Remote-controlled stop of phloem mass flow by biphasic occlusion in Cucurbita maxima. J Exp Bot. 2010:**61**(13):3697–3708. https://doi.org/10.1093/jxb/erq181

Gambardella R, Alfano F. Monoplastidic mitosis in the moss Timmiella barbuloides (bryophyta). Protoplasma. 1990:**156**(1–2):29–38. https://doi.org/10.1007/BF01666503

Gensler W. A hypothesis concerning callose. Plant Signal Behav. 2019:**14**(1):1548878. https://doi.org/10.1080/15592324.2018.1548878

Gigli-Bisceglia N, Engelsdorf T, Hamann T. Plant cell wall integrity maintenance in model plants and crop species-relevant cell wall components and underlying guiding principles. Cell Mol Life Sci. 2020:**77**(11):2049–2077. https://doi.org/10.1007/s00018-019-03388-8

Gohlke S, Muthukrishnan S, Merzendorfer H. In vitro and in vivo studies on the structural organization of chs3 from Saccharomyces cerevisiae. IJMS. 2017:**18**(4):702. https://doi.org/10.3390/ijms18040702

Granato LM, Galdeano DM, D'Alessandre NDR, Breton MC, Machado MA. Callose synthase family genes plays an important role in the Citrus defense response to Candidatus Liberibacter asiaticus. Eur J Plant Pathol. 2019:**155**(1):25–38. https://doi.org/10.1007/s10658-019-01747-6

Guseman JM, Lee JS, Bogenschutz NL, Peterson KM, Virata RE, Xie B, Kanaoka MM, Hong Z, Torii KU. Dysregulation of cell-to-cell connectivity and stomatal patterning by loss-of-function mutation in *Arabidopsis CHORUS* (*GLUCAN SYNTHASE-LIKE 8*). Development. 2010:**137**(10):1731–1741. https://doi.org/10.1242/dev.049197

Hall JD, McCourt RM, Delwiche CF. Patterns of cell division in the filamentous desmidiaceae, close green algal relatives of land plants. Am J Bot. 2008:**95**(6):643–654. https://doi.org/10.3732/ajb.2007210

Harris BJ, Harrison CJ, Hetherington AM, Williams TA. Phylogenomic evidence for the monophyly of bryophytes and the reductive evolution of stomata. Curr Biol. 2020:**30**(11):2001–2012.e2. https://doi.org/10.1016/j.cub.2020.03.048

Herburger K, Holzinger A. Localization and quantification of callose in the streptophyte green Algae *Zygnema* and *Klebsormidium*: correlation with desiccation tolerance. Plant Cell Physiol. 2015:**56**(11):2259–2270. https://doi.org/10.1093/pcp/pcv139

Heslop-Harrison J, Mackenzie A. Autoradiography of soluble [2-"C] thymidine derivatives during meiosis and microsporogenesis in Lilium anthers. J Cell Sci. 1967:**2**(3):387–400. https://doi.org/10.1242/jcs.2.3.387

Hess S, Williams SK, Busch A, Irisarri I, Delwiche CF, de Vries S, Darienko T, Roger AJ, Archibald JM, Buschmann H, et al. A phylogenomically informed five-order system for the closest relatives of land plants. Curr Biol. 2022:**32**(20):4473–4482.e7. https://doi.org/10.1016/j.cub.2022.08.022

Hong Z, Delauney AJ, Verma DPS. A cell plate–specific callose synthase and its interaction with phragmoplastin. Plant Cell. 2001:**13**(14):755–768. https://doi.org/10.1105/tpc.13.4.755

Huang L, Chen X-Y, Rim Y, Han X, Cho WK, Kim S-W, Kim J-Y. Arabidopsis glucan synthase-like 10 functions in male gametogenesis. J Plant Physiol. 2009:**166**(4):344–352. https://doi.org/10.1016/j.jplph.2008.06.010

Jacobs AK, Lipka V, Burton RA, Panstruga R, Strizhov N, Schulze-Lefert P, Fincher GB. An Arabidopsis callose synthase, GSL5, is required for wound and papillary callose formation. Plant Cell. 2003:**15**(11):2503–2513. https://doi.org/10.1105/tpc.016097

Jakobsen MK, Cheng Z, Lam SK, Roth-Johnson E, Barfield RM, Schekman R. Phosphorylation of Chs2p regulates interaction with COPII. J Cell Sci. 2013:**126**(Pt 10):2151–2156.

