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Unbiased segregation of yeast chromatids in *Saccharomyces cerevisiae*

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Abstract The budding yeast *Saccharomyces cerevisiae* is characterized by asymmetric cell division and the asymmetric inheritance of spindle components during normal vegetative growth and during certain specialized cell divisions. There has been a longstanding interest in the possibility that yeast chromosomes segregate non-randomly during mitosis and that some of the differences between mother and daughter cells could be explained by selective chromatid segregation. This review traces the history of the experiments to determine if there is biased chromatid segregation in yeast. The special aspects of spindle morphogenesis and behavior in yeast that could accommodate a mechanism for biased segregation are discussed. Finally, a recent experiment demonstrated that yeast chromatids segregate randomly without mother–daughter bias in a common laboratory strain grown under routine laboratory conditions.

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Abbreviations

ADE3	Adenine biosynthesis gene 3
BrdU	5-bromo-deoxy-uridine
CDC21	Cell division cycle 21
CEN5	Centromere of chromosome 5
CEN15	Centromere of chromosome 15
DED1	ATP dependent DEAD box RNA helicase 1
dUMP	Deoxyuridine monophosphate
ERC	Extra-chromosomal ribosomal DNA circles
HIS3	Histidine biosynthesis gene 3
*MAT***a**	Mating type a
MATα	Mating type α
SCE	Sister chromatid exchange
SPB	Spindle pole body
TK	Thymidine kinase
TUT1	Thymidine utilization 1

Introduction

Non-random segregation of *Saccharomyces cerevisiae* chromatids during mitosis

The immortal strand hypothesis was originally proposed by Cairns to explain the comparatively low frequency of tumors in epithelial tissues which may

be explained by co-segregation of template-containing DNA strands (Cairns 1975). A duplex of DNA after replication contains one DNA strand (the template strand) that is older than the other (the daughter strand). When the duplex is replicated, the sister chromatids are different in that one contains the original template strand and therefore the sisters have different replicative ages. Cairns proposed that co-segregation of chromatids containing the template strands, and hence of the same replicative age, could protect stem cells from potentially deleterious mutations that arise during DNA replication, which is referred to as "the immortal strand hypothesis". Experiments were done using DNA labeling, and the fate of the labeled DNA was followed in subsequent generations. The prediction for co-segregation is that all of the label should be segregated to the same daughter cells in the second generation so that there would be cells that were fully labeled and cells that were unlabeled. The only confounding possibility would be sister chromatid exchange where the sister chromatid is used as a template for DNA repair after DNA replication (Cairns 1975). There were several examples of co-segregation of labeled DNA consistent with the immortal strand hypothesis in a variety of organisms, cultured cells, and fungi at the time that the immortal strand hypothesis was proposed (Lark 1967; Lark 2012; Lark et al. 1966; Rosenberger and Kessel 1968). However, there were also reports that were consistent with random distribution of chromatids at mitosis and therefore the immortal strand hypothesis and the subsequent non-random distribution of chromatids was not a universal mechanism of chromosome segregation (Geard 1973; Fernandez-Gomez et al. 1975). However, it remained possible that the immortal strand hypothesis, as originally proposed, applied in some cells and was especially appealing to invoke the hypothesis for cells undergoing asymmetric cell divisions.

The yeast *S. cerevisiae* is encased in a cell wall that is synthesized every cell cycle in the process of budding (Orlean 2012; Howell and Lew 2012). A cell in the G1 phase of the cell cycle initiates budding and the bud grows during the cell cycle. The mitotic spindle separates the two nuclei after DNA replication, one of which remains in the "mother" cell (defined by the original cell wall) and the other is placed in the "daughter" cell, defined by the new cell wall. This asymmetric division produces a "mother" cell that is different from the daughter cell, not only in the cell wall but in the cell cycle time and in the curious behavior of mating type switching (Howell and Lew 2012; Haber 2012; Strathern and Herskowitz 1979). Williamson and Fennel tested the co-segregation hypothesis in budding yeast and reported their findings at the 16th Alfred Benzon symposium in 1981 (Williamson and Fennell 1981). Their rationale was that co-segregation could be a mechanism underlying asymmetric cell divisions that result in different cell fates in yeast and other cells. Yeast can exist in two mating types (a and α), and mating type is determined by the mating type locus on chromosome III (Haber 2012). *MAT***a** and *MAT*α are alleles and specify the cell type with respect to mating behavior. There are strains of yeast that are capable of switching their mating type (most laboratory strains have a mutation in a gene that prevents this behavior). Strains that are competent to switch mating types have "rules" for switching; mother cells switch and daughter cells do not (Haber 2012; Strathern and Herskowitz 1979). Ira Herskowitz and his graduate students discovered this remarkable property and they proposed that one possible mechanism for the asymmetry was the asymmetric inheritance of chromosome III akin to the immortal strand hypothesis (Strathern and Herskowitz 1979; Hicks and Herskowitz 1976).

