Random Segregation of Chromatids at Mitosis in *Saccharomyces cerevisiae*

Mark W. Neff and Daniel J. Burke

Department of Biology, University of Virginia, Charlottesville, Virginia. 22901

Manuscript received May 17, 1990
Accepted for publication November 17, 1990

ABSTRACT

Previous experiments suggest that mitotic chromosome segregation in some fungi is a nonrandom process in which chromatids of the same replicative age are destined for cosegregation. We have investigated the pattern of chromatid segregation in *Saccharomyces cerevisiae* by labeling the DNA of a strain auxotrophic for thymidine with 5-bromodeoxyuridine. The fate of DNA strands was followed qualitatively by immunofluorescence microscopy and quantitatively by microphotometry using an anti-5-bromodeoxyuridine monoclonal antibody. Chromatids of the same replicative age were distributed randomly to daughter cells at mitosis. Quantitative measurements showed that the amount of fluorescence in the daughter nuclei derived from parents with hemilabeled chromosomes diminished in intensity by one half. The concentration of 5-bromodeoxyuridine used in the experiments had little effect on the frequency of either homologous or sister chromatid exchanges. We infer that the 5-bromodeoxyuridine was distributed randomly due to mitotic segregation of chromatids and not via sister chromatid exchanges.

FAITHFUL replication of DNA and the subsequent distribution of sister chromatids during mitosis is required at every round of cell division to assure the survival of the resulting daughter cells. Several mechanisms ensure the fidelity of DNA synthesis (GOODMAN 1988), but the molecular mechanisms by which sister chromatids disjoin and segregate to opposite poles remain obscure. In the yeast *Saccharomyces cerevisiae*, where the accuracy of mitotic chromosome segregation has been determined by genetic tests, chromosomes are segregated inaccurately once in every 10^5 cell divisions (HARTWELL *et al.* 1982, HARTWELL and SMITH 1985). The frequency of chromosome loss provides a minimal estimate of the fidelity of sister chromatid disjunction. Various models have been proposed to explain how sister chromatids disjoin from each other and segregate toward opposite mitotic poles (LARK 1966; PICKETT-HEAPS, TIPPET and PORTER 1982; MURRAY and SZOSTAK 1985; MCINTOSH and KOONCE 1989). One of the simplest, conceptually, is that the entire set of chromosomes maintains a permanent attachment to the original pole and after DNA synthesis, the unattached set of chromatids must establish attachments and segregate to the newly duplicated (opposite) pole (LARK 1966). Chromatids in G_2 would make their attachments via those DNA strands that had been used as templates in DNA replication. The resulting "nonrandom segregation" would therefore depend upon the "replicative age" of the chromosomes and would assure that sister chromatids are oriented to opposite poles at mitosis.

Chromatid sets of the same replicative age segregate randomly in the majority of eucaryotic cells that have been examined (GEARD 1973; FERNANDEZ-GOMEZ, TORRE and STOCKERT 1975; MAYRON and WISE 1976; MORRIS 1977; ITO and MCGHEE 1987; ITO, McGhee and SCHULTZ 1988). However, there are some examples of nonrandom segregation (LARK 1966, 1967, 1969; LARK, CONSIGLI and MINODIA 1966; ROSENBERGER and KESSLER 1968; WILLIAMSON and FENNELL 1981) and it has been suggested as a possible mechanism for stem cell maintenance during development (CAIRNS 1975; POTTEN *et al.* 1978). A nonrandom model of chromatid segregation was proposed several years ago for the partitioning of the *Escherichia coli* chromosome (LARK 1966), and is supported by recent experiments (HELMSTETTER and LEONARD 1987; OGDEN, PRATT and SCHAECHTER 1988). There is also evidence that the multinucleate fungus *Aspergillus nidulans* segregates DNA strands by a nonrandom mechanism during mitosis (ROSENBERGER and KESSEL 1968). WILLIAMSON and FENNEL (1981) demonstrated nonrandom segregation of mitotic chromosomes in the yeast *S. cerevisiae*. The phenotypes of some yeast mutants, like *ndc1*, which shows a complete failure in chromosome segregation at mitosis and at meiosis II, could be explained by a failure in a nonrandom segregation process (THOMAS and BOTSTEIN 1986). Mating type switching in the yeast *Schizosaccharomyces pombe* is dependent on asymmetric inheritance of DNA strands (KLAR 1987a). The nonrandom segregation of chromosomes in three species of fungi may be explained by some common feature of mitosis in these organisms.

We used a more refined technique to examine

TABLE 1

***Saccharomyces cerevisiae* strains**

Strain	Genotype	Source
Haploids:		
155	*MATα ade1 ade2 leu2-3,112 trp1-289 ura3-52 lys2 can1 cyh2 tmp1-6 tut1* (pJM81) *rho*$^+$	R. SCLAFANI
167	*MAT***a** *ade1 ade2 leu2-3,112 trp1-289 ura1 lys2 tmp1-6 tut1-2* (pJM81) *rho*$^+$	R. SCLAFANI
1101	*MAT***a** *ade1 ade2 leu2-3,112 trp1-289 ura1 lys2 tmp1-6 tut1-2* (pJM81) *rho*$^-$	This study
1102	*MAT***a** *ade1 ade2 leu2-3,112 trp1-289 ura1 lys2 tmp1-6 tut1-2* (pJM81) *rho*^{0}	This study
421	*MAT***a** *leu2-3,112 trp1-289 his7 lys2 rho*$^+$	R. SCLAFANI
5373	*MAT***a** *ade1 ade3 leu2-3,112 trp1-289 ura3-52 can1 cyh2 tmp1-6 tut1-2* SCE::*URA3* (pJM81) *rho*$^+$	This study
8202	*MAT***a** *ade2 ade3 leu1 trp1-289 ura3-52 sap3 can1 cyh2* SCE::*URA3 rho*$^+$	L. KADYK and L. HARTWELL
Diploids:		
721	*MAT***a** *ade2 leu2-3,112 tmp1-6 tut1-2 trp1-289 lys5 cyh2 leu1* / *Matα ade2 leu2-3,112 tmp1-6 tut1-2 trp1-289 LYS5 CYH2 LEU1*(pJM81) *rho*$^+$	This study
5899	*MAT***a** *leu2-3,112 trp1-289 ura3-52 lys5 cyh2* / *MATα leu2-3,112 trp1-289 ura3-52 LYS5 CYH2rho*$^+$	BURKE, GASDASKA, and HARTWELL (1989)
520	*MAT***a** *leu2-3,112 ura3-52* Circular Chr. *III*::pDB30 / *MATα leu2-3,qqw ura3-52* Linear Chr. *III* *rho*$^+$	This study
521	*MAT***a**- *leu2-3,112 ura3-52 ade1 tmp1-6 tut1-2 trp1-289 cyh2* / *MATα leu2-3,112 ura3-52 ade1 tmp1-6 tut1-2 trp1-289 cyh2* Circular Chr. *III*::pDB30 / Linear Chr. *III* (pJM81) *rho*$^+$	This study

segregation of chromatids during mitosis in the yeast *S. cerevisiae* in order to address the molecular mechanism responsible for nonrandom segregation. We used an auxotrophic strain that efficiently utilizes thymidine (TdR) to label the DNA with the thymidine analogue 5-bromodeoxyuridine (BUdR) (SCLAFANI and FANGMAN 1986) and the BUdR was detected by immunofluorescence using a commercially available monoclonal antibody. We show, in contrast to the earlier study, that chromatids of the same replicative age are distributed randomly at mitosis. We used two genetic assays to determine that there is little sister chromatid exchange under our experimental conditions and conclude that the distribution of labeled DNA is due to random segregation of sister chromatids at mitosis and not to sister chromatid recombination. In this regard, mitosis in *S. cerevisiae* is similar to mitosis in higher eucaryotic cells.

