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Supplemental materials and methods

Quantification of the fluorescence intensities at specific cell compartments

The integrated fluorescence at spindle poles, kinetochores, spindle apparatus, and cytosol were measured from five metaphase LLC-PK cells with Metamorph software by drawing a circular region around the specific targets. The background was subtracted and the integrated fluorescence of the circled area was compared with the total integrated fluorescence of the cell.

Laser photobleaching and FRAP

For FRAP experiments, cells were incubated in dye-free DME supplemented with 10 mM Hepes, 10% FCS, and antibiotics. As a control we analyzed fluorescence recovery in an LLC-PK line stably expressing α-tubulin–GFP (a gift from Dr. Patricia Wadsworth, University of Massachusetts, Amherst, MA). FRAP analysis was performed using a ZEISS Axioplan microscope equipped with 63× (N.A. 1.4) or 100× objectives (N.A. 1.4) and a Hamamatsu Orca II CCD camera (Hamamatsu Photonics). Photobleaching was done with a nitrogen-pumped dye laser (Phototonic Instruments Inc.) affixed to the epi-illumination port. Laser light was attenuated with a neutral density slider to a level that did not noticeably affect cell viability or mitotic progression. The laser was aligned above the target region using phase contrast optics and a set of prebleach images was captured. A short series of laser pulses (~10 pulses, each 4 ns) was used to diminish the fluorescence intensity of the target. Time-lapse sequences (time intervals ranging from 1 frame/s to 1 frame every 5 s) were captured using 30–300-ms exposure times per frame.

Statistical analysis of kinetochore and centrosome dynamics

Measurement procedures were adapted from those reported by Howell et al. (Howell, B.J., D.B. Hoffman, G. Fang, A.W. Murray, and E.D. Salmon. 2000. *J. Cell Biol.* 150:1233–1250), Maddox et al. (Maddox, P.S., K.S. Bloom, and E.D. Salmon. 2000. *Nat. Cell Biol.* 2:36–41), and Wadsworth and Salmon (Wadsworth, P., and E.D. Salmon. 1986. *J. Cell Biol.* 102:1032–1038). A small region (R_{kin}) slightly larger than the kinetochore or centrosome of interest was marked over the target area and the integrated fluorescence intensity within the region (F_{kin}) was logged into an Excel spreadsheet from each time point. The background fluorescence was subtracted by placing a larger region (R_{back}) over the smaller target area. Integrated fluorescence within the R_{back} was measured (F_{back}). The following equation was used to obtain integrated fluorescence intensity of a kinetochore minus the background:

$$F_{kin} - F_{back} = \frac{(F_{back} - F_{kin}) \times R_{kin}}{F_{kin} - (R_{back} - R_{kin})}$$

The half-life of Cdc20–GFP fluorescence recovery was calculated using the equation $t_{1/2} = \ln_2/k$. The constant k was derived from fitting the data to a curve generated with the nonlinear perturbation relaxation equation within GraphPad Prism software. The percentage of fluorescence recovery was calculated using the equation 100% $(F_{max} - F[0])/(F_{pre} - F[0])$, where F_{pre} is the intensity of the region before laser irradiation, F_{max} is the intensity of the same region after maximal recovery, and F(0) is the intensity at the first time point after photobleaching. A t test and two-way ANOVA were performed using Microsoft Excel.