There was also reason to suspect that co-segregation of chromatids due to the replicative age of the DNA strands could happen during mitosis in yeast because of the behavior of the spindle pole body (SPB) in normal cells and in cell division cycle (*cdc*) mutants (Jaspersen and Winey 2004). The SPB is the equivalent of the centrosome in yeast and is the sole source of nucleated microtubules (Winey and Bloom 2012; Winey and O'Toole 2001). The SPB consist of three disks (plaques) of darkly staining material that are visible by electron microscopy. The outer plaque faces the cytoplasm and nucleates cytoplasmic microtubules and the inner plaque nucleates the spindle microtubules that will emanate from the SPB and connect to the kinetochores and ultimately segregate the chromatids at mitosis. The central plaque spans the nuclear envelope. Two SPBs form the poles of the bipolar mitotic spindle and function in a "closed mitosis" where the nuclear envelope remains intact throughout mitosis. There is a single SPB embedded in the nuclear envelope of cells in the G1 phase of the cell cycle, prior to DNA replication, and must duplicate in multiple well-characterized steps in G1. Interestingly, there are microtubules associated with the single SPB in G1 and those spindle microtubules appear to remain attached to the inner plaque of the SPB

during SPB duplication. SPB duplication culminates when the newly formed SPB is inserted into the nuclear envelope and microtubules can now form on the new SPB. Almost immediately, the two SPBs are moved to opposite sides of the nucleus as spindle assembly begins in S phase (Byers and Goetsch 1974, 1975; Byers et al. 1978; Winey and O'Toole 2001; Jaspersen and Winey 2004). One intriguing possibility was that the nuclear microtubules connected to the inner plaque of the G1 SPB could be attached to the kinetochores from the previous cell cycle and that they would direct the co-segregation of DNA strands of the same replicative age.

Determining if co-segregation occurred in yeast required some mechanism to label the DNA and distinguish old DNA strands from new DNA strands. The principle is shown in Fig. 1, which shows two chromosomes in G1 with hemi-labeled DNA where the oldest DNA strands are red. The chromosomes are replicated in the absence of label (black) and the chromosomes are shown aligned at metaphase. Co-segregation is shown on the left where the chromatids containing the oldest DNA strands will segregate to the same pole and be in the same nucleus in the subsequent G1 (G1′). The alternative is random segregation where chromatids containing the oldest DNA could segregate to opposite poles. The predictions for the distribution of label for all chromosomes are shown in schematically in the graph below each outcome. If there is co-segregation, then only half of the nuclei will be labeled, but the amount of label per labeled G1′ nucleus will be the same as G1 nuclei. If there is random segregation, all of the G1′ nuclei will be labeled, but the amount of label will be decreased by half.

Labeling DNA directly in yeast using radioactive thymidine is not possible because yeast do not contain thymidine kinase and therefore lack the thymidine salvage pathway that permits efficient uptake of exogenous thymidine and incorporation into DNA during S phase. Instead, yeast synthesize thymidine entirely de novo from dUMP using thymidylate synthase, encoded by *CDC21* (Taylor et al. 1982; Taylor et al. 1987; Bisson and Thorner 1977). Williamson and Fennell (1981) used tritiated adenine to label DNA and assayed the incorporation by emulsion-based autoradiography (Williamson and Fennell 1981). The technique was labor intensive, and each experiment was long (several months of autoradiography). Furthermore, the majority of adenine was incorporated into RNA during the experiment that had to be carefully and completely removed to prevent the result being obscured by background signal from the labeled RNA. Signals were low (usually less than 10 grains per nucleus) that had to be ascertained by precise alignment of the autoradiogram and the concomitant pictures of the cells and their nuclei. They used two experimental procedures to determine if there was co-segregation. Both used asynchronous cells and either continuous labeling for many generations or pulse labeling for a single generation (where generations were estimated from the doubling time). The cells in both procedures were cultured after the labeling in medium lacking label, and cells were sampled over the next three generations. They followed the fate of the label by whole cell autoradiography after high pH treatment and RNase to eliminate labeled RNA. They counted the number of grains per nucleus after autoradiography and found that there was a normal distribution of with a mean of approximately 8 grains/nucleus until the third generation when they saw a bimodal distribution, with half the cells retaining the label (~8 grains per nucleus) and the other half having little to none. These data were consistent with yeast having a mechanism to assure co-segregation of DNA strands of the same replicative age (Fig. 1).

