A Plausible Microtubule-Based Mechanism for Cell Division Orientation in Plant Embryogenesis

Graphical Abstract

Highlights

- Cortical microtubule dynamics is simulated on experimentally extracted cell shapes
- Cell geometry, edge effects, and developmental cues affect microtubule dynamics
- Cortical microtubule array orientation can predict division planes in plant embryos
- Predictions match observations in wild-type and two developmental mutants

Authors
Bandan Chakrabortty, Viola Willemsen, Thijs de Zeeuw, Che-Yang Liao, Dolf Weijers, Bela Mulder, Ben Scheres

Correspondence
mulder@amolf.nl (B.M.), ben.scheres@wur.nl (B.S.)

In Brief
Chakrabortty et al. show that a computational model for dynamic self-organization of cortical microtubules on experimentally extracted cell shapes provides a plausible molecular mechanism for division plane orientation in the first four divisions of early stage A. thaliana embryos, in WT as well as two developmental mutants bodenlos and clasp.
A Plausible Microtubule-Based Mechanism for Cell Division Orientation in Plant Embryogenesis

Bandan Chakrabortty,1,2 Viola Willemsen,1,5 Thjis de Zeeuw,4,5 Che-Yang Liao,4 Dolf Weijers,4 Bela Mulder,2,3,* and Ben Scheres1,6,*

1Plant Developmental Biology, Wageningen University, Wageningen 6708 PB, the Netherlands
2Theory of Biomolecular Matter, Institute AMOLF, Science Park 104, 1098 XG Amsterdam, the Netherlands
3Cell Biology, Wageningen University, Wageningen 6708 PB, the Netherlands
4Laboratory of Biochemistry, Wageningen University, Stippeneng 4, 6708 WE Wageningen, the Netherlands
5These authors contributed equally
6Lead Contact
*Correspondence: mulder@amolf.nl (B.M.), ben.scheres@wur.nl (B.S.)
https://doi.org/10.1016/j.cub.2018.07.025

SUMMARY
Oriented cell divisions are significant in plant morphogenesis because plant cells are embedded in cell walls and cannot relocate. Cell divisions follow various regular orientations, but the underlying mechanisms have not been clarified. We propose that cell-shape-dependent self-organization of cortical microtubule arrays is able to provide a mechanism for determining planes of early tissue-generating divisions and may form the basis for robust control of cell division orientation in the embryo. To show this, we simulate microtubules on actual cell surface shapes, from which we derive a minimal set of three rules for proper array orientation. The first rule captures the effects of cell shape alone on microtubule organization, the second rule describes the regulation of microtubule stability at cell edges, and the third rule includes the differential effect of auxin on local microtubule stability. These rules generate early embryonic division plane orientations and potentially offer a framework for understanding patterned cell divisions in plant morphogenesis.

INTRODUCTION
Plant cell division patterns show striking regularities. A prime example is the early-stage embryo in Arabidopsis [1–3], which starting from the single-cell stage undergoes a few rounds of remarkably robust and geometrically precise internal divisions, setting the stage for further development through differentiation and growth. For already more than a century, these regularities have spurred the formulation of heuristic geometric rules for oriented cell divisions. These rules relate the selection of division planes, e.g., to the principal direction of growth, geometric relations to existing cell walls and the nucleus, or minimum cell surface energy [4–9]. With the advent of modern cell biology, however, division plane orientation in plants has been connected with the orientation of the ordered ensemble of microtubules (MTs) associated with the plasma membrane, the cortical microtubule array (CMA) [10, 11]. Shortly before cell division, the cortical MTs, while keeping their net orientation, become spatially restricted to a plane that is closely associated with the nucleus, forming the so-called pre-prophase band (PPB). The orientation of the PPB and, by extension, that of the CMA is an indicator of cell division orientation [12, 13]. Some variability between PPB and cell plate orientation may occur (e.g., [14]), and recent evidence suggests that the PPB is not strictly required for division plane orientation [15], but nevertheless the link of cell division plane to CMA orientation is maintained. The question of how the CMA is organized is therefore of prime importance to understanding division plane orientation.

Here, we address this question in the early Arabidopsis embryo. Apart from the intrinsic biological interest of the tissue-separating (“formative”) cell divisions that occur in this system, it provides a unique, well-defined, and yet highly non-trivial “laboratory” for studying the interplay between the molecular mechanisms driving CMA organization and the developmental context. This holds a fortiori, as previous analysis of Arabidopsis embryogenesis surprisingly shows that auxin-insensitive embryos misexpressing the bodenlos (bdl) protein do divide consistently with proposed geometric rules, whereas wild-type (WT) divisions seem to require additional control [1], possibly through auxin-mediated CMA regulation. This indicates the need to elucidate to what extent the geometrical rules are in fact consistent with concrete molecular mechanisms.

