Introduction

Cytokinesis, the final step of cell division, requires regionally specialized activities of the microtubule and actin cytoskeleton that are precisely regulated in space and time. We now have a nearly complete “parts list” of cytokinesis proteins (Eggert et al., 2006); however, our understanding of the wiring diagram that interconnects cytokinesis proteins is still rudimentary. For example, we do not know why regulators such as kinesin-6 are selectively delivered on some microtubules and not others. This selectivity presumably requires some unknown biochemical differentiation of microtubules oriented toward the equator. Moreover, most reviews discuss a linear pathway from microtubules to actomyosin (D’Avino et al., 2005; Glotzer, 2005; Yuce et al., 2005). Our data calls this linear view into question and implies the existence of positive feedbacks with a central role in dynamic organization.

Bipolar microtubule organization plays a central role in furrow positioning. The midpoint between two asters tends to organize a furrow (Rappaport, 1996). It was thus a surprise when Canman et al. (2003) showed that a single aster could initiate a furrow on one side of PtK cells, prompting the question of how the symmetric aster could position an asymmetrical furrow. Monopolar mitotic PtK cells tend to cluster chromosomes on one side of the aster and the furrow always forms on the same side as the chromosomes. They proposed that asymmetrically located chromosomes stabilized microtubules in their vicinity and that these stable microtubules triggered furrow assembly. However, this model of signaling from chromosomes was not proven and signaling molecules were not identified. The system developed by Canman et al. (2003) was inflexible in that it required microinjection. We developed a convenient drug-synchronization protocol in HeLa cells. HeLa cells arrest in monopolar mitosis with chromosomes symmetrically distributed around the aster, so there is no preexisting directional bias as in PtK cells. Triggering monopolar cytokinesis caused HeLa cells to break symmetry and polarize essentially every aspect of their internal organization. We investigated polarization mechanisms and discuss their implications for normal cytokinesis.

Results and discussion

Drug-synchronized monopolar cytokinesis in HeLa cells

Combining two established approaches (Canman et al., 2003; Niiya et al., 2005), we developed a convenient monopolar cytokinesis protocol. HeLa cells were accumulated in monopolar mitosis using the kinesin-5 inhibitor S-trityl-L-cysteine (Skoufias et al., 2006) and forced into cytokinesis by adding the potent Cdk1 inhibitor purvalanol A (Gray et al., 1998). Addition of 30 μM purvalanol A caused nearly 100% of monopolar mitotic cells to undergo a series of cytokinesis-like morphological changes over ~30 min at 37°C (Fig. S1, A and B, available at http://www.jcb.org/cgi/content/full/jcb.200711105/DC1). An Mps1 kinase inhibitor, SP600126, which forces mitotic exit by an alternative
preserved after adding purvalanol A. Microtubule elongation and RhoA recruitment to the cortex were uniform in the beginning but strongly polarized in minutes (Fig. 1A, b and c). This symmetry breaking distinguished monopolar cytokinesis in HeLa from that in PtK cells, where the chromosomes were already polarized in mitosis (Canman et al., 2003). We will refer to the biochemically differentiated cortex that recruits furrow markers as “furrow-like cortex.” As the furrow-like cortex polarized, it

Figure 1. Purvalanol A–induced mitotic exit from monopolar mitosis. (A) Summary of morphological changes after exit from monopolar arrest. Monopolar HeLa cells were fixed either before (a) or 5–30 min after (b–h) purvalanol A addition. Images are arranged to simulate a time course. Note the initial recruitment of RhoA to the cortex is approximately symmetric (b) but rapidly becomes asymmetrical (c–e). Similar stages in bipolar mitosis (i) and cytokinesis (j–n) are shown for comparison. [B] Phase-contrast time-lapse sequence that illustrates morphological changes of the cortex (video 1, available at http://www.jcb.org/cgi/content/full/jcb.200711105/DC1). The temperature in time-lapse experiments was 27°C, which slows down the sequence of morphological changes but does not otherwise alter them. Typical cytokinesis blebs occurred on one side of the cell (red arrows). On the opposite side, a protrusion gradually expanded and a furrow (green arrows) developed between it and the cell body. [C] Distribution of microtubules and cytokinesis proteins using spinning disc confocal microscopy. Note the highly polarized microtubules and cytokinetic cortical proteins at the cap. All microtubule-associated cytokinesis proteins, except Aurora B, are concentrated at microtubules tips. Aurora B localizes at the gap region between the cortex and microtubules. Bars, 5 μm.

mechanism (Schmidt et al., 2005), triggered the same morphological changes as purvalanol A (Fig. S1 C), showing they result from forced mitotic exit rather than a nonspecific drug effect.