Jawaid MZ, Sinclair R, Bulone V, Cox DL, Drakakaki G. A biophysical model for plant cell plate maturation based on the contribution of a spreading force. Plant Physiol. 2022:**188**(2):795–806. https://doi.org/10.1093/plphys/kiab552

Kalmbach L, Bourdon M, Belevich I, Safran J, Lemaire A, Heo J, Otero S, Blob B, Pelloux J, Jokitalo E, et al. Putative pectate lyase PLL12 and callose deposition through polar CALS7 are necessary for long-distance phloem transport in Arabidopsis. Curr Biol. 2023:**33**(5):926–939.e9. https://doi.org/10.1016/j.cub.2023.01.038

Kalmbach L, Helariutta Y. Sieve plate pores in the phloem and the unknowns of their formation. Plants. 2019:**8**(2):25. https://doi.org/10.3390/plants8020025

Kanazawa T, Morinaka H, Ebine K, Shimada TL, Ishida S, Minamino N, Yamaguchi K, Shigenobu S, Kohchi T, Nakano A, et al. The liverwort oil body is formed by redirection of the secretory pathway. Nat Commun. 2020:**11**(1):6152. https://doi.org/10.1038/s41467-020-19978-1

Kapoor K, Geitmann A. Pollen tube invasive growth is promoted by callose. Plant Reprod. 2023. https://doi.org/10.1007/s00497-023-00458-7

Katsaros C, Weiss A, Llangos I, Theodorou I, Wichard T. Cell structure and microtubule organisation during gametogenesis of Ulva mutabilis Føyn (Chlorophyta). Botanica Marina. 2017:**60**(2):123–135. https://doi.org/10.1515/bot-2016-0073

Knafler HC, Smaczynska-de Rooij II, Walker LA, Lee KK, Gow NAR, Ayscough KR. AP-2-Dependent endocytic recycling of the chitin synthase chs3 regulates polarized growth in Candida albicans. mBio. 2019:**10**(2):e02421-18. https://doi.org/10.1128/mBio.02421-18

Knoblauch M, van Bel AJE. Sieve tubes in action. Plant Cell. 1998:**10**(1): 35–50. https://doi.org/10.1105/tpc.10.1.35

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**(10):2926–2940. https://doi.org/10.1105/tpc.15.00442

Leijon F, Melzer M, Zhou Q, Srivastava V, Bulone V. Proteomic analysis of plasmodesmata from Populus cell suspension cultures in relation with callose biosynthesis. Front Plant Sci. 2018:**9**(1681). https://doi.org/10.3389/fpls.2018.01681

Li S, Lei L, Somerville CR, Gu Y. Cellulose synthase interactive protein 1 (CSI1) links microtubules and cellulose synthase complexes. Proc Natl Acad Sci USA. 2012a:**109**(1):185–190. https://doi.org/10.1073/pnas.1118560109

Li W, Zhao Y, Liu C, Yao G, Wu S, Hou C, Zhang M, Wang D. Callose deposition at plasmodesmata is a critical factor in restricting the cell-to-cell movement of soybean mosaic virus. Plant Cell Rep. 2012b:**31**(5):905–916. https://doi.org/10.1007/s00299-011-1211-y

Liu F, Zou Z, Fernando W. Characterization of callose deposition and analysis of the callose synthase gene family of Brassica napus in response to leptosphaeria maculans. IJMS. 2018:**19**(12):3769. https://doi.org/10.3390/ijms19123769

Liu J, Liu Y, Wang S, Cui Y, Yan D. Heat stress reduces root meristem size via induction of plasmodesmal callose accumulation inhibiting phloem unloading in Arabidopsis. IJMS. 2022a:**23**(4):2063. https://doi.org/10.3390/ijms23042063

Liu Y, Liu L, Yang S, Liu G, Zeng Q, Liu Y. Molecular characterization and functional analysis of a pathogenesis-related β-1,3-glucanase gene in spruce (Picea asperata). Eur J Plant Pathol. 2022b:**164**(2): 177–192. https://doi.org/10.1007/s10658-022-02547-1