MATERIALS AND METHODS

Yeast strains: Genotypes and sources of the strains used are listed in Table 1. Strains were constructed by standard genetic methods (SHERMAN, FINK and HICKS 1986). Strains auxotrophic for thymidine (*tmp1 tut1*, carrying a viral TK gene) were maintained as described by SCLAFANI and FANGMAN (1986). Isogenic petite strains were isolated after growth in 10 μg/ml of ethidium bromide (SHERMAN, FINK and HICKS 1986). Petite (rho$^-$) strains were detected by their inability to use glycerol as a carbon source, while petite strains lacking mitochondrial DNA (rho^0) were identified as devoid of extranuclear staining with 4′6,-diamidino-2-phenylindole (DAPI) (Sigma) (0.5 μg/ml). In addition, DNA prepared from rho^0 strains did not yield a mitochondrial DNA band after isopycnic centrifugation in cesium chloride gradients containing Bisbenzamide 33258 (GARGOURI 1989). Unequal sister chromatid exchange (SCE) was measured using a construct containing a duplication of *ade3* and *URA3* sequences kindly provided by LISA KADYK and LEE HARTWELL. The SCE construct was confirmed by the presence of the *URA3* phenotype and by gel transfer of genomic DNA and hybridization using a radioactively labeled probe (SAMBROOK, FRITSCH and MANIATIS 1989). Homologous sister chromatid exchange was detected using a circular derivative of chromosome *III* (provided by JIM HABER) as described by HABER, THORBURN and ROGERS (1984). Strains 520 and 521 were constructed by integrating a plasmid (pDB30) containing the *URA3* gene into the circular derivative of chromosome *III* between *LEU2* and the centromere.

Growth and media: YM-1, YEPD, and SC media were used as previously described (HARTWELL 1967; SHERMAN, FINK and HICKS 1986). Media were supplemented with TdR (Sigma) at a final concentration of 100 μg/ml. BUdR (Sigma) was added directly to the TdR-containing medium. Cultures grown in the presence of BUdR were maintained in the dark. Cell counts were performed by microscopy with the aid of a hemocytometer.

Immunofluorescence and flow cytometry: Immunofluorescence was performed similarly to the procedure of ADAMS and PRINGLE (1984) with some modifications. Cells were fixed directly in medium with 3.7% formaldehyde at room temperature for 1–2 hr. They were washed three times in phosphate-buffered saline solution (PBS), pH 7.2, and resuspended in buffer (pH 7.0) containing 1.2 M sorbitol, 0.12 M potassium phosphate, 33 mM citric acid, 25 mM β-mercaptoethanol. An aliquot of 200 μg/ml of zymolyase 100T (Seikagaku Kogyo Co.) was added and the cell walls were removed by incubating at 30° for 30 min to 1 hr. Cells were adhered to 1% polylysine-coated slides, permeabilized by a 30-min treatment with PBS + 0.5% Tween 20 and then transferred successively to 6 N hydrochloric acid for 5 min, 5 M sodium tetraborate, pH 8.5, for 5 min, and 5 M urea for 20 min, all at room temperature. The slides were

washed twice in PBS before the addition of antibody.

A mouse monoclonal anti-BUdR antibody (Becton Dickinson) was diluted 1:10 before use and the monoclonal rat-anti-tubulin antibody Yol 3/4 (Serotec) was diluted 1:250. Rhodamine-conjugated goat-anti-mouse (rat IgG adsorbed) (Jackson Immunoresearch) and fluorescein-conjugated goat-anti-rat (Cappel) secondary antibodies were both diluted 1:400. All antibody dilutions were made with PBS containing 1% bovine serum albumin and 0.1% sodium azide. The primary antibody dilutions were incubated on the slides overnight at 4° while the secondary dilutions were incubated for 2 hr at room temperature. Mounting medium pH 9.0 consisted of 0.5 μg/ml DAPI, 1 mg/ml phenlyenediamine, 90% glycerol, and 10% PBS (ADAMS and PRINGLE 1984). The DAPI was used to stain DNA, but the staining was faint due to the acid, sodium tetraborate and urea treatments of the cells. Cells were viewed and photographed using a Zeiss axiophot microscope equipped with epifluorescence and a 100 Watt mercury-arc lamp. Greater than 100 cells were counted in each experiment to determine the fraction of labeled cells. Flourescence of individual nuclei was quantified according to the manufacturers instructions using a Leitz MPV photometer attached to a Leitz Laborlux D microscope equipped with epifluorescence. Rhodamine fluorescence was detected using filters that resulted in excitation by 546 nM light and emission greater than 590 nM. Flourescein isothicyanate fluorescence was detected using filters that resulted in excitation between 450–490 nM and emission at 520 nM. The DNA content of individual cells was measured by flow cytometry of propidium iodide stained cells as previously described (BURKE, GASDASKA and HARTWELL 1989).

Genetic assays: Mitotic recombination was measured using diploid strains heterozygous for the *cyh2* and *trp5* mutations (strains 5899 and 721). The strains were grown to stationary phase in medium containing TdR or in medium containing TdR and BUdR. The cells were sonicated, diluted in water and dilutions were plated onto YEPD plates containing 100 μg/ml TdR, to obtain the total number of cells and dilutions were plated onto YEPD containing 100 μg/ml of TdR plus 10 μg/ml of cycloheximide to obtain the frequency of cycloheximide resistance. The frequency of unequal exchange between sister chromatids was determined by using strains (8202 and 5373) that contained heteroalleles of *ade3* separated by bacterial plasmid sequences containing the *URA3* gene. Revertants arising by unequal SCE were detected after growth to stationary phase in SC-URA medium containing 100 μg/ml TdR or medium containing TdR and the indicated amount of BUdR. Cells were sonicated, diluted with water and plated onto YEPD containing 100 μg/ml TdR to obtain the total number of cells and revertants were selected by plating dilutions on SC plates lacking histidine (LISA KADYK, personal communication) containing 100 μg/ml of TdR. Equal sister chromatid exchanges were detected by growing cells to stationary phase in YM-1 containing 100 μg/ml TdR or medium containing TdR and BUdR, washing the cells in YM-1, diluting them 100 fold and growing them to stationary phase in YM-1 containing 100 μg/ml TdR. The purpose of the outgrowth in YM-1 plus TdR was to allow for the phenotypic lag associated with loss of *URA3*, although pilot experiments showed no difference in the results when the outgrowth was omitted. Cells were sonicated and dilutions were plated onto YEPD containing 100 μg/ml TdR to obtain the total number of cells. Dilutions were plated onto medium containing 5-fluoro-orotic acid (FOA) to select for monosomes and the monosomes were confirmed by mating.