Fig. 1A summarizes our terminology and compares monopolar to bipolar cytokinesis. The most dramatic aspect of the monopolar version was the rapid symmetry breaking and cell polarization. Microtubules and chromosomes were radially symmetric in mitosis (Fig. 1A, a). This symmetry was initially preserved after adding purvalanol A. Microtubule elongation and RhoA recruitment to the cortex were uniform in the beginning but strongly polarized in minutes (Fig. 1A, b and c). This symmetry breaking distinguished monopolar cytokinesis in HeLa from that in PtK cells, where the chromosomes were already polarized in mitosis (Canman et al., 2003). We will refer to the biochemically differentiated cortex that recruits furrow markers as “furrow-like cortex.” As the furrow-like cortex polarized, it
started to protrude, causing most cells to become pear shaped (Fig. 1 A, d and e). Later, the protrusion enlarged. The furrow-like cortex transformed from dome to ring shaped (Fig. 1 A, f) and started to ingress, forming a furrow between the protrusion and cell body (Fig. 1 A, g and h). Time-lapse microscopy also revealed protrusion and ingress of the furrow-like cortex (Fig. 1 B and video 1, available at http://www.jcb.org/cgi/content/full/jcb.200711105/DC1), as well as transient blebs at the opposite side of the cell (Fig. 1 B, red arrows) similar to those observed at the poles during bipolar cytokinesis.

**Microtubules polarize and assemble into monopolar midzones**

Fig. 1 C shows microtubules and cytokinesis proteins at the pear stage. Note the dramatic organization of microtubules into polarized bundles that extend from the centrosome and chromosomes toward the furrow-like cortex. These bundles did not extend all the way to the cortex, probably because the cortex is rapidly protruding and microtubule plus ends may not be dynamic (see following paragraphs). Thus, a gap opened between polarized microtubule tips and the furrow-like cortex (Fig. 1 A, yellow), allowing us to distinguish protein localization to microtubule tips, the furrow-like cortex, or in between. Making such distinction in normal cytokinesis is difficult because the gap between microtubules and the cortex is less evident. Cytokinesis proteins on the furrow cortex (Myosin II, Anillin, Septin 7, RhoA, and mDia) and midzone (PRC1, MKLP1, and Ect2) were strongly concentrated in a cap at the furrow-like cortex and at the tips of the polarized microtubule bundles, respectively (Fig. 1 C). Two crucial kinases are Plk1 and Aurora B. Plk1 also localized to microtubule tips, presumably bound to PRC1 (Neef et al., 2007). Aurora B, in contrast, localized mainly as small puncta in the gap region (Fig. 1 C), where it probably binds to actin filaments (see Fig. 5 B and related discussion). We suspect this gap region corresponds to the “telophase disc” in bipolar cytokinesis, whose existence was inferred partly on the basis of localization of members of the Aurora B complex (Andreasen et al., 1991; Margolis and Andreasen, 1993).

Thin-section EM of pear (Fig. 2 A) and furrow (Fig. 2 B) stages revealed that microtubules in the midzone-like assemblies were organized into dense bundles polarized toward the protruding cortex. The cortex-proximal ends of the microtubules in a single bundle appeared to terminate within a micrometer of each other, at a discrete site in the cytoplasm often several micrometers from the cortex. We assume that these are plus ends because they are distal to the centrosomes and the plus end-directed kinesin MKLP1 strongly accumulates on them (Fig. 1 C). The tips of these bundles were typically coated with electron-dense material (Fig. 2, arrows), which is similar in appearance to the material of unknown composition that accumulates at overlap regions of midzone and midbody microtubules during normal cytokinesis (Buck and Tisdale, 1962).

The highly polarized microtubules in our system resembled midzone bundles in all aspects of structure and function, except their monopolar organization. We term them “monopolar midzones” and conclude that PRC1, MKLP1, Ect2, and the unknown electron-dense material can all be recruited to microtubule plus ends that lack antiparallel overlap, contrary to most models in the literature. Cytology of bipolar cytokinesis revealed plus ends of midzone microtubules terminating near the equator but apparently not interacting with microtubules of opposite polarity (Shannon et al., 2005), confirming our view that bipolarity is not a prerequisite for midzone assembly and function. Plus ends in the monopolar midzones localize to discrete sites in the cytoplasm. At the EM level, microtubules in a single bundle terminate close together and at the light level many ends...
localize to a single plane. Because dynamic instability randomizes positions of ends, this localization suggests the ends are not dynamic and are most likely capped. Canman et al. (2003) previously showed that microtubules growing toward the furrow were stabilized, which is consistent with this hypothesis. No protein with plus-end capping activity is known.