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:**38**(3):305–315. https://doi.org/10.1016/j.devcel.2016.06.032

Liu Z, Yan J-P, Li D-K, Luo Q, Yan Q, Liu Z-B, Ye L-M, Wang J-M, Li X-F, Yang Y. UDP-Glucosyltransferase71C5, a Major glucosyltransferase, mediates abscisic acid homeostasis in Arabidopsis. Plant Physiol. 2015:**167**(4):1659–1670. https://doi.org/10.1104/pp.15.00053

Livanos P, Müller S. Division plane establishment and cytokinesis. Annu Rev Plant Biol. 2019:**70**(1):239–267. https://doi.org/10.1146/annurev-arplant-050718-100444

Lloyd JP, Seddon AE, Moghe GD, Simenc MC, Shiu S-H. Characteristics of plant essential genes allow for within- and between-Species prediction of lethal mutant phenotypes. Plant Cell. 2015:**27**(8):2133–2147. https://doi.org/10.1105/tpc.15.00051

Lokhorst GM, Sluiman HJ, Star W. The ultrastructure of mitosis and cytokinesis in the sarcinoid chlorokybus atmophyticus (Chlorophyta, Charophyceae) revealed by rapid freeze fixation and freeze substitution. J Phycol. 1988:**24**:237–248. https://doi.org/10.1111/j.1529-8817.1988.tb04239.x

Lora J, Hormaza JI, Herrero M. The diversity of the pollen tube pathway in plants: toward an increasing control by the sporophyte. Front Plant Sci. 2016:**7**(107). https://doi.org/10.3389/fpls.2016.00107

Manton I, Ettl H. Observations on the fine structure of mesostigma viride lauterborn. J Linn Soc Bot. 1965:**59**(378):175–184. https://doi.org/10.1111/j.1095-8339.1965.tb00056.x

Maule A, Faulkner C, Benitez-Alfonso Y. Plasmodesmata "in Communicado". Front Plant Sci. 2012:**3**(30). https://doi.org/10.3389/fpls.2012.00030

McFarlane HE, Mutwil-Anderwald D, Verbančič J, Picard KL, Gookin TE, Froehlich A, Chakravorty D, Trindade LM, Alonso JM, Assmann SM, et al. A G protein-coupled receptor-like module regulates cellulose synthase secretion from the endomembrane system in Arabidopsis. Dev Cell. 2021:**56**(10):1484–1497.e7. https://doi.org/10.1016/j.devcel.2021.03.031

McIntosh K, Pickett-Heaps JD. Cytokinesis in spirogyra: integration of cleavage and cell-plate formation. Int J Plant Sci. 1995:**156**(1):1–8. https://doi.org/10.1086/297222

McMurray MA, Stefan CJ, Wemmer M, Odorizzi G, Thomer J. Genetic interactions with mutations affecting septin assembly reveal ESeRT functions in budding yeast cytokinesis. Biol Chem. 2012:**392**(8–9):699–712. https://doi.org/10.1515/BC.2011.091

McNairn RB, Currier HB. Translocation blockage by sieve plate callose. Planta. 1968:**82**(4):369–380. https://doi.org/10.1007/BF00386439

Miart F, Desprez T, Biot E, Morin H, Belcram K, Höfte H, Gonneau M, Vernhettes S. Spatio-temporal analysis of cellulose synthesis during cell plate formation in Arabidopsis. Plant J. 2014:**77**(1):71–84. https://doi.org/10.1111/tpj.12362

Micali CO, Neumann U, Grunewald D, Panstruga R, O'Connell R. Biogenesis of a specialized plant-fungal interface during host cell internalization of golovinomyces orontii haustoria: plant-fungal interface around powdery mildew haustoria. Cell Microbiol. 2011:**13**(2): 210–226. https://doi.org/10.1111/j.1462-5822.2010.01530.x

Mizuta Y, Higashiyama T. Antisense gene inhibition by phosphorothioate antisense oligonucleotide in Arabidopsis pollen tubes. Plant J. 2014:**78**(3):516–526. https://doi.org/10.1111/tpj.12461

Müller S, Jürgens G. Plant cytokinesis—no ring, no constriction but centrifugal construction of the partitioning membrane. Semin Cell Dev Biol. 2016:**53**(5):10–18. https://doi.org/10.1016/j.semcdb.2015.10.037

