Regulation of cytokinesis by spindle pole bodies

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Abstract

In the fission yeast *Schizosaccharomyces pombe*, cytokinesis is thought to be controlled by the daughter spindle pole body (SPB) through a regulatory pathway, the Septation Initiation Network (SIN). Here we demonstrate that laser ablation of both but not a single SPB results in cytokinesis failure. Ablation of just the daughter SPB often leads to activation of the SIN on the mother and successful cytokinesis. Thus, either SPB can drive cytokinesis.

Cytokinesis in the fission yeast *Schizosaccharomyces pombe* is accomplished by the contraction of the actomyosin ring and formation of the medial septum (a cell wall structure). These concurrent processes are regulated by the Septation Initiation Network (SIN): loss-of-function mutations in this pathway are defective in ring contraction and septation, while constitutive activation of the SIN causes multiple rounds of cytokinesis[1]. Localization studies, as well as biochemical interactions have suggested a model in which SIN components associate with the Spindle Pole Bodies (SPBs, yeast centrosomes) and are active primarily on the daughter SPB[1-4]. However, the requirement of the SPB in cytokinesis remains to be tested directly.

Laser microsurgery is a powerful tool for localized ablation of cellular organelles in a variety of cell types including *S. pombe*[5,6]. Tightly focused laser pulses instantly destroy all molecular components within the irradiated area, with little harm to the rest of the cell[6]. Here, we ablated the SPBs during mitosis, and subsequently monitored effects on cytokinesis by time-lapse microscopy. We used fission yeast strains that express functional GFP-fusion proteins to mark the SPBs (sid2-GFP[7] or cdc7-GFP[8], SIN pathway components) and the contractile ring

(rlc1-GFP[9]) (Table S1; Video S1). Ablations of SPBs during metaphase were carried out in a *mad2*Δ background[10] to avoid potential activation of the spindle assembly checkpoint, while ablations during anaphase were conducted in both wild type and *mad2*Δ strains with similar results. Because only <10% of the total pool of SIN proteins such as sid2p and cdc7p (Fig. S1 and reference[11]) actually reside at the SPB, laser ablation should not significantly decrease the total amount of SIN proteins in the cell (Methods).

Ablation of both SPBs during prometaphase-metaphase (SPB separation < 3 μm) resulted in cytokinesis failure (Fig. 1A, Table S2). The ablation had no immediate effect on the formation of the actomyosin ring; it continued to assemble with normal kinetics and usually was fully formed ~5 min after SPB ablation. However, ring contraction was blocked, and after ~30 min the ring disassembled into pieces (Fig. 1A). There was no detectable sign of septum formation for at least 1 hr. This phenotype is similar to what is seen for the genetic mutants in the SIN pathway[11-14]. Ablation of both SPBs at later stages of mitosis (anaphase B) also led to cytokinesis failures but defects were generally weaker and more heterogeneous (Table 1). Almost all cells initiated but most did not complete septum formation. Laser irradiation of the mitotic spindle, nuclear envelope or the cytoplasm did not cause cytokinesis defects. These results demonstrate that presence of SPBs during metaphase and early anaphase is required for cytokinesis.

We then tested the hypothesis that only the daughter SPB is required for cytokinesis[1]. Unfortunately, the mother and daughter SPBs could not be distinguished during metaphase in our system, since even asymmetric SPB markers such as cdc7-GFP associate equally with both SPBs prior to anaphase[4]. Therefore, we ablated a single randomly chosen SPB in cells expressing sid2-GFP during metaphase, expecting to hit the daughter SPB approximately half the time. However, >90% of cells with a single SPB completed cytokinesis (Fig. 1B, Table S2).

Next, we sought to ablate specifically the daughter SPB labeled with cdc7-GFP. This protein initially accumulates in both SPBs during prometaphase-metaphase, but as the cell progresses through anaphase, cdc7p concentration decreases at the mother while increasing at the daughter SPB (Fig. 1C)[2,4]. We ablated the cdc7-GFP-positive (daughter) SPB during mid-to-late anaphase, just after the asymmetry between the SPBs became apparent. Surprisingly, in 15 of 23 cells, cdc7-GFP reappeared on the mother SPB within minutes after the daughter SPB had been ablated (Fig. 1D, Table S2). The recovery was transient and did not reach the levels typical for daughter SPBs in control cells. Notably, there was a strong correlation between the recovery of cdc7-GFP on the remaining SPB and completion of septation, suggesting that the SIN pathway was activated and functional on the mother SPB (Table S2). Because laser ablation should not significantly alter cytoplasmic concentration of SIN components, the recruitment of cdc7p to the mother SPB implies that the affinity of the mother SPB for this protein increases upon ablation of the daughter. The simplest explanation for this effect is that the daughter SPB normally produces a signal that somehow inhibits SIN activities on the mother. This putative inhibition does not require the SPBs to be connected by microtubules as cutting off the daughter SPB from the spindle with the laser beam did not induce cdc7p recruitment to the mother (Fig. S3).

In summary, our results provide direct proof that the presence of one SPB is required for successful cytokinesis in *S. pombe*. Under normal conditions, the daughter SPB drives cytokinesis and also somehow suppresses SIN activities on the mother SPB. However, the mother can be activated and compensate for the daughter when the latter is incapacitated.

Note: Supplementary Information (including Methods) is available on the Nature Cell Biology website.

ACKNOWLEDGEMENTS

This work was supported by National Institutes of Health grants GMS 59363 (to A.K.), GMS 69670 (to F.C), and by the Human Frontiers Science Program grant RGP0064 (to AK). We thank Drs. D. McCollum and V. Simanis for strains, and Dr. R. Daga for technical assistance.

COMPETING FINANCIAL INTERESTS

The authors declare that they have no competing financial interests.

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Figure Legend

Figure 1. **Effects of SPB ablation on cytokinesis**. **A-B)** Selected images from multi-mode time-lapse recordings of cells expressing the SIN component sid2-GFP (to label SPBs) and rlc1-GFP (to label the contractile ring) and containing a mutation inactivating the spindle assembly checkpoint (*mad2Δ*). Although the contractile ring can sometimes appear overlapping with the SPBs when 3-D fluorescence data are presented as maximal-intensity projections, SPBs can be easily distinguished from the contractile ring in individual focal planes (left images, also see Video S1). **A)** Ablation of both SPBs during metaphase (arrows in 00:00 and 02:45 time points) does not immediately affect formation of the contractile ring (07:45). However, the ring does not contract and later breaks down (39:00 – 60:00). DIC images of the same cell (right) reveal no sign of septum formation for approximately 1 hr. **B)** Ablation of a single SPB during metaphase (*cf*. arrows in 00:00 and 01:30) does not prevent septation. The contractile ring assembles and contracts normally (28:15 – 50:00). DIC images of the same cell (right) reveal normal septum formation (black arrows). Note that laser ablation of one SPB does not affect sid2-GFP association with the other SPB (arrowheads). **C-D)** Maximal-intensity projections of cells expressing cdc7-GFP (SPB) and rlc1-GFP (contractile ring). **A)** In control cells, cdc7p associates with both SPBs until mid anaphase (arrows and arrowheads in 00:00 and 02:00 time points) and later concentrates at the daughter SPB (arrowheads in 04:00 – 26:00). **B)** Upon ablation of the daughter SPB (cf. arrowheads in 01:00 and 02:30), the mother transiently re-accumulates cdc7p (arrows in 02:30 and 04:30 time points), and the cell undergoes cytokinesis (12:45 – 38:45). Time in minutes : seconds. Scale bar = 5 μm.