25. Although the kinetic analysis of the effect of lipoproteins on the number of CFUs of *M. tuberculosis* does not differentiate between microbicidal and microbiostatic mechanisms [Web fig. 6 (*12*)], antimicrobial proteins can mediate activity by either mechanism. Although it is well known that defensins clearly lyse microbial pathogens in vitro, in vivo, inside a cell, it appears that the principal action is growth inhibition (*29*). Over time, the cumulative effect would be to cause death and elimination of the organism. As the infection is contained, T cell responses, including the release of granulysin (*27*), could contribute to killing of the organism.
26. S. Stenger *et al.*, *Science* **276**, 1684 (1997).
27. S. Stenger *et al.*, *Science* **282**, 121 (1998).
28. D. Jullien *et al.*, *J. Immunol.* **158**, 800 (1997).
29. M. A. Couto, L. Liu, R. I. Lehrer, T. Ganz, *Infect. Immun.* **62**, 2375 (1994).

30. This work was supported in part by grants from the National Institutes of Health (AI 22553, AR 40312, AI 47868, AI 07118), the Deutsche Forschungsgemeinschaft (SFB263), and the AIDS Stipendienprogramm of the Ministry of Science and Technology (BMFT), Germany.

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Requirement of a Centrosomal Activity for Cell Cycle Progression Through G_1 into S Phase

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Centrosomes were microsurgically removed from BSC-1 African green monkey kidney cells before the completion of S phase. Karyoplasts (acentrosomal cells) entered and completed mitosis. However, postmitotic karyoplasts arrested before S phase, whereas adjacent control cells divided repeatedly. Postmitotic karyoplasts assembled a microtubule-organizing center containing γ-tubulin and pericentrin, but did not regenerate centrioles. These observations reveal the existence of an activity associated with core centrosomal structures—distinct from elements of the microtubule-organizing center—that is required for the somatic cell cycle to progress through G_1 into S phase. Once the cell is in S phase, these core structures are not needed for the G_2-M phase transition.

The centrosome in mammalian cells consists of a pair of centrioles associated with a cloud of pericentriolar material containing the γ-tubulin ring complexes that nucleate microtubules during interphase and mitosis (*1*). The centrioles, along with their associated structures, represent "core centrosomal structures" that determine the precise one-to-two duplication of the centrosome in preparation for mitosis (*2*). After removal of the centrosome, both somatic and embryonic cells can regenerate a microtubule-organizing center (MTOC) (*3–5*) but do not regenerate centrioles (*2, 4*), even though the cytoplasm (in the case of zygotes) contains enough subunits to assemble many complete centrosomes (*6*).

It has been generally understood that both the duplication of the centrosome and variations in its microtubule-nucleating capacity are driven by cell cycle–dependent changes in the cytoplasmic environment (*7*). The notion that the centrosome is a necessary participant in cell cycle progression through interphase was raised by a report that BSC-1 African green monkey karyoplasts (acentrosomal cells) do not enter mitosis even though they grow to larger than normal size (*4*). This finding, coupled with the observation that cyclin-dependent kinase 1–cyclin B (Cdk1-B) is concentrated at the centrosome (*8*), led to the proposals that the presence or duplication (or both) of an intact centrosome is required for the activation of Cdk1-B and entry into mitosis (*4, 9*). However, these proposals lacked direct experimental support because the karyoplasts were not continuously followed in vivo.

To investigate the role of the centrosome in cell cycle progression, we physically cut BSC-1 cells during interphase between the nucleus and the centrosome to form karyoplasts (*4, 10*) and continuously followed the karyoplasts for several days by time-lapse videomicroscopy (*11*). The fact that the centrosome is slightly separated from the nucleus and lies at the center of a mass of granules makes this cell type favorable for this microsurgery (*12*). We brought the microneedle down at the edge of the nucleus, which displaced the centrosome from the nucleus and segregated it into the anucleate cytoplast as the needle approached the cover slip (Fig. 1A). In no case did we cut or fragment the nucleus. Although we cannot know at what point in the cell cycle the cells were cut, 5-bromo-2′-deoxyuridine (BrdU) incorporation experiments (*13*) revealed that they were cut before the completion of S phase (*14*), consistent with previous findings (*4*). None were cut in early G_1 or in prophase.