Nibbering P, Castilleux R, Wingsle G, Niittyla T. CAGEs are Golgi-localized GT31 enzymes involved in cellulose biosynthesis in Arabidopsis. Plant J. 2022:**110**(5):1271–1285. https://doi.org/10.1111/tpj.15734

Nishikawa S, Zinkl GM, Swanson RJ, Maruyama D, Preuss D. Callose (β-1,3 glucan) is essential for arabidopsispollen wall patterning, but not tube growth. BMC Plant Biol. 2005:**5**(1):22. https://doi.org/10.1186/1471-2229-5-22

Nishiyama T, Sakayama H, de Vries J, Buschmann H, Saint-Marcoux D, Ullrich KK, Haas FB, Vanderstraeten L, Becker D, Lang D, et al. The chara genome: secondary complexity and implications for plant terrestrialization. Cell. 2018:**174**(2):448–464.e24. https://doi.org/10.1016/j.cell.2018.06.033

Ochs J, LaRue T, Tinaz B, Yongue C, Domozych DS. The cortical cytoskeletal network and cell-wall dynamics in the unicellular charophycean green alga penium margaritaceum. Ann Bot. 2014:**114**(6): 1237–1249. https://doi.org/10.1093/aob/mcu013

One Thousand Plant Transcriptomes Initiative. One thousand plant transcriptomes and the phylogenomics of green plants. Nature. 2019:**574**(7780):679–685. https://doi.org/10.1038/s41586-019-1693-2

Park E, Díaz-Moreno SM, Davis DJ, Wilkop TE, Bulone V, Drakakaki G. Endosidin 7 specifically arrests late cytokinesis and inhibits callose biosynthesis, revealing distinct trafficking events during cell plate maturation. Plant Physiol. 2014:**165**(3):1019–1034. https://doi.org/10.1104/pp.114.241497

Park M, Krause C, Karnahl M, Reichardt I, El Kasmi F, Mayer U, Stierhof Y-D, Hiller U, Strompen G, Bayer M, et al. Concerted action of evolutionarily ancient and novel SNARE complexes in flowering-plant cytokinesis. Dev Cell. 2018:**44**(4):500–511.e4. https://doi.org/10.1016/j.devcel.2017.12.027

Parre E, Geitmann A. More than a leak sealant. The mechanical properties of callose in pollen tubes. Plant Physiol. 2005:**137**(1):274–286. https://doi.org/10.1104/pp.104.050773

Perrot T, Pauly M, Ramírez V. Emerging roles of β-glucanases in plant development and adaptative responses. Plants. 2022:**11**(9):1119. https://doi.org/10.3390/plants11091119

Piršelová B, Matušíková I. Callose: the plant cell wall polysaccharide with multiple biological functions. Acta Physiol Plant. 2013:**35**(3): 635–644. https://doi.org/10.1007/s11738-012-1103-y

Purushotham P, Ho R, Zimmer J. Architecture of a catalytically active homotrimeric plant cellulose synthase complex. Science. 2020:**369**-(6507):1089–1094. https://doi.org/10.1126/science.abb2978

Puttick MN, Morris JL, Williams TA, Cox CJ, Edwards D, Kenrick P, Pressel S, Wellman CH, Schneider H, Pisani D, et al. The interrelationships of land plants and the nature of the ancestral embryophyte. Curr Biol. 2018:**28**(5):733–745.e2. https://doi.org/10.1016/j.cub.2018.01.063

Ren Z, Chhetri A, Guan Z, Suo Y, Yokoyama K, Lee S-Y. Structural basis for inhibition and regulation of a chitin synthase from Candida albicans. Nat Struct Mol Biol. 2022:**29**(7):653–664. https://doi.org/10.1038/s41594-022-00791-x

Reyes A, Sanz M, Duran A, Roncero C. Chitin synthase III requires Chs4p-dependent translocation of Chs3p into the plasma membrane. J Cell Sci. 2007:**120**(12):1998–2009. https://doi.org/10.1242/jcs.005124

Rieseberg TP, Dadras A, Fürst-Jansen JMR, Dhabalia Ashok A, Darienko T, de Vries S, Irisarri I, de Vries J. Crossroads in the evolution of plant specialized metabolism. Semin Cell Dev Biol. 2023:**134**(2):37–58. https://doi.org/10.1016/j.semcdb.2022.03.004