There was a problem. Co-segregation could only be detected in respiration-deficient cells that completely lacked mitochondrial DNA. They did not detect a bimodal distribution of grains/nucleus after three generations in cells that were respiration competent. They performed the experiment in the same way, sampling cells each generation. The number of grains/nucleus that they detected for each generation was normally distributed with a mean of ~8 grains per nucleus. They interpreted the data from the respiration-deficient cells to mean that there was co-segregation of DNA strands of the same replicative age in yeast. They explained the apparent random segregation in respiration competent cells as reflective of a high rate of sister chromatid exchanges (SCE). They hypothesized that SCE was induced by the radiolabeled adenine, which obscured the co-segregation in respiration competent cells. They further proposed that SCE was dependent on mitochondrial function and perhaps respiration per se.

Random segregation of *S. cerevisiae* chromatids during mitosis

Sclafani and Fangman (1986) engineered a yeast strain that would be amenable to experiments where DNA was labeled with thymidine and thymidine analogs

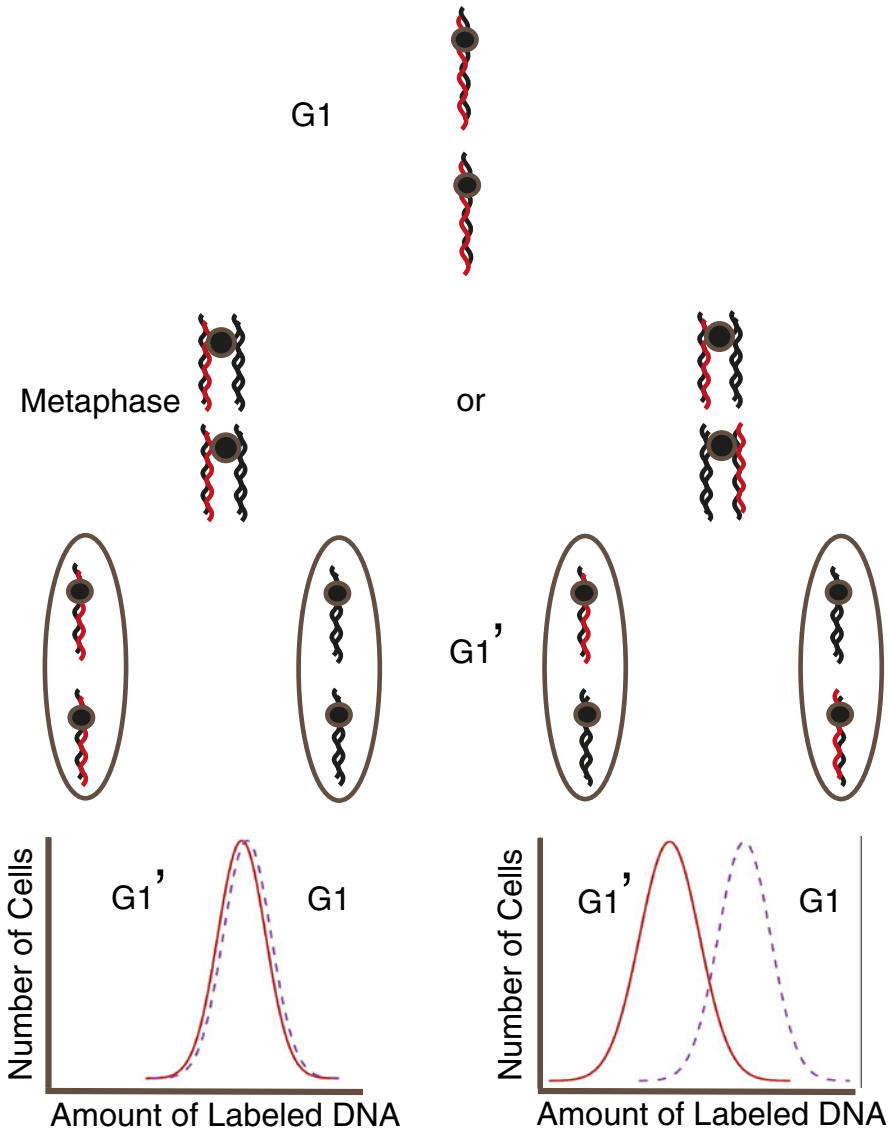

Fig. 1 Strategy for identifying co-segregation of DNA strands of the same replicative age. Cells grown for several generations in labeling medium generates uniformly labeled DNA strands (*red*). The cells are grown in the absence of label (*black*) to produce the hemi-labeled duplexes at the top (G1). Chromosomes at metaphase (Metaphase), following DNA replication in unlabeled medium, have one labeled and one unlabeled chromatid. Co-segregation of the labeled chromatids, shown on the left, produces half labeled and half unlabeled nuclei (G1′). The amount of label per labeled nucleus in G1 and G1′ is shown in the graph and the amounts are identical. Random segregation of chromatids, shown on the right, results in label being present in all nuclei. The amount of label in G1′ nuclei is half what was present in G1 nuclei

such a 5-Bromo-deoxyuridine (BrdU) (Sclafani and Fangman 1986). They used temperature sensitive *cdc21* cells to conditionally inactivate thymidylate synthase and block the de novo thymidine synthesis pathway, making cells dependent on exogenous thymidine. They selected a mutant (*tut1*) that allowed cells to grow on a relatively low concentration of exogenous thymidine and showed that it could be used for BrdU labeling. They used a high copy plasmid to express Herpes Simplex Virus thymidine kinase (TK) fortuitously expressed from the *DED1* promoter 3′ to the *HIS3* gene (McNeil and Friesen 1981). The improved strain permitted Neff and Burke (1991) to repeat the co-segregation experiments