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A Biophysical Model for Plant Cell Plate Development

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Abstract

Plant cell division involves *de novo* formation of a 'cell plate' that partitions the cytoplasm of the dividing cell. Cell plate formation is directed by orchestrated delivery of cytokinetic vesicles via the phragmoplast, vesicle fusion, and membrane maturation to the nascent cell wall by the timely deposition of polysaccharides such as callose, cellulose, and crosslinking glycans. In contrast to the role of endomembrane protein regulators the role of polysaccharides, particularly callose, in cell plate development is poorly understood. It has been suggested that the transient accumulation of callose provides an anisotropic spreading force which helps the transition of earlier, membrane-network cell plate stages into a more mature fenestrated sheet stage. Here we present a biophysical model based on the Helfrich free energy for membranes that models this spreading force. We show that proper cell plate development in the model is possible, depending upon the selection of the bending modulus, with a two-dimensional spreading force parameter of between $2 - 6pN/nm$, an osmotic pressure difference of $2 - 10kPa,$ and a range of spontaneous curvature between $0 - 0.04nm^{-1}$. With these conditions, we can achieve stable membrane conformations in agreement with observed sizes and morphologies corresponding to intermediate stages of cell plate development. Altogether, our mathematical model predicts that a spreading force generated by callose and/or other polysaccharides, coupled with a concurrent decrease in spontaneous curvature, is vital for the transition of a membrane network to a nearly mature cell plate.

Significance Statement

Plant cell division features the development of a unique membrane network called the cell plate that matures to a cell wall which separates the two daughter cells. During cell plate development, callose, a β-1-3 glucan polymer, is transiently synthesized at the cell plate only to be replaced by cellulose in mature stages. The role for this transient callose accumulation at the cell plate is unknown. It has been suggested that callose provides mechanical stability, as well as a spreading force that widens and expands tubular and fenestrated cell plate structures to aid the maturation of the cell plate. Chemical inhibition of callose deposition results in the failure of cell plate development supporting this hypothesis. This publication establishes the need for a spreading force in cell plate development using a biophysical model that predicts cell plate development in the presence and the absence of this force. Such models can potentially be used to decipher for the transition/maturation of membrane networks upon the deposition of polysaccharide polymers.

Main Text

Introduction

Cytokinesis in plants is fundamentally different from that in animals and fungi. In plants, *de novo* formation of a cell plate develops into the new cell wall, partitioning the cytoplasm of the dividing cell. Cell plate formation takes place in multiple stages that involve highly orchestrated vesicle accumulation, fusion and membrane transformation concurrent with the time specific deposition of polysaccharides such as callose, cellulose and cross-linking glycans. This development requires the directed and choreographed accumulation of post-Golgi vesicles via the phragmoplast, which is an assembly of microtubules and microfilaments that helps organize vesicle delivery, to the cell plate assembly matrix, at the division plane.

Cell plate expansion is centrifugal, led by the accumulation and fusion of cytokinetic vesicles to the leading edge. (1–4). The different cell plate development stages are defined morphologically as follows (Fig. 1). During the initial fusion of Golgi vesicles stage (FVS), cytokinetic vesicles, mainly derived from the Golgi, are guided by the phragmoplast to the cell plate assembly matrix where their fusion starts to occur (Fig. 1*A*). Fused vesicles are transformed into dumbbells that undergo conformational changes to form a tubulo-vesicular network (TVN) (Fig. 1*B*), which then expands to the tubular network (TN) (Fig. 1*C*). The TN then expands into a planar fenestrated sheet that ultimately develops into a young cell wall (1, 2). Excess membrane material during the transitional stages is recycled concurrently, along with the accumulation of different polysaccharide materials (2). It is important to note that multiple stages exist simultaneously, adding complexity in dissecting them (5). Although several determinants involved in cell plate formation have been identified (3), a biophysical understanding of the contribution of the molecular and structural players involved in cell plate expansion is not available.

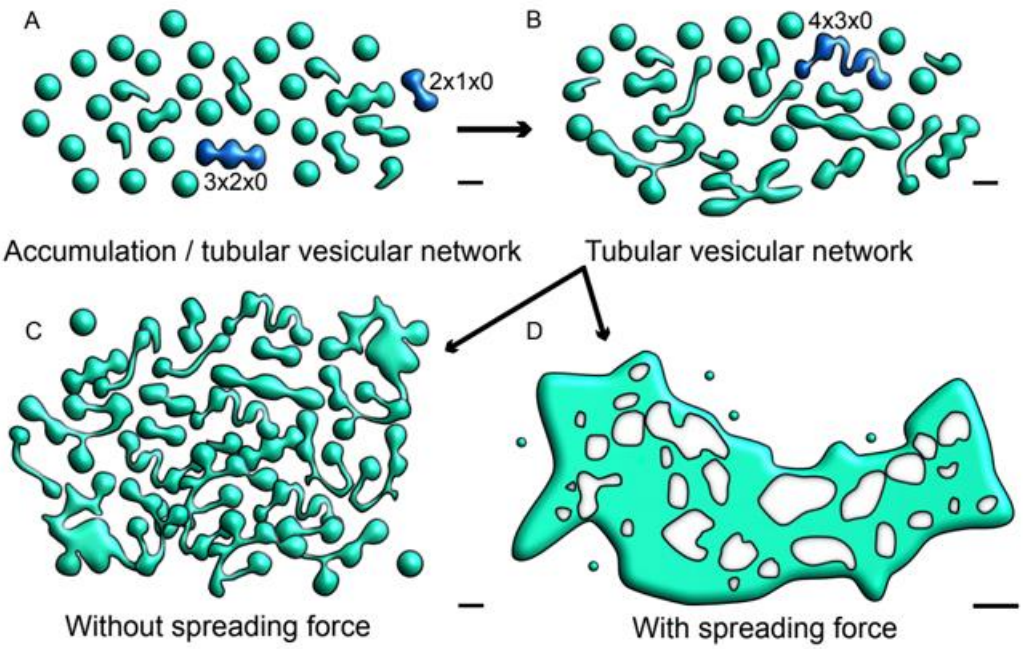


Figure 1. Schematic representation of cell plate development describing the role of a spreading force. Early stages of vesicle accumulation and fusion (*A*), and tubular network structures are shown (*B*). Two different possibilities are projected for stage transition. I) Incomplete/arrested cell plate (*C*). In the absence of a spreading force (*C*), tubular and fenestrated structures are accumulating, and there is a lack of maturation towards a single, complete cell plate structure. II) Normal cell plate transition (*D*). In our calculations, we discover that for expansion/maturation to occur as in (*D*), the presence of a spreading force is required, along with the decrease of spontaneous curvature to a threshold value. This allows for a sheet like cell plate structure (SCP) to form. The structures in this schematic description are adapted from data collected from EM tomography (2) with bars in *A* to *C* = 50nm, *D* = 0.25µm. Dark blue vesicles denote those labeled by the mathematical naming schema as described in Fig. 2. Where in A, 2x1x0 denotes 2 oblate spheroids, 1 tubular connection and 0 holes.