Ross-Elliott TJ, Jensen KH, Haaning KS, Wager BM, Knoblauch J, Howell AH, Mullendore DL, Monteith AG, Paultre D, Yan D, et al. Phloem unloading in Arabidopsis roots is convective and regulated by the phloem-pole pericycle. eLife. 2017:**6**(e24125). https://doi.org/10.7554/eLife.24125

Ruhfel BR, Gitzendanner MA, Soltis PS, Soltis DE, Burleigh J. From algae to angiosperms–inferring the phylogeny of green plants (Viridiplantae) from 360 plastid genomes. BMC Evol Biol. 2014:**14**(1):23. https://doi.org/10.1186/1471-2148-14-23

Ruiz-Herrera J, Ortiz-Castellanos L. Cell wall glucans of fungi. a review. Cell Surf. 2019:**5**(100022). https://doi.org/10.1016/j.tcsw.2019.100022

Saatian B, Austin RS, Tian G, Chen C, Nguyen V, Kohalmi SE, Geelen D, Cui Y. Analysis of a novel mutant allele of GSL8 reveals its key roles in cytokinesis and symplastic trafficking in Arabidopsis. BMC Plant Biol. 2018:**18**(1):295. https://doi.org/10.1186/s12870-018-1515-y

Saatian B, Kohalmi SE, Cui Y. Localization of *Arabidopsis* Glucan Synthase-Like 5, 8, and 12 to plasmodesmata and the GSL8-dependent role of PDLP5 in regulating plasmodesmal permeability. Plant Signal Behav. 2023:**18**(1):2164670. https://doi.org/10.1080/15592324.2022.2164670

Sacristan C, Manzano-Lopez J, Reyes A, Spang A, Muñiz M, Roncero C. Oligomerization of the chitin synthase chs3 is monitored at the Golgi and affects its endocytic recycling: chs3 dimerization and trafficking. Mol Microbiol. 2013:**90**(2):252–266. https://doi.org/10.1111/mmi.12360

Samuels AL, Giddings TH, Staehelin LA. Cytokinesis in tobacco BY-2 and root tip cells: a new model of cell plate formation in higher plants. J Cell Biol. 1995:**130**(6):1345–1357. https://doi.org/10.1083/jcb.130.6.1345

Sánchez N, Roncero C. Chitin synthesis in yeast: a matter of trafficking. IJMS. 2022:**23**(20):12251. https://doi.org/10.3390/ijms232012251

Scherp P, Grotha R, Kutschera U. Occurrence and phylogenetic significance of cytokinesis-related callose in green algae, bryophytes, ferns and seed plants. Plant Cell Rep. 2001:**20**(2):143–149. https://doi.org/10.1007/s002990000301

Scherp P, Grotha R, Kutschera U. Interaction between cytokinesis-related callose and cortical microtubules in dividing cells of the liverwort *Riella helicophylla*. Plant Biol. 2002:**4**(5):619–624. https://doi.org/10.1055/s-2002-35436

Schneider R, Ehrhardt DW, Meyerowitz EM, Sampathkumar A. Tethering of cellulose synthase to microtubules dampens mechano-induced cytoskeletal organization in Arabidopsis pavement cells. Nat Plants. 2022:**8**(9):1064–1073. https://doi.org/10.1038/s41477-022-01218-7

Schuette S, Wood AJ, Geisler M, Geisler-Lee J, Ligrone R, Renzaglia KS. Novel localization of callose in the spores of Physcomitrella patens and phylogenomics of the callose synthase gene family. Ann Bot. 2009:**103**(5):749–756. https://doi.org/10.1093/aob/mcn268

Shi X, Sun X, Zhang Z, Feng D, Zhang Q, Han L, Wu J, Lu T. GLUCAN SYNTHASE-LIKE 5 (GSL5) plays an essential role in male fertility by regulating callose metabolism during microsporogenesis in rice. Plant Cell Physiol. 2015:**56**(3):497–509. https://doi.org/10.1093/pcp/pcu193

Sinclair R, Hsu G, Davis D, Chang M, Rosquete M, Iwasa JH, Drakakaki G. Plant cytokinesis and the construction of new cell wall. FEBS Lett. 2022:**596**(17):2243–2255. https://doi.org/10.1002/1873-3468.14426