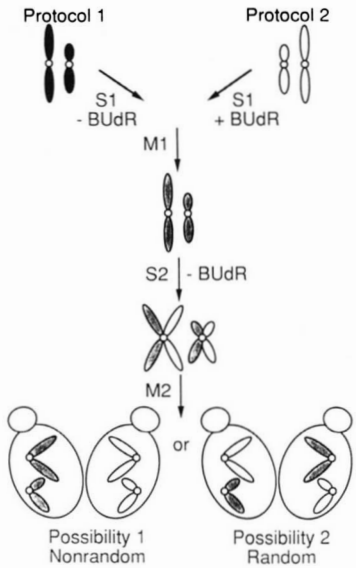

FIGURE 1.—Protocols for labeling chromosomal DNA with BUdR. Two different protocols were used to obtain hemisubstituted chromosomes. For protocol 1, cells were grown continuously for five generations in medium containing 96 μg/ml of thymidine plus 4 μg/ml BUdR. Cells were washed free of BUdR by filtration and resuspended in medium containing 100 μg/ml thymidine and allowed to proceed through two more rounds of replication in the absence of BUdR (− BUdR). In protocol 2, cells were grown in medium containing 100 μg/ml of thymidine and synchronized in the G_1 stage of the cell cycle. BUdR was added to 4 μg/ml and the cells proceeded through one round of DNA synthesis (+ BUdR). The cells were washed free of BUdR and allowed to proceed through another round of DNA synthesis in medium containing 100 μg/ml of thymidine. Both protocols should result in metaphase chromosomes, prior to the second mitosis, with one hemilabeled chromatid containing BUdR (in one of the two DNA strands) and the other unlabeled chromatid containing only thymidine. Three different chromosomes are shown. The dark chromosomes are fully labeled and have BUdR incorporated in both DNA strands. The gray chromosomes are hemi-labeled and have BUdR incorporated in one of the two DNA strands. The clear chromosomes are unlabeled and have only TdR in the DNA strands and are therefore unstained by the anti-BUdR antibody. S1 and S2 refer to the first and second rounds of DNA synthesis; M1 and M2 refer to the first and second mitosis. The two possible outcomes of the second mitosis are that all of the BUdR-containing chromatids are inherited into the same cell (possibility 1) or that the BUdR-containing chromosomes segregate randomly at M2 (possibility 2).

RESULTS

Experimental design and predictions: Our initial experiments were similar in design to those of WILLIAMSON and FENNEL (1981) and analogous to procedures used to obtain chromosomes substituted with BUdR in mammalian tissue culture cells to distinguish sister chromatids (WOLFF and PERRY 1974). Cells were grown for greater than five generations to obtain DNA with BUdR incorporated into both strands (protocol 1, Figure 1). The internal pool of TdR in yeast is small and the degree of BUdR substitution is proportional to the amount of BUdR added to the medium (SCLAFANI and FANGMAN 1986). The BUdR was removed by filtration and the cells grown for two

more rounds of DNA synthesis in medium containing only TdR. The chromosomes at the second metaphase should have one chromatid hemisubstituted with BUdR and the other chromatid containing only TdR in the DNA. If segregation is nonrandom, then one of the daughter cells should inherit all of the BUdR-containing DNA (possibility 1, Figure 1). If the labeled chromatids segregate randomly, then both daughter cells would inherit labeled DNA (possibility 2, Figure 1). Although mitotic chromosomes of *S. cerevisiae* are not visible cytologically (BYERS 1981), we could distinguish between random and nonrandom segregation of chromatids by observing the distribution of BUdR in whole nuclei of daughter cells. The model of nonrandom segregation predicts that one half of the nuclei should contain all of the BUdR after two divisions. In contrast, if chromosomes segregate randomly, then the proportion of cells with no BUdR should be $1/2^{n-1}$ where n = the number of chromosomes. For *S. cerevisiae*, with $n = 16$ (MORTIMER *et al.* 1989), the frequency of unlabeled nuclei should be approximately 10^{-5}. Therefore the predictions in the experiment are that the proportion of unlabeled nuclei will be either $1/2$ or $1/2^{15}$.

BUdR-labeling conditions: The use of thymidine auxotrophs, *tmp1 tut1* strains, for labeling DNA has been described in detail (SCLAFANI and FANGMAN 1986). The *tmp1* mutants cannot synthesize dTMP from dUMP. The strains are transformed with a multicopy plasmid, pJM81, that has a modified Herpes TK gene that provides both thymidine kinase and dTMP kinase activities to permit use of exogenous TdR. *tut1* mutations permit efficient utilization of TdR and BUdR as substrates in DNA synthesis. Our initial objective was to determine BUdR labeling conditions that would provide adequate staining in double-label immunofluorescence. Two control experiments convinced us that the immunofluorescence was due to the BUdR incorporated into DNA. First, cells of strain 421 (*MATa TMP1 TUT1*), grown in medium where 4% of the added nucleoside was BUdR, were unstained by the antibody. Second, cells of strain 167 (*MATa tmp1 tut1*) were unstained if grown in medium containing only TdR. This suggested that the immunofluorescence was due to BUdR incorporation into DNA and dependent on the *tmp1* and *tut1* mutations. Labeling the DNA during our experiments required choosing a concentration of BUdR that was low enough to minimize DNA recombinational repair, especially sister chromatid exchanges (SPEIT and VOGEL 1986; KAUFMAN 1988). However, the immunofluorescence had to be sufficiently sensitive to allow us to detect a 10-fold dilution of the BUdR. If the chromosomes segregated randomly after the second round of DNA synthesis the intensity of BUdR staining would diminish. We grew strain 1102 (*MATa tmp1 tut1 rho^0*) in medium containing various concentrations of BUdR and determined the fluorescence intensity in labeled nuclei by microphotometry. The data, Figure 2, show a linear relationship between fluorescent staining and the amount of BUdR in the medium. We chose to grow the cells in medium containing 96 μg/ml of TdR and 4 μg/ml of BUdR (4% of the added nucleoside as BUdR) for the experiments because we obtained bright, fluorescently labeled nuclei in each cell and we could detect BUdR fluorescence of reduced intensity in cells grown in medium containing 0.4% of the added nucleoside as BUdR. *tmp1 tut1* strains grown in medium containing 4 μg/ml BUdR, 96 μg/ml TdR had a generation time of 250 minutes compared to 200 minutes for congenic *TMP1 TUT1* strains. We estimate that at least 85% of the *tmp1 tut1* cells are cycling when grown in medium containing 4% of the added nucleoside as BUdR.

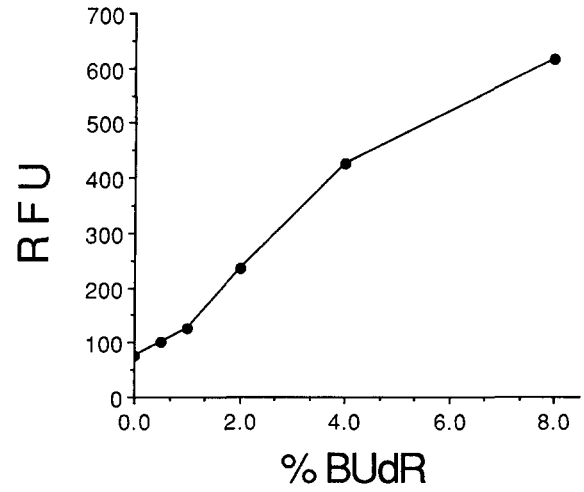

FIGURE 2.—BUdR titration by microphotometry. Cells of strain 1102 (*MATa tmp1 tut1 rho^0*) were grown to stationary phase in medium containing different concentrations of BUdR and stained with the anti-BUdR monoclonal antibody. The cells were stained with a rhodamine conjugated secondary antibody and the amount of rhodamine fluorescence in unbudded cells was determined by microphotometry. Ordinate—relative fluorescent units (RFU); abscissa—percentage of added nucleoside in the medium that is BUdR (%BUdR).

