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Localization of cytokinesis factors to the future cell division site by microtubule-dependent transport

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Summary

The mechanism by which spindle microtubules (MTs) determine the site of cell division in animal cells is still highly controversial. Putative cytokinesis “signals” have been proposed to be positioned by spindle MTs at equatorial cortical regions to increase cortical contractility, and/or at polar regions to decrease contractility (Rappaport 1986; von Dassow 2009). Given the relative paucity of MTs at the future division site, it has not been clear how MTs localize cytokinesis factors there. Here, we test cytokinesis models using computational and experimental approaches. We present a simple lattice-based model in which signal-kinesin complexes move by transient plus-end directed movements on MTs interspersed with occasions of uniform diffusion in the cytoplasm. In simulations, complexes distribute themselves initially at the spindle midzone and then move on astral MTs to accumulate with time at the equatorial cortex. Simulations accurately predict cleavage patterns of cells with different geometries and MT arrangements and elucidate several experimental observations that have defied easy explanation by previous models. We verify this model with experiments on indented sea urchin zygotes showing that cells often divide perpendicular to the spindle at sites distinct from the indentations. These studies support an equatorial stimulation model and provide a simple mechanism explaining how cytokinesis factors localize to the future division site.

Introduction

Cells typically divide at a specific, predictable location. Positioning of the cell division plane in cytokinesis is a fundamental and universal cellular process that has broad relevance to cell morphogenesis, development and tissue architecture. Cell division site determination involves multiple processes; first the spindle (and the interphase nucleus in some cell-types) is oriented along a certain axis in a microtubule (MT) dependent manner. This axis is dictated in many cells by cell shape (Minc et al. 2011) or cell polarity factors (Siller and Doe 2009). In early anaphase, elements of the spindle dictate the placement of the actin-based contractile ring at the cell surface and the subsequent cleavage furrow. Microtubules are critical for this process, while chromosomes and centrosomes can be dispensable. Rappaport and others have postulated that the spindle signals to the cortex through cleavage “signals” (Rappaport 1996).

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Supplementary Information.

Supplementary Materials include a file containing a description of the computational modeling, tables, and 9 supplementary figures, and 4 supplementary movies.

We have no conflicts of interest to declare.

There is still controversy over what these putative signals are, and how they may localize on specific regions of the cortex. One point of contention has been about what part of the spindle is critical for signaling. Prominent models include the equatorial stimulation model and the polar relaxation model (Burgess and Chang 2005; Devore et al. 1989; Rappaport 1996; von Dassow 2009; White and Borisy 1983; Wolpert 1960). In an equatorial stimulation model, MTs localize factors to the equatorial cortex where they actively promote cleavage. Astral MTs may direct the signal to equatorial cortex. Signals may also emanate from the overlapping spindle midzone MTs. In the polar relaxation models, MTs localize factors that inhibit contractility (and hence promote the relaxation of the cortex) to the “polar regions,” which then leads to a relative increase in contractility at the equatorial regions. Rappaport and others have developed a large body of experiments, many of them in echinoderms, that support the equatorial stimulation model over the polar relaxation model (Rappaport 1996). More recently, models invoking both polar relaxation and equatorial stimulation signals have been proposed (Bringmann and Hyman 2005; Dechant and Glotzer 2003; von Dassow et al. 2009).

One challenge of the equatorial stimulation model is that it is not yet clear how MTs localize a cleavage signal (or any other cytokinesis factor) to equatorial cortex. It has been speculated that signals accumulate at the equator because MTs might be more dense (or more overlapping) in this cortical region between the two asters (Devore et al. 1989; Motegi et al. 2006). However, direct imaging of MTs indicate that they may be actually less dense at the equatorial cortex and do not appear to form extensive overlaps (Asnes and Schroeder 1979; Dechant and Glotzer 2003; von Dassow et al. 2009). Increased MT stability at the equatorial cortical region has also been proposed to contribute to the equatorial accumulation of signal (Canman et al. 2003a; Foe and von Dassow 2008), which serves as a basis for a recent agent-based 3D model (Odell and Foe 2008). However, recent experimental findings question whether this selective stabilization occurs in all cell types (von Dassow et al. 2009), and show that MTs with altered dynamics also induce furrowing (Strickland et al. 2005a; von Dassow et al. 2009). Thus, it has not been clear if certain cytokinesis factors at the equatorial position are targeted directly by MT-based transport or if they use some other indirect mechanism.

