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A Mitotic Septin Scaffold Required for Mammalian Chromosome Congression and Segregation

Elias T. Spiliotis¹, Makoto Kinoshita², and W. James Nelson^{1,*}

¹Department of Molecular and Cellular Physiology, Beckman Center for Molecular and Genetic Medicine, Stanford University School of Medicine, Stanford, CA 94305–5435, USA

²Biochemistry and Cell Biology Unit, Horizontal Medical Research Organization, Kyoto University Graduate School of Medicine, Yoshida Konoe, Sakyo, Kyoto 606–8501, Japan

Abstract

Coordination of cytokinesis with chromosome congression and segregation is critical for proper cell division, but the mechanism is unknown. Here, septins, a conserved family of polymerizing guanosine triphosphate-binding proteins, localized to the metaphase plate during mitosis. Septin depletion resulted in chromosome loss from the metaphase plate, lack of chromosome segregation and spindle elongation, and incomplete cytokinesis upon delayed mitotic exit. These defects correlated with loss of the mitotic motor and the checkpoint regulator centromere-associated protein E (CENP-E) from the kinetochores of congressing chromosomes. Mammalian septins may thus form a mitotic scaffold for CENP-E and other effectors to coordinate cytokinesis with chromosome congression and segregation.

In eukaryotic cells, genetic inheritance requires replicated chromosomes to congress within the mitotic spindle at the midplane of the dividing cell and segregate equally into two daughter cells (1). Fidelity in chromosome segregation is achieved by the spindle-assembly checkpoint that signals anaphase when all proper attachments have been made between chromosomes and spindle microtubules (2). Though many molecular details of these events are described, it is unknown how chromosome congression and segregation, anaphase, and the final separation of daughter cells (cytokinesis) are coordinated.

In fungi and animals, septins are a conserved family of guanosine triphosphate (GTP)-binding proteins required for cell division (3). In the budding yeast *Saccharomyces cerevisiae*, spindle assembly and chromosome-microtubule attachments are enclosed within the nuclear envelope; septins assemble into filaments at the cortex of mother-daughter cell neck (4, 5) and regulate cortical localization of proteins involved in cell cycle progression and cytokinesis (6–11).

In mammalian cells, there are at least 12 septin genes (12). Mammalian septins are found at the plasma membrane and in the cytosol, and during cytokinesis, they colocalize with actin in the cleavage furrow and with microtubules in the midbody and central spindle (13–15).

*To whom correspondence should be addressed. wjnelson@stanford.edu.

Supporting Online Material

www.sciencemag.org/cgi/content/full/307/5716/1781/

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Materials and Methods

Figs. S1 to S7

References

Movies S1 to S6

Interference with septin expression results in binucleate cells (13–15), but the septin functions that are disrupted are unknown.

We reassessed the distribution of mammalian septins during mitosis by staining Madin Darby canine kidney (MDCK) and HeLa cells with antibodies against septin 2 (Sept2) and septin 6 (Sept6). Sept2 localized to the mid-body, the ingressing cleavage furrow, and the central spindle of cells undergoing cytokinesis (Fig. 1, A to C). However, in metaphase cells, Sept2 and Sept6 localized within the microtubule spindle (Fig. 1, D to I, and fig. S1, A to F). At the metaphase plate, a network of short interwoven Sept2 filaments was observed in close apposition to the kinetochores of the congressed chromosomes and kinetochore microtubules (Fig. 1, G to I). Similar distribution of septin filaments was observed within the spindle apparatus of living cells (fig. S2). When spindle microtubules were depolymerized, septins remained throughout the metaphase plate (Fig. 1, J to L, and fig. S1, G to I).

To probe for septin function during mitosis, we used RNA interference (RNAi) to deplete septins. High amounts of septin depletion over long periods have pleiotropic effects on microtubule and actin cytoskeleton organization (15, 16). To avoid cell death and the cumulative effects of chronic septin depletion (14), we transfected cells with Sept2 small interfering RNAs (siRNAs) for 18 to 48 hours, yielding a 10 to 65% reduction in Sept2 expression in the cell population whereas α -tubulin expression was unaffected (Fig. 2A). Reduction of Sept6 and Sept7 also occurred upon Sept2 knockdown (16).

