# Mitotic Transmission of Artificial Chromosomes in *cdc* Mutants of the Yeast, *Saccharomyces cerevisiae*

**Robert E. Palmer,*,†,[1] Eileen Hogan† and Douglas Koshland†**

**Department of Population Dynamics, Division of Reproductive Biology, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland 21205, and †Department of Embryology, The Carnegie Institution of Washington, Baltimore, Maryland 21210*

Manuscript received January 11, 1990
Accepted for publication May 2, 1990

ABSTRACT

In the yeast, *Saccharomyces cerevisiae*, cell division cycle (*CDC*) genes have been identified whose products are required for the execution of different steps in the cell cycle. In this study, the fidelity of transmission of a 14-kb circular minichromosome and a 155-kb linear chromosome fragment was examined in cell divisions where specific *CDC* products were temporarily inactivated with either inhibitors, or temperature sensitive mutations in the appropriate *CDC* gene. All of the *cdc* mutants previously shown to induce loss of endogenous linear chromosomes also induced loss of a circular minichromosome and a large linear chromosome fragment in our study (either 1:0 or 2:0 loss events). Therefore, the efficient transmission of these artificial chromosomes depends upon the same *trans* factors that are required for the efficient transmission of endogenous chromosomes. In a subset of *cdc* mutants (*cdc6*, *cdc7* and *cdc16*), the rate of minichromosome loss was significantly greater than the rate of loss of the linear chromosome fragment, suggesting that a structural feature of the minichromosome (nucleotide content, length or topology) makes the minichromosome hypersensitive to the level of function of these *CDC* gene products. In another subset of *cdc* mutants (*cdc7* and *cdc17*), the relative rate of 1:0 events to 2:0 events differed for the minichromosome and chromosome fragment, suggesting that the type of chromosome loss event observed in these mutants was dependent upon chromosome structure. Finally, we show that 2:0 events for the minichromosome can occur by both a *RAD52* dependent and *RAD52* independent mechanism. These results are discussed in the context of the molecular functions of the *CDC* products.

IN the yeast, *Saccharomyces cerevisiae*, the construction and genetic analyses of small circular minichromosomes have been instrumental in understanding the structural features of chromosomes that are essential for their proper replication and segregation. For example, the analysis of minichromosomes has led to identification and characterization of *cis* sequences required for the initiation of DNA replication (HSIAO and CARBON 1979; STINCHCOMB, STRUHL and DAVIS 1979) and for the function of centromeres (CLARKE and CARBON, 1980; STINCHCOMB, MANN and DAVIS 1982). Circular minichromosomes that contain an origin of replication (*ARS*) and a centromere (*CEN*) are transmitted properly in 99% of cell divisions (CLARKE and CARBON, 1980; FITZGERALD-HAYES *et al.* 1982). However, their rate of loss per cell division is still several orders of magnitude greater than endogenous (ESPOSITO *et al.* 1982; HARTWELL *et al.* 1982) or large artificial linear chromosomes (MURRAY, SCHULTES and SZOSTAK 1986; HIETER *et al.* 1985) suggesting that minichromosomes lack a structural feature(s) of endogenous chromosomes (nucleotide content, size or topology) which ensures a high fidelity of transmission during mitotic cell divisions. In the absence of this structural feature, minichromosomes may be acted upon by the same replication and segregation machinery that acts upon endogenous chromosomes but may be poor substrates for this machinery at one or more steps in the cell cycle. Alternatively, the absence of this structural feature in minichromosomes may cause them to be transmitted by secondary replication or segregation processes that are inherently less faithful.

An understanding of the molecular mechanism of replication and segregation will also require the identification and characterization of the *trans* acting factors that comprise the replication and segregation machinery. In *S. cerevisiae*, temperature-sensitive mutants have been identified which arrest at different stages of the cell cycle when they are shifted to the nonpermissive temperature (PRINGLE and HARTWELL 1981). A subset of these cell division cycle (*cdc*) mutants identify genes whose products are required for the execution of discrete steps in $G_1$ (*CDC28*, *CDC4*, *CDC7*), S (*CDC2*, *CDC6*, *CDC9*, *CDC17*), G2-M (*CDC13*, *CDC16*, *CDC20*, *CDC23*) and late M phase

[1] To whom reprint requests should be addressed at the Carnegie Institution.