There is a broad consensus that orientational ordering in the CMA is the collective outcome of the local effects of “collisions” between MTs driven by their dynamical instability mechanism [16]. Moreover, there is evidence gleaned from root epidermis cells that specific geometric features, such as sharps edges between relatively flat cell faces, can influence the orientation of the CMA by effectively blocking the passage of MTs from one cell face to the other and that this effect is possibly modulated by microtubule-associated protein (MAP) CLASP [17]. However, we as yet lack a full understanding of how cell shape in general impacts CMA organization. This is especially salient in the early Arabidopsis embryo, which, in its first 4 division cycles, displays a gamut of cell shapes, ranging from almost spherical to halves of spherical wedges with a sharp opening angle. Recently, tensile stress has also been shown to influence CMA orientation [18], leading to the proposal of a new general rule for division.
Figure 1. Experimental Visualization of CMA and PPB during Arabidopsis Early Embryonic Development

(A) Characteristic divisions lead to the formation of inner and outer tissue layers (left); maximum projection of depth-coded stacks of MTs visualized using a pWOX2::TUA6-GFP reporter line shows an ordered orientation of MT array (right). The look-up table shows color values corresponding to the depth of the image in the z-dimension.

(B) Sequential imaging of cortical MTs and the SR2200 membrane stain allows for extraction of cell shapes for separate embryonic cells. Projection of cortical MT signal on extracted cell shapes shows orientation of CMA that predicts the orientation of PPB (7 cells were analyzed; note that the images are taken from different cells in the same embryo, not from live imaging the same cell over time).

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plane orientation along the direction of maximal wall tension [14]. However, to date, the molecular mechanism by which MTs “sense” the direction of wall tension remains elusive, although the activity of the MT-severing protein katanin appears to be implicated [19]. Finally, as cortical MTs co-align with cellulose microfibrils, growing cells exhibit a complex interplay between CMA orientation, the deposition of microfibrils in the cell wall, and growth [20, 21], so that growth by itself could influence division plane orientation, potentially underpinning one of the phenomenological rules [22].

Here, we utilize a recently developed computational framework that simulates the dynamics of MT ordering on realistic cell shapes [23]. We specifically focused on the impact of shape in a geometrical sense, including the described edge effects, on the collective dynamics of MTs that lead a global orientation of the CMA. This provides a clearly defined default model to which other effects may be added incrementally. As it is at present infeasible to directly measure or predict stress patterns in the embryo, and in the absence of a concrete molecular mechanism to implement the feedback of tension on MT dynamics, we parsimoniously chose to disregard these effects at this stage. Finally, as only a very limited amount of volumetric growth occurs during the considered embryonic developmental phase, potential growth-related effects were also ignored.

Combining our in silico results with high-resolution visualization of both cell outline and cortical MTs in WT Arabidopsis embryos and selected mutants enabled us to show that hitherto unanticipated constraints due to cell shape alone, in combination with cell edge-catastrophe protection and auxin-mediated MT stability, can already provide a sufficient explanation for cell division patterns in 1- to 16-cell stage embryos. Thus, our minimal MT-based molecular statistical mechanism correctly predicts division plane orientations, recapitulates many of the proposed geometric rules, and establishes a basis for specific experimental validations and the incorporation of additional mechanisms when necessary in the future.

RESULTS

First Principle-Based MT Modeling on Realistic Cell Shapes

Our approach for understanding rules of cell division in cells that undergo formative cell divisions was to model cortical MT dynamics during the first four division cycles in the Arabidopsis embryo proper, which have been documented in detail [1, 24–26]. These characteristic divisions lead to the formation of inner and outer tissue layers (Figure 1A, left). Our starting assumption was that embryonic cells, like most cell types previously considered, have a regular CMA orientation that predicts the orientation of cell division. To test this assumption, we utilized an optimized imaging methodology for high-resolution 3D imaging of the CMA, which preserves MT orientation throughout the different stages of Arabidopsis embryogenesis [27]. We expressed TUA6-GFP from the embryo-specific WUSCHEL-RELATED HOMEOBOX 2 (WOX2) promoter. Maximum projections of high-resolution z stacks reveal the overall topology of the MT array, including the CMA, for embryos from the 1-cell stage to globular stage (Figure 1A, right). Cell segmentation allowed the extraction of individual cell surfaces for the different embryonic cells. Analysis of cortical MT signals projected on these extracted cell surfaces shows the ordered orientation of CMA on individual cells. The CMAs collapse to form PPB structures [28], and we found this to likely also be the case in embryos (Figure 1B), thus correctly predicting future division plane orientation (Figure 1C). Our observations validated the early embryo as a suitable model system to investigate how cortical MT ordering is established in cells with varying geometries.

