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Both midzone and astral microtubules are involved in the delivery of cytokinesis signals: insights from the mobility of aurora B

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To address the mechanism that coordinates cytokinesis with mitosis, we have studied the dynamics of aurora B, a chromosomal passenger protein involved in signaling cytokinesis. Photobleaching analyses indicated dynamic exchange of aurora B between a centromeric and a cytoplasmic pool before anaphase onset, and stable associations with microtubules after anaphase onset. Bleaching near centromeres upon anaphase onset affected the subsequent appearance of fluorescence along midzone microtubules, but not that near the lateral equatorial cortex, suggesting that there were centromeric-dependent and -independent pathways that transported aurora B to the equator. The former delivered centromeric aurora B along midzone microtubules, whereas the latter delivered cytoplasmic aurora B along astral microtubules. We suggest that cultured cells use midzone microtubules as the primary signaling pathway for cytokinesis, whereas embryos, with their stockpile of cytoplasmic proteins and large sizes, rely primarily on astral microtubules.

Introduction

Cytokinesis is the final stage of cell division critical for the proper separation of chromosomes and organelles into two daughter cells. Despite much attention, it is still unclear how cytokinesis is coordinated with mitosis. Two major models have been raised for signaling cytokinesis (for reviews see Glotzer, 1997; Oegema and Mitchison, 1997; Glotzer, 2001; Wang, 2001). The first model contends that astral microtubules are primarily responsible for signaling cytokinesis. Its main evidence came from the classical study by Rappaport (1961), who demonstrated the formation of ectopic furrows between two adjacent mitotic spindles in micromanipulated sand dollar embryos. A similar study with artificially fused epithelial cells also found ectopic furrowing between neighboring spindles in a fraction of cells (Rieder et al., 1997). These experiments not only supported a model of stimulation based on overlapping astral microtubules (Rappaport, 1986; Devore et al., 1989; Harris and Gewalt, 1989), but also argued against a direct contribution of chromosomes to cytokinesis.

An equally compelling set of evidence suggests that chromosomes and midzone microtubules, which extend from separated chromosomes toward the equatorial region during late mitosis, play a key role in signaling cytokinesis. For example, cytokinesis is inhibited by a perforation between the equatorial cortex and the spindle, even though the cortex has maintained its access to astral microtubules (Cao and Wang, 1996). In addition, under a number of experimental conditions, cortical ingression is correlated with the organization of midzone microtubules and their proximity to the cortex (Wheatley and Wang, 1996; Eckley et al., 1997; Giansanti et al., 1998). Treatment of cells with topoisomerase II inhibitors further revealed a set of microtubules that emanated laterally from the tangled chromosomes toward the cortex immediately before the ingression (Wheatley et al., 1998). These observations were corroborated by the discovery of a set of chromosomal passenger proteins, which relocate from centromeres to the equatorial region along midzone microtubules after anaphase onset (Martineau-Thuillier et al., 1998; Adams et al., 2001a). At least some of these proteins, including inner centromere protein (INCENP)* and aurora B, were essential for cytokinesis (Schumacher et al., 1998; Kaitna et al., 2000; Severson et al., 2000; Adams et al., 2001b; Giet and Glover, 2001; Leventer et al., 2002; Murata-Hori et al., 2002). However, although these experiments strongly sup-
port a signaling mechanism involving chromosomes and midzone microtubules, curiously ectopic furrows between mitotic spindles also contained a concentration of chromosomal passenger proteins (Savoian et al., 1999), suggesting that the process may be more complex than a simple transport of proteins from the centrosomes or centromeres to the equatorial cortex.

Critical insights into this puzzle may be gained by a detailed understanding of the dynamics of chromosomal passenger proteins. One of these proteins, aurora B, is known to play an essential role in both early and late stages of cell division, including chromosome congression, spindle checkpoint, chromosome segregation, and cytokinesis (Schuhammer et al., 1998; Kaitna et al., 2000; Severson et al., 2000; Adams et al., 2001b; Giet and Glover, 2001; Kallio et al., 2002; Tanaka et al., 2002; Leversen et al., 2002; Murata-Hori and Wang, 2002; Murata-Hori et al., 2002). In the present study, we have applied FRAP, micromanipulation, and drug treatment to probe the dynamics of aurora B in dividing normal rat kidney (NRK) cells. Our data suggested that centromeric aurora B exchanges dynamically with a cytoplasmic pool during early mitosis. This exchange process stops upon anaphase onset, when centromeric aurora B becomes relocated to midzone microtubules and cytoplasmic aurora B is localized to astral microtubules. We further suggested that both pathways contribute to the signaling of cytokinesis, and that the differences in their relative contributions may explain the apparently conflicting views of cytokinesis signaling.

