Sister Chromatids Segregate at Mitosis Without Mother–Daughter Bias in *Saccharomyces cerevisiae*

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ABSTRACT There is evidence accumulating for nonrandom segregation of one or more chromosomes during mitosis in different cell types. We use cell synchrony and two methods to show that all chromatids of budding yeast segregate randomly and that there is no mother–daughter bias with respect to Watson and Crick-containing strands of DNA.

THE immortal strand hypothesis was proposed by J. Cairns as a mechanism to preserve genome integrity during development and was postulated to be especially important for stem cells (Cairns 1975). According to the model, when stem cells undergo asymmetric cell division, one daughter (the self-renewing stem cell) selectively retains the older template DNA strand from each chromosome, avoiding mutations introduced during DNA replication (Cairns 1975; Rando 2007; Tajbakhsh 2008). The model has been tested in a large number of cells from yeast to humans with mixed results and much debate (Neff and Burke 1991; Booth *et al.* 2002; Merok *et al.* 2002; Potten *et al.* 2002; Karpowicz *et al.* 2005; Conboy *et al.* 2007; Lansdorp 2007; Rando 2007; Fei and Huttner 2009; Walters 2009; Escobar *et al.* 2011; Schepers *et al.* 2011; Yadlapalli *et al.* 2011). The experiments often utilize halogenated deoxyribonucleotides to label DNA and determine if the label is retained over successive divisions. This protocol was applied to yeast by labeling cells for several generations with 5-bromo-deoxyuridine (BrdU), followed by two rounds of cell division in the presence of unlabeled thymidine to obtain cells in the second mitosis with

one unlabeled chromatid and one hemi-labeled chromatid (Neff and Burke 1991). Immunofluorescence was used to follow the fate of the hemi-labeled chromatids after the second mitosis. The immortal strand hypothesis predicts that the oldest (labeled) DNA strands would be segregated to the same daughter; therefore, half of the cells would be labeled and half unlabeled. Random segregation predicts that all of the cells are labeled with each cell containing half as much BrdU. Our results were consistent with random segregation in that all the cells were labeled and the amount of BrdU per cell decreased by half between the first and second division. Sister-chromatid recombination was minimal, and the data could not be explained by nonrandom segregation coupled with sister-chromatid exchange (Neff and Burke 1991).

More recently, a different model for nonrandom chromosome segregation on a chromosome-by-chromosome basis was proposed and called “strand-specific imprinting and patterned segregation” (SSIS) (Klar 2007). The model proposes that epigenetic imprinting during DNA replication marks the sister chromatids as different and that differential inheritance of the imprinted chromatids results in different cell fates in the daughter cells (Klar 2007; Tajbakhsh 2008). Chromatid imprinting during DNA replication underlies mating-type switching in *Schizosaccharomyces pombe* (Klar 1987, 2007; Yamada-Inagawa *et al.* 2007). SSIS was proposed as the explanation for nonrandom chromosome segregation in mouse embryonic stem cells where chromosome 7 segregates nonrandomly in a cell-type-specific manner that is dependent on a dynein motor protein (Armakolas and Klar 2006, 2007; Klar 2007; Armakolas *et al.* 2010). Supporting evidence for nonrandom segregation of a subset

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Figure 1 Watson and Crick-containing chromatids are not exclusively segregated to mother or daughter cells. (A) The labeling protocol is shown with the BrdU-containing strands of DNA in red and the unlabeled DNA in black. The original Watson (W) and Crick (C) strands are indicated, as are centromeres (circles). Cells are labeled with BrdU in the first cell cycle, and mothers and daughters are separated and grown for a second cell cycle in the presence of unlabeled thymidine (TdR). Mothers derived from the first mother (MM) are purified from the daughters (MD) and similarly for the mothers derived from the first daughter (DM) and the corresponding daughter (DD) after the second cell cycle. (B) Short and long exposures of the Southwestern blot to detect BrdU. All experiments were performed in strain CVY63 *MATa ade2-1 trp1-1 can1-100 leu2-3,112, his3-11,15 bar1::hisG LEU2:BrdU-inc*, which is isogenic with W303a and was kindly supplied by Oscar Aparicio. All methods are in [File S1](#).

of chromosomes in intestinal crypt cells was demonstrated using a fluorescence *in situ* hybridization strategy and is consistent with SSIS operating on a subset of chromosomes in intestinal cells (Falconer *et al.* 2010). Previous experiments to test the Cairns hypothesis in yeast had insufficient resolution to detect SSIS (Neff and Burke 1991). Selective nonrandom segregation of a single yeast chromosome, especially one of the smaller chromosomes, would have been difficult to distinguish from completely random segregation solely on the basis of immunofluorescence. Sister chromatids of yeast chromosome 5 are randomly segregated in mitosis but that cannot be said with certainty for the other 15 chromosomes (Chua and Jinks-Robertson 1991).