The development of new cell walls during cell division is of profound importance in all plant life, yet fundamental questions about this process remain unanswered, including: How does callose contribute to cell plate expansion? Currently, it is not known why callose is deposited in the developing cell plate during intermediate stages (namely, the TVN, TN and PFS), only to be replaced by cellulose (a β-1-4 glucan polymer) close to maturation to a complete cell wall (1). It is widely believed that callose provides mechanical stability in addition to a rapid transient spreading force that helps transition the membrane network to a more developed stage (1) (Fig. S1). However, there is no biophysical understanding of whether a spreading force is necessary for cell plate expansion in the first place.

Studies have revealed that this energetically costly temporal inclusion of callose is vital to cell plate development (6, 7). Genetic mutations in the cytokinesis specific callose synthase (glucan synthase like 8, GSL8) are lethal (6, 8, 9). In addition, selective chemical inhibition of callose synthase by the small molecule Endosin 7 (ES7) arrests late stage cytokinesis (7).

We hypothesize that callose provides a necessary time-dependent spreading force, which we can model by developing a free energy for the cell plate surface, and by adopting a variational approach to locally minimize it in time. We thus assume that we are near an equilibrium, which is constantly redefined as vesicles are added at the cell plate boundary. This enables us to use the cell plate area as a proxy for time. We demonstrate semi-quantitatively that by assuming a late time onset of a spreading force followed by the reduction of membrane spontaneous curvature, that we can reproduce the observed morphological time course of the cell plate.

Results and Discussion:

In order to establish the role of callose and other polysaccharides in cell plate expansion we modeled the free energy for the cell plate surface by adopting the Helfrich energy (10) with the addition of a novel term to model the presence of a spreading force. The free energy is defined as follows:

$$E = E_{bending} + E_{pressure} + E_{tension} + E_{gaussian} + E_{spreading} \quad (1)$$

The first term describes the bending energy over the closed membrane surface(s) of the cell plate. It is given by:

$$E_{bend} = \frac{K_B}{2} \oint (H_1 + H_2 - c_o)^2 \, dA \quad (2)$$

Where K_B is the bending modulus for the membrane surface, H_1 and H_2 are the principle curvatures at a point on the surface, and c_o is the spontaneous curvature, or the preferred curvature for the membrane. For simplicity, we assume that the bending modulus is time independent. We allow for the spontaneous curvature, c_o, to be time dependent, which reflects potential differences in membrane composition during cell plate evolution.

The next term is the pressure energy which results from the difference in osmotic pressure between the inside and the outside of the cell plate, such that $\Delta p = p_{out} - p_{in}$. It is given by:

$$E_{pressure} = \int \Delta p \, dV \quad (3)$$

The third term is the energy associated with the surface tension of the membrane, given by:

$$E_{tension} = \oint \gamma \, dA \quad (4)$$

We assume that the surface tension given by γ is a constant. Since we assume that our system locally equilibrates in time at a constant area, this term only adds a constant energy equal to the area at that time, multiplied with the surface tension.

Finally, we introduce the novel element of a spreading force, which is analogous to a two-dimensional pressure that acts against the periphery of the cell plate structure along the equatorial plane. In this interpretation, λ has units of force/length, with the energy representation as follows:

$$E_{spreading} = -\int_{plane} \lambda \, dA \quad (5)$$

As with the spontaneous curvature, we allow for the spreading force coefficient λ to be time dependent, representing, e.g., the “turning on” of callose production in an expanding plate.

The last term is the Gaussian bending energy term, given by:

$$E_{gaussian} = 4\pi K_G (1 - g) \quad (6)$$

Here, K_G is the Gaussian bending modulus, and g is the genus of the surface. This is a result of the Gauss-Bonnet theorem (11). Finally, we consider the surface area of the cell plate as a proxy for cell plate development time and then we minimize this energy for a given surface area.

We use established values for the bending modulus K_B (12), as well as the Gaussian bending modulus K_G (13), and the surface tension γ (14). However, the pressure difference Δp was phenomenologically

determined. The bending modulus is sensitive to the environment of the cell as well as the membrane type and conformation (12), therefore there is a range of available literature values. We decided to test over the full range of literature values ranging from about 62.5pNnm to 200pNnm (roughly corresponding to a range of 15k_bT to 50k_bT).

As described in the materials and methods section, we can model cell plate structures as combinations of oblate spheroids and elliptic hyperboloids. Oblate spheroids can be used to model vesicles, as well as an almost complete cell plate (in the case that $a \gg c$), while combinations of oblate spheroids and elliptical hyperboloids can be used to model various tubular and fenestrated structures as shown in Figs. 1-2, S2. We identify our structures using a naming convention of (A_nxB_nxC_n), where A_n is the number of oblate spheroids (or vesicles), B_n the number of hyperboloids (or tubes) and C_n the number of fenestrations (or perforations). This methodology can also be used to provide a basis for the quantitative assessment of membrane structures found in the endoplasmic reticulum and the Golgi apparatus.