During the first 1 to 3 hours after the operation, the cytoplasmic granules became organized into a spherical mass at the center of the cytoplast, indicative of the presence of the centrosome, while the granules in the karyoplast remained randomly distributed in the vicinity of the nucleus (Fig. 1B). Normally we removed the cytoplast with the microneedle so that it would not interfere with observations of karyoplast behavior. Within an hour of the microsurgery, karyoplasts extended lamellipodia and resumed movement across the cover slip (Fig. 2A). Later, they grew in area and regenerated their Golgi apparatus to control levels, as judged by in vivo labeling with Bodipy FL C_5-ceramide (*4, 12, 15*).

In 37 experiments, 32 karyoplasts entered mitosis (Fig. 2A), four remained in interphase until the recordings were terminated 24 hours after the microsurgery, and one died within 12 hours. The interval from the microsurgical operation to the onset of mitosis was on average 12.5 hours (range 4 to 24 hours), which is within the normal interphase duration for control cells in our preparations (average 15.5 hours, range 11 to 26 hours, N = 25). In mitosis, karyoplasts aligned chromosomes into a metaphase plate, separated two groups of chromosomes in anaphase, and formed a cleavage furrow (*14*). This indicates that karyoplasts organized a functional, albeit acentrosomal, bipolar spindle [see also (*5, 16*)]. Karyoplasts spent a longer and a more variable amount of time in mitosis (average 197 min, range 68 to 557 min) than did control cells (average 56 min, range 24 to 99 min; N = 40), presumably because of the need for extra time to organize an acentrosomal spindle. In telophase all karyoplasts initiated bipolar cleavage. However, in 13 of 32 cases (41%), the cleavage furrow regressed and the karyoplasts exited mitosis as a single cell with one or more nuclei (*12, 17*).

We unexpectedly found that in 28 of 32 experiments, the postmitotic karyoplasts—whether they divided or not—arrested in interphase for the duration of the observations, up to 60 hours after mitosis (Fig. 2A) (*12*). This was not attributable to loss of cell viability in our preparations, because the karyoplasts showed continuous lamellipod extension, cell motility, and movement of phase-dense granules toward

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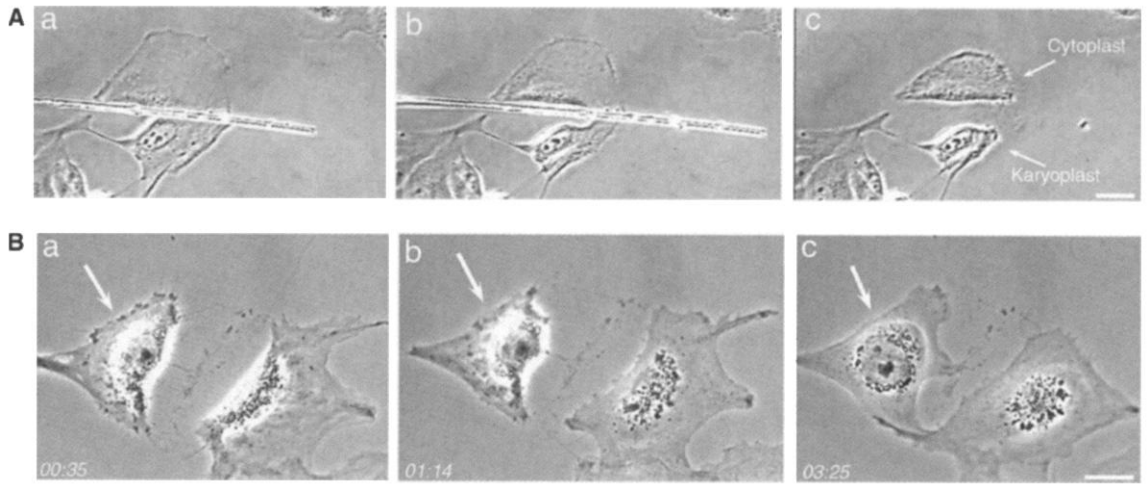