Smertenko A, Assaad F, Baluška F, Bezanilla M, Buschmann H, Drakakaki G, Hauser M-T, Janson M, Mineyuki Y, Moore I, et al. Plant cytokinesis: terminology for structures and processes. Trends Cell Biol. 2017:**27**(12):885–894. https://doi.org/10.1016/j.tcb.2017.08.008

Song L, Wang R, Zhang L, Wang Y, Yao S. *CRR1* Encoding callose synthase functions in ovary expansion by affecting vascular cell patterning in rice. Plant J. 2016:**88**(4):620–632. https://doi.org/10.1111/tpj.13287

Starr TL, Pagant S, Wang C-W, Schekman R. Sorting signals that mediate traffic of chitin synthase III between the TGN/endosomes and to the plasma membrane in yeast. PLoS One. 2012:**7**(10):e46386. https://doi.org/10.1371/journal.pone.0046386

Stieglitz H. Role of β-1,3-glucanase in postmeiotic microspore release. Dev Biol. 1977:**57**(1):87–97. https://doi.org/10.1016/0012-1606(77)90356-6

Stull GW, Qu X-J, Parins-Fukuchi C, Yang Y-Y, Yang J-B, Yang Z-Y, Hu Y, Ma H, Soltis PS, Soltis DE, et al. Gene duplications and phylogenomic conflict underlie major pulses of phenotypic evolution in gymnosperms. Nat Plants. 2021:**7**(8):1015–1025. https://doi.org/10.1038/s41477-021-00964-4

Thiele K, Wanner G, Kindzierski V, Jürgens G, Mayer U, Pachl F, Assaad FF. The timely deposition of callose is essential for cytokinesis in Arabidopsis: callose is essential for plant cytokinesis. Plant J. 2009:**58**(1):13–26. https://doi.org/10.1111/j.1365-313X.2008.03760.x

Töller A, Brownfield L, Neu C, Twell D, Schulze-Lefert P. Dual function of Arabidopsis glucan synthase-like genes GSL8 and GSL10 in male gametophyte development and plant growth. Plant J. 2008:**54**(5):911–923. https://doi.org/10.1111/j.1365-313X.2008.03462.x

Tomczynska I, Stumpe M, Doan TG, Mauch F. A *Phytophthora* effector protein promotes symplastic cell-to-cell trafficking by physical interaction with plasmodesmata-localised callose synthases. New Phytol. 2020:**227**(5):1467–1478. https://doi.org/10.1111/nph.16653

Vain T, Crowell EF, Timpano H, Biot E, Desprez T, Mansoori N, Trindade LM, Pagant S, Robert S, Höfte H, et al. The cellulase KORRIGAN is part of the cellulose synthase Complex. Plant Physiol. 2014:**165**(4):1521–1532. https://doi.org/10.1104/pp.114.241216

Vatén A, Dettmer J, Wu S, Stierhof Y-D, Miyashima S, Yadav SR, Roberts CJ, Campilho A, Bulone V, Lichtenberger R, et al. Callose biosynthesis regulates symplastic trafficking during root development. Dev Cell. 2011:**21**(6):1144–1155. https://doi.org/10.1016/j.devcel.2011.10.006

Vellosillo T, Dinneny JR, Somerville CR, Ehrhardt DW. TRANVIA (TVA) facilitates cellulose synthase trafficking and delivery to the plasma membrane. Proc Natl Acad Sci USA. 2021:**118**(30):e2021790118. https://doi.org/10.1073/pnas.2021790118

Verma DPS, Hong Z. Plant callose synthase complexes. Plant Mol Biol. 2001:**47**(6):693–701. https://doi.org/10.1023/A:1013679111111

Vogler H, Draeger C, Weber A, Felekis D, Eichenberger C, Routier-Kierzkowska A-L, Boisson-Dernier A, Ringli C, Nelson BJ, Smith RS, et al. The pollen tube: a soft shell with a hard core. Plant J. 2013:**73**(4):617–627. https://doi.org/10.1111/tpj.12061

von der Heyde EL, Hallmann A. Molecular and cellular dynamics of early embryonic cell divisions in *Volvox carteri*. Plant Cell. 2022:**34**(4):1326–1353. https://doi.org/10.1093/plcell/koac004