### Results

**Aurora B is a dynamic component of the centromere during early mitosis**

We first applied FRAP analysis to investigate the mobility of aurora B at centromeres during prometaphase. Cells expressing aurora B fused to GFP (aurora B–GFP; Murata-Hori et al., 2002) were photobleached at centromeres with a small laser beam, and the recovery rate assessed from time-lapse images. The bleaching caused a ~4% decrease in total cellular fluorescence, with no detectable effect on mitosis or cytokinesis. As shown in Fig. 1 (Video 1, available at http://www.jcb.org/cgi/content/full/jcb.200207014/DC1), fluorescence returned to bleached centromeres with a relatively high rate ($t_{1/2} = 47 \pm 24$ s, $n = 13$) and extent (78%; Table I), indicating that most aurora B at centromeres was able to exchange with a noncentromeric pool. Similar results were obtained whether the bleached centromere was isolated or within a group of centromeres.

To assess if microtubules affect the turnover of aurora B at centromeres, we performed FRAP experiments after the disassembly of microtubules with 1 μM nocodazole (Fig. 2 A; Video 2, available at http://www.jcb.org/cgi/content/full/jcb.200207014/DC1). Fluorescence returned to bleached centromeres with a relatively high rate ($t_{1/2} = 62 \pm 19$ s, $n = 20$; Table I), indicating that most aurora B at centromeres was able to exchange with a noncentromeric pool. Similar results were obtained whether the bleached centromere was isolated or within a group of centromeres.

Table I. Rate and extent of FRAP aurora B–GFP or the kinase inactive mutant of aurora B, aurora B(K-R)-GFP

|                  | Slope  | $t_{1/2}$ | Recovery | $n$ |
|------------------|--------|-----------|----------|-----|
| Aurora B–GFP     |        |           |          |     |
| Prometaphase centromeres | 0.017 ± 0.007 | 47 ± 24  | 78 ± 13  | 13  |
| Nocodazole centromeres | 0.037 ± 0.005 | 62 ± 19  | 59 ± 12  | 20  |
| Aurora B(K-R)-GFP |        |           |          |     |
| Prometaphase centromeres | 0.009 ± 0.003 | 84 ± 35  | 48 ± 14  | 13  |

The rate constant $k$ was measured as the negative slope of $\ln(i_{\infty} - i)$ versus time after photobleaching. Half-time was calculated as $t_{1/2} = \ln2^*(-1/k)$, as described in Materials and methods. Values are shown ± SD.

In the present study, we have applied FRAP, micromanipulation, and drug treatment to probe the dynamics of aurora B in dividing normal rat kidney (NRK) cells. Our data suggested that centromeric aurora B exchanges dynamically with a cytoplasmic pool during early mitosis. This exchange process stops upon anaphase onset, when centromeric aurora B becomes relocated to midzone microtubules and cytoplasmic aurora B is localized to astral microtubules. We further suggested that both pathways contribute to the signaling of cytokinesis, and that the differences in their relative contributions may explain the apparently conflicting views of cytokinesis signaling.

**Figure 1.** FRAP analysis of aurora B–GFP turnover at centromeres of prometaphase cells. Fluorescence images of the cells were acquired before (a) and after (b–e) photobleaching a small number of centromeres (arrows). Time is shown in mins. The fluorescence intensity gradually increased at bleached centromeres. Graphs show the fluorescence intensity at the indicated centromere. Bar, 10 μm.
ity, which is essential for maintaining motor proteins on
the kinetochores during prometaphase (Murata-Hori and
Wang, 2002). FRAP analysis of GFP-tagged, kinase-inac-
tive mutant of aurora B (aurora B[K-R]-GFP; Fig. 2 B;
Video 3, available at http://www.jcb.org/cgi/content/full/
jcb.200207014/DC1) at centromeres indicated a significant
difference from wild-type aurora B-GFP in both the turn-
over rate ($t_{1/2} = 84$ s vs. 47 s, $P < 0.01$; Table I), and the
mobile fraction (48% vs. 78%, $P < 0.01$; Table I). These re-
results suggested a limited dependence of the turnover of cen-
tromeric aurora B-GFP on microtubules and a stronger de-
pendence on its kinase activity.