Fig. 1. (**A**) A BSC-1 cell being cut between the nucleus and the centrosome. The resultant karyoplast and cytoplast are shown in frame c. Phase contrast microscopy; scale bar, 10 μm. (**B**) The immediate postsurgery behavior of a karyoplast-cytoplast pair. Note that in the karyoplast (arrow) the cytoplasmic granules are randomly distributed in the vicinity of the nucleus. The centrosome lies at the focus of the radially arrayed granules in the center of the cytoplast. Elapsed time after microsurgery (hours:minutes) is shown in the lower left corner of each frame. Phase contrast optics; scale bar, 10 μm.

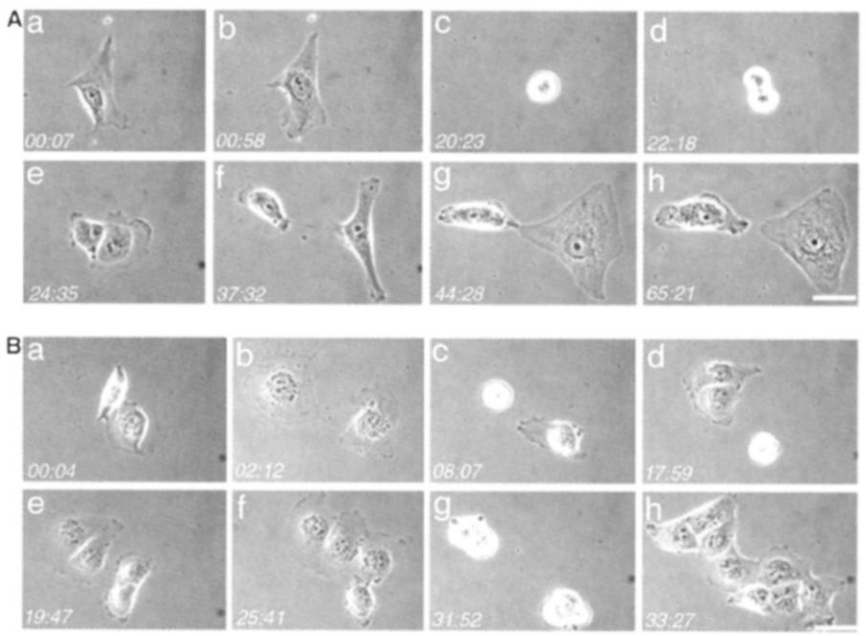

Fig. 2. (**A**) Mitosis and interphase arrest for a karyoplast that completes cytokinesis. (a and b) The karyoplast flattens and resumes motility after surgery; the edge of the cut is on the lower left of the cell. (c and d) The karyoplast enters mitosis and divides into two. (e to h) The daughter karyoplasts move apart and remain in interphase for at least 66 hours. Scale bar, 10 μm. (**B**) Two control-amputated cells divide twice within 33 hours of the microsurgery. (a and b) Both cells flatten and resume motility after the microsurgery. (c to e) Both cells enter mitosis and divide. (f to h) Second mitosis and division into eight daughter cells. Scale bar, 10 μm.

the microtubule focus at the nucleus. In 32 experiments, only one postmitotic karyoplast underwent apoptosis. Control cells in the same preparations divided repeatedly until the observations were terminated.