Distribution of *tmp1 tut1* cells within the cell cycle: The experimental design required that BUdR-labeled cells be grown for two rounds of DNA synthesis in medium containing TdR to determine if chromatid segregation was random with respect to replicative age. Cells that were in the G_1 stage of the cell cycle when the BUdR was removed would require two cell divisions to complete two rounds of DNA synthesis. However, cells that had initiated DNA synthesis (or post-S phase) at the time BUdR was removed must divide once before initiating the first round of DNA synthesis in the absence of BUdR and therefore require three divisions to complete two rounds of DNA synthesis. We measured the distribution of cells within the cell cycle to determine the number of cell

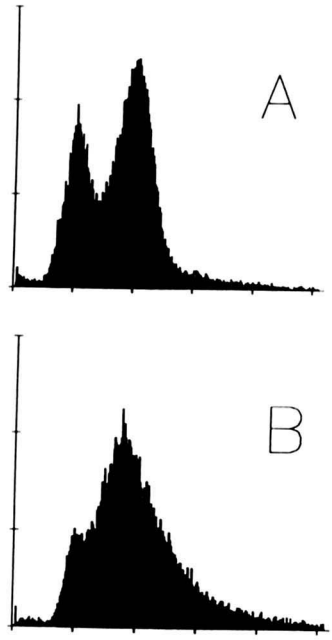

FIGURE 3.—Distribution of *tmp1 tut1* cells in the cell cycle. Asynchronous populations of cells were fixed, stained with propidium iodide, and the relative fluorescence per cell determined by flow cytometry. A, *TMP1 TUT1* cells (haploid strain 421) grown in YM-1 medium. B, *tmp1 tut1* cells (haploid strain 167) grown in YM-1 medium containing 96 μg/ml thymidine and 4 μg/ml BUdR. Ordinate—number of cells; abscissa—relative fluorescence.

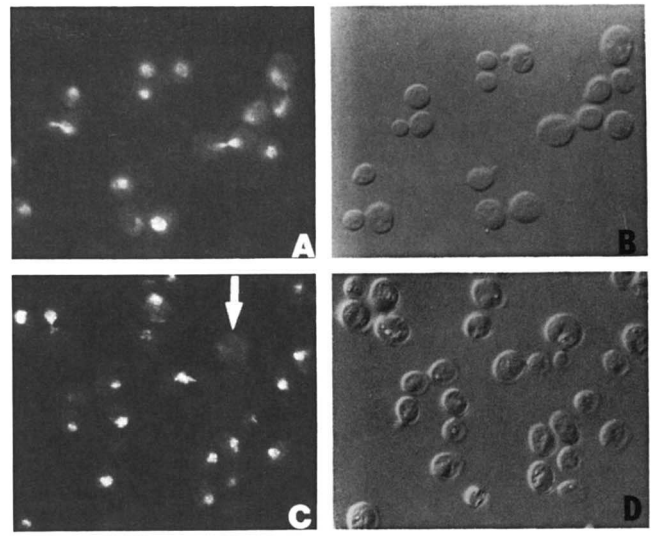

FIGURE 4.—Immunofluorescent staining of nuclei with BUdR-containing DNA of strain 1101(*MAT***a** *tmp1 tut1 rho*$^-$) by protocol 1. A and B, Cells grown in BUdR for five generations. C and D, Cells after three divisions in the absence of BUdR. Anti-BUdR staining is in A and C, and differential interference contrast images of the same cells are in B and D. The cell indicated by the arrow in panel C stained faintly with the anti-BUdR antibody and was unstained with YOL/34, an antitubulin antibody.

divisions required after removing the BUdR. Cells from strains with *tmp1 tut1* mutations grow slowly compared to cells of a congenic wild type strain (SCLAFANI and FANGMAN 1986). We determined, using phase contrast microscopy, that only 12% of the cells were unbudded, a characteristic of cells in the G_1 phase of the cell cycle (PRINGLE and HARTWELL 1981). We analyzed the DNA content of individual cells by flow cytometry. Figure 3 shows the fluorescence distribution among cells of a wild type strain and among a *tmp1 tut1* strain grown in medium containing 4% of the added nucleoside as BUdR. Wild-type cells show two peaks of fluorescence corresponding to the DNA content of cells in the G_1 phase of the cell cycle prior to DNA synthesis or cells in the G_2/M phases that have completed DNA replication but have not yet divided. The two populations are separated by a number of cells that are in the S phase of the cell cycle. The *tmp1 tut1* strain had fewer cells with a 1n content of DNA compared to wild type and the majority in either S phase or later in the cell cycle. These data confirm the cytological observations and suggest that the majority of cells (approximately 90%) would require three cell divisions to complete two rounds of DNA synthesis when BUdR was removed.

BUdR is distributed randomly after three divisions: We grew haploid strain 155 (*MATα tmp1 tut1*) and strain 167 (*MAT***a** *tmp1 tut1*) for at least five generations in medium containing 4% of the added nucleoside as BUdR to obtain fully substituted DNA strands (protocol 1, Figure 1). After cells reached a cell density of 10^6/ml, they were washed extensively by filtration, resuspended in medium containing only TdR and grown for three divisions (final cell density approximately 1×10^7 per ml). Cells were sampled after each doubling and prepared for immunofluorescence. A representative photograph of a *tmp1 tut1* strain (strain 1101) is shown in Figure 4. Cells fully labeled with BUdR stain with the anti-BUdR antibody (Figure 4A). Greater than 95% of the cells sampled after the third division (Figure 4C) stained with the anti-BUdR antibody. There was always a small proportion of unlabeled nuclei in the samples (arrow in Figure 4C), even among the cells grown continuously in BUdR. To determine if these cells were impermeable to the antibodies, we included an antitubulin antibody to detect intranuclear microtubules as a control for nuclear permeability (data not shown). Cells that were unstained with the anti-BUdR were likewise unstained with the tubulin antibody confirming that the lack of staining in these cells was due to impermeability.

WILLIAMSON and FENNEL (1981) reported nonrandom segregation using a respiratory deficient (*rho* $^-$) strain but the pattern appeared random in an isogenic respiratory proficient (*rho*$^+$) strain. We constructed the isogenic petite strain 1101 (*MAT***a** *tmp1 tut1 rho*$^-$) and repeated our experiments. We found, as before, that the BUdR was distributed to over 94% of the daughter cells after three divisions (Figure 4). In total, the fraction of labeled nuclei after two rounds of DNA

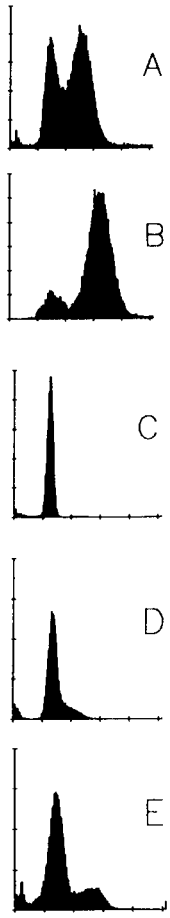

FIGURE 5.—Cell synchrony obtained for labeling DNA by protocol 2. Populations of cells were stained with propidium iodide and analyzed by flow cytometry. A, Asynchronous *TMP1TUT1* cells from strain 421. B, *TMP1 TUT1* cells from strain 421 treated with 15 μg/ml nocodazole. C, Small unbudded cells, from strain 1102 (*MATa tmp1 tut1 rho*^{0}), isolated by centrifugal elutriation. D, Small unbudded cells from C isolated after one round of DNA synthesis in medium containing 4% of the nucleoside as BUdR. E, Cells from D arrested in the following cell cycle with α-factor.