To enable the exploration of a number of factors that, given observations in other cell types, are likely to govern CMA orientation in the early embryo, we designed a computational framework to simulate the key properties of MT dynamics on arbitrary shaped cellular surfaces. CMAs in animal cells have been shown to be sensitive to cell shape [29]. Therefore, we used the actual embryonic cell shapes obtained by segmenting high-resolution confocal images of fluorescently stained Arabidopsis embryos to extract cell surfaces, approximated through fine-grained triangulation (Figure 2A). To simulate MT dynamics on individual planar triangles, we adapted a previously developed event-driven algorithm [31, 32] that implements all key MT properties with biophysical significance, such as nucleation and dynamic instability [33]. In the latter mechanism, MTs alternate between states of steady growth or shrinkage, stochastically switching between these states at constant rates in events termed catastrophes and rescues, respectively. We also encoded three possible results from MT-MT collisions that have been observed [16], zippering, in which an impinging growing MT bends and continues growing along an obstructing MT; crossover, in which an impinging MT simply passes over an obstructing MT; and induced-catastrophe, in which the impinging MT switches to a shrinking state and retreats from the obstructing MT (Figure 2B). Additional factors are known to influence MT dynamics, most notably directional nucleation from existing MTs [34] and severing by katanin [19, 35]. At present, however, data are lacking to what extent these factors play a role in the early embryo. Also, previous modeling work has shown that these additional effects mainly modify the range of parameters for which ordered arrays develop spontaneously but have less impact on the nature of the ordered state per se [35–37]. They are therefore not expected to influence the results we present here, which involve the effect cell geometry and other developmental factors has on the global orientation of an otherwise spontaneously aligned MT array. For simplicity’s sake, we therefore choose to omit these features at this stage but stress that they are all implemented in our simulation framework [35, 36]. Likewise, MTs that are nucleated in the bulk cytosol but become entrained to the cortex by binding to the cell membrane (see, e.g., [38])

(C) PPBs predicts the location and orientation of the division plane: (1) representative single cell from 2-cell stage and 4-cell stage embryo and representative single cell from upper tier and lower tier of 8-cell stage embryo are highlighted; (2) projection of PPB on the corresponding cell templates during 2- to 4-cell stage (3 cells were analyzed), 4- to 8-cell stage (5 cells were analyzed), and 8- to 16-cell stage (5 cells were analyzed) transitions; and (3) pair of daughter cells during 2- to 4-, 4- to 8-, and 8- to 16-cell stage transitions. Figures S1A–S1C show the cortical localization of MTs.
Figure 2. MT Modeling on Arabidopsis Early Embryonic Cell Templates
(A) The confocal image of embryonic 1-cell shape is extracted as a triangulated surface mesh via image segmentation software MorphoGraphX [30]. The edge of the mesh is tagged (red color) by assigning all triangles belonging to this edge with a unique edge-tag index. The flat-bottom face (faint blue) and the curved-top face (faint gray) are tagged by assigning all their triangles with a unique face-tag index. Edge tags and face tags are used for simulation implementation of edge-catastrophe and face-specific MT stabilization.

(B) Basic MT-MT interactions. Two-dimensional attachment of MTs to cell cortex allows MTs to interact with each other via collisions. Shallow angles (≤ 40°) of collision lead to zippering, and steeper angles (> 40°) of collision lead to crossover or induced-catastrophe.

(C) Order parameter to quantify the degree of MT order and array orientation. A disordered state results in Q(2) = 0 without formation of MT array. An ordered state indicates the formation of MT array with significant degree of MT order, Q(2) ≈ 1. Perpendicular to the MT array, a vector (\( \vec{U} \)) is defined that quantifies the orientation of the MT array. The tip of this vector will be used to represent the MT array orientation.

(D) A schematic overview of the simulation approach, where geometrically correct propagation of growing MT ends between the neighboring triangles lead to the reconstruction of MT dynamics on the cell surfaces.

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effectively only increase the nucleation rate. Parsimony therefore led us to omit these features here. The simulations on individual triangles are linked together by ensuring that the trajectories of MTs crossing from a triangle to a neighboring one are propagated in a manner consistent with the 3D geometry of the cell surface, as illustrated in Figure 2D. The technical details of our simulation procedure, its validation against known results, and illustrative background results on the interplay between geometry and CMA organization on simple shapes are more fully explained in a recently published method paper [23].