The protruding cortex at the pear stage was highly convoluted (Fig. 1 B; and Fig. 2, A and C). Surface area is known to expand four- to sixfold as tissue culture cells undergo cytokinesis (Boucrot and Kirchhausen, 2007). We noted a large accumulation of internal vesicles near the furrow-like cortex in thin sections (Fig. 2, c and e), some of which may be endosomes preparing the fuse with the expanding membrane. Unexpectedly, other membrane-bound organelles, including cisternae of the rough ER (appearing linear in cross section; Fig. 2 b) and mitochondria (Fig. 2, a and d), also accumulated in this region. This dramatic polarization of internal membrane systems may be a consequence of polarization of both the microtubule and actin cytoskeletons.

**Cytoskeletal requirements for polarization**

To observe how the microtubule cytoskeleton broke symmetry, we performed time-lapse confocal microscopy on HeLa cells stably expressing GFP-β-tubulin (Fig. 3 and video 2, available at http://www.jcb.org/cgi/content/full/jcb.200711105/DC1). After purvalanol A addition, microtubules initially grew out to the cortex with radial symmetry (Fig. 3, 10 and 20 min). Over time, microtubule density increased in one direction and decreased in others (Fig. 3, 30 min). This polarization continued as the cell became pear shaped (Fig. 3, 40–89 min). Thus, symmetry breaking occurs only after microtubules reach the cortex.

To test if microtubules were required for symmetry breaking of cortical components, we accumulated cells in mitosis, using nocodazole in addition to S-trityl-L-cysteine. After forced mitotic exit, RhoA and Aurora B were not recruited to the cortex in the absence of microtubules. Anillin was recruited as variable-size puncta uniformly distributed (Fig. 4 A), indicating a complete block to polarization in the absence of microtubules.

To test the role of cortical components in cell polarization, we inhibited RhoA, actin, and myosin II before purvalanol A addition. The RhoA-specific toxin C3 prevented recruitment of RhoA, Anillin, and Aurora B to the cortex and blocked all signs of polarization (Fig. 4 A). Microtubules still grew out to the cortex normally and uniformly but their subsequent polarization was completely blocked and cell shape stayed rounded.

Latrunculin B disassembled most F-actin but some small aggregates of filaments persisted, even after prolonged drug exposure (Fig. 4 B, latrunculin B). As expected, latrunculin B caused large changes in cell morphology, with mitotic cells often appearing star shaped rather than spherical. Latrunculin B–treated cells failed to generate a single polarity axis after purvalanol A addition. Instead, multiple foci assembled where cytokinesis proteins accumulated near the tips of microtubule bundles. These foci were also enriched in residual F-actin. Latrunculin B had no significant effect on recruitment of PRC1 and Aurora B to the tips of microtubule bundles. We did not observe Aurora B on the cortex. The gap region did not open up in latrunculin B, making it impossible to observe if Aurora B still localized there.

Myosin II inhibitor (−)-blebbistatin also blocked all evidence of polarization (Fig. 4 B, blebbistatin). The inactive enantiomer (+)-blebbistatin as a control had no effect (unpublished data). The cortical proteins F-actin, RhoA, Anillin, and mDia all accumulated at the cortex in (−)-blebbistatin but in a nonpolarized manner. PRC1 still localized toward microtubule plus ends but the distribution was broader than in control cells. Aurora B delocalized from both microtubules and the cortex, as in C3 treatment. These data indicated a requirement for Myosin II activity for normal localization of PRC1 and Aurora B to midzone microtubules. We conclude that Myosin II activity is required for polarization and also for normal recruitment of PRC1 to microtubules. This is surprising because previous studies in bipolar cytokinesis have shown that Myosin II activity is not required for midzone assembly or for recruitment of furrow components (Straight et al., 2003). We suspect that the monopolar system is less robust to perturbation and, thus, reveals partially redundant pathways that had been missed in bipoles.
A requirement for RhoA, F-actin, and Myosin II activity for organization of monopolar midzones implies feedback from the cortex back to microtubules that are missed in the standard view, in which signals are carried unidirectionally from microtubules to the cortex by kinesins. This is not a novel conclusion. Giansanti et al., (1998) reported that perturbation of the actin