Here, we use computational modeling to examine the behavior of a putative cleavage signal. We assume that the “signal” is a stable particle that contains a plus-end directed kinesin. Simulations show that spindle MTs guide the movement of this signal to the predicted site of cleavage at the equatorial cortex. We also test this model further with additional experiments in which we manipulate the shape of sea urchin embryos using micro-fabricated chambers. These studies provide important support to the equatorial stimulation model favored by Rappaport by showing how spindle MTs can localize such a signal to the equatorial cortex.

Results

Model for microtubule-dependent transport

We developed a coarse-grained 2D model to study the motor-based transport of putative cytokinesis signaling proteins (Figure 1A; see Supplementary Information for a full description of the model). We assume that factors exist in stable complexes that include a plus-end directed motor protein. This particle can move on a MT for short distances or diffuse freely in the cytoplasm. We base the properties of the motor on behaviors of kinesin motors described previously, such as the centralspindlin motor kinesin-6 (Howard 2001; Hutterer et al. 2009). Movement of particles is simulated as discrete jumps from their current box to their surrounding nearest neighbors (left, right, up or down). In the cytoplasm, particles diffuse in a random manner. The particles in MT-containing regions diffuse in a biased manner towards plus end to emulate the effect of a plus-end directed motor

movement (Figure 1A; Supplementary Movie 1). If the particle falls into a MT region, in the next iteration, its probability weight is increased in the direction of pre-assigned average orientation of MTs. This directional drive of particles in MT regions is defined by a single parameter termed MTD (for **MT Drive**), which is simply the amplitude of the vector designated for local average effective MT orientation and density (Figure S1). We increased the probability weights in the direction (and by the amount) of the components of this vector to create the bias. To determine the value of MTD, we fit the particle density profiles from our simulations to those of unbound molecular motors obtained through microscopic models with known analytical solutions (Nedelec et al. 2001) (see Supplementary Information). In the simulations, we generally start with a uniform distribution of particles and monitor their collective behavior over iterations.

To characterize the system, we first ran some control simulations. In a parallel array of MTs, particles accumulate in the cytoplasm near the plus ends of the longest MTs in the array (Figure 1B). The positive relationship of signal with MT length is non-linear and exhibited an exponential character. With a centered MT aster with uniform angular distribution, the particles accumulate evenly near the cortex. If the MTs are of different density, then the signal accumulates at areas of highest MT plus end density (Figure 1C, D). With a MT aster that is asymmetrically localized, the particles accumulate more around in the cortical region opposite the aster pole, which is associated with long MTs with low MT density rather than in areas of short MTs with high density (Figure 1E). Thus, our algorithm for simple movements can recapitulate the MT length and density dependent distribution of unbound motors (Klumpp et al. 2005; Nedelec et al. 2001).

Spindle microtubules can guide a factor to the equatorial cortex

We next simulated the transport of signaling particles in dividing cells. In general, we used the organization of the spindle MTs and cellular dimensions observed in sea urchin embryonic cell divisions as templates (Strickland et al. 2005a; von Dassow et al. 2009). In Figure 2A, we consider a sea urchin cell at the time of cleavage induction during anaphase, with MTs arranged with two asters and a spindle midzone. We assume that the MTs in the spindle midzone partially overlap in an anti-parallel manner with a uniform cross sectional density, and that asters exhibit a uniform angular distribution of a given number of MTs. Astral MTs emanate from the spindle poles to the cortex or end at the midline without overlapping. The spindle poles are closer to the polar region than the equatorial region, and the MT are more dense and short in the area of the polar cortex than at the equator. Because chromosomes are not required for cytokinesis, they are not included in the simulations. For the astral and midzone MT components, we assigned them their own MTD values, MTD_a and MTD_b , respectively, using separate fits for each component (see Supplementary Information).