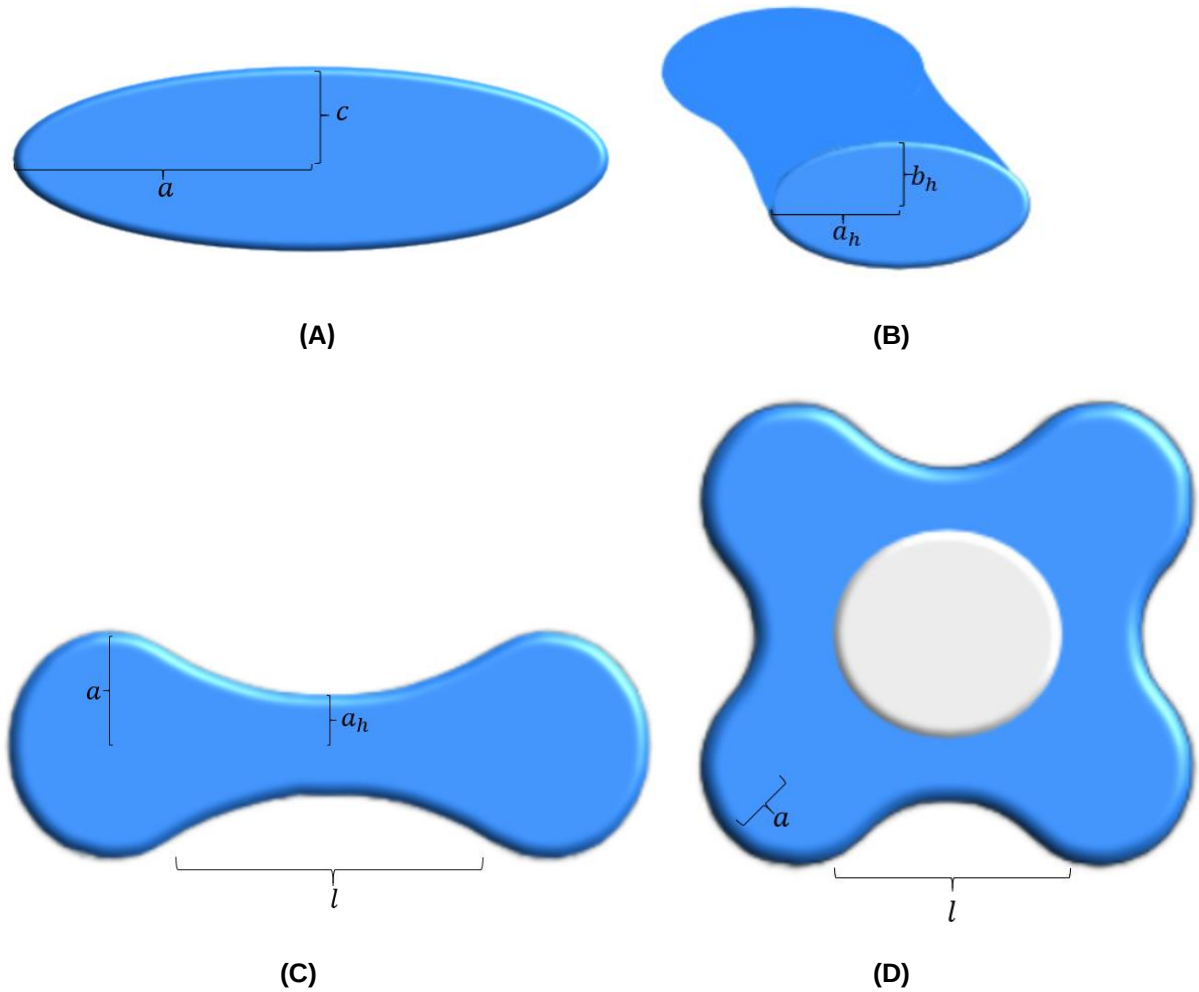


Figure 2. Examples of membrane structure parameterizations used for modeling. (*A*) Cross section of an oblate spheroid through the polar axis. The major axis radius is labelled a and the minor axis radius is labelled c. This structure is used to model vesicles, or larger cell plate structures in the case where $a \gg c$. (*B*) A cross section of an elliptic hyperboloid at its center, showing the skirt radii. The hyperboloid can be parameterized by its length l and its skirt radius in the equatorial plane a_h, the skirt radius in the axial plane is given by b_h, which can be written as a function of the other parameters listed as shown in Eq. (S3) (*C*) An example of a tubulo-vesicular structure parameterized by two oblate spheroids connected by a single elliptic hyperboloid (referred to as a 2x1x0 structure). Only the top view is shown. (*D*) An example of a 4x4x1 conformation that models a transition to a fenestrated network with genus $g = 1$.

Within this approach, we first calculated results for multiple types of structures to determine a range of parameters that would yield structures matching the experimentally observed cell plate sizes/thicknesses. From electron tomography Cryo-EM images of a developing cell plate, we determined that the thickness of a cell plate in various stages of development, with or without the presence of callose, was approximately 40-120nm (2). Therefore, we tuned the free parameters in our energy model such that the thickness of any of our conformations across the equatorial plane was in the range of 40-120nm. We determined that, depending on the choice of the bending modulus, the allowed values of the spreading force parameter λ should be between 0.0-6.0pN/nm, the spontaneous curvature c_o between 0-0.04nm^{-1}, and a finite pressure difference Δp around 2-10kPa. A deviation from these ranges results in structures that are either too thick or too thin to exist in intermediate stages of cell plate development based on literature. An application on a single oblate spheroid structure is shown in Fig. 3, and a summary of the full range of parameter values are given in Table 1.

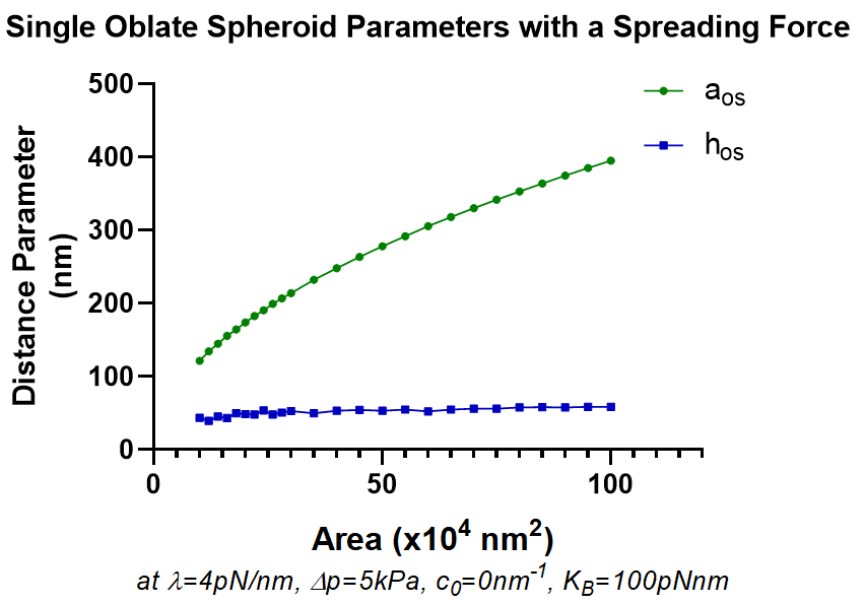


Figure 3. Evolution of single oblate spheroid parameters in the presence of a spreading force. Here, h_{os} = $2c$ (a_{os}, c shown in Fig. 2*A*), thereby representing the overall height, or thickness, of the oblate spheroid. In the presence of a spreading force, we see that the thickness of the oblate spheroid remains in the 40-80nm range despite the increase in area. This reflects the thicknesses and growth patterns found in large vesicles in intermediate cell plate stages (1). In the absence of a spreading force, h_{os}, or the thickness, would grow to values that are not observed in experiment. For reference, an area of $10^4 nm^2$ is roughly equal to that of a single vesicle.