Wang B, Andargie M, Fang R. The function and biosynthesis of callose in high plants. Heliyon. 2022:**8**:e09248. https://doi.org/10.1016/j.heliyon.2022.e09248

Wang Y, Zhao Y, Wang X, Zhong L, Fan Q, Lan Z, Ye X, Huang Y, Li Z, Cui Z. Functional characterization of the novel laminaripentaose-producing β-1,3-glucanase *Mo* GluB and its biocontrol of *Magnaporthe oryzae*. J Agric Food Chem. 2021:**69**(33):9571–9584. https://doi.org/10.1021/acs.jafc.1c03072

Wawrzynska A, Rodibaugh NL, Innes RW. Synergistic activation of defense responses in *Arabidopsis* by simultaneous loss of the GSL5 callose synthase and the EDR1 protein kinase. MPMI. 2010:**23**(5):578–584. https://doi.org/10.1094/MPMI-23-5-0578

Williams JH. Novelties of the flowering plant pollen tube underlie diversification of a key life history stage. Proc Natl Acad Sci USA. 2008:**105**-(32):11259–11263. https://doi.org/10.1073/pnas.0800036105

Xie B, Wang X, Zhu M, Zhang Z, Hong Z. *Cals7* encodes a callose synthase responsible for callose deposition in the phloem: *a phloem-specific callose synthase*. Plant J. 2011:**65**(1):1–14. https://doi.org/10.1111/j.1365-313X.2010.04399.x

Xu X, Feng Y, Fang S, Xu J, Wang X, Guo W. Genome-wide characterization of the β-1,3-glucanase gene family in Gossypium by comparative analysis. Sci Rep. 2016:**6**(1):29044. https://doi.org/10.1038/srep29044

Yamaguchi T, Hayashi T, Nakayama K, Koike S. Expression analysis of genes for callose synthases and rho-type small GTP-binding proteins that are related to callose synthesis in rice anther. Biosci Biotechnol Biochem. 2006:**70**(3):639–645. https://doi.org/10.1271/bbb.70.639

Yim K-O, Bradford KJ. Callose deposition is responsible for apoplastic semipermeability of the endosperm envelope of muskmelon seeds1. Plant Physiol. 1998:**118**(1):83–90. https://doi.org/10.1104/pp.118.1.83

Yu Y, Jiao L, Fu S, Yin L, Zhang Y, Lu J. Callose synthase family genes involved in the grapevine defense response to downy mildew disease. Phytopathology. 2016:**106**(1):56–64. https://doi.org/10.1094/PHYTO-07-15-0166-R

Zavaliev R, Levy A, Gera A, Epel BL. Subcellular dynamics and role of *Arabidopsis* β-1,3-glucanases in cell-to-cell movement of tobamoviruses. MPMI. 2013:**26**(9):1016–1030. https://doi.org/10.1094/MPMI-03-13-0062-R

Záveská Drábková L, Honys D. Evolutionary history of callose synthases in terrestrial plants with emphasis on proteins involved in male gametophyte development. PLoS One. 2017:**12**(11):e0187331. https://doi.org/10.1371/journal.pone.0187331

Zhang B, Gao Y, Zhang L, Zhou Y. The plant cell wall: biosynthesis, construction, and functions. J Integr Plant Biol. 2021a:**63**(1):251–272. https://doi.org/10.1111/jipb.13055

Zhang Y, Nikolovski N, Sorieul M, Vellosillo T, McFarlane HE, Dupree R, Kesten C, Schneider R, Driemeier C, Lathe R, et al. Golgi-localized STELLO proteins regulate the assembly and trafficking of cellulose synthase complexes in Arabidopsis. Nat Commun. 2016:**7**(1):11656. https://doi.org/10.1038/ncomms11656

Zhang Y, Yu J, Wang X, Durachko DM, Zhang S, Cosgrove DJ. Molecular insights into the complex mechanics of plant epidermal cell walls. Science. 2021b:**372**(6543):706–711. https://doi.org/10.1126/science.abf2824

Zhou H, von Schwartzenberg K. Zygnematophyceae: from living algae collections to the establishment of future models. J Exp Bot. 2020:**71**-(11):3296–3304. https://doi.org/10.1093/jxb/eraa091