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Article

Budding Yeast Mitotic Chromosomes Have an Intrinsic Bias to Biorient on the Spindle

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Summary

Background: Chromosomes must biorient on the mitotic spindle, with the two sisters attached to opposite spindle poles. The spindle checkpoint detects unattached chromosomes and monitors biorientation by detecting the lack of tension between two sisters attached to the same pole. After the spindle has been depolymerized and allowed to reform, budding yeast *sgo1* mutants fail to biorient their sister chromatids and die as cells divide.
Results: In *sgo1* mutants, chromosomes attach to microtubules normally but cannot reorient if both sisters attach to the same pole. The mutants' fate depends on the position of the spindle poles when the chromosomes attach to microtubules. If the poles have separated, sister chromatids biorient, but if the poles are still close, sister chromatids often attach to the same pole, missegregate, and cause cell death.
Conclusions: These observations argue that budding yeast mitotic chromosomes have an intrinsic, geometric bias to biorient on the spindle. When the poles have already separated, attaching one kinetochore to one pole predisposes its sister to attach to the opposite pole, allowing the cells to segregate the chromosomes correctly. When the poles have not separated, the second kinetochore eventually attaches to either of the two poles randomly, causing orientation errors that are corrected in the wild-type but not in *sgo1* mutants. In the absence of spindle damage, *sgo1* cells divide successfully, suggesting that kinetochores only make stable attachments to microtubules after the cells have entered mitosis and separated their spindle poles.

Introduction

Natural selection asks organisms to balance speed and accuracy. A unicellular organism whose mitosis is slow and precise will be outcompeted by mutants with a faster and sloppier mitosis as long as the benefit of faster reproduction exceeds the cost of producing more dead or defective progeny. Combining a modest intrinsic accuracy with cell-cycle checkpoints is an attractive solution to this problem. Cells that attach their chromosomes to the spindle correctly on their first attempt can go quickly through mitosis, whereas those that make mistakes activate a cell-cycle checkpoint that has two roles: arresting the cell cycle and trying to correct attachment errors.

The spindle checkpoint [1] monitors chromosome orientation on the spindle [2] and increases the fidelity of chromosome segregation. Microtubules make up the spindle, and chromosomes attach to them with kinetochores, specialized structures that assemble on the centromeric DNA. For the chromosomes to segregate properly, the kinetochores of sister chromatids must attach to microtubules that emanate from opposite poles of the spindle. In this bioriented state, the kinetochores are pulled toward the poles, but the chromosome arms are held together by proteinaceous links (cohesin [3, 4]); these opposing forces stretch the linkage between sister kinetochores to a distance of 1 to 2 μm [5–7]. If sister kinetochores attach to the same pole (mono-orientation [strictly speaking, *syntelic* mono-orientation]), there is no tension between them, and the spindle checkpoint destabilizes their binding to microtubules and delays chromosome segregation (anaphase) to allow additional attempts at biorientation [2, 8–10]. The spindle checkpoint arrests the cell cycle by inhibiting the anaphase-promoting complex (APC) [1, 11], whose activation ultimately triggers sister separation.

Little is known about how sister chromatids biorient and reach a position midway between the spindle poles (metaphase). Kinetochore geometry could promote biorientation. If sister kinetochores are forced to point in opposite directions, a microtubule that captures one kinetochore biases its sister to attach to a microtubule originating from the opposite pole. Such geometric constraints are not essential in budding yeast [12], but they could act as one of many overlapping mechanisms that promote accurate chromosome segregation, especially in cells whose kinetochores can simultaneously bind microtubules from both spindle poles [13].

A puzzling observation led us to ask whether geometric constraints promote accurate chromosome segregation in yeast. Although the *sgo1-100* mutant [14] cannot respond to a lack of tension between sister chromatids, it arrests in mitosis in response to microtubule depolymerization. But when the microtubule poisons are removed, the cells missegregate their chromosomes and die, even though they produce viable progeny when mitosis is unperturbed [14]. This observation prompted us to ask whether the defect in *sgo1-100* was the inability of to let go of incorrectly attached chromosomes and whether the difference between these situations reflected different initial patterns of chromosome attachment. Our experiments support this hypothesis. Apart from being unable to let go of microtubules when they are not under tension, the kinetochores of *sgo1-100* cells interact normally with microtubules, and these cells only mono-orient their chromosomes when spindle-pole separation is delayed. We argue that yeast chromosomes have an intrinsic bias to biorient on the spindle,

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and this might have predated controls that detect and correct errors in chromosome alignment.