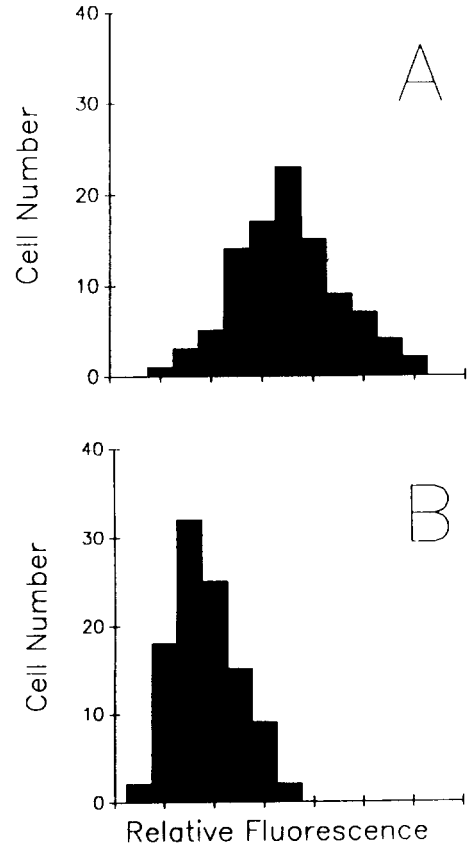

FIGURE 6.—Quantitative immunofluorescence of individual nuclei by microphotometry. Cells from strain 1102(*MATa tmp1 tut1 rho*^{0}) labeled with BUdR by protocol 2 were stained with the anti-BUdR antibody and detected with a rhodamine conjugated secondary antibody. A, The distribution of fluorescence in the G1 cells (Figure 4D) with hemisubstituted DNA strands. B, The distribution of fluorescence in G_1 cells (Figure 4E) derived from A by a single round of cell division.

synthesis in the absence of BUdR was 97 ± 2.5% (five experiments, four with grandes and one with petites)

The distribution of BUdR is random: The previous experiment approximates, as closely as possible, that of WILLIAMSON and FENNEL (1981) and yet we obtained different results. We were concerned that the difference arose in some way because of experimental design. In particular, we had a population of cells distributed randomly in the cell cycle and hence required different numbers of cell divisions to complete two rounds of DNA synthesis. In addition, our immunofluorescence assay for the proportion of labeled cells was a qualitative measure of the distribution of the DNA labeled with BUdR. We repeated the experiment using protocol 2 (Figure 1), which was a more precise experiment that used synchronized cells so that all of the cells could be followed through exactly two rounds of DNA synthesis. In addition, we used microphotometry to quantify the intensity of staining in daughter cells from parents with hemilabeled chromatids. If the BUdR was randomly distributed the fluorescence intensity of the stained cells should be decreased by one half. We constructed strain 1102 (*MATa tmp1 tut1 rho*^{0}) that completely lacked mitochondrial DNA to eliminate any chance that the random segregation was due to some effect of the mitochondrial genome. We grew strain 1102 in medium containing only TdR, isolated unbudded cells by centrifugal elutriation (CROSS and SMITH 1988) and a sample of the cells was prepared for flow cytometry to confirm the 1n content of DNA (Figure 5C). The unbudded cells were grown in medium containing 4% of the added nucleoside as BUdR until the cell number doubled and the smallest cells were isolated by centrifugal elutriation. A sample was prepared for flow cytometry (Figure 5D) to confirm the 1*n* content of DNA. These unbudded cells were in the G_1 phase of the cell cycle with hemisubstituted chromosomes. After one more round of DNA synthesis in the absence of BUdR, the cells would contain chromosomes that have one hemisubstituted and one

TABLE 2

Effects of BUdR substitution on recombination

Genotype and (% of nucleoside as BUdR)	Mitotic[a] Recombination $Cyh^r \times 10^{-5}$	Unequal sister[b] Chromatid exchange $His^+ \times 10^{-5}$	Equal sister[c] Chromatid exchange $FOA^r \times 10^{-5}$
TMP1 TUT1 (0%)	0.4 (0.2)	2.3 (1.5)	3.9 (0.1)
tmp1 tut1 (0%)	0.8 (0.1)	2.8 (1.4)	3.1 (1.0)
tmp1 tut1 (4%)	1.5 (0.8)	3.5 (1.3)	2.7 (0.8)
tmp1 tut1 (25%)	3.2 (1.7)	10.5 (3.3)	3.4 (0.9)

These values are the means obtained from at least three independent trials and their (standard deviations).
[a] Diploid strains 5899 and 721 containing heterozygous markers on chromosome *VII* were used.
[b] Haploid strains 8202 and 5373 containing duplicate heteroalleles of *ade3* in tandem were used.
[c] Diploid strains 520 and 520 carrying a circular derivative of chromosome *III* were used.

unlabeled chromatid. The BUdR was removed by several washes, the cells were suspended in medium containing only TdR and grown until 90% of the cells had formed small buds. The mating pheromone, alpha factor, was added to a final concentration of 3 μM to arrest the cells in the G_1 stage of the following cell cycle (PRINGLE and HARTWELL 1981). Samples of cells were prepared for flow cytometry to confirm that the majority of cells were arrested by α-factor had the 1n content of DNA (Figure 5E). We prepared samples of BUdR labeled cells for immunofluorescence and measured the amount of fluorescence per cell by photometry. The prediction for nonrandom segregation of chromosomes is that one half of the cells would inherit all of the label while the prediction for random segregation is that all of the cells would be labeled but that the intensity would diminish by half. The data, presented in Figure 6, show that the relative fluorescence intensity is reduced by one half from a mean value of 333 to 167 relative fluorescence units. These data are consistent with the model of random segregation of chromatids. However, the data might be explained by nonrandom segregation of chromatids with a high rate of sister chromatid exchange.

BUdR had little effect on recombination: When cells of a *tmp1 tut1* strain are grown vegetatively, they spend a large portion of the cell cycle either during or after the time of DNA synthesis which could increase the opportunity for DNA exchanges to occur between chromosomes (PAINTER 1980). We assayed mitotic homologous exchange using a diploid strain (strain 721) heterozygous for a mutation that confers resistance to cycloheximide and selected for resistant cells after growth in different concentrations of BUdR. The data, Table 2, show that mitotic exchanges are stimulated eight fold after growth in 25 μg/ml BUdR, 75 μg/ml TdR, a sublethal concentration of BUdR that results in slow growth (data not shown). However, the frequency of mitotic recombination after growth in medium where 4% of the added nucleoside was BUdR was slightly elevated (less than fourfold) compared to the frequency obtained with a wild-type strain.

It was possible that BUdR had a slight effect on stimulating mitotic recombination but a dramatic effect on stimulating sister chromatid exchanges. A circular variant of chromosome *III* can be used to estimate the frequency of sister chromatid exchanges because a reciprocal recombination event between sister chromatids anywhere on the circular chromosome produces an unstable dicentric chromosome (HABER, THORBURN and ROGERS 1984). Chromosome instability can be detected by loss of the *MAT* allele (*MATa* for our strains) on the circular chromosome *III* resulting in a change in phenotype from nonmating **a**/α diploids to fertlile monosomics of the α mating type. We inserted the *URA3* gene into the ring chromosome adjacent to the *LEU2* gene so that losses could be selected using FOA (BOEKE, LACROUTE and FINK 1984) and confirmed the FOA-resistant cells as being monosomics that mated as α. We compared the frequency of loss of the circular chromosome *III* in a *TMP1 TUT1* strain with and without selection for FOA resistance. *MAT*α-maters were present at a frequency of 3.9×10^{-3} and FOA-resistant cells that were *MAT*α-maters were present at a frequency of 2.4×10^{-3}, frequencies quite comparable to each other and to that previously reported by HABER, THORBURN and ROGERS (1984), suggesting that FOA does not stimulate sister chromatid exchanges and does not affect the recovery of cells that have lost a chromosome. The effect of BUdR substitution on the loss of the circular chromosome *III* is shown in Table 2. The frequency of loss of the circular chromosome *III* is the same in the *tmp1 tut1* strain and the *TMP1 TUT1* strain and the frequency is not increased by growth in BUdR. The circular derivative of chromosome *III* is approximately 2.5% of the total genome (MORTIMER *et al.* 1989) and the rate of loss is 1.2×10^{-3} per cell division (HABER, THORBURN and ROGERS 1984). Assuming that all of the losses are due to reciprocal sister chromatid exchanges and that the rate of exchanges for chromosome *III* reflects the rate for the entire genome, the total number of reciprocal sister chromatid exchanges per genome per cell division is