of Williamson and Fennell (1981) with several improvements (Neff and Burke 1991). Most importantly, they labeled DNA with BrdU and detected it by immunoflourescence using a monoclonal anti-BrdU antibody so that the experiments were faster and more sensitive. They showed that the strain delayed their progress through S phase, presumably because the cells were somewhat starved for thymidine. They performed the experiments on synchronously grown strains using a combination of centrifugal elutriation and α-factor arrest and release to follow cells for a single cell cycle (Manukyan et al. 2011). They repeated the Williamson and Fennel experiments with an experimental design

similar to that shown in Fig. 1. They prepared cells for immunoflourescence, counted the fraction of labeled nuclei, and quantified the fluorescence using microphotometry. The microphotometer was a homemade instrument built in the 1960s, mounted on a microscope from the same era, and was considerably less sensitive than modern quantitative fluorescence microscopy systems. Nevertheless, they detected BrdU in every cell and the amount of fluorescence per nucleus, after the critical mitosis, was half as predicted by random segregation (Fig. 1). They were unable to detect co-segregation even in cells that lacked mitochondria.

One concern was that BrdU induced a high rate of SCE, which accounted for the difference in the results compared to those of Williamson and Fennell (1981). Neff and Burke (1991) measured the extent of SCE in response to growth in the presence of BrdU using two quantitative genetic assays. One relied on unequal SCE in haploid cells between repeated sequences that resulted in the reconstitution of an auxotrophic marker, *ADE3,* that could be selected by growth on medium lacking adenine (Kadyk and Hartwell 1992). The other utilized diploid cells heterozygous for a circular derivative of chromosome III where sister chromatid exchange between the circular sister chromatids would generate a dicentric chromosome that is unstable (Haber et al. 1984). The diploid cell loses the circular chromosome III at the following mitosis producing a viable diploid that is monosomic for chromosome III. The monosomic cell loses the allele of the mating type locus located on the circular chromosome III and changes from a non-mating to a mating diploid, which is easily measured quantitatively (Dutcher and Hartwell 1983). Neff and Burke (1991) showed that there was less than one SCE per cell per division when cells were grown in the presence of BrdU. Therefore, biased co-segregation of chromatids accompanied by a high rate of SCE could not explain the random distribution of the BrdU, and the discrepancy in the results between the two labs. The discrepancy has never been explained.