(*CDC14*, *CDC15*) of the cell cycle (PRINGLE and HARTWELL 1981). Given their cell cycle phenotype, these *CDC* genes are likely candidates for encoding *trans* factors required for proper chromosome transmission. In fact, when the *cdc* mutants were incubated at a semi-permissive temperature, conditions that partially inactivate the *CDC* gene product, many of these mutants lose endogenous chromosome at elevated rates demonstrating that these *CDC* products are involved in ensuring proper chromosome transmission (HARTWELL and SMITH 1985).

The molecular functions of some of the *CDC* gene products have been identified by cytological and biochemical analysis of arrested cells, while the activity of other *CDC* products have been inferred from amino acid similarities with other better characterized proteins. The *CDC28* and *CDC7* genes encode protein kinases (LORINCZ and REED 1984; PATTERSON *et al.* 1986); the former shows extensive similarity to the kinase subunit of maturation promoting factor, an essential regulator of the G2 to M transition in many organisms (ARION *et al.* 1988; BEACH, DURKACZ and NURSE 1982; GAUTIER *et al.* 1988). The *CDC4* product shares homology to the β-adrenergic receptor (YOCHEM and BYERS 1987). *CDC2* and *CDC17* encode DNA polymerases (SITNEY, BUDD and CAMPBELL 1989; BOULET *et al.* 1989; CARSON 1987), while *CDC9* encodes DNA ligase (BARKER and JOHNSTON 1983). However, the function of many of the *CDC* gene products remains to be elucidated.

In this paper, we examined the effect of temporarily reducing the function of different *CDC* gene products on the mitotic transmission of two artificial chromosomes, a 14-kb circular minichromosome and a 155-kb linear chromosome fragment. Both an increase or decrease in the ploidy of these test chromosomes could be detected in individual cell divisions through the use of a visual assay for chromosome ploidy (KOSHLAND, KENT and HARTWELL 1985; KOSHLAND and HIETER 1987). In this study, we identified mutations in *cdc* genes that reduce the fidelity of minichromosome transmission as evidenced by an increase in the rate of aberrant cell divisions where ploidy of the minichromosome changed. For each *cdc* mutant, we determined the types of changes in minichromosome ploidy that were observed and their rate of occurrence. Finally, we compared the fidelity of transmission of the circular minichromosome and large linear chromosome fragment in a subset of *cdc* mutants. These experiments provide insights into the role of *CDC* products in the transmission of minichromosomes, the cause of the high basal instability of minichromosomes, and the ability of different *CDC* products to transmit chromosomes with different structure. These insights are discussed in the context of the molecular functions of the *CDC* products and the effect of *cdc* mutations on endogenous chromosome transmission (HARTWELL and SMITH 1985).

## MATERIALS AND METHODS

**Strains:** All of the *cdc* mutations used in this study were obtained by back crossing at least five times the original *cdc* mutations isolated in A364A to strains congenic with the original A364A parent. All other markers were obtained by back crossing the mutation from another *Saccharomyces cerevisiae* strain into the A364A congenic background by at least ten back crosses. The haploid strains used in this study (Table 1) were constructed by standard genetic methods (SHERMAN, FINK and HICKS 1986) and then transformed (ITO *et al.* 1983) with the minichromosome, pDK243 (KOSHLAND, KENT and HARTWELL 1985). The homozygous *cdc* diploid strains containing the chromosome fragment (Table 1) were constructed as follows. First, the chromosome fragment, CF352 (Figure 1), was constructed in the haploid strain, 4513-121, by the chromosome fragmentation protocol (GERRING, CONNELLY and HIETER 1989; VOLLRATH *et al.* 1988). This strain was then crossed with a set of congenic haploid *cdc* strains (Table 1), and a set of congenic haploid *cdc* strains were obtained that contained the chromosome fragment. These strains were then mated with the haploid *cdc* strains listed in Table 1 to generate homozygous *cdc* diploids with 1 copy of the chromosome fragment.