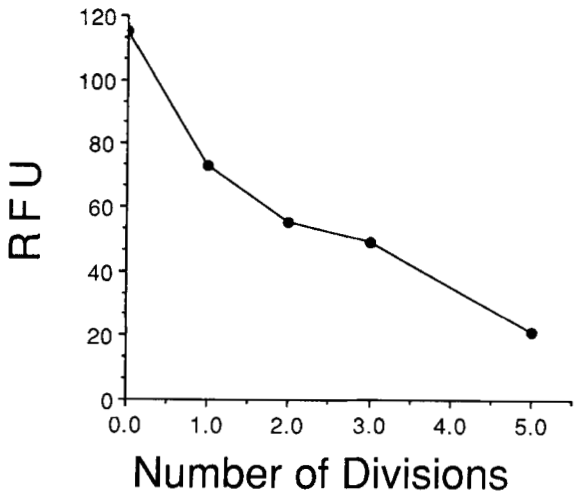

FIGURE 7.—Quantitative immunofluorescence of diploid nuclei following protocol 1. Cells of diploid strain 721(*Mata/Matα tmp1/tmp1 tut1/tut1*) were grown continuously in medium containing 96 μg/ml TdR and 4 μg/ml BUdR. The BUdR was removed and the cells grown in medium containing 100 μg/ml TdR. Cells were removed at times after removal of BUdR, stained with the anti-BUdR antibody and detected with a rhodamine conjugated secondary antibody. RFU = relative fluorescent units. Number of divisions = number of cell divisions in medium containing 100 μg/ml TdR.

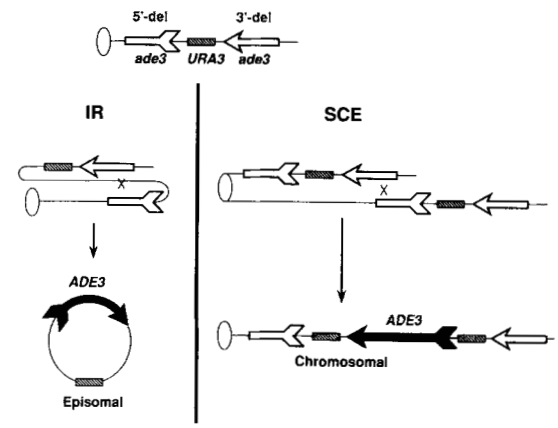

FIGURE 8.—Assay for unequal sister chromatid exchange. Strains 8202 and 5373 have duplicated heteroalleles (arrows) of *ade3*, one a 5′deletion (5′-del) and the other a 3′ deletion (3′-del) that are marked with the *URA3* gene (shaded square) integrated into chromosome *III*. The strains carry *ade3* mutations which result in requirements for both adenine and histidine. Two types of intrachromosomal events, selectable by histidine prototrophy, can restore wild type *ADE3* sequences. The first is intrachromatid recombination (IR), which occurs between the repeated sequences in the two *ade3* alleles and results in an episomal copy of *ADE3* that cannot be maintained because there is no origin of replication. The second type of recombination is between the repeated sequences on the sister chromatids (SCE) produced from a single round of DNA replication. The *ADE3* gene resulting from SCE is chromosomal and stable and therefore, only the products of SCE are detected in the assay.

0.05, less than 1 sister chromatid exchange event per genome per division.

BUdR did not stimulate sister chromatid exchanges to an extent that could account for the random distribution of BUdR via recombination rather than random chromosome segregation during mitosis. However, we were concerned that all of the segregation experiments performed by us and WILLIAMSON and FENNEL (1981) were done with haploid strains but that we assayed recombination using diploid strains. If haploids had a high rate of sister chromatid exchanges that obscured the nonrandom segregation and if the recombination was suppressed in diploids, then repeating the immunoflourescence experiments in diploids would reveal the non-random chromosome segregation. We repeated the BUdR labeling experiment using the diploid strain 721 (*Mata/Matα tmp1/tmp1 tut1/tut1*) following protocol 1 (Figure 1). After three cell divisions, the labeled nuclei were distributed to greater than 90% of the daughter cells. We quantified the amount of fluorescence per nucleus as a function of the number of cell divisions in TdR. The data, Figure 7, show that the average amount of fluorescence per nucleus diminished by one half at each division. The rate of mitotic reciprocal recombination between the *CYH2* locus and the centromere is 10^{-4} per division (D. BURKE unpublished observation) and the CYH2 to centromere distance is 80 cM (MORTIMER *et al.* 1989) or 2% of the genome. Assuming that the rate of mitotic recombination between *CYH2* and the centromere is representative of the entire genome, there is 0.005 recombination event/genome per division. Therefore the effect of BUdR on stimulating mitotic recombination (Table 2) would elevate the number of mitotic homologous exchanges to 0.018, approximately equal to the number of sister chromatid exchanges. The combined rate of homologous and sister chromatid recombination is insufficient to account for the random distribution of BUdR and we conclude chromatids segregate randomly at mitosis in diploid cells.

FASULLO and DAVIS (1987) reported a simple genetic test that can detect sister chromatid exchanges in haploids. We tested the effect of BUdR on sister chromatid exchange in haploids by using a similar test in strains 5373 and 8202 based on *ade3* heteroalleles, one a 5′ deletion and the other a 3′ deletion, in tandem and separated by bacterial plasmid DNA containing the *URA3* gene (constructed by LISA KADYK, personal communication). The *ade3* mutants were auxotrophic for both adenine and histidine but reverted to prototrophy by intrachromosomal (unequal sister chromatid) recombination (Figure 8). We measured the reversion to histidine prototrophy in both strains and tested the effect of growth in different concentrations of BUdR. The data, Table 2, show that unequal sister chromatid exchanges are stimulated by growth in 25% BUdR but not significantly by growth in medium where 4% of the added nucleoside was BUdR. We conclude that sister chromatid recombination cannot account for the random distri-

bution of BUdR in both haploids and diploids in our experiments and is due instead to random segregation of the chromosomes.

DISCUSSION

We have presented evidence that chromatids of the same replicative age segregate randomly at mitosis in the yeast *S. cerevisiae*. Our results differ from those of WILLIAMSON and FENNEL (1981), which may be explained in two ways. Firstly, we have used immunofluorescence, which offers greater resolution than whole cell autoradiography, to follow the fate of the DNA strands. WILLIAMSON and FENNEL (1981) used strains that were auxotrophic for adenine to label the DNA, followed by autoradiography. It is possible that incorporation of radioactive adenine was not restricted to DNA and therefore was measuring the fate of more than one macromolecule. A second possible explanation is that the different results in our experiments are explained by strain differences. WILLIAMSON and FENNEL (1981) reported variability of random *vs.* nonrandom segregation within their strains. Chromosomes appeared to segregate randomly in a respiratory proficient (*rho*$^+$) strain and nonrandomly in a respiratory deficient (*rho*$^-$) strain, which they explained as an effect of respiration on sister chromatid exchange. We have measured the extent of sister chromatid exchange by genetic methods and detect no difference in frequency between *rho*$^+$ and *rho*$^-$ strains (M. NEFF, unpublished observations). Perhaps there was something unique about the *rho*$^-$ strain used in the previous experiment that showed nonrandom segregation. There is also a chance that the strain differences in our experiments can be explained by differences in ploidy. Our data do not measure the extent of homologous sister chromatid exchanges in haploids and we cannot be certain of the relationship between the frequency of unequal sister chromatid exchanges and equal sister chromatid exchanges. Haploids and diploids differ in rates of mitotic recombination (FRIIS and ROMAN 1968) and it is possible that there are also differences in the rates of equal sister chromatid recombination. If the rate of sister chromatid exchanges in haploids grown in BUdR were at least twenty-five fold higher than in diploids grown in the same medium, then the BUdR could have been randomized by a recombinational mechanism. Unequal sister chromatid exchanges would have to be insensitive to this level of stimulation to be unaffected in the assay. Regardless of the explanation, either differences in strains or a fundamental difference between haploids and diploids, the phenomenon observed by WILLIAMSON and FENNEL (1981) is not an obligatory part of mitosis in *S. cerevisiae*.