In the simulation, particles accumulate at maximal levels (red in the heat maps) at the equatorial regions of the cortex (Figure 2A; Supplementary Movie 2). The sites of maximal accumulation correspond with the site of the predicted division plane (shown by arrows) (Figure 2A). This distribution is similar to a broad band of activated Rho at the future site of division seen in vivo (Bement et al. 2005). Representative frames from a simulated time course show this pattern arising starting from a uniform distribution at $t=0$ (Figure 2B). Initially, particles accumulate at the spindle midzone where MTs overlap. They accumulate here because of the high density of MTs and their anti-parallel orientation. Then, they accumulate at regions around the equatorial cortex. This equatorial pattern is established within 1–5 min (depending on diffusion constants and cell size parameters used) and is maintained at steady state. This timing is consistent with the timing of initial cleavage occurring in 5–10 min upon anaphase onset in vivo (Shuster and Burgess 2002).

To understand how MTs influence the movement of particles, we examined tracks taken by individual particles in the simulation. Particles initially accumulate in the spindle midzone due to the high density of MTs and move back and forth between MTs in the anti-parallel arrays. With time, they escape the midzone region, diffuse in the cytoplasm and move on MTs in a plus-end directed manner towards the equatorial cortex (Figure 2C). Consistent with random diffusion making a large part of the transport, paths are not unidirectional. Particles do not, for instance, move from the centrosome to the cortex in a unidirectional path. One view is that the MTs help to drive a flow of particles away from the region of the spindle poles. They influence the direction of the flow and corral the particles in the region towards a region of the cortex. More particles accumulate at the equatorial cortex because the set of MTs facing this region collects and guides the particles over a larger cytoplasmic volume than the MTs facing the polar cortex (Figure S4). In this mechanism, the primary feature that dictates the final cortical pattern is the organization of the MT array, rather than the density or length of MTs.

Next, we investigated the effects of varying parameters. We found that varying initial conditions, of starting with particles in a uniform distribution to starting with particles in the middle of the cell or from the centers of the asters, did not change the localization pattern (Figure S5). The distributions were generally robust to variations in motor behavior as shown by varying MTD values (Figure S6). Varying the run lengths from 1 μm (used in the previous simulations above) to 10 μm showed that longer run lengths produce more intense accumulation at the equator (Figure S7). Although our initial simulations used non-dynamic MTs (von Dassow et al. 2009), simulations incorporating dynamic MTs generated similar patterns (Figure S8; Supplementary Movie 3). We confirmed our 2D model by developing a genuine 3D microscopic model with similar microscopic parameters and dimensions used in our 2D model; these show similar cortical distributions as the 2D model (our unpublished observations).

We also considered how proteins might accumulate at the polar cortical regions, as specified in the polar relaxation model. Proteins that affect cytokinesis include the MT plus end protein EB1, which is thought to primarily bind directly to the plus end of the MTs, and EB-interacting proteins such as a Rho-GEF (Rogers et al. 2004; Strickland et al. 2005b). Such proteins are predicted not to accumulate at the equatorial site, but may concentrate to the regions of greatest density of MTs or MT plus ends, at the spindle midzone and near the polar regions of the cell.

Testing the model on spindle variants

In the study of cytokinesis, there is a long history of experiments in which perturbations to cell geometry, the spindle, and MTs have been tested for their effects on cleavage furrow formation and placement (Rappaport 1986; Rappaport 1996). Many of these experiments have focused on distinguishing the roles of different MT structures, such as the astral MTs versus the spindle midzone. Several results have been especially difficult to reconcile with some current models for cytokinesis. We therefore ran simulations of such experiments to gain insight into these conditions and to test our model.