**Growth conditions of exponentially growing cells and temporarily arrested cells:** The *cdc* mutant strains (Table 1) were grown at 23° to saturation in YPD (SHERMAN, FINK and HICKS 1986) supplemented with adenine. These cultures were used to inoculate YPD. When cells reached mid-log phase ($5 \times 10^6$ cells/ml), an aliquot was diluted with water approximately 1000-fold and spread onto YPD plates. The remaining cells were temporarily arrested by shifting them to the nonpermissive condition for 3 hr. The *cdc* mutants were temporarily arrested by incubating them at 36° for 3 hr. Wild-type cells were temporarily arrested by incubating them in the presence of nocodazole or hydroxyurea (a final concentration of 0.1 mM and 0.1 M, respectively) for 3 hr at 23° and 36°. The time spent at the nonpermissive condition was sufficient to inactivate most of the *CDC* gene product in the majority of cells as evidenced by the fact that greater than 90% of the cells were arrested at the appropriate stage of the cell cycle. After 3 hr at the nonpermissive condition, arrested cells were diluted with water and plated on YPD plates at 23° where they reentered the cell cycle and formed a colony. The dilution and plating were done rapidly to ensure that arrested cells did not complete division before they were plated.

**Half-sector analysis of minichromosome and chromosome fragment ploidy:** The ploidy of the minichromosome and chromosome fragment were monitored in the wild-type and *cdc* strains by a visual assay for chromosome ploidy in which cells that contained 0, 1 or 2 copies of these artificial chromosomes were white, pink and red, respectively (KOSHLAND, KENT and HARTWELL 1985). The change in the ploidy of the chromosome fragment was monitored in diploids instead of haploid strains because the distinction between the red and pink color of colonies was easier to make in diploids strains. Five to seven days after exponentially growing cells and temporarily arrested cells were plated, the colony size and color were sufficiently developed to determine the ploidy of the artificial chromosomes in the colony. Each colony was scored as white, pink, red, or as pink:white, red:white, or pink:red half-sectored. The different type of half-sectored colonies reflected 1:0, 2:0, and 2:1 events