**Aurora B shows a very slow turnover along midzone
microtubules during late mitosis**

Aurora B is known to relocate from centromeres to midzone
microtubules after anaphase onset, and eventually to the
midbody during telophase (Schumacher et al., 1998; Adams
et al., 2001b; Giet and Glover, 2001; Murata-Hori et al.,
2002). To determine if aurora B underwent similar turnover
at the midbody as at centromeres, we bleached half of
the midbody with a small laser beam. As shown in Fig. 3
(Video 4, available at http://www.jcb.org/cgi/content/full/
jcb.200207014/DC1), there was only a very limited recov-
er of aurora B-GFP in the bleached region, and a corre-
sponding limited loss of fluorescence in the unbleached re-
gion, during the period of observation. In both regions the
rate of change was too low for a reliable measurement of the
halftime. These results suggested that aurora B was stably as-
associated with the midbody.

The turnover rate of aurora B during anaphase was more
difficult to measure, due to the rapid, extensive reorganiza-
tion of spindle structures and the relocation of chromosomai
passenger proteins. Therefore, we performed bleaching im-
mediately after anaphase onset, when most aurora B in the
equatorial region was still localized either at centromeres or

![Figure 2. Effects of microtubule disassembly and kinase activity on the turnover of aurora B.](http://www.jcb.org/cgi/content/full/jcb.200207014/DC1)
along adjacent midzone microtubules. This allowed us to bleach nearly all aurora B at or near centromeres with a large laser beam, and to ask if midzone microtubules subsequently became fluorescent as in nonbleached cells. The bleaching caused an ~30% decrease in total cellular fluorescence; however diffuse, cytoplasmic fluorescence returned to the bleached region within 10 s. As shown in Fig. 4
(Video 5, available at http://www.jcb.org/cgi/content/full/jcb.200207014/DC1), except for the region near the lateral equatorial cortex (discussed below), no bright fluorescent structures were subsequently found in the equatorial region at the onset of cytokinesis. This was in contrast to the striking concentration of fluorescence along the array of midzone microtubules seen in unbleached cells (n = 20; Fig. 4, C and C'). The extent of equatorial localization remained much lower in bleached cells than that in unbleached cells throughout cytokinesis over a period of 5 min (Fig. 4, D and D'). Furthermore, when aurora B–GFP was partially bleached around centromeres, reduced fluorescence was found along the corresponding portion of midzone microtubules during cytokinesis (unpublished data). Together, these results suggest that aurora B relocated directly from centromeres to midzone microtubules with a very limited turnover.

Cytoplasmic aurora B is transported to the equatorial cortex along astral microtubules
Surprisingly, despite the extensive bleaching of fluorescence at central equatorial region, and the large decrease of aurora B–GFP fluorescence along midzone microtubules in the previous experiment, concentration of aurora B–GFP developed near the lateral equatorial cortex (Fig. 4, C and D, arrows), suggesting that there might be a centromere-independent pathway that delivered cytoplasmic aurora B to the equatorial cortex. To determine if astral microtubules played a role in this process, NRK cells expressing aurora B–GFP were treated with a low dose of nocodazole (150 nM), which causes a preferential disassembly of astral microtubules (O’Connell and Wang, 2000). Compared with control cells (Fig. 5 A, right), nocodazole-treated cells showed a pronounced reduction of aurora B along the lateral equatorial cortex (Fig. 5 A, left), whereas midzone microtubules maintained a strong accumulation of aurora B (Fig. 5 A, right; Murata-Hori et al., 2002).

As an alternative approach, a perforation was generated between the central spindle and the equatorial cortex (Fig. 5 B). As was shown previously (Cao and Wang, 1996), the region between the perforation and the lateral cortex contained only astral microtubules. Immunofluorescence staining of aurora B indicated a small but detectable amount of aurora B along the cortex of this region (4/5, Fig. 5 B, inset), suggesting that astral microtubules were able to mediate the transport of a minor fraction of aurora B to the equatorial cortex.