First, we examined furrow induction by asters only, without a midzone. There is a long history of experiments suggesting that asters, without chromosomes or midzone, can induce furrowing (see (Rappaport 1986)). Von Dassow and Bement (2009) found that two asters can induce furrow formation only if the asters are far enough apart (see also (Rappaport 1985)). For reasons that have been unclear, two asters close together do not result in furrowing, or only weak furrowing. Our simulations reproduced this effect in Figure 3A. If the center of asters were far apart, the signal accumulated at the equator, but if the asters were close together, the signal concentration was more spread. We plotted the ratio of

equatorial signal (Eq) to polar signal (Po) as a function of the ratio of the distance between the asters (D) to cell radius (R). Eq/Po becomes greater than 2 when the separation of asters is greater than the cell radius. This result can be explained by the fact that if the asters are further apart, the MTs facing the equatorial cortex cover a relatively larger region and thus are able to move more particles to the equatorial cortex.

Second, we stimulated cytokinesis in the absence of astral MTs. Cytokinesis still occurs when centrosomes and astral MTs are inhibited by laser ablation, drugs, or genetic mutation (Alsop and Zhang 2003; Bonaccorsi et al. 1998; Dechant and Glotzer 2003; Khodjakov et al. 2000; Megraw et al. 2001; von Dassow et al. 2009). These cases have been used to suggest that signals from the spindle midzone are sufficient to induce cleavage. This mechanism may be dependent on the size of the midzone structure relative to the size of the cell (Wang 2001). In large cells with a relative small midzone, as in the first division of sea urchin embryos, cleavage occurs robustly only if the remainder of the spindle is moved to the cortex (Shuster and Burgess 2002; Strickland et al. 2005a) or if the cortex is brought closer to the spindle by changing cell shape (Rappaport and Rappaport 1984). Also, in some cell types where the spindle midzone is much more prominent, the midzone appears to be the primary regulator of cytokinesis (Inoue et al. 2004; Kawamura 1960). In our simulations with midzone MTs only, we found that particles accumulated strongly on the overlapping spindle midzone MTs, and then diffused in the cytoplasm to form a cytoplasmic diffusion gradient that extends towards the equatorial cortex (Figure 3B). Interestingly, even though this gradient is generated by random diffusion, it is not radial in shape, but extends from the middle of the midzone in an elongated distribution with its long axis perpendicular to the spindle axis. This may be because particles in the vicinity of the spindle poles have a propensity to be “sucked up” by the midzone and sent out from the MT plus ends around the middle of the spindle midzone. Thus, even without astral MTs, this simulation shows how diffusion from spindle midzone can send particles out in a direction perpendicular the spindle axis. We plotted the effects of varying the ratio between cell size and spindle size, which showed a strong inverse non-linear effect; cortical signals are relatively stronger at the equatorial cortex in smaller cells (Figure 3B). These observations provide a view that the same signals that normally travel on astral MTs to the equator can accumulate on the spindle midzone MTs and induce furrow formation from there via free diffusion if the midzone is sufficiently large compared to cell size.

Third, we modeled the division of cells with monopolar spindles, in which MTs emanate out from a single aster-like pole (Canman et al. 2003a; Hu et al. 2008) see also (Rappaport 1985)(Figure 3C). The simulations show that the signaling particles accumulate in the cytoplasm and cortex in the part of cell opposite from the MT aster, near the ends of the longest MTs. This localization pattern predicts the accumulation of particles distal to the centrosome, similar to accumulation of cytokinesis factors and sequent division plane observed in monopolar cells in vivo.