**TABLE 1**

**Strains and genotypes used in this study**

| Strains | Genotype |
|---|---|
| BP5063 | *Mata leu2/Matα leu2 ade2/ade2 ade3/ade3 HIS3/his3 his7/His7 cdc6-1/cdc6-1 can1/can1 sap3/sap3* [cf352 chrIII (D8Bleft) *CEN6 LEU2 ade3*-2p] |
| BP5519 | *Mata leu2/Matα leu2 ade2/ade2 ade3/ade3 his7/his7 URA1/ura1 cdc7-1/cdc7-1 can1/can1 sap3/sap3* [cf352 chrIII (D8Bleft) *CEN6 LEU2 ade3*-2p] |
| EH5205 | *Mata leu2/Matα leu2 ade2/ade2 ade3/ade3 his7/His7 cdc14-1/cdc14-1 can1/can1 sap3/sap3* [cf352 chrII (D8Bleft) *CEN6 LEU2 ade3*-2p] |
| BP5010 | *Mata leu2/Matα leu2 ade2/ade2 ade3/ade3 HIS3/his3 his7/HIS7 cdc16-1/cdc16-1 can1/can1 sap3/sap3* [cf352 chrIII (D8Bleft) *CEN6 LEU2 ade3*-2p] |
| BP5524 | *Mata leu2/Matα leu2 ade2/ade2 ade3/ade3 his7/his7 URA1/ura1 cdc17-1/cdc17-1 can1/can1 sap3/sap3* [cf352 chrIII (D8Bleft) *CEN6 LEU2 ade3*-2p] |
| BP5061 | *MATa leu2/MATα leu2 ade2/ade2 ade3/ade3 his3/his3 his7/HIS7 cdc20-1/ cdc20-can1/can1 sap3/sap3* [cf352 chrIII (D8Bleft) *CEN6 LEU2 ade3*-2p] |
| 4521-001/pDK243 | *Mata leu2 ade2 ade3 can1 sap3 ura1 his7/LEU2 ade3*-2p |
| 4522-281/pDK243 | *MATa cdc28-1 leu2 ade2 ade3 can1 sap3 his7/LEU2 ade3*-2p |
| 4523-041/pDK243 | *Mata cdc4-1 leu2 ade2 ade3 can1 sap3 his7/LEU2 ade3*-2p |
| 4524-1-3/pDK243 | *Mata cdc7-1 leu2 ade2 ade3 can1 sap3 ura1 his7/LEU2 ade3*-2p |
| 4525-061/pDK243 | *Mata cdc6-1 leu2 ade2 ade3 can1 sap3 his7/LEU2 ade3*-2p |
| 4527-021/pDK243 | *Mata cdc2-1 leu2 ade2 ade3 can1 sap3 his7/LEU2 ade3*-2p |
| 4528-091/pDK243 | *Mata cdc9-1 leu2 ade2 ade3 can1 sap3 ura1 his7/LEU2 ade3*-2p |
| 4529-131/pDK243 | *Mata cdc13-1 leu2 ade2 ade3 can1 sap3 his7/LEU2 ade3*-2p |
| 4530-161/pDK243 | *Mata cdc16-1 leu2 ade2 ade3 can1 sap3 his7/LEU2 ade3*-2p |
| 4531-4-2/pDK243 | *Mata cdc20-1 leu2 ade2 ade3 can1 sap3 ura1 his7/LEU2 ade3*-2p |
| 4532-171/pDK243 | *Mata cdc17-1 leu2 ade2 ade3 can1 sap3 ura1 his7/LEU2 ade3*-2p |
| 4535-141/pDK243 | *Mata cdc14-1 leu2 ade2 ade3 can1 sap3 his7/LEU2 ade3*-2p |
| 4536-151/pDK243 | *Mata cdc15-1 leu2 ade2 ade3 can1 sap3 ura1 his7/LEU2 ade3*-2p |
| 4541-8-1/pDK243 | *Mata TOPII leu2 ade2 ade3 can1 sap3 ura1 his7/LEU2 ade3*-2p |
| 4563-9-2/pDK243 | *MATa cdc13-1 his7 ade2 ade3 rad52 can1 sap3/LEU2 ade3*-2p |
| 4564-9-1/pDK243 | *MATa cdc16-1 his7 leu2 ade2 ade3 rad52 can1* sap3/*LEU2 ade3*-2p |
| 4567-11-4/pDK243 | *MATa cdc17-1 his7 leu2 ade2 ade3 rad52 can1 sap3/LEU2 ade3*-2p |
| 4571-8-2/pDK243 | *MATa cdc7-1 his7 leu2 ade2 ade3 rad52 can1* sap3/*LEU2 ade3*-2p |
| 4565-11-1/pDK243 | *MATα his7 leu2 ade2 ade3 rad52 can1* sap3/*LEU2 ade3*-2p |

Each strain contained a *leu2* marker used in transformation and crosses, and *ade2* and *ade3* markers which were necessary for monitoring the ploidy of chromosomes harboring the *ADE3*-2p marker by the visual assay for chromosome ploidy. All markers after the slash are markers contributed by the minichromosome, pDK243 (see Figure 1).

which occurred in the first division after plating (see Figure 1). The rate of these events per cell division for cells containing 1 copy of the artificial chromosome was calculated as the number of half-sectored colonies divided by the number of pink colonies plus the number of half-sectored colonies (the total number of cells with one copy prior to plating). The scoring of red-white (2:0) and pink-white (1:0) half-sectored colonies was usually unambiguous. However, the difference between the red and pink color did vary in the different *cdc* mutants.