To control for the microsurgical operation and loss of cytoplasm, we amputated equivalent areas of cytoplasm with the cut located in the granule mass on the side of the centrosome away from the nucleus. In all cases these control cells divided at least twice (Fig. 2B). The interval from control amputation to first mitosis was 9.5 hours on average (N = 4, range 7 to 13 hours), and the time from first to second mitosis for the daughter cells averaged 18 hours (N = 8, range 16 to 22 hours), which is about 16% longer than the average normal interphase. Thus, even though some growth may be needed after mitosis for cut cells (karyoplasts or control amputees) to reach sufficient size to transit the following interphase, exceptionally long periods of growth are not required.

To determine where in the cell cycle postmitotic karyoplasts arrest, we introduced BrdU into the medium just after mitosis (*13*) and fixed them for immunofluorescence 12 or 28 hours later (*18*); these times approach or exceed the average total cell cycle duration for control cells. For six postmitotic karyoplasts fixed at 12 hours and three fixed at 28 hours after mitosis, we found no BrdU incorporation into any nuclei (Fig. 3), indicating that postmitotic karyoplasts arrest before S phase. Control cells on the same cover slips showed robust nuclear staining for BrdU (Fig. 3).

To test whether our karyoplasts arrested in interphase because they spent extra time in mitosis (*19*), we treated BSC-1 cells with low doses of Taxol (*20*), which prolongs mitosis in PtK (rat kangaroo kidney) cells to a variable extent but nonetheless allows them to divide in a normal fashion (*21*). We found that 79% of Taxol-treated cells, which spend at least as much time in mitosis as do the karyoplasts, divided two or more times. Also, seven karyoplasts went through mitosis in 68 to 97 min, which is within the normal range of mitotic duration, yet all arrested in interphase. Control cells that spent the same amount of time in mitosis continued to divide two or more times (*12*). Thus, the interphase arrest observed in karyoplasts appears not to result from extra time they spent in mitosis.

All postmitotic karyoplasts reformed a single microtubule focus next to the nucleus that collected phase-dense granules to the same extent as did the focus in control cells. To test whether this microtubule focus is organized by a MTOC, we fixed postmitotic karyoplasts 4 to 60 hours after mitosis and double-labeled them with antibodies to α-tubulin and either γ-tubulin or pericentrin, proteins integral to the pericentriolar material (*18*). In all cases, the quantity and distribution of microtubules were qualitatively the same as those in normal cells (Fig. 4). Both γ-tubulin (N = 4) and pericentrin (N = 3) immunoreactivities in karyoplasts localized to the center of the microtubule focus (Fig. 4). However, karyoplasts do not contain complete centrosomes, because serial semi-thick