Both midzone and astral microtubules contribute to the signaling of cytokinesis
To determine the functional role of astral microtubules in cytokinesis, we examined the cytokinesis of cells treated with 150 nM nocodazole to disassemble preferentially astral microtubules as described above. As reported previously, these cells showed a high frequency of mispositioned spindles (O’Connell and Wang, 2000). We found that cytokinesis became highly asymmetric when the spindle was located closer to one side of the lateral cortex than the other side by >50% (Fig. 6 A). Deep ingestion occurred on the side with a short distance, whereas no ingestion or shallow ingestion took place on the other side (77%, n = 13). Thus, midzone microtubules by themselves were not able to signal cytokinesis over a long distance, and astral microtubules were likely involved in the early phase of cytokinesis before the cortex was brought near the spindle by the ingestion. However, astral microtubules by themselves were insufficient for stimulating cortical ingestion in NRK cells, as indicated by the previous observation that a perforation imposed between the cortex and the spindle blocks local ingestion despite the presence of astral microtubules and some aurora B in the region (0/9, Fig. 5 B; Video 6, available at http://www.jcb.org/cgi/content/full/jcb.200207014/DC1; Cao and Wang, 1996).

To test the possibility that the effectiveness of astral microtubules in stimulating cytokinesis is determined by the amount of aurora B delivered by these microtubules, we performed the perforation experiment with cells expressing a
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A high level of aurora B–GFP (Fig. 6 B). In contrast to nontransfected cells (Fig. 5 B), these cells showed a variable degree of ingression along the equatorial cortex blocked from the spindle (15/18, Figs. 6 B, arrow, and S1; Video 7, available at http://www.jcb.org/cgi/content/full/jcb.200207014/DC1). Immunofluorescence confirmed the presence of a larger amount of aurora B along the cortex than in untransfected cells (Fig. 6 B). Together, these experiments indicate that both astral and midzone microtubules played a role in signaling cytokinesis.

Discussion

Dynamics of aurora B during early mitosis

Previous studies have demonstrated an essential role of aurora B and other chromosomal passenger proteins in signaling cytokinesis. These proteins are localized at centromeres in early mitosis, and along midzone microtubules during late mitosis (Adams et al., 2001a). Thus, the simplest model for the stimulation of cytokinesis would involve a transport mechanism, which delivers these proteins from centromeres to the equatorial cortex along midzone microtubules. However, although this model provides a plausible explanation for the spatial and temporal coordination of cytokinesis with mitosis, it does not easily explain the observations that convincingly demonstrated the involvement of astral microtubules (Rappaport, 1961; Rieder et al., 1997).

A more comprehensive understanding of this signaling process may be achieved through a better characterization of the behavior of the chromosomal passenger proteins during various stages of cell division. In the present study, we have applied a combination of FRAP, drug treatment and micromanipulation to study the dynamics of aurora B. The first significant finding is that aurora B, and likely other chromosomal passenger proteins, is not a stable passenger of the chromosomes, but constantly hops on and off centromeres prior to anaphase onset. Moreover, because the turnover rate is not significantly affected by the disassembly of microtubules, the process likely involves a cytoplasmic pool that interacts directly with the centromeres.

The half recovery time of aurora B at centromeres, 47 ± 24 s, was not drastically different from that of the checkpoint protein Mad2 (24–28 s; Howell et al., 2000). Moreover, nocodazole had a similar detectable effect on the mobile fraction of both proteins at centromeres (Howell et al., 2000). The similar behavior of aurora B and Mad2 during prometaphase raises the possibility that checkpoint proteins and chromosomal passenger proteins may form a complex during early stages of mitosis. A mechanism linked to the spindle checkpoint may maintain the integrity of the complex and keep aurora B from associating with microtubules upon anaphase onset. Consistent with this idea, we found that the kinase activity of aurora B is involved in maintaining the binding of Mad2 and microtubule motor proteins at prometaphase kinetochores (Murata-Hori and Wang, 2002; Murata-Hori et al., 2002). In addition, the possibility of a kinase-regulated complex is supported by the dependence of the mobility of aurora B on its kinase activity (Table I), and by the dependence of Mad2 turnover on ATP (Howell et al., 2000).