Fourth, we simulated cases seen in which both astral MTs and spindle midzone MTs appear to induce contractility. We also investigated the multiple furrows seen in *C. elegans* embryos defective in spindle positioning, such as in a *zyg-9* mutant (XMAP215 orthologue)(Figure 3D)(Werner et al. 2007a). Here, there appears to be a combination of effects from astral MTs and midzone MTs. A similar situation is seen in sea urchin cells manipulated into a cone shape (Figure 3E). We simulated Rappaport’s torus experiment, in which two spindles establish three furrows in a torus-shaped cell (Rappaport 1961)(see also (Baruni et al. 2008; Rieder et al. 1997; Sanger et al. 1998)). Our simulation closely predicts the multiple furrow patterns seen in vivo (Figure 3G). We also simulated experiments in *C. elegans* and sea urchins, where a laser cut to one side of the spindle midzone (Figure 3G) or damaging one of the centrosomes (Figure 3H) produces a situation in which cells initially divide at a site

dictated by astral MTs, and then appear to cleave again at the site of the spindle midzone (Bringmann and Hyman 2005; von Dassow et al. 2009). These findings illustrate how a single signal may determine furrow position from either astral MTs and/or the midzone.

Prediction why astral MTs do not cross the midline

In large embryonic cells such as sea urchin and *Xenopus* oocytes, astral MTs have been noted to be largely restricted to their half of the cell and not overlap with MTs from the other pole (Wuhr et al. 2010); there thus may be a mid-line barrier between the two halves of the spindle at this point in the mitotic cycle that somehow blocks MTs from extending into the other half of the cell. The molecular basis for this MT organization and its possible function are not known. We simulated the effect of removing this barrier, so that MTs grow uniformly around each aster and extend into the opposite side of the cell (see also (Odell and Foe 2008)). Our simulation predicts that these cells will fail to focus signal onto the equatorial cortex (Figure 3G) and therefore will likely to fail in cytokinesis as well. Thus, a mid-line barrier may be critical for cytokinesis.

Experiments on division patterns of indented cells

To test the model further with experimental results, we manipulated the shape of sea urchin zygotes by inserting them into PDMS wells of specific shape just after fertilization and followed their division behavior using time-lapse microscopy (Minc et al. 2011). In these experiments, we generated cells with artificial indentations, either two symmetric indentations (shaped like a symmetrically dividing cell), or two asymmetrically placed indentations (Figure 4A)(see also (Rappaport and Rappaport 1984)). Models postulating simple diffusion from the spindle midzone predict that the signal would accumulate at the indented regions of the cortex, causing the cell to divide there because these cortical regions are the closest to the spindle midzone. In another model, negative membrane curvature, which might recruit curvature-sensing proteins like F-Bar membrane proteins for instance (Roberts-Galbraith and Gould 2010), may be used as a spatial cue for the division site (Shlomovitz and Gov 2008; Wang 2001); this model also predicts division at the indentations.

We found that cells did not necessarily divide at the indented regions. We categorized the division patterns in four types (Types I–IV) based upon where the furrow appeared to originate (Figure 4A; Supplementary Movie 4). In wells with symmetric indentations, cells divided at the indentations only about 50% of the time (Type I; n=54 cells; Figure 4B). Others divided at different angles and from other regions of the cortex. In the asymmetric indented cells, only about 25% of the cells divided at both indentation, while over 50% divided in a pattern in which one end of the furrow formed at one of the indents, and the other end of the furrow at some other cortical region (Type II; n=65 cells; Figure 4B).

Measurements of furrow placement showed no strong bias in the angles of the furrows relative to the indents. However, we found that division almost always occurred in a perpendicular direction to the spindle axis, regardless of the orientation of the spindle to the indentations (Figure 4C). Thus, spindle orientation appeared to have a stronger effect on the orientation of the division plane than the local proximity of the cortex imposed by cell shape.