Our data only allow us to rule out the most extreme model of nonrandom segregation, namely that all chromatids of the same replicative age co-segregate. Therefore the phenotype of mutants that diploidize, such as *ndc1*, cannot be explained by a failure to mark the chromosomes for nonrandom segregation (THOMAS and BOTSTEIN 1986). We cannot rule out the possibility that one (or a small number) of different chromatids of the same replicative age segregate to a specific spindle pole. There is precedent for some form of genomic imprinting in yeast. Asymmetric inheritance of the ability to switch mating type in *S. pombe* is due to imprinting a single chromosomal locus for at least one chromosome (KLAR 1987a). KLAR (1987b) tested if asymmetric mating type switching in *S. cerevisiae* could be explained by inheritance, in the mother cell, of a transcriptionally competent *HO* gene. His data suggest that there is no chromosomal imprinting at the HO locus that accounts for the pattern of mating type switching. It is still possible that switching in *S. cerevisiae* requires the action of some other gene that is asymmetrically expressed in mother cells due to chromosomal imprinting. Our experiments do not address asymmetric inheritance of a single chromosome.

Random segregation of chromatids has been documented in a variety of other eucaryotic organisms (GEARD 1973; FERNANDEZ-GOMEZ, TORRE and STOCKERT 1975; MAYRON and WISE 1976; MORRIS 1977; ITO and MCGHEE 1987; ITO, MCGHEE and SCHULTZ 1988). Models that propose to explain mitosis in these cells (PICKETT-HEAPS, TIPPET and PORTER 1982; MURRAY and SZOSTAK 1985; MCINTOSH and KOONCE 1989) must account for the random nature of chromatid disjunction. There must be a mechanism which determines that sister chromatids are oriented toward opposite poles at mitosis to assure proper disjunction and to account for the high fidelity of chromosome transmission (HARTWELL *et al.* 1982; HARTWELL and SMITH 1985). MURRAY and SZOSTAK (1985) proposed that the topology of the DNA (catenation) plays some role in orienting chromatids at mitosis in *S. cerevisiae*, but physical analysis of minichromosomes during the cell cycle does not support this model (KOSHLAND and HARTWELL 1987). Observations from other cell types suggest that chromatid orientation is achieved during prometaphase as a consequence of opposing forces applied to the kinetochore from the spindle (reviewed in PICKETT-HEAPS, TIPPET and PORTER 1982; MURRAY and SZOSTAK 1985). Chromatids improperly aligned on the spindle (kinetochores attached to the same pole) are unstable. Once opposing forces are applied and stabilized (kinetochores attached to the opposite poles), the chromatids disjoin and anaphase ensues. A molecular mechanism that senses the forces at the kinetochore has not been identified.

We have measured some genetic effects of BUdR and determined that BUdR is mutagenic to both

nuclear and mitochondrial genomes (MARK NEFF, unpublished observations). Low levels of BUdR had little effect on either homologous or sister chromatid recombination. Internal pools of deoxynucleotides influence rates of both mutagenesis (BARCLAY and LITTLE 1981; KUNZ 1988) and DNA recombinational repair (PAINTER 1980) and BUdR is mutagenic in a variety of other organisms (KAUFMAN 1988). The combination of altered thymidine pools and the presence of BUdR in the DNA of our *tmp1 tut1* strains may account for the effects on mutagenesis.

We did not observe an increased frequency of mitotic recombination in our *tmp1 tut1* strains, although thymidine starvation is reported to induce mitotic exchanges (KUNZ *et al.* 1980, HARTWELL and SMITH 1985). For example, HARTWELL and SMITH (1985) observed a tenfold increase in the rate of mitotic recombination in strains limited for *cdc8* function compared to wild-type strains. Since *CDC8* is the structural gene for thymidylate kinase (SCLAFANI and FANGMAN 1984), limiting the cells for *cdc8* function is equivalent to lowering the internal thymidine pools. KUNZ *et al.* (1980) also reported a tenfold increase in the frequency of mitotic recombination in a *cdc21* (thymidylate synthase) mutant. In contrast, there was a dramatic increase in mitotic recombination (up to 400-fold) in response to thymidine starvation using antifolate drugs. The differences in the data may reflect the extent of thymidine starvation in the strains used in the different experiments. We infer that the *tmp1 tut1* strains that we used were mildly starved for thymidine and therefore showed little stimulation of mitotic recombination.

Finally, our experiments demonstrate that BUdR can be utilized effectively for cytological observations to visualize DNA in *S. cerevisiae*. The fate of hemilabeled chromatids, derived from a single round of DNA synthesis, can be followed and quantified through subsequent cell divisions. This capability extends the uses of thymidine analogs in *S. cerevisiae* for studies of mutagenesis, DNA repair, density labeling of DNA, mutant selection and facile cloning of chromosomal DNA sequences (KUNZ *et al.* 1980; BARCLAY and LITTLE 1981; SCLAFANI and FANGMAN 1986).

We thank BOB SCLAFANI, LISA KADYK and JIM HABER for providing strains and MITCH SMITH for providing assistance with centrifugal elutriation. We thank MIKE WORMINGTON, MITCH SMITH, LISA KADYK and LEE HARTWELL for comments on the manuscript. We also thank TOM PETES and JIM HABER for suggesting the use of the Circular chromosome *III* for measuring reciprocal sister chromatid exchanges. This work was supported by the National Institutes of Health (GM 40334-02) and by a March of Dimes Birth Defects Foundation, Basil O'Connor Scholarship.

LITERATURE CITED

ADAMS, A. E. M., and J. R. PRINGLE, 1984 Relationship of actin and tubulin distribution to bud growth in wild type and morphogenetic mutants of *Saccharomyces cerevisiae*. J. Cell Biol. **98:** 934–945.

BARCLAY, B. J., and J. G. LITTLE, 1981 Mutation induction in yeast by deoxythymidine monophosphate: a model. Mol. Gen. Genet. **181:** 279–281.

BOEKE, J. D., F. LACROUTE and G. R. FINK, 1984 A positive selection for mutants lacking orotidine 5′ phosphate decarboxylase activity in yeast: 5-fluoroorotic acid resistance. Mol. Gen. Genet. **181:** 288–291.

BURKE, D., P. GASDASKA and L. H. HARTWELL, 1989 Dominant effects of tubulin overexpression in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **9:** 1049–1059.

BYERS, B., 1981 Cytology of the yeast life cycle, pp. 59–96 in *The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance*, edited by J. STRATHERN, E. W. JONES and J. R. BROACH. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

CAIRNS, J., 1975 Mutation selection and the natural history of cancer. Nature **255:** 197–200.

CROSS, S. L. and M. M. SMITH, 1988 Comparison of the structure and cell cycle expression of mRNAs encoded by two histone H3 and H4 loci in *Saccharomyces cerevisiae*. Mol. Cell Biol. **8:** 945–954.

FASULLO, M. T., and R. W. DAVIS, 1987 Recombinational substrates designed to study recombination between unique and repetitive sequences. Proc. Natl. Acad. Sci. USA **78:** 6334–6338.