Dynamics of aurora B during late mitosis and cytokinesis

Upon anaphase onset, aurora B is transferred from centromeres to midzone microtubules. The concentration and limited turnover of fluorescence at the midbody suggested a change from dynamic association at centromeres, to stable association with a highly possessive motor that migrated along microtubules to the midbody. However, the most interesting finding was the identification of two discrete pathways that deliver aurora B to the equatorial region. First, bleaching of aurora B–GFP at central equatorial region during early anaphase caused a strong reduction in the subsequent concentration of fluorescence along midzone microtubules. The observation is most easily explained by a direct relocation of centromeric aurora B to midzone microtubules during early anaphase, coupled to a limited exchange of...
microtubule-associated aurora B with cytoplasmic aurora B–GFP. However, some fluorescent aurora B did appear near the lateral equatorial cortex in treated cells, while it was localized along both structures in control cells (arrows). (B) Immunofluorescence of endogenous aurora B after cell perforation (arrow). The perforation, positioned near the left edge of the metaphase plate, caused inhibition of cytokinesis on the side blocked from the mitotic spindle (boxed region). The perforation migrated toward the upper right as a result of biased ingression activities (arrow; Cao and Wang, 1996). The cell was subsequently fixed and stained with anti–aurora B antibodies. Most aurora B was detected on the cleaving side. However, very faint staining of aurora B was observed along the cortex of the perforated side upon enhancement of the image (inset). Bars, 10 μm.

Therefore, the most plausible explanation is that the cytoplasmic pool of aurora B, which is involved in the exchange with the centromeric pool during prometaphase, is recruited preferentially to astral microtubules after anaphase onset and subsequently transported to the cortex by a similar mechanism that transports centromeric aurora B along midzone microtubules. The preferential recruitment of cytoplasmic aurora B to astral microtubules implies that midzone microtubules may differ qualitatively from astral microtubules, as suggested also by their differential sensitivity to nocodazole (Wheatley and Wang, 1996; O’Connell and Wang, 2000). Alternatively, the localization to astral microtubules may require specific entry points such as the spindle pole.

Signaling of cytokinesis in animal cells

We suggest that there are two mechanisms that deliver cleavage signals to the equatorial cortex. The first mechanism is mediated by cytoplasmic aurora B and astral microtubules. It functions primarily during the early stage of cytokinesis, before the cortex is brought to the vicinity of the mitotic spindle by ingression. In addition, it is capable of delivering the signal not only to the equator but also to other regions accessible to astral microtubules, including the polar cortex. Thus, this mechanism is responsible for the ectopic furrowing between two adjacent mitotic spindles, as seen in the experiments of Rappaport (1961) and Rieder et al. (1997). The second mechanism is mediated by centromeric aurora B and midzone microtubules.
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The relative contribution of the two pathways likely varies among different cell types. In NRK cells, the astral microtubule pathway delivers only a limited amount of aurora B to the cortex, and is insufficient for stimulating cytokinesis as indicated by the failure of ingression in perforated cells. However, it plays a synergistic role with the midzone microtubule pathway and becomes increasingly important as the spindle is further separated from the cortex, as indicated by the experiments with low dose nocodazole. This mechanism also explains the somewhat equivocal role of centrosomes in cytokinesis, as suggested by an increased failure in cytokinesis upon the ablation of centrosomes (Khodjakov and Rieder, 2001).

The contribution of the astral microtubule versus midzone microtubule pathway is likely determined by the relative amount of centromeric and cytoplasmic aurora B at anaphase onset, the length of astral microtubules, and the distance between the spindle and the cortex. We found that the effect of astral microtubule pathway increased with the level of expression of aurora B in NRK cells, eventually overcoming the inhibitory effect of perforation. This pathway likely plays a major role in early embryos, where aurora B may exist in stockpiles and the spindle is separated from the cortex by up to several hundred microns.

Although our results readily reconcile the two apparently conflicting models of cytokinesis, many important questions remain. Of prime importance is the mechanism that regulates the localization and dynamics of aurora B at early anaphase, and the mechanism that transports aurora B to the cortex. Equally important are the targets of aurora B both before and after anaphase onset. The diverse effects of the disruption of aurora B suggest that there are multiple targets at various structures including centromeres and the cortex. Moreover, as an increase in aurora B is sufficient to overcome the inhibitory effect of perforation, it is reasonable to assume that aurora B indeed represents a key component in the signaling mechanism of cytokinesis.