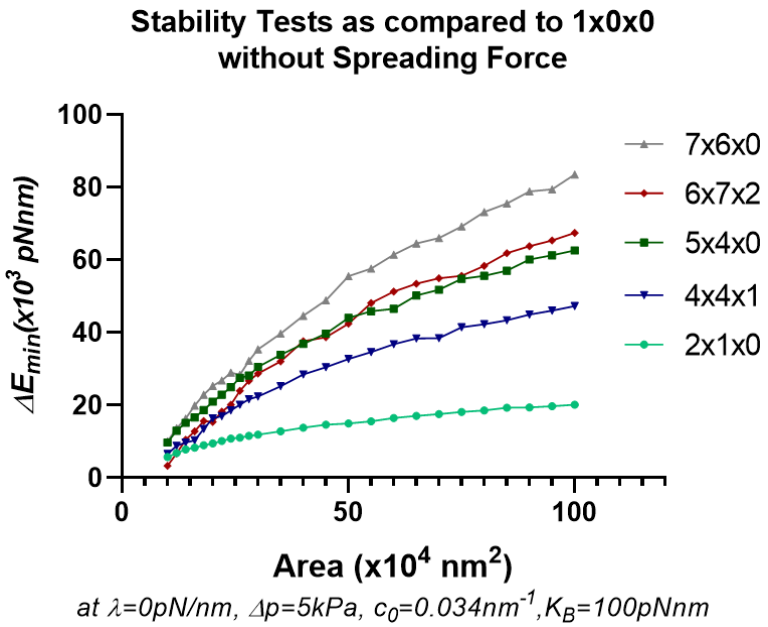
PARAMETER		VALUE
Bending Modulus	$\boldsymbol{K_B}$	62.5-200$pNnm$
Spontaneous Curvature	$\boldsymbol{c_o}$	0-0.04nm^{-1}
Pressure Difference	$\boldsymbol{\Delta p}$	2-10kPa
Spreading Force Parameter	$\boldsymbol{\lambda}$	0-6pN/nm
Gaussian Bending Modulus	$\boldsymbol{K_G}$	-0.8K_B
Surface Tension Parameter	$\boldsymbol{\gamma}$	1.6pN/nm

Table 1. Model Parameter Ranges

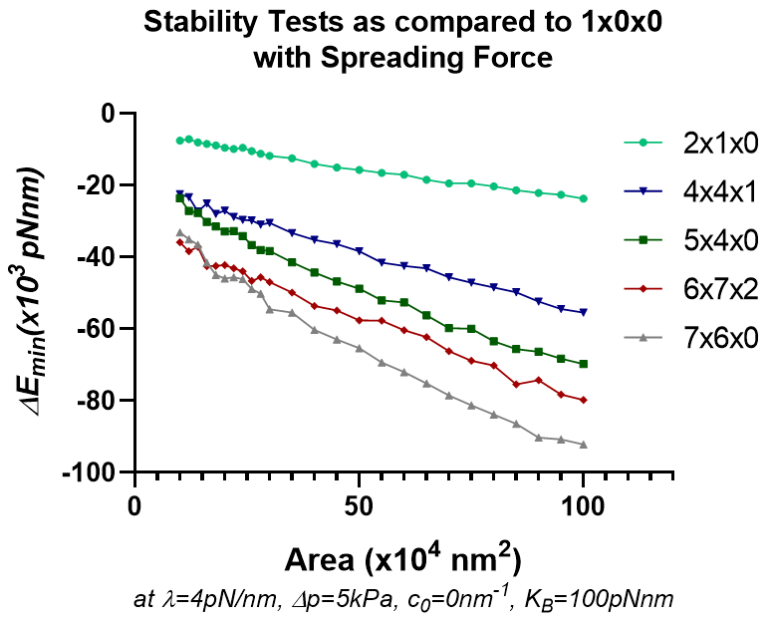
Although the range of values for the pressure difference and the planar spreading force parameter were phenomenologically determined, they are within reasonable bounds. For instance, the spreading force due to callose over a length of a nanometer is around 2-6pN, which is comparable to the ratchet forces of a microtubule (15). We can also extract the solute concentration difference between the interior and the exterior of the cell plate by employing the van't Hoff equation, which yields a solute concentration difference between $8\text{x}10^{-4} mol/l$ and $4\text{x}10^{-3} mol/l$, comparable to protein solute concentration differences in higher plant cells.

Our goal was to assess within the modeled free energy of Eq. (1), as to whether a spreading force is essential for the necessary transitions from a combination of tubulo-vesicular network to a fenestrated sheet and finally to a single mature cell plate structure. From an energy perspective, we identified that in the absence of a spreading force, single tubulo-vesicular and fenestrated structures have a lower value of energy at minima and are more stable than a single late stage cell plate structure (resembled by a single oblate spheroid) of the same area. Fig. *4A* shows the energy minima of some tubular and fenestrated structures (7x6x0,...) compared to those of a single oblate spheroid (1x0x0). ΔE_{min} represents the difference of the energy minima value of the labelled structure with that of a single oblate spheroid, so that ΔE_{min}(2x1x0)= E_{min}(1x0x0)$-E_{min}$(2x1x0). Thus, positive values indicate the relative stability of the labelled conformation. The general trend seems to indicate that in the absence of a spreading force, increasing tubularity is preferred with the increase in area. Interestingly, some fenestrated structures are more stable than a single oblate spheroid at the same area as well. Experimental results confirm that in the absence of callose, the maturation of the cell plate is arrested. Treatment with ES7, which inhibits callose deposition at the cell plate (7), leads to an abnormal transition of cell plate development such that it fails to mature into a cross wall. Fig. *5E, F* show an example of arrested cell plate development in the absence of callose, in contrast to gradual cell plate expansion concomitant with callose deposition as in Fig. *5A, B*. Cumulatively, the data supports the model in which callose provides the spreading force and mechanical support necessary for cell plate development.

In the presence of a spreading force, we examined the possibility of a transition from tubulo-vesicular networks to a single oblate spheroid. Within the theory and the variational approach, we find that this is possible if the spontaneous curvature decreases to a threshold value (about $0.015 nm^{-1}$) with larger cell plate area, i.e. in the presence of a spreading force. From an energy perspective, that would mean that tubulo-vesicular networks, as well as fenestrated sheets, should be unstable as compared to a single oblate spheroid, thereby preferring to change their morphology to one that is without tubes or fenestrations. While a finite spontaneous curvature is necessary to explain the origin of stability of the incoming vesicles (16), one can expect a change in the spontaneous curvature of the membrane due to the expected changes in membrane composition and protein activity that occurs during cell plate development (17). Fig. 4*B* shows the relative instability of selected tubular and fenestrated structures as compared to a single oblate spheroid in the presence of a spreading force and zero spontaneous curvature. Less tubular structures are now energetically favorable than highly tubular or fenestrated structures, with a single, complete structure being the most favorable. This represents the transition of a vesicle network to an expanding and mature cell plate (Fig. 5 *A* and *B-D*) in the presence of callose. It is noteworthy that callose accumulation starts at the center of the cell plate (Fig. 5 *A, B,* arrow), overlapping with later cell plate stages that transition from a vesicular network to a fenestrated sheet. For structures with genus zero (such as 2x1x0 or 3x2x0), we can also map a path to a single oblate spheroid if we relax the parameter restrictions that were initially imposed during the variational calculation. Fig. 4*B* shows results with the parameter restrictions in place. Fig. S2 shows data for a fenestrated structure in the absence (Fig. S2*A*) and the presence (Fig. S2*B*) of a spreading force, leading to fenestration shrinkage with the parameter restrictions in place.