Earlier work has shown that all parameters of individual MT behavior, excluding collision parameters, can be absorbed in a single dimensionless control parameter (G) that controls the frequency of MT interactions [37, 39],

\[ G = \left( \frac{2(v_+ - v_m)(v_+ + v_m)}{r_n(v_+ + v_-)} \right)^{1/3} \left( \frac{r_c}{v_+ - v_m} - \frac{r_c}{v_+ - v_m} \right), \]

where \( v_+ \), \( v_- \), and \( v_m \) are the growth, shrinkage, and minus-end treadmilling speeds of the MTs, \( r_n \) and \( r_c \) the catastrophe- and rescue rates, and \( r_c \) the rate of MT nucleations per unit of cell surface area. By convention, the sign of G is chosen such that G < 0 when the MTs are in the biologically relevant bounded growth regime. If G increases beyond a threshold value, the interacting cortical MTs will spontaneously form an ordered array. G can be increased (i.e., brought closer to 0) in several ways. For example, the higher nucleation rate \( r_n \), the more MTs are present, which increases the frequency of collisions in the cortex. Likewise, decreasing the catastrophe rate \( r_c \) makes the MTs longer on average, which makes them more likely to interact through collisions. As the value of G can be freely chosen on each triangle, we are able to modulate MT behavior on selected areas of the cell surface, corresponding to biologically controlled effects, e.g., developmentally distinct cell faces and/or highly curved edge regions between cell faces.

To read out the order of the steady-state CMA, we employed a tensorial order parameter \( Q^2 \) (for details of its definition, see [23]), whose absolutely smallest eigenvalue \( Q^{(2)} \) characterizes the degree of ordering: \( Q^{(2)} = 0 \) when MT orientation is random and \( Q^{(2)} \sim 1 \) when MTs are almost aligned. The normalized eigenvector \( \mathbf{Q} \) associated with the eigenvalue \( Q^{(2)} \) characterizes the global orientation of the MT array. Its direction is perpendicular to the plane in which on average the MTs are pointing, i.e., if the MTs form a dense band around the “equator” of a cell, \( \mathbf{Q} \) will point to one of the “poles.” In all simulation images, \( \mathbf{Q} \) is represented by a dot marking the end point of the vector (Figure 2C).

**Shape and Edge-Catastrophe Can Explain Early Embryo Cell Division Patterns in an Auxin Response Mutant**

We performed \( \approx 1,000 \) stochastically independent simulations of MT dynamics per cell on cell surfaces extracted from WT embryos from 1- to 8-cell stage, ensuring throughout that a steady state was reached. The resulting CMAs displayed a diverse, but not randomly distributed, orientation (Figure 2E: quantification in Figure 7A). Thus, cell shape significantly impacted but could not uniquely specify the orientation of the CMAs on a given cell template. It has been reported that cell edges may hinder the propagation of MTs in plant cells depending on their degree of curvature [17]. We were curious whether this reported property of MT behavior could further reduce the diversity of CMA orientations in our simulations. We implemented a local enhanced catastrophe in those cell edges that have high curvature. In the various WT cells, this implementation robustly reduced the possible CMA orientations to a single sharply defined cluster for each cell stage (Figure 2F). However, the resultant inferred division patterns were non-WT and instead appeared to match those of the auxin-insensitive embryos ectopically expressing bdl protein (RPS5A>>bdl) [1]. Moreover, the simulations predicted a preference for slightly oblique division at the 4-cell stage in auxin-insensitive embryos, hitherto not described. We experimentally verified this preference of oblique division for 4- to 8-cell stage transition in RPS5A>>bdl embryos as well as their aberrant divisions in 8- to 16-cell stage transition [1] (Figure S2). Note that, at the 1- to 2-cell transition, all simulations yielded horizontal divisions, whereas only 18% of the original bdl mutant (non-overexpressed) divides in this direction [40]. To exclude that the mapping to bdl division patterns was a coincidental effect of using WT cell templates, we extracted cell templates from 4- to 16-cell stages RPS5A>>bdl embryos, reconstructed progenitor 2- to 8-cell stage cell surfaces by merging corresponding daughter cell pairs, and ran simulations on these reconstructed “mother cells” (Figure 3A). The steady-state CMA orientation in all these simulations correctly predicted the division plane, confirming that shape effects and edge-catastrophe were sufficient to explain bdl divisions between 2- and 16-cell stages (Figure 3B). For a quantification of the predicted division plane angles with respect to the basal plane compared to experimentally measured values, see Figure 7B.

**Face Stability and Edge-Catastrophe Reduction Explain WT Early Embryo Cell Division Patterns**

The highly characteristic WT division pattern at the 8- to 16-cell stage transition that separates inner and outer cell layers did not follow the rules based on cell shape and edge-catastrophe only. To search for an additional rule that might explain the WT division pattern, we first focused on the divisions between 2- and 16-cell stages, which were correctly predicted in bdl mutant cells. We asked whether the observed defects with respect to WT were associated with aberrant CMA formation. In RPS5A>bdl embryos, the cytosolic (non-polymerized) TUA6:GFP fraction was significantly higher, suggesting that MTs were more often depolymerized compared to WT. This indicates that reduced auxin signaling affects MT polymerization or stability in embryo cells (Figure S1D). We implemented this observation in our simulations by allowing auxin-dependent, cell-face-specific changes in the G parameter, leading to locally