Random segregation of individual *S. cerevisiae* chromatids at mitosis

The experiments of Neff and Burke (1991) utilized indirect immunoflourescence of nuclei and argued against co-segregation of all chromatids during a single mitosis. However, they pointed out that their approach did not have the sensitivity to determine if there was non-random segregation for a subset of chromosomes. Quantifying the amount of BrdU by indirect immunoflourescence using microphotometry is far less precise than the methods used today for quantitative fluorescence microscopy, but even modern detection methods may not be sensitive enough to identify non-random segregation of a single chromosome. If one of the 16 chromosomes of yeast were non-randomly segregated, then the prediction from the experiment diagrammed in Fig. 1 would be that all of the nuclei would be labeled after the critical mitosis, but the label would be bimodal with means separated by the amount of fluorescence attributable to one chromosome. Detecting such a subtle difference, even today, would be challenging, especially if there was non-random segregation of one of the smaller yeast chromosomes.

There is good reason to suspect that non-random segregation of sister chromatids could be the biological basis for cellular asymmetry as has been clearly demonstrated in fission yeast. Analysis of mating type switching in the fission yeast showed that switching is asymmetric and follows the “one on four” rule (Klar 2007). *Schizzosaccharomyces pombe* are rod-shaped cells that grow by polarized tip extension and divide symmetrically by medial septum formation (Perez and Rincon 2010). Therefore, *S. pombe* produces two nearly identical daughter cells so the designation of mother and daughter, as it is used in reference to budding yeast, is meaningless. An *S. pombe* cell of one mating type divides to produce two cells of the same mating type as the mother but with different fates (Klar 2007). One cell divides to produce two identical cells and the other divides to produce one cell that switches mating types. Therefore, switching happens in one of the four granddaughters (the “one in four” rule) (Klar 1990). This means that the sister chromatids, produced by replication in the original parental strain (the grandparent), are non-equivalent. The asymmetric pattern of switching in *S. pombe* is controlled by an epigenetic mark that is imprinted onto one of the two chromatids during DNA replication (Dalgaard and Klar 2001; Klar 1990; Vengrova and Dalgaard 2004; Vengrova and Dalgaard 2006). Switching happens only in cells that inherit the imprinted chromatid. Amazingly, this mechanism is evolutionarily conserved to highly diverged yeast species (Yu et al. 2013). It has been proposed that such imprinting, followed by non-random segregation of chromatids, could be the basis of some cell fate

decisions in fungi and higher eukaryotic cells including humans (Armakolas et al. 2010). Therefore, the question changed from Cairn's original immortal strand hypothesis of non-random segregation of all chromosomes to a different model for non-random chromosome segregation on a chromosome-by-chromosome basis that has been proposed and called "strand-specific imprinting and patterned segregation" (SSIS) (Armakolas et al. 2010). The model proposes that epigenetic imprinting during DNA replication marks the sister chromatids as being different, and differential inheritance of the imprinted chromatids results in different cell fates in the daughter cells. Is it possible that there is non-random segregation of individual chromosomes in budding yeast?

Early studies showed that there was preferential segregation of some artificial chromosomes to mother cells, a so-called mother–daughter bias (Murray and Szostak 1983; Murray and Szostak 1985). Perhaps SSIS operates in yeast, and the asymmetry reflects an inherent bias in segregating some yeast chromosomes that becomes obvious in artificial chromosomes. Yeast cells have a limited capacity for cell division and a mother cell produces a limited number of daughters in a replicative aging process (Kaeberlein 2010; Longo et al. 2012). One mechanism that sets the capacity for division potential of aging mother cells is the accumulation of extra-chromosomal rDNA circles (ERCs) (Sinclair and Guarente 1997). ERCs result from homologous recombination between repeats of the ribosomal DNA repeats in the mother cell to produce an episomal circular molecule with an autonomously replicating sequence lacking a centromere (Sinclair and Guarente 1997). The ERCs replicate once every cell cycle during S phase but are maintained in the mother cell and therefore show mother–daughter bias in a manner similar to artificial chromosomes. Although there is asymmetric segregation of the artificial chromosomes and ERCs, the mechanism does not appear to be through SSIS. It has been proposed that the asymmetric segregation of plasmids and ERCs reflects tethering to nuclear pores that are asymmetrically distributed (Shcheprova et al. 2008). Recent modeling and experiments suggest that the bias is due to nuclear architecture and rapid mitosis that creates barriers to diffusion (Gehlen et al. 2011; Khmelinskii et al. 2011). The biased segregation of artificial chromosomes and ERCs is unlikely to reflect an inherent mechanism of non-random chromosome segregation.