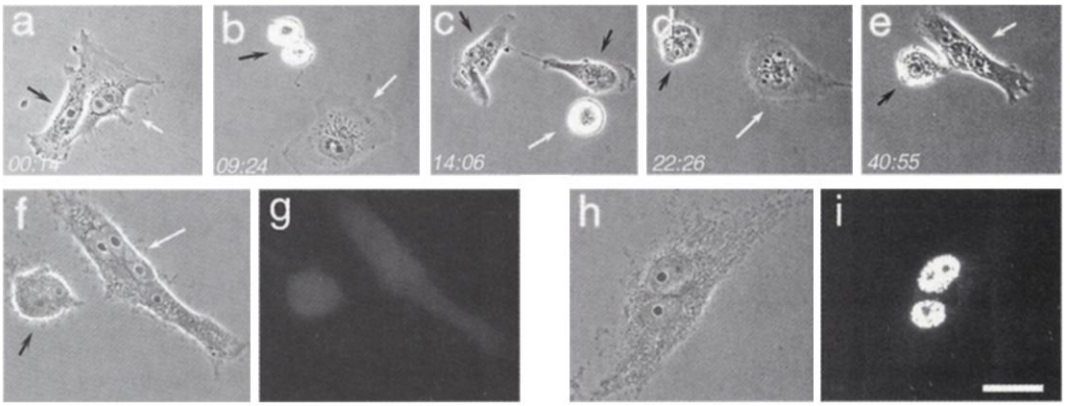

Fig. 3. Postmitotic karyoplasts do not enter S phase. (**a**) Two karyoplasts (indicated by black and white arrows) shortly after microsurgery. (**b** and **c**) The karyoplast at the left has nearly completed mitosis; it divides at 10:30 after microsurgery. BrdU is added 1 hour later at 11:30. The karyoplast at the right enters mitosis at 13:45. (**d**) The karyoplast at the right exits mitosis as a single cell at 16:00. One daughter of the karyoplast at the left crawls off the field. (**e**) Both postmitotic karyoplasts before fixation. (**f**) Progeny of both karyoplasts after fixation (28 hours after BrdU addition). (**g**) No incorporation of BrdU into karyoplast nuclei; exposure and contrast are increased to reveal the cell outlines. (**h**) Fixed control cells in the same preparation. (**i**) Their nuclei incorporate BrdU. Scale bar, 10 μm.

section ultrastructural reconstruction (*22*) of a postmitotic karyoplast revealed that there were no centrioles present in the MTOC 10 hours after mitosis (*12*). Also, all other karyoplasts behaved as if they lacked core centrosomal components; their MTOCs never doubled, either before or after mitosis. In addition, when a karyoplast divided into two, each daughter contained a single microtubule focus, whereas karyoplasts that failed to cleave organized only one microtubule focus, never two. Because centrosome number and ability to duplicate are determined by centrioles (*2, 23*), both behaviors are characteristic of the lack of centrioles.

Our finding that BSC-1 karyoplasts enter mitosis demonstrates that, once committed to the cell cycle, these cells do not require the presence or duplication of the intact centrosome for the G_2-M transition, as had been proposed (*4, 9*). Our data also reveal that a heretofore unrecognized activity associated with the centrosome is required for primate somatic cells to progress through G_1 into S phase. This activity is evidently not the microtubule-mediated accumulation (or dispersal) of cellular structures or molecules, because postmitotic karyoplasts reestablish a single MTOC of seemingly normal composition and function, yet arrest before S phase. The fact that centrioles do not regenerate in karyoplasts suggests that this activity is physically associated with core centrosomal structures, such as the centrioles and/or centriole-associated structures. The phenomena we observe here may be specific to animal somatic cells, because embryonic systems with abbreviated cell cycles—such as frog egg extracts and early mouse zygotes, as well as plant cells—enter the cell cycle without centrioles (*24*).

There are two possible explanations for why karyoplasts arrest before S phase. Perhaps BSC-1 cells have a checkpoint that monitors centrosome duplication, and this checkpoint remains activated in the absence of the core centrosomal components necessary for centrosome duplication (*2*). Alternatively, core centrosomal structures could bind cell cycle regulatory molecules in a way that activates their function or raises their local concentration to the point that essential reactions occur in a timely fashion.