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(E and F) Simulated orientation of MT array on 1- to 8-cell stage WT cell templates: (E) default cell shapes, which showed a diverse, but not randomly distributed, MT array orientation, and (F) with edge-catastrophe in MT dynamics, which showed one unique cluster of MT array orientation in each cell stage. Simulations were performed for \( \approx 1,000 \) independent configurations of stochastic MT dynamics. The arrow vectors represent mean orientation of the associated MT array. See also Figure S3.
enhanced average MT stability (Figure S3). The resulting simulations therefore implemented, next to the basic rules for MT dynamics, effects of two biological control parameters: auxin-regulated face stability (Figure 4A) and edge-catastrophe. We investigated multiple combinations of the strength of these two effects yielding different behavioral regimes. A complete match with WT division patterns (Figure 4B; quantification in Figures 7C and S4) was obtained by simulating MT dynamics on “original” or “reconstructed mother” 2- to 8-cell stage cell templates under the following adaptations of our initial rules: (1) enhanced cell face stability that is strongest in recent division faces and (2) reduced edge-catastrophe (for simulation parameters, see Figure S3). These specific rules conceivably result from concrete molecular processes. First, auxin-mediated enhanced face stability could be transiently established at each new division site. Second, edge-catastrophe could be reduced, for example, by the activity of proteins that stabilize bending MTs [17].

As an initial test of the requirement of edge-catastrophe reduction for obtaining correctly oriented CMAs between 2- and 16-cell stages in WT, we analyzed embryos homozygous for two different mutations in the CLASP gene, reported to influence MT edge-catastrophe [17]. To support our assumption that edge-catastrophe is regulated in WT embryos by CLASP, its mutations should affect division plane orientation in a manner predicted by our simulations. Indeed, in both mutant alleles, division orientations were severely skewed compared to WT (Figure 5A for 4- to 8-cell stage; Figure S5 for all 1- to 16-cell stage). To test whether we could correctly predict division planes in clasp mutant embryos, we first focused on the resulting sister cells of 4- to 8-cell stage transition in the clasp mutant to reconstruct mother cell and then simulated CMA orientation on the resulting cell shape. We used WT settings for auxin-mediated face stability, assumed to be unaffected, but introduced increased edge-catastrophes to simulate loss of CLASP. Under these conditions, the

Figure 3. Recapitulation of bdl Division Patterns in Arabidopsis Early Embryonic Development
(A) Reconstruction of mother cell by merging the corresponding daughter cell pair. In this example, the daughter pair of both upper and lower tier of Arabidopsis bdl 16-cell template was merged to reconstruct the corresponding mother 8-cell template. Such re-created mother cells were separated out for simulating MT dynamics.
(B) Simulated MT arrays on 2- to 6-cell stage of bdl cell template, which were reconstructed by merging the corresponding daughter cell pair of the next cell stage. Simulations were performed with edge-catastrophe, yielding one unique cluster of MT array orientation in each cell stage, which correctly predicted the corresponding bdl division plane orientation. Cell edges are colored red to indicate that, in simulation, MTs were subjected to edge-catastrophes.
See also Figures S2, S3, and S7.
observed CMA orientations indeed robustly predicted the observed division planes in the clasp mutant (Figure 5B; quantification in Figure 7D). In conclusion, the model assumptions that result in WT division pattern also yield, mutatis mutandis, the aberrant division pattern of clasp at the critical 4- and 8-cell stage transition.

Figure 4. WT Division Pattern in Arabidopsis Early Embryonic Development
(A) Developmental age of cell faces $F_5 > F_4 > F_3 > F_2 > F_1$, as depicted by the color scale. MT dynamic catastrophe rate increases with developmental age, yielding a corresponding decrease in average MT length, i.e., lower MT stability.
(B) Simulated orientation of MT array on WT cell templates taken from different cell stages: (1) 2-cell stage with reduced edge-catastrophe in MT dynamics and enhanced MT stabilization at developmentally new cell faces, resulting in one unique cluster of MT array orientation correctly predicting the WT division plane orientation; (2) 4-cell stage without edge-catastrophe in MT dynamics and enhanced MT stabilization at developmentally new cell faces, resulting in two clusters of MT array orientation correctly predicting the two observed phenotypes of WT division plane orientation; and (3) 8-cell stage without edge-catastrophe in MT dynamics and enhanced MT stabilization at developmentally new cell faces, resulting in one unique cluster of MT array orientation for both upper and lower tier cells correctly predicting the WT division plane orientation. See also Figures S1D, S3, and S4.