Importantly, our computational model predicted the locations of the observed division planes. In simulations with this particular geometry, particles accumulate at cortical sites consistent with observed division planes. As an example, Figure 4D and 4E shows the output from the simulations applied onto type III cells. Our model thus suggests a mechanism for positioning the furrow perpendicular to the spindle axis. In contrast, the

experimental results were not consistent with the alternative simple midzone diffusion model or membrane curvature model. In a simulation of a midzone diffusion model, the averaged signal density of particles simply diffusing from the midzone has the highest cortical value at the tips of indentations (Figure 4F). To test whether membrane curvature marks the division plane (Shlomovitz and Gov 2008), we generated a curvature map of the cell cortex, which shows, not surprisingly, that the cortical regions of greatest negative curvature in these cells are at the tips of the indentations. Predicted outcomes of a polar relaxation model in these indented cells are not clear. Thus, these experimental data support our MT based model, but not these other models.

Discussion

We develop here a computational model for the behavior of putative signaling protein complexes driven by MT motors in dividing cells. A simple mechanism of a signal moving transiently on spindle MTs and diffusing in the cytoplasm can lead to accumulation at the equatorial cortex in a broad band. The distribution of this broad band is reminiscent of the equatorial broad band of activated Rho at the cortex (Bement et al. 2005). Subsequent compaction of contractile ring components driven by myosin-dependent membrane flow (Cao and Wang 1990; Fishkind and Wang 1993; Ng et al. 2005) may translate this broad band into a narrow band of the furrow. These findings thus support an equatorial stimulation model, although it does not rule out that other mechanisms may also be functioning. It has been previously suggested that greater MT density, and/or distance to the near cortex are critical for equatorial accumulation. Our results suggest that the organization of spindle MTs at this stage in mitosis is a key factor in determining where signaling particles accumulate.

A striking finding of this work is that this single, simple mechanism can explain a large variety of division behaviors observed in cells with different MT configurations. Recent studies have postulated that there may be multiple, molecularly distinct cytokinetic signals that are each targeted to distinct regions of the spindle (Bringmann and Hyman 2005; Werner et al. 2007a). Although we certainly do not rule out other types of factors (such as a polar relaxation factors), our results show that a much more simple mechanism in which a single stimulatory signal with a plus end directed motor can explain all these division behaviors.

Equatorial accumulation can be achieved in a variety of situations, for instance, with asters alone, or with the spindle midzone alone, and thus it is possible that different cell types may use variations of these mechanisms. Our results with indented cells provide a clear demonstration how the spindle axis dictates the division plane perpendicular to the spindle axis, regardless of cell shape and location of the nearest cortex.

Our model differs in several important aspects from a similar 3D agent-based model on this same process (Odell and Foe 2008). In this Odell model, the MTs are organized without a mid-line barrier; as with our findings, this configuration does not produce equatorial accumulation of motors. Only the addition of an extra assumption of selective MT stabilization at the equatorial cortex produces equatorial localization, although recent evidence questions whether this stabilization occurs in vivo. This model also leaves open the question of how this stabilization zone may be established in the first place and does not address the behavior in spindle variants.

These studies help to develop a general model for how motor proteins move in the cell. We note that the dynamic localization patterns from midzone to equatorial cortex seen in our simulations are highly reminiscent of those of chromosomal passenger complex (CPC) components, such as Aurora B kinase (Earnshaw and Cooke 1991; Murata-Hori and Wang

2002; Vagnarelli and Earnshaw 2004). Centralspindlin, which includes a kinesin-6 plus end directed motor and a Rac-GAP protein (Mishima et al. 2002), is also a candidate stimulus factor. However, it has been observed primarily at the spindle midzone, and is normally not detectable or enriched at the equatorial cortex. How these protein complexes move from one location to another is not understood, and it has been speculated that these patterns arise from binding to distinct proteins at each location. Our work shows that these localization patterns may arise simply from movements guided by spindle MTs. However it still remains to be shown whether the centralspindlin complex and/or the CPC complex are elements of this putative cleavage signal responsible for division site placement. Indeed, recent experiments in *C. elegans* suggest that although these complexes contribute to cytokinesis, they are not essential for the placement of the furrow (Lewellyn et al. 2011). The ultimate test of Rappaport's view will come with the identification and characterization of the putative cytokinesis signals.