This still does not rule out the possibility that there is non-random segregation of one or more of the endogenous yeast chromosomes. There was a recent demonstration of asymmetric segregation of kinetochore proteins in the first vegetative cell divisions after meiosis (Thorpe et al. 2008; Thorpe et al. 2009). Selected kinetochore genes in diploid cells were tagged with fluorescent proteins such that one of the two alleles was tagged. The cells were induced into meiosis and the spores were isolated by tetrad dissection. Quantitative microscopy and pedigree analysis was used to determine the abundance of proteins inherited in the mother and daughter cells in the first few divisions in cells derived from the germinating spores. Importantly, only the spores that did not have the tagged gene were selected. These spores inherited a pool of fluorescent kinetochore protein from the heterozygous cell that was induced into meiosis, and differences in the levels of fluorescence between the mother and daughter cells could not be attributed to differential transcription of the tagged gene encoding the kinetochore protein. Fluorescent histone H2A distributed equally to the mother and daughter cells in the cell divisions after meiosis. Strikingly, four different kinetochore proteins, members of different kinetochore sub-complexes, showed an asymmetric pattern of distribution that was restricted to the mother cell lineage. This asymmetry was not seen during vegetative growth but was restricted to the cell divisions following meiosis. Clearly, the kinetochores between mothers and daughters are different in the mitotically dividing cells that are formed immediately after meiosis. It is not known if the chromosomes are segregated in a non-random fashion with respect to replicative age of the DNA during mitosis in those cells.

There is clearly non-random segregation of the SPB during vegetative growth as the "old" SPB is preferentially segregated into the bud (Jaspersen and Winey 2004). Biased SPB segregation might result because cytoplasmic microtubules are nucleated on the cytoplasmic face of the old SPB during SPB duplication and the microtubules are therefore preferred to position the nucleus and move it into the bud. Any chromatid preferentially attached to the old SPB would show non-random segregation with mother–daughter bias (Winey and O'Toole 2001). Perhaps some form of SSIS assures the attachment of one or more kinetochores, assembled in the previous cell cycle, to the old

spindle pole and directs its segregation to the daughter cell.

Tandem copies of the tet or lac operator form arrays that have been used extensively to observe behavior of individual centromeres and chromosomes in yeast (Dewar et al. 2004; Goshima and Yanagida 2000; Straight et al. 1997). Arrays can be precisely directed, by site-directed recombination, to sequences adjacent to individual centromeres in cells expressing fluorescently tagged tet and lac repressors. The fluorescent repressors bind tightly to the arrays at the centromere-linked sequences and can be observed in real time. Tanaka and colleagues have used this extensively to study the behavior of individual centromeres including a study to directly test if centromeres remain continuously attached to the old SPB (Dewar et al. 2004; Kitamura et al. 2007; Tanaka et al. 2000; Tanaka et al. 2002). They tagged *CEN5* and *CEN15* with fluorescent proteins and showed that they associate with the SPB early in the cell cycle, as expected. They showed that there is transient detachment of the centromeres from the SPB early in S phase that lasts for 1–2 min and is dependent on DNA replication. Two outer kinetochore proteins transiently lose their association with *CEN5* and *CEN15* DNA, suggesting that the outer kinetochore is disassembled upon DNA replication. The authors suggest that kinetochores disassemble upon DNA replication at the time when centromeric DNA is replicated early in the S phase (Kitamura et al. 2007). The kinetochores rapidly reassemble and the centromeres re-associate with the SPB. The authors argue compellingly that this re-association is with the old SPB. Overall, the disassembly and reassembly of kinetochores would provide a mechanism for random segregation of sister chromatids if the reassembled kinetochores attached to the old SPB in a random manner. However, it does not disprove the possibility of biased segregation for some chromosomes. The experiment could not distinguish the replicative age of the chromatids of chromosome V and chromosome XV. In addition, only 2 of the 16 yeast chromosomes were analyzed, and it is possible that one or more kinetochores are not disassembled in the same manner. Furthermore, it is easy to imagine an SSIS mechanism that marks a centromere DNA sequence or the chromatin associated with the centromere during DNA replication, making the sister centromeres different. If the mark resulted in different rates of kinetochore assembly on the sisters, then the kinetochore that assembled first would attach to the old SPB and would be preferentially segregated to the daughter cell.