After 18 hours, the percentage of mitotic cells in Sept2 siRNA-treated cells was higher than in the control (14% versus 5%) (Fig. 2B). Half of Sept2 siRNA-treated cells accumulated in prometaphase (Fig. 2B), with some chromosomes aligned at the metaphase plate and some near the spindle poles (Fig. 2D). The same phenotype was observed in cells transfected with plasmid DNAs encoding hairpin siRNAs to Sept2 and Sept7 (fig. S3). In Sept2 siRNA-treated cells, Sept2 was depleted from the metaphase plate, and the nocodazole-resistant septin network was not detected (Fig. 2, D and E). The overall spindle length and bipolarity and formation of stable kinetochore microtubules remained unaffected (Fig. 2C and fig. S4). However, real-time imaging of histone B–green fluorescent protein (GFP)–labeled chromosomes showed failure in chromosome congression during early prometaphase (movie S1) (17) and loss of chromosome maintenance at the metaphase plate in later stages of prometaphase; chromosomes gradually dislocated from the metaphase plate and accumulated near the spindle poles (Fig. 2, F to H, and movies S2 and S3).

Treatment of cells with Sept2 siRNAs for 36 hours resulted in binucleate cells (14% versus 5% in controls) (Fig. 3, A to C). Because 18 hours earlier there was only a high percentage of prometaphase cells with no difference in the percentage of binucleate cells (5% versus 5%) (Fig. 3C), we reasoned that chronically arrested prometaphase cells had exited mitosis and progressed straight to binucleate cells. Indeed, misaligned HisB-GFP–tagged chromosomes decondensed into two attached nuclei (Fig. 3D). Nuclear envelope formation (Fig. 3A) and kinetochore distribution into two, albeit attached to each other, nuclear masses (fig. S5, J to L) indicated that these cells had entered anaphase. However, chromosome segregation remained incomplete because of lack of spindle elongation (Fig. 3, E and F); the spindle apparatus maintained the same length for at least twice as long as control cells before forming an interphase-like network (Fig. 3G). This abnormal transition in microtubule morphology was accompanied by defective cytokinesis (cleavage furrow regression) (movie S6). Cytokinetic defects have been seen in septin-depleted cells (13–15), but our data identify an earlier defect involving chromosome congression and segregation.

In yeast, septins are required for the maintenance of several proteins at the site of cell cleavage (4). To examine whether mammalian septins have a similar function within the mitotic spindle apparatus, we probed for the localization of CENP-E (centromere-associated protein E) and MCAK (mitotic centromere-associated kinesin) motor proteins. CENP-E, a protein without an apparent homolog in yeast, is a mitotic kinesin required for stable kinetochore binding to spindle microtubules (18–20). CENP-E deletion results in chromosome misalignment and loss of a sustainable mitotic checkpoint (20–22). Similarly, depletion of MCAK, a protein of the KinI family of microtubule depolymerases, leads to defects in chromosome congression and segregation (23, 24).

In Sept2 siRNA-treated prometaphase cells, the percentage of CENP-E-containing kinetochores was significantly reduced ($23\% \pm 3\%$ versus $45\% \pm 3\%$) (Fig. 4, A and E), and large CENP-E aggregates were often observed near the spindle poles (Fig. 4A). In contrast, no significant change in MCAK-containing kinetochores was observed ($39\% \pm 3\%$ versus $41\% \pm 3\%$) (Fig. 4, B and E). To test whether septins are required for CENP-E localization in the absence of microtubule-kinetochore attachments, we incubated mock and Sept2 siRNA-treated cells with nocodazole for 2 hours (Fig. 4D). In siRNA-treated cells, large CENP-E aggregates overlapped with a few kinetochores ($20\% \pm 5\%$) (Fig. 4, D and E), whereas in mock-treated cells, where the septin network remained intact, CENP-E was enriched on many individual kinetochores ($51\% \pm 4\%$) (Fig. 4, D and E). Thus, septins are required for proper CENP-E localization at kinetochores in the absence of microtubule-kinetochore attachments.

Septins may thus be required at the metaphase plate for CENP-E maintenance at the kinetochore ends of disassembling microtubules during chromosome congression. Indeed, loss of CENP-E from kinetochores resulted in chromosome dislocation from the metaphase plate and in a high number of mono-oriented, unattached chromosomes (Fig. 2, F to H). In addition, an increased number of kinetochores contained the mitotic checkpoint protein Mad2 [$46\% \pm 4\%$ compared with $36\% \pm 3\%$ (Fig. 4, C and E)], which accumulates on unattached kinetochores (25).

In CENP-E-depleted cells where recruitment of CENP-E is affected, chromosomes did not dislocate from the metaphase plate (fig. S6) (26). Moreover, when both septins and CENP-E were depleted by siRNA, an additive phenotype was observed (fig. S7). Thus, septin depletion appears to affect not only maintenance of CENP-E localization but also some of the redundant mechanisms responsible for partial chromosome congression in the absence of CENP-E (26).