Methods

Shaping sea urchin cells using micro-fabricated wells

Sea urchin experiments were performed using similar approaches as described in Minc et al., 2011 (Minc et al. 2011). To form a PDMS replica of the wells, a SU-8 positive master was created by utilizing Heidelberg μ PG 101 Laser Writer (Heidelberg Instruments). Then a 10:1 mixture of PDMS Sylgard 184 silicone elastomer and curing agent was poured onto the master and baked at 65°C for 4 hr. The replica was cut, peeled and plasma etched for 45 seconds (Harrick Scientific). Wells were about 140 μ m in diameter with a surface area of about 16,000 μ m², so that sea urchin cells, which are normally about 100 μ m in diameter, are slightly flattened to approximately 50 μ m thick in the wells. Sea urchins *Lytechinus pictus* were obtained from Marinus Scientific. Fertilized sea urchin eggs, which were denuded of their fertilization envelopes, were placed onto the PDMS replica in seawater and allowed to sediment. The eggs were gently pushed into the wells by placing a glass coverslip on top of the suspension and wicking the seawater from the sides of the coverslip. Cells were imaged at about 17°C.

Computer modeling

The programming of the 2D coarse grained model was done in MATLAB. First regions of the cell body were virtually created and discretized, and then microtubule containing regions and their density and orientations were assigned to created pixels. Large numbers of particles (approximately thousands) were assigned to initial positions, allowed to move according to the algorithm, and their positions were updated in a time loop. The positions of the particles at each time point were recorded, and from these data, time-averaged densities were computed for each pixel, which gave the final output as the signal density.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Model for microtubule-based transport

(A) Behavior of a individual particle containing a plus end directed microtubule motor that exhibits transient directed runs on the MT interspersed with diffusive behavior in the cytoplasm. Below: Control simulation of particles that start from a uniform distribution and accumulate near the plus ends of MTs. See Supplementary Movie 1.

(B) Simulation showing accumulation of the particles at the ends of longest MTs in a parallel array. MT plus ends are all aligned on the right. The density color map shows the predicted concentration of particles in the cytoplasm (particles on the MT are not shown). The color scale bar applies proportionally to all the density color maps in the figures. Red = maximal concentration.

(C) Simulation in case of a centered radial array of MTs with unequal cross sectional density, with MT plus ends facing outwards.

(D) Simulation of centered radially symmetric array of MTs with MT plus ends facing outwards, shown as a control case.

(E) Simulation of an MT aster shifted towards one side of the cell with MT plus ends facing outwards. Signal accumulates opposite the center of the aster.

Figure 2. Movement of a cytokinesis factor to the future division plane

(A) Simulation of a cell in anaphase with MTs arranged as shown. MTs are organized with minus ends at the two spindle poles. Initial signal density is uniform, and the distribution shown is the average for 7 min real time run. The signal accumulates at the equatorial cortex with an approximate ratio of 2:1, equator to polar, respectively. The heat map shows the predicted concentration of particles in the cytoplasm, with red representing highest concentration. See Supplementary Movie 2.

(B) Time course in the simulation. Note that the signaling factor accumulates first in the spindle midzone, followed by accumulation at the equatorial cortex.

(C) Traces of individual particles in the simulation, moving from the middle of the cell to the equatorial cortex. Tracks reveal that movement is dominated by random diffusion with the MT providing a “corralling function” to guide the particles in a certain area. Scale Bars = 20 μm

Figure 3. Testing the model with spindle variants

Results of simulations of cells with spindle variants, using the templates as shown. The heat maps show the predicted concentration of particles in the cytoplasm, with red representing highest concentration. Arrowheads generally represent predicted sites of cleavage at cortical sites where the particles are at maximal concentrations.