(A)



(B)

Figure 4. Stability tests determined by ΔE_{min} vs. Area for different conformations as compared to a single oblate spheroid at the labelled area. Here, a positive value of ΔE_{min} indicates relative stability of the labelled conformation as compared to a single oblate spheroid (1x0x0). (*A*) Relative stability of tubular and fenestrated structures in the absence of a spreading force with a finite spontaneous curvature. (*B*) Stability of a single oblate spheroid over tubular (2x1x0, 5x4x0, 7x6x0) and fenestrated (4x4x1, 6x7x2) structures in the presence of a spreading force and with zero spontaneous curvature. Note that in (*B*) a decrease of spontaneous curvature to a threshold value close to 0.015nm^{-1} yields similar results.

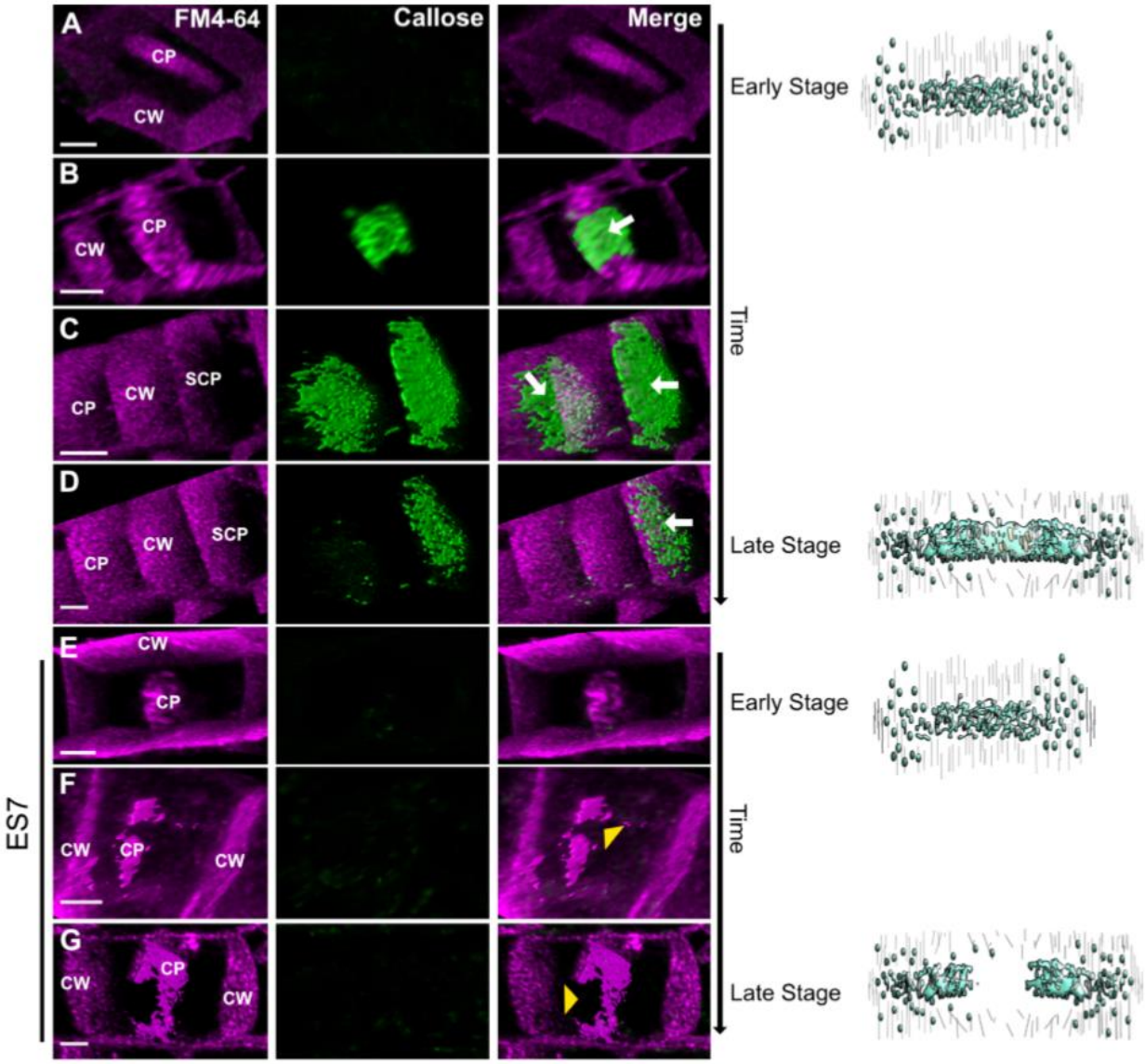
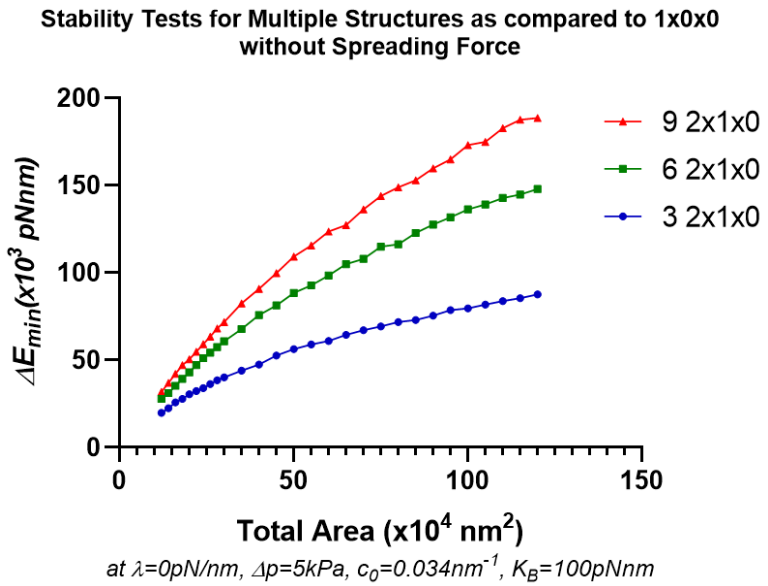