## RESULTS

**Rationale for experimental design:** The amount of functional *CDC* gene product in cells could be reduced during a single cell division because these products could be temporarily inactivated either by briefly exposing wild-type cells to the appropriate inhibitors or by briefly exposing *cdc* mutants to the nonpermissive temperature (see MATERIALS AND METHODS). Wild-type and *cdc* cells contained a nonessential test chromosome, either a 14-kb circular minichromosome, pDK243, or a 155 kb linear chromosome fragment, *CF*352 (Figure 1A). Aberrant transmission of these chromosomes occurred when the ploidy of the chromosome in one or both of the progeny cells differed from its ploidy in the parental cell. These aberrant events led to half-sectored colonies when the two primary progeny inherited different numbers of the chromosome (Figure 1B). The ploidy of the test chromosome in these half-sectored colonies were identified by a visual assay for chromosome ploidy (KOSHLAND, KENT and HARTWELL 1985). The frequency of half-sectored colonies among the total colonies was a direct measure of the rate of aberrant events in the first cell division after cells were plated. When wild-type cells or *cdc* cells were plated prior to their shift to the nonpermissive conditions, the frequency of half-sectored colonies reflected the rate of aberrant events per cell division of exponentially growing wild-type or *cdc* mutant cells. When wild-type or mutant cells were plated just after being released from arrest, then the first division of these cells on the plate was the completion of a division in which cells had been previously arrested. Therefore, the frequency of half-sectored colonies produced by these cells reflected the rate of aberrant events in cell