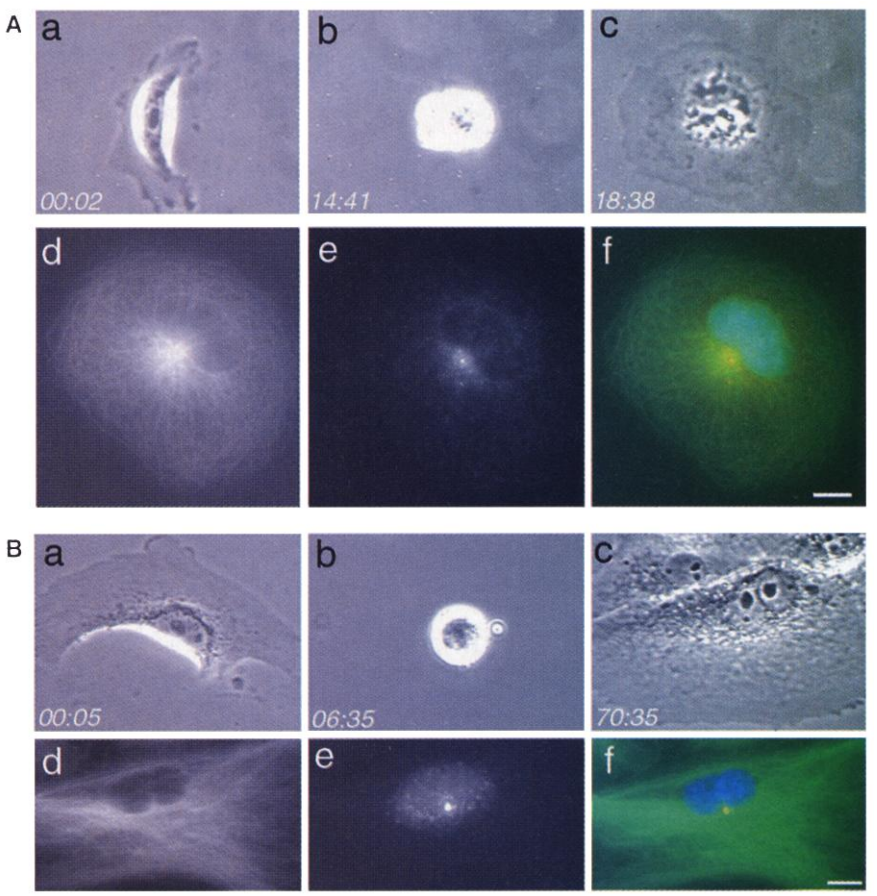

Fig. 4. (**A**) Distribution of microtubules and γ-tubulin in a postmitotic karyoplast. (a to c) Karyoplast enters mitosis and exits as a single cell with three nuclei. It is fixed 19 hours after microsurgery. (d) α-Tubulin distribution in the same karyoplast. (e) γ-Tubulin distribution. (f) Merged image of α-tubulin, γ-tubulin, and DNA. Scale bar, 10 μm. (**B**) Distribution of microtubules and pericentrin in a postmitotic karyoplast. (a to c) Karyoplast completes mitosis, exits mitosis as a single cell with one nucleus, and is fixed 70.5 hours after the microsurgery. (d) α-Tubulin distribution in the same karyoplast. (e) Pericentrin distribution. (f) Merged image of α-tubulin, pericentrin, and DNA. Scale bar, 10 μm.

References and Notes

1. R. E. Palazzo, J. M. Vogel, B. J. Schnackenberg, D. R. Hull, X. Wu, *Curr. Top. Dev. Biol.* **49**, 449 (2000).
2. G. Sluder, C. L. Rieder, *J. Cell Biol.* **100**, 887 (1985); W. F. Marshall, J. L. Rosenbaum, *Curr. Biol.* **9**, R218 (1999).