We suggest that mammalian septins form a novel scaffold at the midplane of the mitotic spindle that coordinates several key steps in mammalian mitosis. First, at metaphase, the septin scaffold is required to maintain CENP-E at kinetochores and consequently chromosome congression. Second, the septin scaffold may be involved indirectly in the timing of chromosome segregation, because maintenance of CENP-E localization at kinetochores is critical for activation of the mitotic checkpoint. Third, during anaphase, the septin scaffold remains within the central spindle, where it is required for chromosome segregation and spindle elongation. Indeed, CENP-E also redistributes to the central spindle of anaphase cells (18), and in septin-depleted cells, chromosomal passenger proteins failed to redistribute to the central spindle or the midbody (fig. S5). Thus, the septin scaffold may be involved in integrating molecular information necessary to coordinate chromosome congression and segregation with positioning and activation of the division plane and the site of cytokinesis.

These functions of mammalian septins are similar to those of yeast septins, which are required for proper positioning of the spindle apparatus (27), spatial coordination of cytokinesis (11), and proper activation of the mitotic exit network (9). Hence, septin functions may have been conserved during transition from a closed (yeast) to open (mammals) mitosis by adapting roles within the mitotic spindle apparatus. Septin abnormalities have been found in human tumors (28). Disruption of septin function in different stages of mitosis could potentially lead to chromosome instability and changes in ploidy common to cancers.

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Fig. 1. (A to I) Fixed MDCK and HeLa cells stained (17) with Sept2, Sept6, α -tubulin, CREST (kinetochores) antibodies and 4',6'-diamidino-2-phenylindole (DAPI) (nuclei). Insets show magnified images of the metaphase plate. Arrows indicate Sept2 filaments coaligning with kinetochore microtubules; arrowheads, tubulin-free Sept2 structures. (J to L) Cells treated with 35 μ M nocodazole (noc) for 20 min at room temperature. Scale bars indicate ~1 μ m.

Fig. 2.

(A) HeLa and MDCK cells treated with mock (blue) and Sept2 siRNAs (red). Cell extracts were immunoblotted with α-tubulin, Sept2, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, loading control) antibodies. Histograms show percent change in protein expression after normalization to 100% for the control. (B) Percentage of mitotic MDCK cells from mock ($n = 1409$) and Sept2 siRNA-treated ($n = 1124$) samples and percentage of prophase, prometaphase, metaphase, and anaphase/telophase cells within their respective mitotic populations. (C) Mean distance between the two γ-tubulin-labeled spindle poles of metaphase (mock, $n = 36$) and prometaphase (Sept2 siRNA, $n = 49$) MDCK cells and percentage of cells (mock, $n = 83$; Sept2 siRNA, $n = 83$) with a bipolar spindle. (D and E) Untreated and nocodazole-treated MDCK cells stained with Sept2 antibodies and DAPI. Sept2 fluorescence intensities were measured along the axis of the metaphase plate of untreated mock (solid blue) and Sept2 siRNA (solid red) cells. Dotted lines represent background fluorescence in the respective cells. Mean relative fluorescence intensity was 69 ± 10 in mock ($n = 19$) and 34 ± 9 in Sept2 siRNA ($n = 12$) cells. (F) MDCK cells transfected with *His2B-GFP* and mock/Sept2 siRNAs for 20 hours and imaged every 2 min. Arrows, mono-oriented chromosomes dislocated from the metaphase plate; arrowheads, mono-oriented chromosomes that move toward the metaphase plate. (G and H) The distance of individual *His2B-GFP* spots was measured from the long axis of the metaphase plate and plotted as a function of time, and mean distances were calculated and plotted versus time.

Fig. 3.

(A) MDCK cells transfected with Sept2 siRNAs for 36 hours and stained with Sept2 and lamin A/C antibodies. Arrows, Sept2-depleted cell with two attached nuclei. (B and C) Histograms show mean Sept2 fluorescence intensities in cells with one (blue, $n = 32$) and two (red, $n = 13$) attached nuclei and the percentage of binucleate cells within a random population of cells ($n = 500$) treated with mock (blue) and Sept2 (red) siRNAs. (D) MDCK cells transfected with His2B-GFP and Sept2 siRNAs and imaged every 5 min. (E to G) MDCK- α -tub-GFP cells transfected with mock (E) and Sept2 siRNAs (F) for 20 hours and imaged every 4 min. Spindle pole distances were measured for each time point and plotted as a function of time (movies S1 to S6).

Fig. 4. MDCK cells transfected with mock (blue) and Sept2 siRNAs (red) for 18 hours. Scale bars, ~5 μ m. **(A to C)** Cells were stained with DAPI and CREST, CENP-E, MCAK, and Mad2 antibodies. **(D)** Cells were incubated with 35 μ M nocodazole (nzd) for 2 hours at 37°C. **(E)** Percentages of CENP-E-, MCAK-, and Mad2-positive kinetochores per cell. An average of 110 kinetochores were counted per cell (17); the number of cells counted is shown within each bar. Error bars represent the 95% confidence interval of the mean value according to the unpaired Student's *t* test.