We have tested the SSIS model for mother–daughter bias and nonrandom segregation of chromatids in budding yeast using two different strategies. Both depended on a yeast strain engineered to permit BrdU labeling and on a simple method to

purify mother cells from daughters (Park *et al.* 2002; Viggiani and Aparicio 2006). Cells were arrested with α -factor, and the cell surface was biotinylated. Cells were released into the cell cycle, allowed to divide, and arrested prior to budding in the second cell cycle by adding α -factor again to the culture. The biotinylated mother cells were purified from the unlabeled daughters using streptavidin-coated magnetic beads. The first strategy to determine if there was nonrandom segregation of individual chromosomes is shown in Figure 1A. Cells were labeled with BrdU in the first cell cycle before separating the mothers (M) from the daughters (D). The daughter cells were biotinylated, and both populations were grown for one cell cycle in the absence of BrdU and arrested with α -factor, and mothers were separated from daughters (MM, MD and DM, DD). Figure 1 shows the prediction for the SSIS model with the hypothesis that the mother cells inherit the parental Watson-containing strand and the daughter cells inherit the

Figure 2 There is no mother–daughter bias in the segregation of Watson and Crick-containing chromatids during mitosis. (A) The labeling protocol is shown with the BrdU-containing strands of DNA in red and the unlabeled DNA in black. The original Watson (W) and Crick (C) strands are indicated, as are centromeres (circles). (B) Normalized mean raw intensities for individual genes on the Watson strand (red) or the Crick strand (blue) of chromosome 5 vs. the position along the chromosome. (C) The log2 ratios of intensities for every gene on the Watson strand (red) and the Crick strand (blue) vs. the position along the chromosome. (D) Q-Q plot of the log2 ratio for the Watson strand of chromosome 5. (E) Plot of the distribution of the intensities of log2 ratios for the Watson strand (red) and the Crick strand (blue) for chromosome 5.

parental Crick-containing strand. The label is expected to be in two of the four cell types if there is complete nonrandom segregation of chromatids. We assayed the inheritance by separating chromosomes in a contour-clamped homogeneous electric field (CHEF) gel and by detecting the BrdU by Southwestern blots (Figure 1B). We saw no evidence of completely nonrandom segregation of chromatids for any chromosome.

We used an independent method that was highly quantitative and had sufficient resolution to determine if there was any mother–daughter bias associated with sister-

chromatid segregation (Figure 2A). Cells were arrested in α -factor and biotinylated as described above. Cells were released to the cell cycle, and BrdU was incorporated into newly synthesized DNA strands (W' and C' in Figure 2A). α -Factor was added to arrest the cells after cell division, prior to budding in the subsequent cell cycle, and mothers were separated from daughters. DNA was purified and denatured, and the BrdU-containing strands were recovered by immunoprecipitation and eluted by competition with BrdU. The complementary strand was biotin-labeled *in vitro*, and

the biotinylated DNA was hybridized to Affymetrix Yeast Genome 2.0 microarrays containing probes representing both the Watson and Crick strands of DNA for all chromosomes (<http://www.affymetrix.com/estore/>).

The experiment was performed in duplicate, and scatter plots show the reproducibility (Supporting Information, Figure S1). The mean intensity of hybridization to the genes on the Watson and Crick strands for chromosome 5 in the mother cell are shown in Figure 2B. The mean intensity of labeling for Watson and Crick strands for all chromosomes in the mother cells is shown in Figure S2. The mean intensity of labeling for the Watson and Crick strands for all chromosomes in daughter cells is shown in Figure S3. If chromatids were randomly segregated, the signal for hybridization to Watson and Crick probes should be in equal amounts (50:50) in both the mother and daughter cells. Any deviation from 50:50 would be evidence of mother–daughter bias. We calculated the ratio of the log₂-transformed mean intensities of genes on the Watson and Crick strands for mothers vs. daughters for each probe on every chromosome. If chromatid segregation were random, the log₂ ratio would be zero. The data for the log₂ ratios of all probes for the Watson and Crick strands of chromosome 5 are shown in Figure 2C. The data for all chromosomes are shown in Figure S4. The log₂ ratios for both Watson and Crick probes for all chromosomes were close to zero. The Q-Q plot for the data for the Watson strand of chromosome 5 is in Figure 2D; Q-Q plots for the Watson strand for all chromosomes are in Figure S5, and the Q-Q plots for the Crick strand for all chromosomes are in Figure S6. The Q-Q plots show that the log₂ ratios for all chromosomes fit normal distributions. The distributions of the log₂ ratios for Watson strands and Crick strands for chromosome 5 are shown in Figure 2E, and the distributions for all chromosomes are shown in Figure S7. The overlapping distributions for the Watson and Crick strands centered on zero strongly suggest that all 16 of the chromosomes segregate randomly without mother–daughter bias. We performed a Wilcoxon ranked sign test to test the null hypothesis that the mean of the distribution of the mother–daughter ratios for probes to the Watson and Crick strands for each chromosome were equal to zero. The results are shown in Table S1. We found no significant *P*-values (*P* < 0.05) and conclude that all 16 chromosomes of yeast segregate randomly in mitosis without mother–daughter bias.

There is some evidence of nonrandom distribution of kinetochore proteins in the first cell division after sporulation and germination that may reflect nonrandom segregation of yeast chromosomes during a specialized cell division (Thorpe *et al.* 2009). The experiments described here could be modified to test this more directly and could be applied to any other conditions or specialized cell divisions in budding yeast.