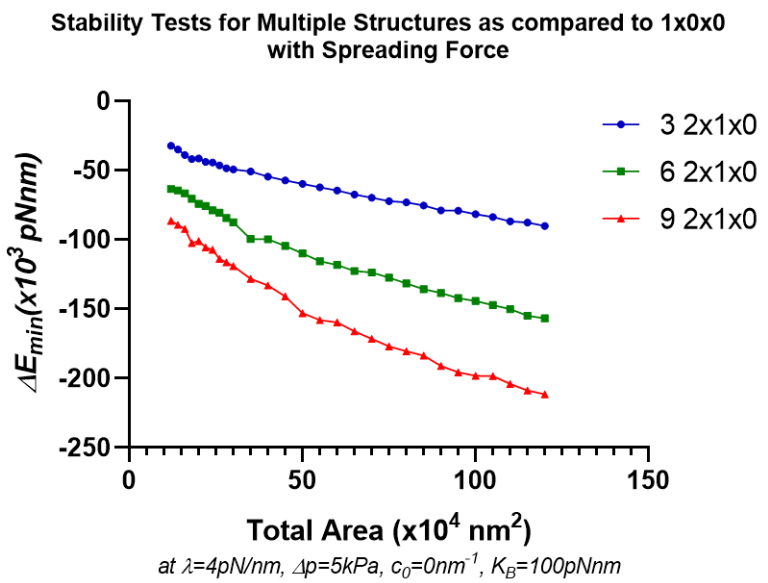
Figure 5. Progression of cell plate in the presence and absence of callose. (*A*-*D*) Cell plate progression in the presence of callose. (*A*) shows an early stage cell plate before the accumulation of callose, similar to that depicted in Fig 1*A, B*, while *B*-*D* represent later cell plate stages including sheet like cell plates (SCP) as indicated in Fig 1*D*. Cell plate progression is shown by plasma membrane staining by FM4-64 (magenta), and callose accumulation staining with Aniline Blue fluorochrome (green). Note the transient accumulation of callose in later stages leading to the maturation of cell plate during normal cytokinesis (*B*-*D*). In *C,* two cell plates are shown, and as maturation continues to *D,* callose is removed from one cell plate indicating its transition to a mature cell plate. (*E*-*G*) depict the progression of cytokinesis under ES7 that inhibits callose deposition. Note, early cell plate development is not affected with ES7 (*E*). However, in late stages of cell plate development under ES7 treatments, the absence of callose prevents the transition of membrane network into a stable single structure, leading to characteristic 'cell plate stubs' (*F* and *G*). CP, indicates cell plate, SCP, indicates sheet-like cell plate as depicted in Fig 1*D*. CW indicates parental cell wall. Arrows indicate callose accumulation at the cell plate. Yellow arrowheads denote lack of callose at cell plate breakage points. Scale bar = 3µm

To better represent a biological system, we compared multiple 2x1x0 structures (approximating accumulated fused vesicles forming a network) to a single oblate spheroid of the same combined area. Similar to our earlier calculations, we find that in the absence of a spreading force, a single oblate spheroid is less stable, as in Fig. 6*A*. The relative instability is magnified with the increase of area, and with the increase in the number of tubular structures. In the presence of a spreading force and a decreased spontaneous curvature, as in Fig 6*B*, the inverse is true, favoring fewer complex structures. When comparing multiple structures to a single mature structure of the same area, there is no need to enforce the decrease in spontaneous curvature. However, for consistency, results with a zero spontaneous curvature in the presence of a spreading force, and a finite spontaneous curvature in the absence of a spreading force are shown.

The exact values of pressure difference, spreading force, and spontaneous curvature that are needed for a transition to a complete cell plate structure depend on the choice of bending modulus. Additional calculations with different choices of bending modulus are given in the supplemental figures (Figs. S3-S7). In general, a stiffer membrane, one that is represented by a larger bending modulus, requires a stronger spreading force in addition to a higher pressure difference to transition to a mature cell plate structure. By testing over the full range of literature values for the bending modulus, we also account for the possible differences in the bending modulus that may arise in different regions of the cell plate due to varying thickness and rigidity. However, as shown in Figs. S3-S7, the need for a spreading force is always there, regardless of the choice of the bending modulus. We also show calculations for larger, highly tubulated fenestrated structures in Figs. S8-S9, which are stable in the absence of a spreading force, but unstable as compared to a single oblate spheroid in the presence of a spreading force, further supporting our hypothesis.



(A)



(B)

Figure 6. Stability test for multiple 2x1x0 structures as compared to a single oblate spheroid at the labelled area. (*A*) Relative stability of multiple 2x1x0 structures as compared to a single oblate spheroid in the absence of a spreading force. At a labelled area, a larger number of structures have a higher, more positive value of ΔE_{min} collectively, thereby indicating that in the absence of a spreading force, tubular structures as well as emerging fenestrated/network structures (as inferred by the results of Fig 4.) would tend to accumulate as in Fig 1*C*. (*B*) Stability of a single oblate spheroid as compared to multiple 2x1x0 structures in the presence of a spreading force and with zero spontaneous curvature. At a labelled area, a larger number of structures have a lower, more negative value of ΔE_{min} collectively, thereby indicating the energetic favorability of structures fusing to form larger, more mature structure(s) in the presence of a spreading force.

While other force generating proteins, including but not limited to, dynamin-like springs and clathrin are involved in the tubulation and membrane material recycling processes (18–20), the hypothesized planar spreading force ensures that the resulting tubulo-vesicular and fenestrated structures do not collapse onto themselves, thereby enabling them to transition to more mature cell plate structures. This is evidenced in Fig 4*A*, where we show that existing fenestrated and tubulo-vesicular structures are more stable compared to a single mature cell plate structure of the same area in the absence of a spreading force.

From an energy minimization analysis, we have shown that a planar spreading force is vital for cell plates to transition from tubular and fenestrated networks to complete, late stage/mature cell plate structures. Given that spatio-temporal experimental data in the absence of callose show the failure of cell plates to mature beyond tubular and fenestrated networks, our model supports the hypothesis that callose provides a spreading force facilitating these transitions. The need for this spreading force is magnified when we compare single cell plate structures to multiple smaller structures of the same total area. We still do not know what the precise molecular scale mechanism for this spreading force is. The development and adaptation of advanced analytical methods to study biomechanics at subcellular resolution can shed light on the nature of this spreading force and its regulation.

One possibility is that callose might be forming chains that tether to the cell plate boundary that constrict expansion in the direction of the minor axis, thereby resulting in a effective planar spreading force. The presence of callose near the surface rather the center of the forming cell plate (1), especially in later cell plate development stages further supports this hypothesis. This raises the intriguing possibility of a common origin to the decrease in spontaneous curvature and onset of a spreading force. In the model results, the spreading force is relevant when there is sufficient connection of individual oblate spheroidal vesicles, and it is at this stage that we shut off the spontaneous curvature. The nanoscale surface topography can potentially serve as a direct biochemical signal to activate this process (21). The possible tethering of the callose to the membrane could concomitantly induce spreading and reduce spontaneous curvature by modifying the membrane mechanics.