FENANDEZ-GOMEZ, M. E., C. DE AL TORRE and J. C. STOCKERT, 1975 Random segregation of sister chromatids in meristematic cells. Exp. Cell. Res. **96:** 156–160.

FRIS, J., and H. ROMAN, 1968 The effect of the mating type alleles on intragenic recombination in yeast. Genetics **59:** 33–46.

GARGOURI, A., 1989 A rapid and simple method for extracting yeast mitochondrial DNA. Curr. Genet. **15:** 235–237.

GEARD, C. R., 1973 Chromatid distribution at mitosis in cultured *Wallabia bicolor* cells. Chromosoma **44:** 301–308.

GOODMAN, M. F., 1988 DNA replication fidelity: kinetics and thermodynamics. Mutat. Res. **200:** 11–20.

HABER, J., P. C. THORBURN and D. ROGERS, 1984 Meiotic and mitotic behavior of dicentric chromosomes in *Saccharomyces cerevisiae*. Genetics **106:** 185–205.

HARTWELL, L. H., 1967 Macromolecular synthesis in temperature-sensitive mutants of yeast. J. Bacteriol. **93:** 1662–1670.

HARTWELL, L. H. and D. SMITH, 1985 Altered fidelity of mitotic chromosome transmission in cell cycle mutants of *Saccharomyces cerevisiae*. Genetics **110:** 381–395.

HARTWELL, L. H., S. DUTCHER, J. WOOD and B. GARVIK, 1982 The fidelity of mitotic chromosome reproduction in *Saccharomyces cerevisiae*. Recent Adv. Yeast Mol. Biol. **1:** 28–38.

HELMSTETTER, C. E., and A. C. LEONARD, 1987 Mechanism for chromosome and minichromosome segregation in *Escherichia coli*. J. Mol. Biol. **197:** 195–204.

ITO, K., and J. D. MCGHEE, 1987 Parental strands segregate randomly during embryonic development of *Caenorhabditis elegans*. Cell **49:** 329–336.

ITO, K., J. D. MCGHEE and G. A. SCHULTZ, 1988 Paternal DNA strands segregate to both trophectoderm and inner cell mass of the developing mouse embryo. Genes Dev. **2:** 929–936.

KAUFMAN, E. R., 1988 The role of deoxynucleotide metabolism in 5-bromo-2′deoxyuridine mutagenesis in mammalian cells. Mutat. Res. **200:** 149–155.

KLAR, A., 1987a Differential parental DNA strands confer developmental asymmetry on daughter cells in fission yeast. Nature **326:** 466–470.

KLAR, A., 1987b The mother-daughter mating type switching asymmetry of budding yeast is not conferred by the segregation of parental *HO* strands. Genes Dev. **1:** 1059–1064.

KOSHLAND, D., and L. H. HARTWELL, 1987 The structure of

sister minichromosome DNA before anaphase in *Saccharomyces cerevisiae*. Science **238:** 1713–1716.

KUNZ, B., 1988 Mutagenesis and deoxyribonucleotide pool imbalance. Mutat. Res. **200:** 133–147.

KUNZ, B. A., B. J. BARCLAY, J. C. GAME, J. G. LITTLE and R. H. HAYNES, 1980 Induction of mitotic recombination in yeast by starvation for thymine nucleotides. Proc. Natl. Acad. Sci. USA **77:** 6057–6061.

LARK, K., 1966 Regulation of chromosome replication and SEGREGATION IN BACTERIA. BACTERIOL. REV. **30:** 3–32.

LARK, K., 1967 Non-random segregation of sister chromatids in *Vicia faba* and *Triticum boeoticum*. Proc. Natl. Acad. Sci. USA **58:** 352–359.

LARK, K., 1969 Sister chromatid segregation during mitosis in polyploid wheat. Genetics **62:** 289–305.

LARK, K. G., R. A. CONSIGLI and H. C. MINODIA, 1966 Segregation of sister chromatids in mammalian cells. Science **154:** 1202–1205.

MAYRON, R., and D. WISE, 1976 Random distribution of controlled regions at mitosis in cultured cells of *Muntiacus muntjak*. Chromosoma **55:** 69–74.

MCINTOSH, J. R., and M. P. KOONCE, 1989 Mitosis. Science **296:** 622–628.

MORRIS, V. B., 1977 Random segregation of sister chromatids in developing chick retinal cells demonstrated *in vivo* using the fluorescence plus giemsa technique. Chromosoma **60:** 139–145.

MORTIMER, R. K., D. SCHILD, E. R. CONTOPOULOU and J. A. KANS, 1989 Genetic map of *Saccharomyces cerevisiae*, Edition 10. Yeast **5:** 321–403.

MURRAY, A. W., and J. W. SZOSTAK, 1985 Chromosome segregation in mitosis and meiosis. Annu. Rev. Cell Biol. **1:** 289–315.

OGDEN, G. B., M. J. PRATT and M. SCHAECHTER, 1988 The replicative origin of the *Eschericia coli* chromosome binds to cell membranes only when hemi-methylated. Cell **54:** 127–135.

PAINTER, R. B., 1980 A replication model for sister-chromatid exchange. Mutat. Res. **70:** 337–41.

PICKETT-HEAPS, J. D., D. H. TIPPIT and K. R. PORTER, 1982 Rethinking mitosis. Cell **29:** 729–744.

Potten, C. S., W. Hume, P. Reed and J. CAIRNS, 1978 The segregation of DNA in epithelial stem cells. Cell **15:** 899–906.

PRINGLE, J. R., and L. H. HARTWELL, 1981 The *Saccharomyces cerevisiae* cell cycle, pp. 97–142 in *The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance*, edited by J. STRATHERN, E. W. JONES and J. R. BROACH. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

ROSENBERGER, R. F., and M. KESSEL, 1968 Non-random sister chromatid segregation and nuclear migration in hyphae of *Aspergillus nidulans*. J. Bacterol. **96:** 1208–1213.

SAMBROOK, J., E. F. FRITSCH and T. MANIATIS, 1989 *Molecular Cloning, A Laboratory Manual*, Ed. 2, Vol. 1–3. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

SCLAFANI, R. A., and W. C. FANGMAN, 1984 Yeast gene *CDC8* encodes thymidylate kinase and is complemented by the herpes thymidine kinase gene *TK*. Proc. Natl. Acad. Sci. USA **81:** 5821–5825.

SCLAFANI, R. A., and W. C. FANGMAN 1986 Thymidine utilization by *TUT* mutants and facile cloning of mutant alleles by plasmid conversion in *Saccharomyces cerevisiae*. Genetics **114:** 753–767.

SHERMAN, F., G. R. FINK and J. HICKS, 1986 *Laboratory Course Manual for Yeast Genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

SPEIT, G., and W. VOGEL, 1986 Detection of bromodeoxyuridine incorporation in mammalian chromosomes by a bromodeoxyuridine antibody. II. Demonstration of sister chromatid exchanges. Chromosoma **94:** 103–106.

THOMAS, J. H., and D. BOTSTEIN, 1986 A gene required for the separation of chromosomes on the spindle apparatus in yeast. Cell **44:** 65–76.

WILLIAMSON, D. H., and D. J. FENNELL, 1981 Non-random assortment of sister chromatids in yeast mitosis, pp. 89–102 in *Molecular Genetics in Yeast* (Alfred Benzon Symposium, Vol. 16), edited by D. VON WETTSTEIN *et al.* Copenhagen, Munksgaard.

WOLFF, S., and P. PERRY, 1974 Differential giemsa staining of sister chromatids and the study of sister chromatid exchanges without autoradiography. Chromosoma **48:** 431–353.

Communicating editor: E. W. JONES