Tuning of Face Stability and Edge-Catastrophe Allows Prediction of All Early Embryo Cell Division Patterns
Our model so far predicted correct WT division patterns between the 2- and 16-cell stage when both auxin-mediated face stability and CLASP-mediated edge-catastrophe reduction are implemented. This same implementation of the model also
correctly predicted the vertical orientation of the cell division plane at the 1- to 2-cell transition, although, due to the high degree of cylindrical symmetry, it does so without fixing its angle around the symmetry axis of the embryo (Figure S6A for WT cell template and Figure S6B for clasp cell template). Notably, even in the presence of edge-catastrophe (simulated as the clasp mutant), the correct vertical orientation is maintained (Figure 6A for WT cell template and Figure 6B for clasp cell template). Moreover, with moderate edge-catastrophe, the division axis was constrained to a single specific direction, reminiscent of the true WT situation. This led us to hypothesize that CLASP is less active at the 1-cell stage than at later stages. Strikingly, when we implemented this stage-specific reduced CLASP activity without the auxin-mediated face stability rule (i.e., simulating a bdl mutant under this new condition), we obtained a proportion of horizontal and vertical divisions that closely matched the 18% horizontal divisions observed in the original bdl mutant (Figure 6C). In conclusion, auxin-mediated face stability from the 1-cell stage onward and CLASP-mediated edge-catastrophe reduction from the 2-cell stage onward can robustly and consistently recapitulate cell division orientations between the 1- and 16-cell stage in WT and bdl embryos and between the 1- and 8-cell stage in clasp embryos.

It is important to note that our quantification of the observed variation in the simulated CMA orientation (Figure 7) confirms the experimentally observed variation in division plane orientation in other cell types where PPB formation had been disturbed using trm678 mutant, i.e., in epidermis, cortex, and endodermis [15]. Finally, we noted that, under these rules, only a fraction of division predictions for the 8- to 16-cell transition of clasp mutants was correct. To assess this discrepancy, we looked at the growth rates of cells at this stage, inferred from the calculated cell volume increases between cell stages (Figures S6D and S6E). We observed that the correctness of prediction negatively correlated with the growth rates of cell volume. When, instead of using a re-created mother cell of the 4-cell stage embryo (by merging the corresponding daughter cell pair from 8-cell stage and hence after growth), we simulated MT dynamics on a true single cell of the 4-cell stage (hence, before growth), the prediction of cell division plane orientation correlated better with the experimentally observed ones (Figure S6C). This result indicated that, in clasp mutants, post-division cell growth skews our division orientation predictions. Therefore, tissue growth rates define an upper bound, above which the rules here described cannot be directly validated based on post-division cell shapes in the absence of additional growth data. This leaves open the interesting question whether mechanisms that influence MT stability depending on direction of growth-induced mechanical stress may become dominant factors in CMA orientation [41].

**DISCUSSION**

Here, we used a modeling framework on actual cell surface shapes and combined this with high-resolution imaging of both cell outline and MTs to derive a plausible model that recovers the orientation of formative cell divisions in the *Arabidopsis* embryo from first principles on a molecular basis. When comparing predictions of division plane orientation based on basic MT-MT interaction rules and edge-catastrophe with the predictions of the heuristic geometrical rules, it is striking that our minimal set of rules that couple intrinsic MT dynamics to the local and global geometry of the cell are by themselves already sufficient to correctly predict divisions in auxin-insensitive bdl cells (Figure S7). The difference between bdl and WT division patterns indicates that, in addition, auxin-mediated regulation is crucial to establish the WT division pattern in *Arabidopsis* embryos. In order to explain the orientation of cell division up to the 16-cell stage of WT, we parsimoniously implemented, besides the basic MT dynamics, two other factors influencing MT stability that are under biological control.

The first biological control mechanism is an auxin-dependent face stability rule, derived from our observed MT patterns on bdl mutant cells. The assumption that transient cortical MT stability is most strongly associated with new cell walls is not without precedence. Several observations indicate that peripheral marks instrumental for cell division orientation can transiently accumulate at specific cell faces in different organisms. In plants, *Arabidopsis* BASL marks are positioned away from
In order to assess to what extent this hypothesis leads to a robust mechanism, we have performed an extensive, and computationally intensive, parameter analysis (see Figure S3 B). This revealed that the transient stability mechanism yields consistently correct predictions over an appreciable range of parameter values.

The second biological control mechanism is a CLASP-dependent edge-catastrophe reduction rule, based on published analyses of MTs at cell edges [17] and our own analysis of division planes in clasp mutant embryos. CLASP-like proteins in mammalian cells provide resistance to MTs under traction [44], and it is therefore conceivable that plant CLASPs in similar ways stabilize MT under torsion. Our parameter analysis (Figure S3A) indicates that, in contrast to the auxin-based mechanism, the activity of CLASP must be tightly regulated in order to achieve the correct divisions. It will therefore be interesting to investigate whether mutations in transcription factors that specifically change early embryo division patterns, such as BDL [1] and WOX2 [45], can be explained by their transcriptional control of MT regulators.