Callose is produced in β-1-3 linear glucan polymers that show a low degree of crystallinity (22). The proposed conformation of inter-molecular associated forms of callose that include triple helices along with loop regions supports a semi flexible network formation (23, 24). The properties exhibited by callose aggregates in such a network are ideal for generating structural molds that can effectively produce planar forces. Detailed experiments are necessary to determine how the possible callose conformations contribute to different magnitudes of spreading force, an analysis which is the focus of our future studies. Callose could also serve as a scaffold into which other more permanent polysaccharides and proteins are later deposited (22, 25). Callose aggregates would allow insertion or co-gelation with other polymers at the cell plate which together may contribute to the spreading force and change of curvature. The properties of callose as a plasticizer allowing for deformation without breakage as opposed to pure cellulose (26) support its transient accumulation during the transition of a membrane network stage to a fenestrated sheet and a mature cell plate. Furthermore, the potential transient interaction with cellulose may also contribute to a flexible yet strong polymer that supports the maturation of the cell plate (26). Taken together, our model provides a basis for understanding how membrane structures evolve in the presence of a spreading force and will likely shed light in such transitions that occur beyond cytokinesis.

Materials and Methods:

Energy Minimization:
For our energy minimization calculations, we treat the membrane boundary of the cell plate as an incompressible two-dimensional surface to a first approximation, with certain defined characteristics. In reality, each lipid bilayer has a finite thickness of 4-6nm (27), however, it is justified to treat the surface of the cell plate as two-dimensional due to the smallness of its thickness compared to the sizes of the overall structures in the cell plate. It can also be shown that lipid bilayers present a high level of incompressibility due to the energy penalty associated with areal stretching being significantly higher compared to membrane deformations due to bending (28). This is similar to the approach taken by Choksi et al. (29) and Sarasij et al. (30).

At a specified area, we define a free energy of our membrane surface that is essentially the Helfrich energy (10) with the addition of a novel term to model the callose spreading force, as given in Eq. (1). Finally, we consider the surface area of the cell plate as a proxy for cell plate development time and then we minimize this energy for a given surface area.

Due to the complexity of this research problem, we decided to look for energy minima within a parameterized restrictive geometry basis set, thereby adopting a variational approach. We found that existing adaptive mesh approaches such as Surface Evolver, while in principle more accurate and flexible, were not readily amenable for application in our study (31).

Shape Approximation:
Structures that are found within the vesicular cloud can be approximated using oblate spheroids, where the two defining radii can be used as variational parameters as shown in Fig. 2*A*. The oblate spheroid can also be used to model the expanded/late stage cell plate close to completion, as a very large oblate spheroid with $a \gg c$.

Similarly, structures found within the fenestrated sheet and the tubulo-vesicular network stages can be approximated using a combination of elliptic hyperboloids and oblate spheroids, such that the hyperboloids are continuous at the oblate spheroid boundaries. These elliptic hyperboloids can be parameterized by their length, hereafter referred to as l, and their skirt radius in the equatorial plane, hereafter referred to as a_h. The other parameters needed to define an elliptic hyperboloid can be written as a function of the two parameters already listed due to boundary conditions that arise from mandating continuity. Fig. 2*B* shows a cross section of an elliptic hyperboloid, while Fig. 2*C* shows an example of two vesicles joined by a single tube.

Naming convention of different approximated conformations:
The naming convention for different conformations was defined as described in the following example: A conformation that is labeled as 6x7x2, represents a conformation that has 6 oblate spheroids (or vesicles), 7 hyperboloids (or tubes), and 2 holes (or fenestrations, with $g = 2$). A 2x1x0 conformation is shown in Fig. *2C*, and an example of a 4x4x1 conformation with a single fenestration is shown in Fig. *2D*.

The area of any given conformation was calculated by numerical integration methods, and a corresponding parameter space of a given area was found. For simplicity, we analyzed conformations that shared the same parameters for each of the oblate spheroids, and each of the hyperboloids with examples shown in Fig. *2* with additional examples in Fig. 1. Therefore, we found a four-dimensional parameter space (a, c, a_h, l) that corresponded to a given area up to an error tolerance (<0.05%) for each conformation of interest, and then calculated the energies of Eq. (1) within that parameter space. The energy minimum was then extrapolated from that parameter space. Additional information about

extrapolating a four-dimensional parameter space is given in the supplemental information (Eq. (S1-S4)), and a full list of the parameters involved are shown in Fig. S10.

Plant Growth:

Arabidopsis seedlings of Col-0 were used in this study. Seeds were sterilized using 30% (v/v) sodium chlorate in ethanol (absolute) with 0.06% (v/v) of Triton X-100 (Sigma-Aldrich). Seeds were plated on 0.25 Murashige and Skoog medium (1.15 g L^{-1} Murashige and Skoog minimal organics salt, 10 g L^{-1} Suc, 5 g L^{-1} Phytagel (Sigma-Aldrich), and cold vernalized for 48 h at 4°C in the dark, after which plates were transferred to a plant growth chamber for seedling growth. Plants were grown in temperature- and photoperiod-controlled environments, set to long-day (16-h-light/8-h-dark cycle) conditions, using fluorescent light (at 100 to 150 mmol quanta photosynthetically active radiation [PAR] m^{-2} s^{-1}) at 22 to 24°C.

Chemical treatment and Imaging:

Four day old Arabidopsis seedlings were treated with 25 µM ES7 and DMSO in 0.25 MS medium for two hours as previously described in Park et al 2014 (7).

A Leica SP8 confocal microscope was used for imaging. Fluorescence signals of, Callose stained by Aniline blue fluorochrome (Biosupplies Australia) (excitation 350 nm, emission 450-510 nm), and FM4-64 (ThermoFisher Scientific) (excitation 488 nm, emission 652-759) were collected with 40x (water), 63x (oil) and 100x (oil) objectives. Z stacks were generated across the volume of full cell and were subsequently deconvolved with Huygens (SVI). 3D reconstructions were prepared using Imaris, Bitplane and figures were assembled using Adobe Illustrator.

Author Contributions:

D.C. and G.D. designed research. M.Z.J. did computational work and R.S. did experimental work. M.Z.J. wrote the paper, with significant revisions from D.C. and G.D. R.S. contributed to the materials and methods section, as well as figure illustrations and captions.

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References

1. Samuels, A.L., T.H. Giddings, and L.A. Staehelin. 1995. Cytokinesis in tobacco BY-2 and root tip cells: A new model of cell plate formation in higher plants. *J. Cell Biol.* 130:1345–1357.

2. Segui-Simarro, J.M. 2004. Electron Tomographic Analysis of Somatic Cell Plate Formation in Meristematic Cells of Arabidopsis Preserved by High-Pressure Freezing. *PLANT CELL ONLINE*.

3. Smertenko, A., F. Assaad, F. Baluška, M. Bezanilla, H. Buschmann, G. Drakakaki, M.T. Hauser, M. Janson, Y. Mineyuki, I. Moore, S. Müller, T. Murata, M.S. Otegui, E. Panteris, C. Rasmussen, A.C. Schmit, J. Šamaj, L. Samuels, L.A. Staehelin, D. Van Damme, G. Wasteneys, and V. Žárský. 2017. Plant Cytokinesis: Terminology for Structures and Processes. *Trends Cell Biol.* 27:885–894.