Figure 4. Microtubules and cortical components are required for polarization. (A) Both nocodazole and C3 prevented the morphological changes associated with mitotic exit. Anillin localized to the cortex after mitotic exit in nocodazole but was not polarized. C3 blocked all evidence of polarization and also recruitment of Anillin to the cortex. (B) Monopolar HeLa cells were pretreated with latrunculin B or (-)-blebbistatin before purvalanol A addition. Boxed regions are magnified below. Some F-actin remained after latrunculin B treatment. Each local cluster of F-actin was associated with cytokinesis proteins but global polarization was blocked. Blebbistatin blocked global polarization but not recruiements of cytokinesis proteins to the cortex or microtubule tips. Bars, 5 μm.
Their organization is reminiscent of the actin cables that polarize budding yeast cells. Costaining with labeled phalloidin revealed Aurora B in the gap colocalized with these cables (Fig. 5B), suggesting it binds to F-actins. The Dictyostelium discoideum homologue of INCENP, a member of the complex, has been proposed to bind to the actin cytoskeleton (Chen et al., 2007). Treatment of cells with detergent before fixation had little effect on Aurora B localization (Fig. S2B), suggesting that it binds to the cytoskeleton rather than to membranes.

To test the role of Aurora B kinase activity in monopolar cytokinesis, we used the potent inhibitor VX680 (Harrington et al., 2004). Cells were treated with VX680 and purvalanol A simultaneously to avoid the complication of mitotic exit induced by VX680. VX680 potently inhibited cell polarization and furrowing (Fig. 5C). Polarization was blocked in 78% of the cells by 200 nM VX680 (Fig. S3, available at http://www.jcb.org/cgi/content/full/jcb.200711105/DC1). The cytological response to VX680 was different than to C3 or blebbistatin. Microtubules were significantly shorter and rarely elongated to the cortex (Fig. 5C, nonpolarized). Aurora B is a negative regulator of microtubule organization in Drosophila melanogaster cytokinesis tends to inhibit normal midzone assembly, which is consistent with the feedback loops we infer. The importance of cortex-to-microtubule feedback may have been masked in mammalian cytokinesis by redundant pathways in the normal bipolar situation. In the monopolar case this feedback is absolutely required for polarization. Involvement of Myosin II (Fig. 4B) and the progressive relocalization of microtubules after they reach the cortex (Fig. 3A) suggest that cortical flow may help organize microtubules.

Aurora B complex may signal between microtubules and the cortex

The function of Aurora B in cytokinesis has been enigmatic. Aurora B complex is the only cytokinesis protein examined that localized to the gap region (Fig. 1C and Fig. S2A, available at http://www.jcb.org/cgi/content/full/jcb.200711105/DC1) and, presumably, is involved in the signaling between microtubules and the cortex. We observed actin cables extend from the furrow-like cortex to chromosomes, throughout the cytoplasm in the gap region and running parallel to microtubule bundles (Fig. 5A). Their organization is reminiscent of the actin cables that polarize budding yeast cells. Costaining with labeled phalloidin revealed Aurora B in the gap colocalized with these cables (Fig. 5B), suggesting it binds to F-actins. The Dictyostelium discoideum homologue of INCENP, a member of the complex, has been proposed to bind to the actin cytoskeleton (Chen et al., 2007). Treatment of cells with detergent before fixation had little effect on Aurora B localization (Fig. S2B), suggesting that it binds to the cytoskeleton rather than to membranes.

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of catastrophe-promoting kinesins, which might account for this effect of VX680 (Sampath et al., 2004). Aurora B was mostly dispersed in the cytoplasm in VX680-treated cells, though it sometimes was observed to associate with chromosome surfaces. PRC1 still accumulated at microtubule tips, revealing that they terminated close to the chromosomes. MKLP1, in contrast, did not localize on microtubules in nonpolarized cells, probably because its phosphorylation by Aurora B is required for microtubule binding (Guse et al., 2005). F-actin accumulated on the cortex but did not polarize. mDia recruitment to the cortex was strongly inhibited. These results suggest that Aurora B plays crucial roles in regulating microtubule dynamics and several aspects of cell polarization. Given that Aurora B localizes to chromosomes in mitosis, it is a good candidate for the factor proposed by Canman et al. (2003) for stabilizing microtubules selectively on the chromosome side of the monopole. Approximately 22% of the monopolar cells did succeed in polarizing in VX680 (Fig. S3, available at http://www.jcb.org/cgi/content/full/jcb.200711105/DC1). However, localizations of cytokinesis proteins were abnormal. Polarized microtubules appeared to be shorter and less bundled, with PRC1 and MKLP1 less evident at their tips (Fig. 5 C, polarized). Some polarized F-actin assem- blled and Aurora B still localized in puncta between microtubule tips and the cortex. Thus, Aurora B localization to F-actin does not appear to depend on its kinase activity. Because MKLP1 and mDia were completely dispersed in the cytoplasm, as in nonpolarized cells, localization of these proteins probably depends more strongly on kinase activity.