Results

sgo1-100 Cells Misorient Their Chromosomes after Spindle Damage

We asked whether chromosome missegregation in *sgo1-100* cells [14] is due to errors in chromosome orientation. We introduced the *sgo1-100* mutation into a strain that has tandem repeats of the Lac operator (*lacO*) inserted near the centromere on chromosome XV (*CEN15*) [5]. The array is seen as a fluorescent dot in cells expressing the Lac repressor fused to green fluorescent protein (GFP) [15]. This strain also has the *CDC20* gene under the *MET3* promoter (P_{MET3}-*CDC20*); in the presence of methionine, cells do not express Cdc20, a protein required to activate the APC and promote anaphase [11]. Thus, they arrest in mitosis even if they lack the spindle checkpoint. Wild-type and *sgo1-100* cells were arrested in G1, methionine was added (so that *CDC20* could be repressed), and the cells were then released from the G1 arrest into medium that contained methionine (so that the repression of *CDC20* could be maintained), benomyl, and nocodazole (so that microtubules could be depolymerized) (Figure 1A). After 3 hr, the benomyl and nocodazole were washed out. Because *CDC20* was still repressed, the cells were arrested in mitosis, and we scored chromosome XV as bioriented if we saw two GFP dots, representing a stretched pair of sister centromeres [5, 6, 16]. Chromosome orientation was defective in *sgo1-100* cells: Biorientation was slower and reached lower final levels (Figure 1B).

These errors could reflect kinetochore defects that make microtubule attachments weaker or less stable or an inability to correct mono-orientation. We distinguished these possibilities by watching the GFP-labeled *CEN15* as cells recovered from spindle depolymerization. We followed individual cells and scored them as showing one (no sister-kinetochore stretch) or two (biorientation) GFP dots. In animal cells, the stretch between sister kinetochores varies over time even though they are continuously bioriented [17]; we believe the same variation makes yeast cells oscillate between showing one and two GFP dots (Figure 1C) [5, 6].

Every wild-type cell stretched its kinetochores at least once during the experiment. More than half of the *sgo1-100* cells never separated their kinetochores (Figure 1C). However, the remainder did biorient chromosome XV, and the dynamics of kinetochore stretching did not differ significantly from that of wild-type cells, suggesting that the only defect in *sgo1-100* is that kinetochores that lack tension cannot release from microtubules: Chromosomes that biorient behave normally, but those that initially mono-orient are trapped in this state.

We modified this experiment to follow chromosome segregation after wild-type and *sgo1-100* cells had recovered from spindle depolymerization. We marked the spindle pole body (SPB, the fungal microtubule-organizing center) by fusing an SPB protein, Spc42, to mCherry (a monomeric variant of red fluorescent protein [RFP]). After cells had been arrested in mitosis without spindles for 3 hr, we removed the microtubule poisons and induced *CDC20*, allowing them to reform their spindles and enter anaphase (Figure 1A). We defined anaphase as a permanent increase in the distance between the SPBs. Videomicroscopy of *CEN15* and Spc42 revealed that about half (60%) of the *sgo1-100* cells mono-oriented chromosome XV and segregated both copies to one spindle pole in anaphase (Figure 1D), supporting the hypothesis that *sgo1-100* cells cannot realign mono-oriented chromosomes.

Unreplicated *sgo1-100* Chromosomes Remain Attached to the Old Spindle Pole Body

Kinetochores can lack tension because two sisters are attached to a single pole or because only one kinetochore has bound a microtubule. We examined chromosomes that had not duplicated and thus lacked a sister. We placed the *sgo1-100* mutant into strains in which DNA replication had been made conditional by placing *CDC6*, an essential initiation factor [18, 19], under the glucose-repressible *GAL1* promoter. In the absence of Cdc6, cells cannot initiate replication. They still enter mitosis, but their sisterless chromosomes segregate randomly to one of the two spindle poles [19]. The absence of sister chromatids, and therefore tension, activates the spindle checkpoint [20, 21], destabilizing kinetochore-microtubule binding and arresting the cell cycle. All three responses are abolished in *ipl1* mutants [7, 21]. Because the sisterless kinetochores cannot release from microtubules, they stay attached to the old SPB, which segregates to the daughter cell, dragging most of the chromosomes with it [7].

We followed the behavior of an unreplicated, LacO-marked chromosome IV in wild-type and *sgo1-100* cells. Cells were arrested in G1 in the presence of galactose (Cdc6 expressed), released into medium with glucose (Cdc6 repressed), and examined by videomicroscopy. Enough Cdc6 persisted to allow normal replication and segregation in both wild-type and *sgo1-100* cells during the first cell cycle (data not shown), confirming that *sgo1-100* cells do not show significant defects in chromosome biorientation during an undisturbed cell cycle. No replication occurred during the second cell cycle, and the sisterless chromosome IV segregated to a single spindle pole (see below). Even in wild-type cells, there was some bias for this chromosome to enter the daughter cell (Figure 2) [22]. We interpret this result as reflecting an initial bias for the unreplicated chromosome to stay bound to the old SPB. The chromosome's fate depends on a race between detaching from the old SPB and SPB separation. If the kinetochore detaches first, it is equally likely to reattach to the old and new SPBs, but if the poles separate first, the detached chromosome will be closer to the pole it was first attached to and more likely to reattach to it. Without DNA replication, there are no linked sister chromatids to restrain the repulsive forces that move the two SPBs apart. Thus, the chromosomes in Cdc6-deficient cells are likely to be allowed very few detachment and release cycles before the poles are so far apart that a kinetochore released from one pole has little chance of attaching to the opposite one.

The chromosomes of *sgo1-100* have an even higher bias to remain attached to the old SPB and end up in the daughter cell (Figure 2). We conclude that the kinetochores of the *sgo1-100* cells form and maintain stable