Materials and methods

Cell culture, microscopy, and image processing

NRK epithelial cells (NRK-52E; American Type Culture Collection) were cultured in Kainh’s modified F12 (F12K) medium supplemented with 10% FBS (JRH Bioscience), 50 U/ml penicillin, and 50 μg/ml streptomycin, on glass chamber dishes as previously described (McKenna and Wang, 1989). The cells were maintained at 37°C in a stage incubator built on top of a Zeiss Axiovert 510TV or an Axiovert 35 inverted microscope.
and viewed with a 100×, NA 1.30 Fluor lens or 40×, NA 0.75 Fluor lens. All images were acquired with a cooled charge-coupled device camera (ST133 controller and CCD57 chip; Roper Scientific) and processed with custom software for background subtraction.

**Transfection, drug treatment, and micromanipulation of the cells**

NRK cells were plated on a coverslip chamber dish and incubated for 18–24 h. Immediately before transfection, the cells were rinsed once in Opti-MEM I medium (Life Technologies). The cells were transfected with the DNA constructs (1 to 2 µg) using LipofectAMINE according to manufacturer’s instructions (Life Technologies). After 4 h incubation, the medium containing DNA-LipofectAMINE was replaced with the F12K medium containing 10% FBS, and the cells were cultured for an additional 14–16 h. Nocodazole (Sigma-Aldrich) was stored at −20°C as 10 000-fold DMSO and diluted into prewarmed medium before application to cells. Cell perforation was performed as described previously (Cao and Wang, 1996).

**Laser photobleaching and data analysis**

Photobleaching experiments were performed with 476.5 nm Argon ion laser at 50 mW for bleaching small areas, or 200 mW for bleaching large areas. The laser beam was passed through a combined spatial filter–beam expander (Newport Corp.) and was focused with an f = 475 mm plano-convex lens (Optics for Research) to the plane of the epi-illuminator field diaphragm of a Zeiss Axiovert 35 microscope. All bleaching experiments were performed with a 100×, NA 1.30 Fluor lens and with 50-ms laser pulses. The size of the laser beam was controlled by moving the exit lens of the spatial filter–beam expander. The full-width half-maximal size of the laser beam on the objective plane, measured by imaging the attenuated beam with a thin layer of fluorescent solution, was 0.6 µm for small beams and 5.7 µm for large beams. The actual size of the bleached region was measured by dividing the images of the cell immediately before and after bleaching, and determining the diameter where the ratio drops by 50%. The small beam typically created a bleached region 3.4 µm in diameter, whereas the large beam created a spot 11.3 µm in diameter.

A mirror mounted on a computer-controlled, rotating wheel controlled the entry of laser versus the illumination beam from an attenuated 101W mercury arc lamp into the microscope. The computer, with custom programs, also controlled the acquisition of images before and after bleaching. The dichroic mirror and emission filter for the epi-illuminator were from a Chroma Technology 51004 v2 SZB double-band filter set for fluorescein and tetramethylrhodamine. The excitation filter was a 490 nm, 7.3-nm band-pass filter (Coherent Ealing), mounted with a BG-38 heat filter in front of the arc lamp. Images of fluorescence recovery were captured every 5–30 s. The measurement of the fluorescence intensity was performed with custom software. Because the image acquisition process caused no significant fluorescence photobleaching (~3% over the period of acquisition), the images were corrected only for the camera background.

The turnover rate was determined by plotting ln (i - i0) versus time, where i0 is the fluorescence intensity in the bleached area at time infinity and i is the fluorescence intensity at time t. The rate constant k was measured as the negative slope of the plot and the halftime was calculated as t1/2 = ln2/k. The percent mobile fraction was calculated from i0, the intensity immediately after bleaching i0, and the prebleaching fluorescence intensity i0: 100%(i - i0)/i. The value of i0 was calculated by extrapolating the plot of In(i0/i) versus t at t = 0. The significance of the results was assessed using analysis of variance and Student’s t test in Microsoft Excel.