Biased segregation of budding yeast chromosomes was tested recently using a highly quantitative assay that followed the fate of all 16 chromosomes and their distribution to mother and daughter cells (Keyes et al. 2012). The experiments took advantage of improved strains for exogenous thymidine labeling where the cells utilize thymidine with greater efficiency (Lengronne et al. 2001; Viggiani and Aparicio 2006). The cells do not delay in S phase as did previous strains, and they have been used for a variety of experiments (Viggiani et al. 2010; Schwob et al. 2009). The experimental design for testing biased chromosome segregation is shown in Fig. 2. *MAT***a** cells, sensitive to the mating pheromone, were arrested in G1 by exogenous addition of α-factor. The cell surfaces were labeled covalently with biotin, the α-factor was removed, and the cells were released to the cell cycle in the presence of BrdU. α-factor was re-added once cells budded to limit the experiment to a single cell cycle. The new buds of the daughter cells were distinct from the mother cells because they were not biotinylated. Therefore, the cells arrested in the subsequent G1 with hemi-labeled DNA and with biotinylated mothers and un-biotinylated daughters. Mothers were purified from the daughters using magnetic streptavidin-containing beads to get pure populations. The DNA was extracted, denatured, and the BrdU-containing strands produced during DNA replication were purified by immunoprecipitation. The DNA was labeled and hybridized to Affymetrix microarrays commonly used for gene expression analysis. Yeast genes are densely packed in the yeast genome and are oriented 5′ to 3′ on either the Watson or Crick strand and are therefore transcribed from either strand. Expression arrays interrogate both the Watson and Crick strands. The question was whether the Watson strand (or Crick strand) for any chromosome was equally distributed to the mother and daughter cells. One way to represent the data is to plot the distribution of the log2 ratios of intensities for each gene in mothers and daughters (log2(mother/daughter)) for both the Watson and Crick strands. An example is shown for chromosome IV in Fig. 2b. Random segregation of chromatids predicts that the mean of the log2 ratio (mother/daughter) should be centered on zero, as is apparent for chromosome IV. Any deviation from log2 ratios of zero is evidence of