(A) Cells with a pair of asters and no spindle midzone. Simulations reveal that asters need to be a certain distance apart for a robust signal concentration, consistent with experimental results (von Dassow et al. 2009). The quantification of the maps is shown as a plot of the ratio of equator (Eq) to polar (Po) signal density versus the ratio of aster separation (D) to cell radius (R). Arrowheads mark the predicted site of furrowing in cases in which the relative concentration of the particles on the equatorial cortex is high (arbitrarily set as $Eq/Po > 2$).

(B) Cells with spindle midzone without astral MTs. The midzone generates a diffusive pattern that is biased in an axis perpendicular to the spindle axis. The concentration on the equatorial cortex is highly dependent on relative size of the spindle to the cell. The quantification of the data is shown as a plot of Eq/Po versus the ratio of cell size to spindle size. Arrowheads predicted division sites (maximal cortical signal) where $Eq/Po > 2$.

(C) Cell with a monopolar aster. The predicted division plane is opposing to the spindle pole, consistent with experimental findings (Canman et al. 2003b)

(D) Cell with misplaced spindle. The predicted division planes are consistent with the T shaped furrow seen experimentally (Werner et al. 2007b).

(E) Conical cell. Similar to example above. Predicted division planes are consistent with T-shaped furrow seen experimentally (Rappaport and Rappaport 1994)

(F) Torus-shaped cell with two mitotic spindles. There are three predicted division planes, as seen experimentally (Rappaport 1961).

(G) Cell in which the spindle has been cut between the bottom pole and midzone by a laser. This leads to a furrow slightly below midline, and a subsequent furrow near the midzone (Bringmann and Hyman 2005).

(H) Cell in which a spindle pole (bottom) has been inactivated by laser ablation. The predicted division planes (similar to seen above) are similar to those seen experimentally (Bringmann and Hyman 2005).

(I) Cells lacking a midline barrier. Our simulations predict that the overlap of MTs across the midline leads to less equatorial concentration of the signal. Scale Bars = 20 μm .

Figure 4. Cytokinesis in indented sea urchin zygotes

(A) Sea urchin zygotes were inserted into PDMS micro-wells with artificial indentations after fertilization, and were imaged as they progress from interphase through mitosis and cytokinesis. Representative trans-illumination time-lapse images are shown. Black dots mark the position of the spindle poles. The patterns of furrow formation are categorized into 4 types: both ends of the furrow are at indentations (Type I), one end of the furrow is at indentation, and the other end is elsewhere on the cortex (Type II), neither end of the furrow is at indentation (Type III), and multiple furrows formed (Type IV). See Supplementary Movie 4. Scale Bar = 50 μ m.

(B) Percentage of cells showing a particular division pattern. $n=54$ cells with symmetric indents; $n=65$ cells with asymmetric indents.

(C) Measurements of angles of spindle orientation and furrow orientation. θ is the orientation of the midzone spindle; β is the orientation of the furrow with respect to symmetry axis; $\theta+\beta$ is the angle between the spindle and furrow.

(D) A representative simulation from our model applied onto cell shown in A, with asymmetric indents, type III. Arrows marked the predicted division plane, which is the same as that observed experimentally.

(E) A simulation from our model applied onto the cell shown in A with symmetric indents, type III. Arrows marked the predicted division plane, which is the same as that observed experimentally.

F) Predictions by a model in which the signal emanates from a point source at the middle of the midzone and diffuses in a MT independent manner. Cell shown is same as in (E). The signal at the cortex is highest at the tips of the indentations, predicting that the cell of this shape would divide at the indentations.

G) Testing a membrane curvature model, in which the site of maximal negative membrane curvature marks the division site. Cell shown is same as in (E). In curvature map of the cortex (scale is in μm^{-1}), the highest negative curvature (blue) is at the tips of the indentation, predicting that the cell of this shape will divide at the indentations.