3. M. A. McNiven, M. Wang, K. R. Porter, *Cell* **37**, 753 (1984); V. I. Rodionov, G. G. Borisy, *Nature* **386**, 170 (1997).
4. A. Maniotis, M. Schliwa, *Cell* **67**, 495 (1991).
5. A. Khodjakov, R. W. Cole, B. R. Oakley, C. L. Rieder, *Curr. Biol.* **10**, 59 (2000).
6. G. Sluder, F. J. Miller, R. Cole, C. L. Rieder, *J. Cell Biol.* **110**, 2025 (1990); D. L. Gard, S. Hafezi, T. Zhang, S. J. Doxsey, *J. Cell Biol.* **110**, 2033 (1990).
7. E. H. Hinchcliffe, G. O. Cassels, C. L. Rieder, G. Sluder, *J. Cell Biol.* **140**, 1417 (1998); A. Khodjakov, C. L. Rieder, *J. Cell Biol.* **146**, 585 (1999); E. H. Hinchcliffe, C. Li, E. A. Thompson, J. L. Maller, G. Sluder, *Science* **283**, 851 (1999).
8. E. Bailly, M. Doree, P. Nurse, M. Bornens, *EMBO J.* **8**, 3985 (1989); S. M. Pockwinse *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 3022 (1997).
9. E. Bailly, M. Bornens, *Nature* **355**, 300 (1992).
10. BSC-1 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing fetal bovine serum (10%) and 12 mM Hepes (pH 7.2 in 5% CO_2). Cover slips bearing cells were assembled into a micromanipulation chamber filled with DMEM and FC-47 [G. Sluder, F. J. Miller, E. H. Hinchcliffe, *Methods Cell Biol.* **61**, 439 (1999)]. Microsurgery was done at 37°C with a piezoelectric micromanipulator on a Zeiss ACM microscope equipped with phase contrast optics. Karyoplast position on the cover slip was marked with a diamond scribe mounted in the nosepiece of the microscope before the preparation was moved to the prewarmed videomicroscope system.
11. Karyoplasts were observed with Zeiss Universal or WL microscopes equipped with phase contrast optics and shuttered illumination pathways. Microscopes were maintained at 37°C in a cardboard box with a proportional temperature control system (Omega Engineering, Stamford, CT). Images were taken with a charge-coupled device (CCD) camera (Orca; Hamamatsu, East Bridgewater, NJ), and video sequences were written to the hard drive of a PC using C-imaging software (Compix Inc., Brandywine, PA).
12. For supplementary figures, see *Science* Online (www.sciencemag.org/cgi/content/full/291/5508/1547/DC1).
13. Within 1 hour after mitosis for the karyoplast of interest, the medium was replaced with DMEM containing BrdU (10 μg/ml) and the preparation was returned to the microscope. When 12 or 28 hours had elapsed since mitosis, the karyoplasts were fixed in methanol for 5 min at −20°C, then immediately transferred to 4 M HCl at room temperature for 2 hours and processed for immunofluorescence microscopy [N. Gunduz, *Cytometry* **6**, 597 (1985)] using a mouse BrdU monoclonal antibody (mAb) (Boehringer Mannheim).
14. E. H. Hinchcliffe, F. J. Miller, M. Cham, A. Khodjakov, G. Sluder, data not shown.
15. Karyoplasts were labeled in vivo with Bodipy FL C_5-ceramide (Molecular Probes, Eugene, OR) as described [R. E. Pagano, C.-S. Chen, *Ann. N.Y. Acad. Sci.* **845**, 152 (1998)].
16. W. Steffen, H. Fuge, R. Dietz, M. Bastmeyer, G. Muller, *J. Cell Biol.* **102**, 1679 (1986); R. Heald *et al.*, *Nature* **382**, 420 (1996).
17. M. Piel, J. Nordberg, U. Euteneuer, M. Bornens, *Science* **291**, 1550 (2001).
18. Karyoplasts were fixed in –20°C methanol and processed for immunofluorescence microscopy. We used α-tubulin mAb (Sigma) and polyclonal antibodies to γ-tubulin (Sigma) or to pericentrin. Observations were made with Zeiss Axiophot or Leica DMR microscopes equipped for epifluorescence. Images were recorded with an Orca CCD camera and stored using C-Imaging software. Each karyoplast was relocated using the scribed circle on the cover slip.
19. J. S. Lanni, T. Jacks, *Mol. Cell. Biol.* **18**, 1055 (1998).
20. Taxol (Sigma) was used in DMEM at 200 to 500 nM.
21. C. L. Rieder, A. Schultz, R. Cole, G. Sluder, *J. Cell Biol.* **127**, 1301 (1994).
22. Postmitotic karyoplasts were located, fixed in 2.5% glutaraldehyde, and processed for serial section ultrastructural analysis [C. L. Rieder, G. Cassels, *Methods Cell Biol.* **61**, 297 (1999)].
23. G. Sluder, E. H. Hinchcliffe, *Biol. Cell* **91**, 413 (1999).
24. A. W. Murray, M. W. Kirschner, *Nature* **339**, 275 (1989); G. Schatten, C. Simerly, H. Schatten, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 4152 (1985); K. C. Vaughn, J. D. Harper, *Int. Rev. Cytol.* **181**, 75 (1998).
25. We thank A. Murray and C. Rieder for helpful discussions, S. Doxsey for antibody to pericentrin, and S. Lambert, W. Theurkauf, and D. McCollum for critically reading the manuscript. Supported by grants from NIH (G.S. and A.K.) and the Trustees of the Worcester Foundation (G.S.) and by a postdoctoral fellowship from the American Cancer Society, Massachusetts Division (E.H.H.).