The localization of Aurora B complex and the effect of inhibiting it strongly suggest it participates in signaling between monopolar midzones and the furrow-like cortex. It is currently unclear if it participates in outward flow of spatial information from microtubules to the cortex, in feedback communication from the cortex to microtubule organization, or both. Outward information flow requires a mechanism for transport of signals over microtubule-free gaps between microtubules and the cortex, as implied by our observations and suggested by previous work on signaling by midzones (Bringmann and Hyman, 2005). Our data suggest that Aurora B complex might travel outwards on actin filaments, perhaps transported by a myosin. However, they are equally consistent with another hypothesis that Aurora B partici- pates in the feedback from the cortex that positions microtubules. In this model, Aurora B might travel inwards by hitchhiking on actin cables polymerizing at the membrane. Live imaging of Aurora B complex should help distinguish these models.

Implications for normal cytokinesis

Symmetry breaking and polarization are perhaps the most dra- matic aspects of monopolar cytokinesis in HeLa cells, and they involve essentially every cytokinesis system, including actin, myosin, microtubules, Aurora B kinase, and vesicles. Symmetry breaking typically requires positive feedback to amplify small initial asymmetries, and our data indicate that one such feed- back may operate from actomyosin to microtubule organization. In normal bipolar cytokinesis, the symmetry is already broken by the position of the asters relative to each other and to the chromo- somes, so there is no immediately obvious role for polarization.

We hypothesize that positive feedback and polarization are im- portant in three aspects of normal cytokinesis.

**Furrow focusing.** It is still a puzzle how the highly dynamic actomyosin ring can be assembled and maintained in a tightly focused region at the equatorial cortex. Blebbistatin blocked polarization in monopoles (Fig. 4 B). In bipoles, it does not block Myosin II recruitment to the furrow but does prevent its focusing into a narrow region (Straight et al., 2003). Polarization during monopolar cytokinesis and furrow focusing during bipolar cytokinesis may both depend on Myosin II pulling itself, F-actin, and associated proteins into contractile foci.

**Microtubule differentiation.** Microtubule differ- entiation is the poorly understood process by which one set of microtubules is differentiated to send positive signals to the cor- tex at the equator, while another set probably sends negative signals to the cortex at the poles (Eggert et al. 2006). It is currently thought that the positive signals may be RhoA regulators carried by kinesin-6, but why these are delivered selectively by microtubules that point to the equator is unclear. We hypothesize that a positive feedback loop exists in which kinesins carry RhoA regu- lators to the cortex, where they promote actomyosin assembly. The resulting specialized cortex feeds back to microtubules in its vicinity by an unknown mechanism to promote stabilization or bundling, and the resulting differentiation helps these micro- tubules generate or carry the outward signal more effectively. In the monopolar case, this feedback loop helps break symmetry. In the bipolar case, it converts the preexisting asymmetry in microtubule position into differentiation of their organization, dynamics, and function. Chromosomes could play a role in biasing differen- tiation by providing a local source of the stabilizing influence, as hypothesized by Canman et al. (2003), and the stabilizing influ- ence might be Aurora B complex.

**Role of actin cables.** It is puzzling why a specific actin nucleator, mDia, is absolutely required for furrowing in cell types where other nucleating mechanisms are present in the cor- tex. We hypothesize that mDia is required to nucleate actin cables that span the gap region and that these cables transport signals from the microtubules to the cortex and, perhaps, in the other direction. One of these signals is probably Aurora B complex. mDia-driven nucleation of actin cables that help transport mDia activating factors to the cortex would create a positive feedback very similar to one of the pathways that polarizes budding yeast (Wedlich-Soldner et al., 2003). To delve deeper into the role of feedback loops in cytokinesis, it will be important to visualize transport of signaling factors in living cells, including the Aurora B complex. The monopolar system, where the gap between micro- tubules and the cortex is exaggerated, may be useful for this.