**Immunofluorescence**

Cells were rinsed with warm cytoskeleton buffer and fixed with 4% para-formaldehyde (EM Science) in warm cytoskeleton buffer for 10 min (Wehleley and Wang, 1996). They were then rinsed thoroughly in the cytoskeleton buffer and permeabilized by incubation with 0.5% Triton X-100 in cytoskeleton buffer for 5 min. Fixed cells were rinsed with the cytoskeleton buffer, blocked for 10 min with 1% BSA (Boehringer Mannheim) in PBS, and then incubated with anti-AIM-1 monoclonal antibodies (Murata-Hori et al., 2000) at a dilution of 1/200 in PBS with 1% BSA for 45 min at 37°C. After washing with PBS/BSA thoroughly, the cells were incubated with Alexa 546-conjugated goat anti-mouse antibodies (Molecular Probes) at a dilution of 1/100 for 30 min at 37°C.

**Online supplemental material**

Videos of fluorescence images corresponding to Figs. 1–4 (Videos 1–4) and 5 B (Video 5), Fig. S1 (Video 6), and a video recorded under the same condition as that for Fig. 6 B (Video 7), are available at http://www.jcb.org/cgi/content/full/jcb.200207104/DC1.

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**References**

Adams, R.R., M. Carmena, and W.C. Earnshaw. 2001a. Chromosomal passengers and the (aurora) ABCs of mitosis. Trends Cell Biol. 11:49–54.

Adams, R.R., H. Maiato, W.C. Earnshaw, and M. Carmena. 2001b. Essential roles of *Drosophila* inner centromere protein (INCENP) and aurora B in histone H3 phosphorylation metaphase chromosome alignment, kinetochore disjunction, and chromosome segregation. J. Cell Biol. 153:865–879.

Cao, L.-G., and Y.-I. Wang. 1996. Signals from the spindle midzone are required for the stimulation of cytokinesis in cultured epithelial cells. Mol. Biol. Cell. 7:225–232.

Devere, J.J., G.W. Conrad, and R. Rappaport. 1989. A model for astral stimulation of cytokinesis in animal cells. J. Cell Biol. 109:2225–2232.

Eckley, D.M., A.M. Amsstein, A.M. Mackay, I.G. Goldberg, and W.C. Earn- sha. 1997. Chromosomal proteins and cytokinesis: patterns of cleavage furrow formation and inner centromere protein positioning in mitotic heterokaryons and mid-anaphase cells. J. Cell Biol. 136:1169–1183.

Giet, R., and M. Glover. 2001. *Drosophila* aurora B kinase is required for histone H3 phosphorylation and condensing recruitment during chromosome condensation and to organize the central spindle during cytokinesis. J. Cell Biol. 152:669–681.

Giansanti, M.G., S. Bonaccorsi, B. Williams, E.V. Williams, C. Santolamazza, M.L. Goldberg, and M. Gatti. 1998. Cooperative interactions between the central spindle and the contractile ring during *Drosophila* cytokinesis. Genes Dev. 12:396–410.

Glotzer, M. 1997. The mechanism and control of cytokinesis. Curr. Opin. Cell Biol. 9:815–823.

Glotzer, M. 2001. Animal cell cytokinesis. Annu. Rev. Cell Dev. Biol. 17:351–386.

Harris, A.K., and S.L. Gewalt. 1989. Simulation testing of mechanisms for induc- ing the formation of the contractile ring in cytokinesis. J. Cell Biol. 109:2215–2223.

Howell, B.J., D.H. Hoffman, G. Fang, A.W. Murray, and E.D. Salmon. 2000. Vi- sualization of Mad2 dynamics at kinetochores, along spindle fibers and spindle poles in living cells. J. Cell Biol. 150:1233–1249.

Kaina, S., M. Mendoza, V. Jantsch-Plunger, and M. Glotzer. 2000. Incenp and an Aurora-like kinase form a complex essential for chromosome segregation and efficient completion of cytokinesis. Curr. Biol. 10:1172–1181.

Kallio, M.J., M.L. McCeland, P.T. Stukenberg, and G.J. Gorbsky. 2002. Inhibi- tion of aurora B kinase blocks chromosome segregation, overrides the spindle checkpoint, and perturbs microurbule dynamics in mitosis. Curr. Biol. 12:900–905.

Khodjakov, A., and C.L. Rieder. 2001. Centrosomes enhance the fidelity of cyto- kinesis in vertebrates and are required for cell cycle progression. J. Cell Biol. 153:237–242.