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Figure S1 Scatter plots of the normalized intensities between the samples from mother cells (purple) and daughter cells (green) from Affymetix Yeast Genome 2.0 microarrays. Correlation coefficients for both plots are 0.9861505 for the mothers and 0.9861781 for the daughters.

Figure S2 The signal intensities for the Watson and Crick probes for all chromosomes in mother cells. The average normalized intensities are plotted relative to the normalized chromosome position.

Figure S3 The signal intensities for the Watson and Crick probes for all chromosomes in daughter cells. The average normalized intensities are plotted relative to the normalized chromosome position.

Figure S4 Plots of the log2 ratios for probes for the Watson and Crick strands for all chromosomes. The data for the log2 ratios all probes for the Watson and Crick strands for each chromosome are plotted relative to chromosome location.

Figure S5 Q-Q plots of the log2 ratios for probes on the Watson strand are normally distributed for all 16 chromosomes. Quantiles for the observed data are plotted against theoretical quantiles from normally distributed data.

Figure S6 Q-Q plots of the log₂ ratios for probes on the Crick strand are normally distributed for all 16 chromosomes. Quantiles for the observed data are plotted against theoretical quantiles from normally distributed data.

Figure S7 There is no mother-daughter bias in chromatid segregation. The distributions of the log₂ ratios of intensities for Watson (red) and Crick (blue) strands for all chromosomes are shown.

Methods

Cells were grown at 30 degrees C in 50 mls of YPD to a density of 2×10^7 cells/ml and α -factor was added at 1 μ M. Cells were incubated for 2.5 hours until greater than 90% of the cells were unbudded with mating projections. Cells were collected by centrifugation and washed twice with water and twice with phosphate buffered saline (PBS) and resuspended in 2 mls of PBS. Cells were sonicated and 24 mg of EZ-biotin (Sulfo-NHS-LC-Biotin, Thermo-Scientific) was added and incubated for 15 min at room temperature with gentle mixing. Cells were washed three times in 50 mls of water and a sample was stained with FITC streptavidin to confirm biotinylation of the cell surface by fluorescence microscopy. Cells were resuspended in 50 mls of YPD containing 20 mg of 5-Bromo-deoxyuridine (Sigma) and incubated in the dark for 1 hour at 30 degrees C until greater than 95% of the cells were budded at which time α -factor was added at 1 μ M. Cells were incubated for 2 hours until greater than 90% of the cells were unbudded with mating projections. Cells were washed twice in 50 ml of water and twice in PBS, sonicated and aliquoted into 1ml aliquots of 10^8 cells in eppendorf tubes. Cells were concentrated by centrifugation and resuspended in 400 μ l of PBS-washed Streptavidin dynabeads and incubate with rotation for 15 minutes. The beads were recovered using a magnet and washed three times. The unbound samples (daughters) were pooled and an aliquot stained with calcofluor and FITC streptavidin. The cells were greater than 90% daughter cells as determined by fluorescence microscopy and identifying the fraction of cells that were stained with both dyes (mothers). In the first protocol, mother and daughter cells were returned separately to YPD medium and incubated in the dark for 1 hour at 30 degrees C until greater than 95% of the cells were budded at which time α -factor was added at 1 μ M. The cultures were treated as described above so that the mothers and daughters from the culture of mother cells (MM and MD) were isolated and the mothers and daughters from the culture of daughter cells (DM and DD) were isolated. DNA was extracted and subjected to electrophoresis using a Bio-Rad CHEF-mapper XA system according to manufacturers instructions. The gel was subjected to Southern blot following standard procedures and the BrdU was detected by immunoblotting using mouse anti-BrdU (G3/G4). In the second protocol, DNA was purified from the BrdU-labeled mothers and daughters and heated to 95 degrees C for 5 min in 360 μ l distilled water and then placed on ice. Forty μ l of 10X PBS was added and 20 μ l of 1 mg/ml mouse anti-BrdU (G3/G4) and the mixture was incubated for an hour. The immuno-complexes were recovered by adding 50 μ l of sheep anti-mouse dyanbeads. The unbound fraction was analyzed by slot blot and greater than 95% of the BrdU was bound to the beads. The BrdU containing DNA was recovered by resuspending the beads in 100 μ l of 1.7 mM BrdU. The recovered DNA was extracted with phenol chloroform isoamyl alcohol and concentrated by ethanol precipitation. DNA was labeled with random priming (In Vitrogen) with using biotin-dCTP according to manufacturers instructions and used for hybridization to Affymetrix arrays following manufacturers instructions.

Table S1 Wilcoxon ranked sign test

Null hypothesis: that the mean of the distribution of the mother-daughter ratios for probes to the Watson and Crick strands for each chromosome is equal to zero.

Chromosome	P Value
1	0.36
2	0.70
3	0.51
4	0.62
5	0.17
6	0.96
7	0.82
8	0.62
9	0.52
10	0.77
11	0.07
12	0.39
13	0.69
14	0.78
15	0.29
16	0.67