Our results clearly only provide evidence for sufficiency of the proposed rules and, hence, at most arguments for their plausibility. However, the fact that we were able to obtain these results without invoking other likely mechanisms, such as the role of

**Figure 6. Fine-Tuning of the 1- to 2-Cell Stage WT, clasp, and bdl Division Patterns in Arabidopsis Early Embryos**

(A) Simulations on WT cell template with reduced edge-catastrophe in MT dynamics and enhanced MT stabilization at developmentally new cell faces, showing a large cluster of a unique vertical MT array orientation (≈ 92%) that matched with the WT division plane orientation (top). A sparse cluster of horizontal MT array orientation (≈ 8%) was also observed (bottom).

(B) Simulations on the clasp (clasp 2) cell template combining reduced edge-catastrophe and enhanced MT stabilization at developmentally new cell faces showed a majority cluster of vertical MT array orientations (≈ 75%) matching the clasp division plane orientation (top) and a sparse cluster of horizontal MT array orientations (≈ 25%), indicating stochasticity in division plane orientation under genetic perturbation (bottom).

(C) Simulations on the bdl cell template with reduced edge-catastrophe resulted in a dominant horizontal orientation of MT array recapitulating the observed fraction (≈ 18%) of horizontal division plane orientation (left). Combination of reduced edge-catastrophe and enhanced MT stabilization at developmentally new cell faces produced a large cluster of MT array orientation (≈ 80%), which reflects the experimentally observed major vertical and unique division plane orientation (right, top) observed in bdl mutant. The remaining horizontal arrays (≈ 20%) indicate possible stochasticity in division plane orientation (right, bottom) under genetic perturbation, as actually observed and quantified [26]. Pink edges indicate reduced edge-catastrophes, and color coding of the cell faces represents the degree of MT stabilization.

See also Figures S3 and S6.
Cell faces are classified in two types: inner faces (flat faces) and outer face (curved face). The basal face is bordered in dashed line, as this face will serve the reference for quantifying the division orientation. Different orientations are classified in four categories: (1) vertical—a plane perpendicular to the basal face and intersects the outer curved face; (2) layer—a plane perpendicular to the basal face but does not intersect the outer curved face; (3) horizontal—a plane parallel to the basal face and intersects the outer curved face; and (4) tilted—a plane other than the horizontal, vertical, and layer. Orientations were measured as the angle between the normal to the basal face (black dashed arrow) and the normal to the division plane for experimental case or MT-array order parameter vector for simulation case (solid magenta arrow).

(A) Simulated MT array orientation for default shape (i.e., considering basic MT-MT collision rules only) between 2- and 16-cell stage. For each cell stage, multiple clusters of MT array orientation were detected.

(B–D) Additional rules lead to convergence of the multiple clusters into a single cluster for each cell stage, which is compared with experimentally observed division phenotype: (B) bd1 phenotype between 2- and 16-cell stage transitions; (C) WT phenotype between 2- and 16-cell stage transitions; and (D) clasp phenotype between the critical 4- and 8-cell stage transitions.
ultimately provide a complete picture of cell division control in plants and potentially the means to modify these divisions for agricultural purposes.

**STAR METHODS**

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **CONTACT FOR REAGENT AND RESOURCE SHARING**
- **EXPERIMENTAL MODEL AND SUBJECT DETAILS**
- **METHOD DETAILS**
  - 3D stacks
  - cortical MT
  - Measurement of cytosolic mGFP: TUA6 signal in individual cells
- **QUANTIFICATION AND STATISTICAL ANALYSIS**

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes seven figures and can be found with this article online at https://doi.org/10.1016/j.cub.2018.07.025.

**ACKNOWLEDGMENTS**

We thank Thomas Laux and Martin Hülskamp for critical reading of an earlier version of the manuscript. The work of B.C. was supported by a Wageningen University Graduate School grant. The work of B.M. is part of the research program of the Netherlands Organisation for Scientific Research (NWO). Simulations were carried out on the Dutch national e-infrastructure with support of the SURF Foundation.

**AUTHOR CONTRIBUTIONS**

B.C. developed the computational framework and performed the simulations. B.S. and B.M. developed the questions and conceptual approach underlying the study. V.W. produced 3D stacks for WT and clasp and analyzed cell division phenotypes. T.d.Z. and C.-Y.L. produced 3D stacks of *bdl* embryos; performed MT imaging in WT and *bdl*; and analyzed the relation between MT, PPB, and cell division orientation. B.C., V.W., T.d.Z., C.-Y.L., D.W., B.M., and B.S. wrote the paper.