Leverson, J.D., H.-K. Huang, S.L. Forsburg, and T. Hunter. 2002. The Schizosac- charomyces pombe Aurora-related kinase Ark1 interacts with the inner centromere protein Pic1 and mediates chromosome segregation and cytokinesis. Mol. Biol. Cell. 13:1132–1143.

McKenna, N.M., and Y.-I. Wang. 1989. Culturing cells on the microscope stage. Methods Cell Biol. 29:195–205.

Marquis-Thibault, S., P.R. Andreassen, and R.L. Margolis. 1998. Colocalization of TD-60 and INCENP throughout G2 and mitosis: evidence for their pos- sible interaction in signaling cytokinesis. Chromosoma. 107:461–470.

Murata-Hori, M., and Y.-I. Wang. 2002. The kinase activity of aurora B is required for kinetochore-microtubule interactions during mitosis. Curr. Biol. 12: 894–899.

Murata-Hori, M., K. Fumoto, Y. Fukuta, A. Ikuchi, M. Tatsuoka, and H. Hosoya. 2000. Myosin II regulatory light chain as a novel substrate for AIM-1, an au-
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Murata-Hori, M., M. Tatsuka, and Y.-l. Wang. 2002. Probing the dynamics and functions of aurora B kinase in living cells during mitosis and cytokinesis. Mol. Biol. Cell. 13:1099–1108.

O’Connell, C.B., and Y.-l. Wang. 2000. Mammalian spindle orientation and position respond to change in cell shape in a dynein-dependent fashion. Mol. Biol. Cell. 11:1765–1774.

Oegema, K., and T. Mitchison. 1997. Rappaport rules: cleavage furrow induction in animal cells. Proc. Natl. Acad. Sci. USA. 94:4317–4220.

Rappaport, R. 1961. Experiments concerning the cleavage stimulus in sand dollar eggs. J. Exp. Zool. 148:81–89.

Rappaport, R. 1986. Establishment of the mechanisms of cytokinesis in animal cells. Int. Rev. Cytol. 105:245–281.

Rieder, C.L., A. Khodjakov, L.V. Paliulis, T.M. Fortier, R.W. Cole, and G. Sluder. 1997. Mitosis in vertebrate somatic cells with two spindles: implications for the metaphase/anaphase transition checkpoint and cleavage. Proc. Natl. Acad. Sci. USA. 94:5107–5112.

Schumacher, J.M., A. Golden, and P.J. Donovan. 1998. AIR-2: an Aurora/Ipl1-related protein kinase associated with chromosomes and midbody microtubules is required for polar body extrusion and cytokinesis in Caenorhabditis elegans embryos. J. Cell Biol. 143:1635–1646.

Severson, A.F., D.R. Hamill, L.C. Carter, J. Schumacher, and B. Bowerman. 2000. The aurora-related kinase AIR-2 recruits ZEN-4/CeMKLP1 to the mitotic spindle at metaphase and is required for cytokinesis. Curr. Biol. 10:1162–1171.

Savoian, M.S., W.C. Earnshaw, A. Khodjakov, and C.L. Rieder. 1999. Cleavage furrows formed between centromeres lacking an intervening spindle and chromosomes contain microtubule bundles, INCENP, and CHO1 but not CENP-E. Mol. Cell. Biol. 19:297–311.

Tanaka, T.U., K. Rachidi, C. Janke, G. Pereira, M. Galova, and K. Nasmyth. 2002. Evidence that Ipl1-Sli15 (Aurora kinase-INCENP) complex promotes chromosome bi-orientation by altering kinetochore-spindle pole connections. Cell. 108:317–329.

Wang, Y.-l. 2001. The mechanisms of cytokinesis: reconsideration and reconciliation. Cell Struct. Funct. 26:635–638.

Wheatley, S.P., and Y.-l. Wang. 1996. Midzone microtubule bundles are continuously required for cytokinesis in cultured epithelial cells. J. Cell Biol. 135:981–989.

Wheatley, S.P., C.B. O’Connell, and Y.-l. Wang. 1998. Inhibition of chromosomal separation provides insights into cleavage furrow stimulation in cultured epithelial cells. Mol. Biol. Cell. 9:2173–2184.