4. Lee, Y.R.J., and B. Liu. 2013. The rise and fall of the phragmoplast microtubule array. *Curr. Opin. Plant Biol.* 16:757–763.

5. Drakakaki, G. 2015. Polysaccharide deposition during cytokinesis: Challenges and future perspectives. *Plant Sci.* 236:177–184.

6. Thiele, K., G. Wanner, V. Kindzierski, G. Jürgens, U. Mayer, F. Pachl, and F.F. Assaad. 2009. The timely deposition of callose is essential for cytokinesis in Arabidopsis. *Plant J.* 58:13–26.

7. Park, E., S.M. Diaz-Moreno, D.J. Davis, T.E. Wilkop, V. Bulone, and G. Drakakaki. 2014. Endosidin 7 Specifically Arrests Late Cytokinesis and Inhibits Callose Biosynthesis, Revealing Distinct Trafficking Events during Cell Plate Maturation. *PLANT Physiol.*

8. Chen, X.Y., L. Liu, E.K. Lee, X. Han, Y. Rim, H. Chu, S.W. Kim, F. Sack, and J.Y. Kim. 2009. The arabidopsis callose synthase gene gsl8 is required for cytokinesis and cell patterning. *Plant Physiol.* 150:105–113.

9. Guseman, J.M., J.S. Lee, N.L. Bogenschutz, K.M. Peterson, R.E. Virata, B. Xie, M.M. Kanaoka, Z. Hong, and K.U. Torii. 2010. Dysregulation of cell-to-cell connectivity and stomatal patterning by loss-of-function mutation in Arabidopsis chorus (GLUCAN SYNTHASE-LIKE 8). *Development*. 137:1731–1741.

10. Helfrich, W. 1973. Elastic Properties of Lipid Bilayers: Theory and Possible Experiments. *Zeitschrift fur Naturforsch. - Sect. C J. Biosci.*

11. Lee, J.M. 1997. The Gauss-Bonnet Theorem. . pp. 155–172.

12. Dimova, R. 2014. Recent developments in the field of bending rigidity measurements on membranes. *Adv. Colloid Interface Sci.* 208:225–234.

13. Hu, M., J.J. Briguglio, and M. Deserno. 2012. Determining the Gaussian curvature modulus of lipid membranes in simulations. *Biophys. J.* 102:1403–1410.

14. Fischer-Friedrich, E., A.A. Hyman, F. Jülicher, D.J. Müller, and J. Helenius. 2014. Quantification of surface tension and internal pressure generated by single mitotic cells. *Sci. Rep.* 4.

15. Kent, I.A., and T.P. Lele. 2017. Microtubule-based force generation. *Wiley Interdiscip. Rev. Nanomedicine Nanobiotechnology*.

16. Jung, H.T., B. Coldren, J.A. Zasadzinski, D.J. Iampietro, and E.W. Kaler. 2001. The origins of stability of spontaneous vesicles. *Proc. Natl. Acad. Sci. U. S. A.* 98:1353–1357.

17. McMahon, H.T., and E. Boucrot. 2015. Membrane curvature at a glance. *J. Cell Sci.*

18. Ahn, G., H. Kim, D.H. Kim, H. Hanh, Y. Yoon, I. Singaram, K.J. Wijesinghe, K.A. Johnson, X. Zhuang, Z. Liang, R. V. Stahelin, L. Jiang, W. Cho, B.H. Kang, and I. Hwang. 2017. SH3 domain-containing protein 2 plays a crucial role at the step of membrane tubulation during cell plate formation. *Plant Cell*. 29:1388–1405.

19. McMichael, C.M., and S.Y. Bednarek. 2013. Cytoskeletal and membrane dynamics during higher plant cytokinesis. *New Phytol.* 197:1039–1057.

20. MS, O., M. DN, K. BH, B. SY, and S. LA. 2001. Three-dimensional Analysis of Syncytial-Type Cell Plates During Endosperm Cellularization Visualized by High Resolution Electron Tomography. *Plant Cell*. 13.

21. Lou, H.Y., W. Zhao, X. Li, L. Duan, A. Powers, M. Akamatsu, F. Santoro, A.F. McGuire, Y. Cui, D.G. Drubin, and B. Cui. 2019. Membrane curvature underlies actin reorganization in response to nanoscale surface topography. *Proc. Natl. Acad. Sci. U. S. A.* 116:23143–23151.

22. Him, J.L.K., L. Pelosi, H. Chanzy, J.L. Putaux, and V. Bulone. 2001. Biosynthesis of (1→3)-β-D-glucan (callose) by detergent extracts of a microsomal fraction from Arabidopsis thaliana. *Eur. J. Biochem.* 268:4628–4638.

23. Michael J. Gidley, and Katsuyoshi Nishinari. 2009. Physico-Chemistry of (1,3)-ß-Glucans. In: Bacic A, GB Fincher, BA Stone, editors. Chemistry, Biochemistry, and Biology of 1-3 Beta Glucans and Related Polysaccharides. AcademicPress, Boston.

24. Falch, B.H., and B.T. Stokke. 2001. Structural stability of (1→3)-β-D-glucan macrocycles. *Carbohydr. Polym.* 44:113–121.

25. Stone, B.A., and A.E. Clarke. 1992. Chemistry and biology of (1 --> 3)-[beta]-glucans - Version details - Trove. La Trobe University Press .

26. Abou-Saleh, R.H., M.C. Hernandez-Gomez, S. Amsbury, C. Paniagua, M. Bourdon, S. Miyashima, Y. Helariutta, M. Fuller, T. Budtova, S.D. Connell, M.E. Ries, and Y. Benitez-Alfonso. 2018. Interactions between callose and cellulose revealed through the analysis of biopolymer mixtures. *Nat. Commun.* 9.

27. Rawicz, W., K.C. Olbrich, T. McIntosh, D. Needham, and E.A. Evans. 2000. Effect of chain length and unsaturation on elasticity of lipid bilayers. *Biophys. J.* 79:328–339.

28. Lázaro, G.R., I. Pagonabarraga, and A. Hernández-Machado. 2015. Phase-field theories for mathematical modeling of biological membranes. *Chem. Phys. Lipids*. 185:46–60.

29. Choksi, R., M. Morandotti, and M. Veneroni. 2012. Global minimizers for axisymmetric multiphase membranes. .

30. Sarasij, R.C., S. Mayor, and M. Rao. 2007. Chirality-induced budding: A raft-mediated mechanism for endocytosis and morphology of caveolae? *Biophys. J.* 92:3140–3158.

31. Brakke, K.A. 1992. The surface evolver. *Exp. Math.* 1:141–165.