**DECLARATION OF INTERESTS**

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER       |
|---------------------|--------|------------------|
| Biological Samples  |        |                  |
| Col-0               | This Lab | N/A              |
| clasp 1             | This Lab | SALK_120061      |
| clasp 2             | This Lab | SALK_83034       |
| pWOX2::TUA6-GFP     | This Lab | ACE-W15          |
| Chemicals, Peptides, and Recombinant Proteins |        |                  |
| SCRi Renaissance Stain 2200 | Renaissance Chemicals | http://www.renchem.co.uk/index.php/specialty-chemicals-division/item/48-selected-fluorescent-dyes-and-brighteners-for-microscopists |
| Software and Algorithms |        |                  |
| Simulation code     | custom made C++ code | available on request |
| Simulation analyzing code | custom made python scripts | available on request |
| MorphoGrapX         | freely available | http://www.mpipz.mpg.de/MorphoGraphX |
| meshLab             | freely available | http://www.meshlab.net/ |

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed and will be fulfilled by the Lead Contact, Ben Scheres (ben.scheres@wur.nl).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Arabidopsis seeds were surface-sterilized and dried seeds were subsequently sown on half-strength Murashige and Skoog (MS) medium. After a 48 hour cold treatment at 4°C without light, seedlings were grown at 22°C in standard long-day (16:8 h light:dark) growth conditions. After two weeks of growth, seedlings were transferred to soil and further grown under the same conditions. Siliques were harvested from mature plants for imaging. pRPS5A>>bdl embryos were generated by pollination of homozygous RPS5A-GAL4 pistils [51] with homozygous UAS-bdl [52] pollen. For bdl mutant MT-imaging, pWOX2::TUA6-GFP was transformed into the RPSSA-GAL4 line. Homozygous RPSSA-GAL4 X pWOX2::TUA6-GFP pistils were subsequently pollinated using either Col-0 or UAS-bdl.

METHOD DETAILS

3D stacks
Arabidopsis ovules of Col-0, clasp 1 and clasp 2 have been isolated from siliques in SCRi Renaissance 2200 solution on an objective slide [53, 54]. The embryos have been popped out of the ovules by applying gentle pressure on the coverslip. 3D stacks of the embryos have been made using of Zeiss LSM710 with the 405nm laser. For generating 3D stacks of bdl embryos, images were collected on a Leica SP5 confocal laser scanning microscope after staining according to [1].

cortical MT
Arabidopsis siliques were cut open using a fine needle and ovules were mounted and stained in a MT imaging solution (10% glucose, 10 μM Taxol, 0.1 M Pipes, pH 6.8, 1 mM EGTA, 1 mM MgSO4) containing 0.1% Renaissance (SR2200; Renaissance Chemicals; stock solution of the supplier was considered as 100%) cell wall counter-stain. Embryos were separated from ovules by gently pressing the coverslip. Images were taken within 30 minutes after release from the ovule. Sequential images were taken with 0.2 μm intervals to create high-resolution Z stacks. Images were obtained using the Leica SP5 confocal laser scanning microscope equipped with photon-counting HyD detector and x63 water immersion objective. GFP and SR2200 signals were detected at 488nm excitation/500-530nm emission or 405nm excitation/520-560nm emission wavelength, respectively.

Measurement of cytosolic mGFP: TUA6 signal in individual cells
The optical section containing the great circle of the nucleus was visually defined, a maximum projection containing 5 optical sections, 2 above, 2 beneath the optical section with the great circle, and the optical section containing the great circle, was then generated from the raw image stack. A 0.2 to 0.4 μm² region of interest (ROI) in the cell of interest was given at where no distinguishable
microtubule structure was found. The average mGFP fluorescence signal intensity in this ROI was documented and defined as the cytosolic mGFP: TUA6 signal of the cell of interest. The difference in average cytosolic mGFP: TUA6 signals were tested via two-tailed Student’s t test with MS Excel.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

Cell segmentation and cortical MT projection were performed in MorphoGraphX (version 2.0) [30]. SR2200 stacks (tiff) were Gaussian blurred using Sigma 0.3 μm. Blurred stacks were segmented using ITK watershed auto seeded segmentation with levels ranging from 500-1500. From segmented cells, meshes were created using Marching cubes 3D with Cube size in the range 0.5-1.0. cortical MT (pWOX2::TUA6-GFP) stacks (tiff) were loaded and overlaid with created corresponding cell meshes. Absolute cortical MT signal with a distance of 0-1 μm relative to the cell surface was projected on the cell mesh. Number of embryonic cells used in the simulations are tabulated below.

| Phenotype | 1-cell stage | 2-cell stage | 4-cell stage | 8-cell stage |
|-----------|--------------|--------------|--------------|--------------|
| WT        | 4            | 4            | 4            | 4            |
| bdl       | 4            | 4            | 4            | 4            |
| clasp 1   | 2            | 2            | 2            |              |
| clasp 2   | 1            | 4            | 4            |              |