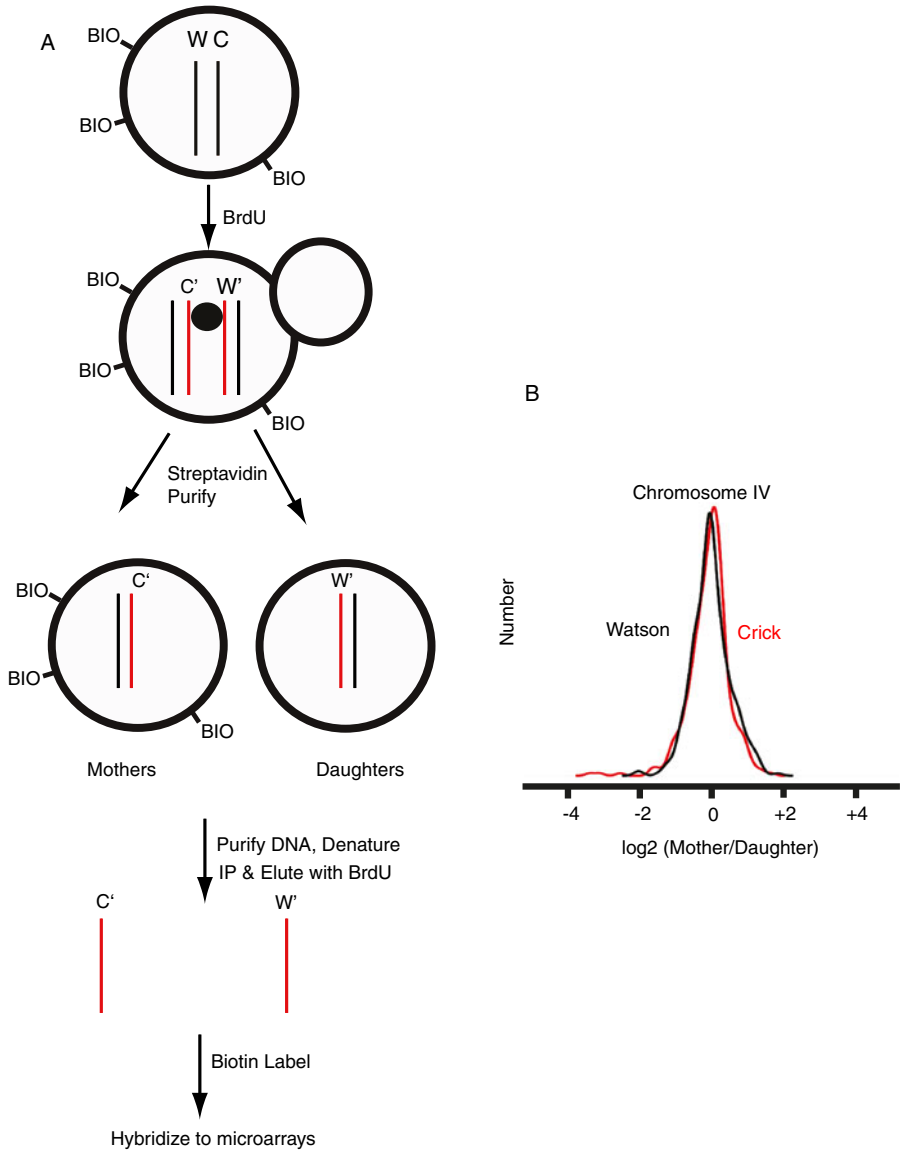

Fig. 2 Strategy for determining if there is mother–daughter bias in chromatid segregation in yeast. **a** Cells arrested in G1 by growth in the presence of pheromone are labeled in vitro with biotin (BIO) and released to the cell cycle in the presence of BrdU (*red*) to generate hemi-labeled DNA strands. The original Watson and Crick strands are indicated (W and C) as are the newly synthesized strands (W′ and C′). Pheromone is added during S phase to limit the experiment to a single cell cycle. Daughters in the second cell cycle are unlabeled with respect to biotin and are separated from the mothers using streptavidin beads. DNA from mothers and daughters are isolated separately, denatured, and the BrdU strand recovered by immunoprecipitation. The DNA is labeled and hybridized to microarrays containing probes to the Watson and Crick strands of DNA. **b** The amount of hybridization to Watson-containing probes and Crick-containing probes is quantified and the distribution is plotted showing the log2 transformed ratio of intensities for mothers/daughters for the Watson (*black*) and Crick (*red*) strands. The data for chromosome IV is shown and a mean of zero is indicative of random segregation

non-random segregation of a chromatid. Plots for all 16 chromosomes were similar to that for chromosome IV, suggesting that all chromosomes segregated randomly. The hypothesis was confirmed with a Wilcoxon signed-rank sign test to test the null hypothesis that the mean of the distribution of the log2 mother/daughter ratios for probes to the Watson and Crick strands for each chromosome were equal to zero. The data clearly demonstrated, with greater than 95 % confidence, that there is random segregation of chromatids with no mother–

daughter bias for all 16 chromosomes in yeast grown under standard laboratory conditions.

There are other cell divisions in the life cycle of budding yeast that may be more specialized including the fist cell divisions of cells derived from a spore after meiosis. The differential behavior of cells with respect to asymmetric distribution of kinetochore proteins to the mother and daughter cell may reflect a "stem-like" quality of yeast cells imparted during meiosis (Thorpe et al. 2008). There is a similar time in the life cycle of yeast cells that may be "stem-like". Haploid cells that mate produce a diploid zygote that buds to produce the first daughter cell that is diploid. It is tempting to speculate that this division is akin to self-renewable stem cells. The immortal strand hypothesis does not operate in the vegetative growth of cells of a commonly used yeast strain (W303) under standard laboratory conditions. But the idea is not dead and the question not fully resolved. The methodology outlined in Fig. 2 should be adaptable to determine if there is biased segregation of chromatids during mitosis of those and any other yeast cell divisions.

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