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Centrosome-Dependent Exit of Cytokinesis in Animal Cells

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As an organelle coupling nuclear and cytoplasmic divisions, the centrosome is essential to mitotic fidelity, and its inheritance could be critical to understanding cell transformation. Investigating the behavior of the centrosome in living mitotic cells, we documented a transient and remarkable postanaphase repositioning of this organelle, which apparently controls the release of central microtubules from the midbody and the completion of cell division. We also observed that the absence of the centrosome leads to defects in cytokinesis. Together with recent results in yeasts, our data point to a conserved centrosome-dependent pathway that integrates spatial controls into the decision of completing cell division, which requires the repositioning of the centrosome organelle.

The checkpoint concept (*1, 2*) was introduced to describe mechanisms controlling the progression of the cell division cycle at critical steps that impose delays to allow corrections or repairs, or even to trigger cell death. Many of the key genes involved in these processes are not essential but, when defective, can be oncogenic (*3*). In *Saccharomyces cerevisiae*, a checkpoint mechanism monitors the coupling between nuclear and cytoplasmic division and relies on the spatial proximity of interacting components. A guanosine triphosphatase (GTPase) is concentrated at the spindle pole body (SPB), whereas the corresponding exchange factor is concentrated in the bud. Therefore, the migration of one SPB to the neck is necessary to activate the GTPase and to trigger mitotic exit (*4–7*). This ensures that cytokinesis does not take place before the nucleus has entered the bud.

We investigated whether similar spatial controls involving the centrosome (the functional equivalent of the SPB) might operate in vertebrate cells, which are much larger than yeast cells. We made time-lapse recordings of cell division in cell lines stably expressing the centrin protein coupled to the green fluorescent protein (GFP) as a centrosomal marker (*8*). During mitosis, cells possess two centrosomes located at the spindle poles, one of which is inherited by each of the two daughter cells. Each centrosome is made up of a daughter centriole that was assembled during the previous S phase and a mother centriole that was assembled during a previous cycle. The two centrioles can be distinguished ultrastructurally (*9*) and biochemically (*10*). In vivo, the mother centriole is also more strongly labeled by centrin-GFP than is the daughter centriole during the first hours of the G_1 phase (*10*).

After furrow ingression is completed, there is a period (from 1.5 to 5 hours) during which daughter cells are still linked by a cytoplasmic bridge before cytokinesis is complete (abscission). We observed that the first centrosomal event after formation of the cleavage furrow was the separation of the two centrioles in each daughter cell (Fig. 1, A and B, panel b), the daughter centriole being more motile than the mother centriole, which bears the microtubule (MT) aster and sits near the cell center (*10*). Just before abscission, the mother centriole in one (70% of the cases) (Fig. 1) or in both daughter cells [Web movie 3, part 1 (*11*)] transiently left its central position and moved straight to the intercellular bridge [Fig. 1, A and B, panels c and d; Fig. 1C; and Web fig. 1 (*11*)]. This movement was as fast as 10 μm/min (2 μm/min on average). When the mother centriole moved back to the cell center, cytokinesis was completed. This unexpected movement was observed in 45 of 50 HeLa cells. In most cases (75%), the mother centriole stayed near the bridge for <1 hour (15 min on average). Altogether, the asynchrony of abscission and the correlative movement of the mother centriole to the

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