**Materials and methods**

**Cell culture and drug treatments**

HeLa cells on glass coverslips were cultured in DME with 10% FBS at 37°C with a 5% CO2 incubator. The GFP-β-tubulin stable HeLa cell line was a gift from P. Chang (Massachusetts Institute of Technology, Cambridge, MA 02139). To generate monopolar mitotic arrested cells, cells were treated with 2 μM Sh-β-catenin (Sigma-Aldrich) for 12 h. For Cdk1 in- hibition, purvalanol A (Tocris Bioscience) was used at 50 μM for 15 min unless specified. Mps1 was inhibited by SP600125 (Sigma-Aldrich) at 10 μM to inactivate spindle assembly checkpoint. Nocodazole (Sigma-Aldrich)
was used at 30 μM for 12 h to disassemble microtubules. To block RhoA activation, cells were treated with 500 mg/ml of cell-permeable C3 (Cyto-skeleton, Inc.) for 6 h without serum before switching to medium with serum and S-trityl-cysteine for 12 h. To disassemble actin, latrunculin B was used at 5 μM for 15 min. To block Myosin II ATPase activity, blebbistatin (Tocris Bioscience) was used at 100 μM for 30 min. To inhibit Aurora B kinase, VX680 (gift from N. Gray, Harvard Medical School, Boston, MA 02115) was used at 200 μM with 30 μM purvalanol A for 15 min unless specified.

Immunofluorescence

HeLa cells on coverslips were fixed by either 10% TCA on ice for 15 min or 3.7% formaldehyde at room temperature for 10 min and then blocked and incubated with primary and secondary antibodies for 45 min each. Sources of antibodies used in this study are the following: Anillin, Myosin, Aurora B, and Septin 7 (T. Mitchison and C. Field, Harvard Medical School, Boston, MA 02115); mDia2 (SC10889), PRC1 (SC9342), RhoA (SC179), EzCl (SC1005), and MKLP1 (SC867; Santa Cruz Biotechnology, Inc.); Survivin (AF886; R&D Systems); INCNCP (HIC-00060; Bethyl Laboratories, Inc.); Pkl1 (6b–813; Millipore); and Aurora B (AB3635; Abcam); FITC-conjugated monoclonal anti–α-tubulin (DM1A) and TRITC-conjugated phalloidin (Sigma-Aldrich); and Alexa 488– or 594–labeled donkey anti-rabbit and –goat antibodies (Invitrogen). DNA was stained by HOECHST (Sigma-Aldrich).

Microscopy and imaging

Fluorescent images in Figs. 1 A and S1 C were acquired by a microscope (90i; Nikon) with a 1.4 NA 60× oil-immersion objective. All other imaging was done by a spinning-disk confocal microscope (TE-2000; Nikon) with a 1.4 NA 100× oil-immersion objective. Images were captured on a charge-coupled device camera (MicroMax [Princeton Instruments] or Orca ER [Hamamatsu Photoind]) using Metamorph imaging software (MDS Analytical Technologies). Z-series optical sections through each cell were obtained at 0.3- to 0.5-μm steps, controlled by Metamorph software through the stepping motor on the TE-2000. Projections of z-series image stacks were generated by Metamorph software.

EM

HeLa cells were cultured on Aclar coverslips. The drug was aspirated and the cells were fixed in prewarmed 3% glutaraldehyde in BRBB0 buffer for 20 min. After two rinses in BRBB0 and two in 0.05 M cacodylate buffer, pH 7.0, samples were postfixed in 1% osmium with 0.8% K4Fe(CN)6 in cacodylate buffer for 15 min on ice. After three rinses in cacodylate and two rinses in water, samples were stained with 1% uranyl acetate overnight. Samples were rinsed in water and dehydrated with an ethanol series from 35% to 100% ethanol while progressively lowering the temperature from 4°C to −40°C. Samples were embedded in epon-araldite and select cells were serial thin sectioned, stained with uranyl acetate and lead citrate, examined, and imaged on a transmission electron microscope (G2 Spirit BioTWIN; FEI Tecnai).

Online supplemental material

Fig. S1 shows the time and concentration dependence of purvalanol A and SP600125 for monopolar mitotic exit. Fig. S2 shows the colocalization of passenger proteins in the gap region. Fig. S3 shows the correlation between VX680 concentrations and polarization blocking. Video 1 shows the blebbing and furrowing during monopolar cytokinesis. Video 2 shows the symmetric cortical breaking of microtubule in monopolar cytokinesis. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.200711105/DC1

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