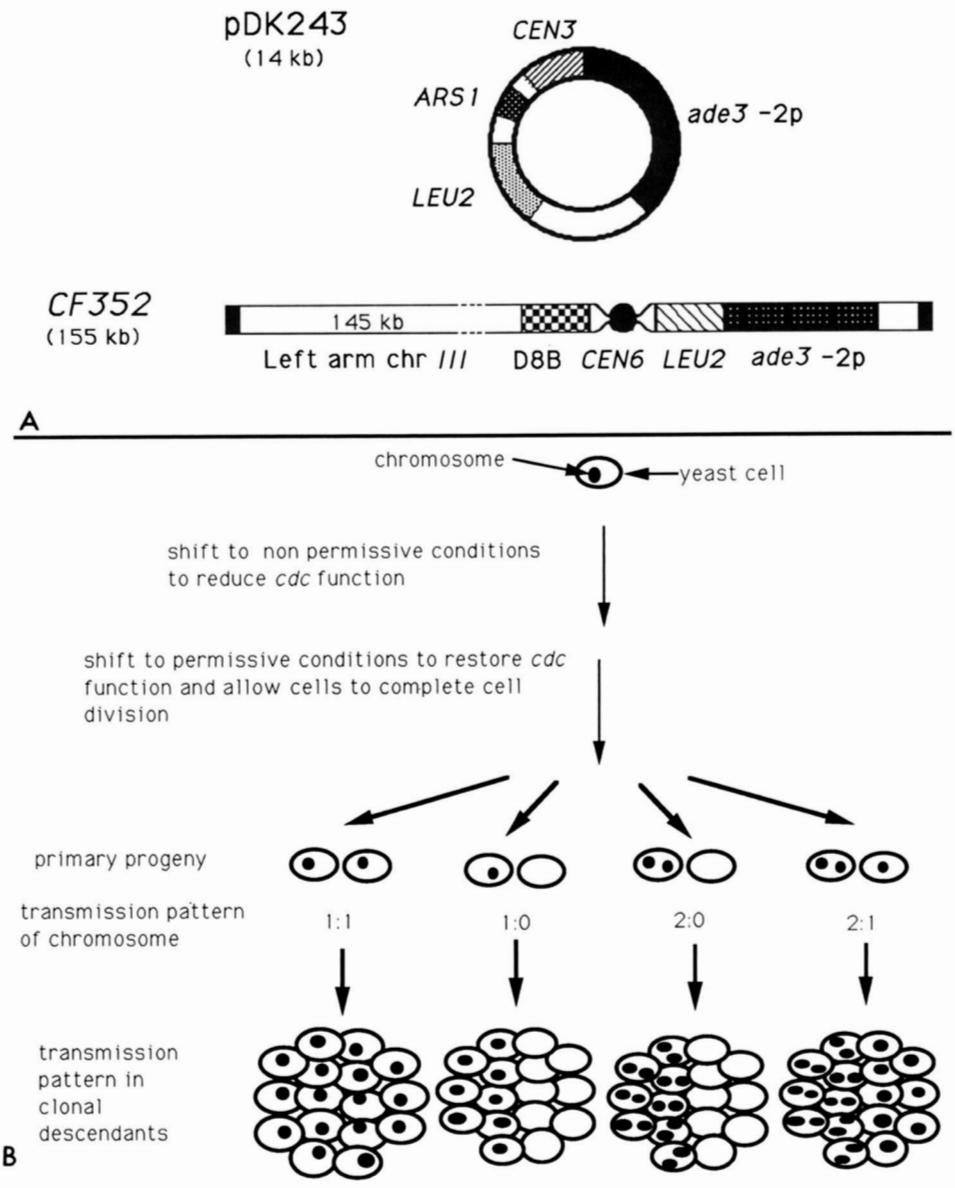


FIGURE 1.—Structure of artificial chromosomes and experimental design to follow changes in their ploidy in *cdc* mutants. A, Diagram (not drawn to scale) of the two artificial test chromosomes, the 155-kb linear chromosome fragment, *CF*352, and the 14-kb circular minichromosome, pDK243, whose ploidy is monitored in this study. The centromere DNA of the chromosome fragment (*CEN6*) is derived from chromosome *VI*. The right arm of the chromosome fragment contains a telomere (solid black), pBR322 sequences, the *ADE3*-2p gene which was used as a color marker to follow the ploidy of the chromosome (see MATERIALS AND METHODS) and the *LEU2* gene which was used as a selectable marker in crosses and transformation. The left arm of the chromosome fragment contains 145 kb of sequences derived from the left arm of chromosome III, starting at the chromosome III sequence, D8B (10 kb from the centromere), and extending to the telomere at the left end. The minichromosome, pDK243 (KOSHLAND, KENT and HARTWELL 1985), contains the origin of replication, *ARS1*, the centromere DNA sequences from chromosome III, *CEN3*, the *LEU2* gene for selection during crosses and transformation, and the *ADE3*-2p for following changes in its ploidy. B, The function of different *CDC* gene products was reduced temporarily as described in the text. Since cells that were temporarily arrested were plated before they have a chance to complete cell division, the primary progeny of these cells were physically juxtapose next to each other on the plate. In cells where the reduction in *CDC* function had no affect on the transmission of the test chromosome (indicated by a black dot), a 1:1 segregation pattern was produced in the primary progeny. Since most of the clonal descendants of the primary progeny contained 1 copy, a colony was formed in which most of the cells contained 1 copy of the minichromosome. Alternatively, the reduction in a given *CDC* function led to aberrant segregation patterns in the primary progeny (1:0, 2:0 and 2:1 events). Since all divisions after the first division on the plate occurred at the permissive conditions, the change of ploidy, generated when cells were temporarily arrested, was stably perpetuated in the clonal descendants, giving rise to half-sectored colonies. Because the artificial chromosomes were marked with the *ADE3*-2p gene and the *cdc* strains were also *ade2 ade3*, these half-sectored colonies could be detected by the visual assay for chromosome ploidy (see MATERIALS AND METHODS).

divisions where *CDC* function was temporarily reduced. Any difference in the absolute rate of 1:0 and 2:0 events, or in the fold induction, that was 3-fold greater than wild-type values was considered significant (see legend of Figure 2 and Figure 3). The occurrence of 2:1 events was so rare in exponentially growing cells and in cells with reduced *CDC* function that no statistically significant conclusions could be drawn about their induction.

**Basal rate of 1:0 and 2:0 events in exponentially dividing wild-type cells and *cdc* mutants:** The rates of 1:0 and 2:0 events for the minichromosome per wild-type cell division were 0.01 and 0.005, respectively (Figure 2, A and B). These values are similar to

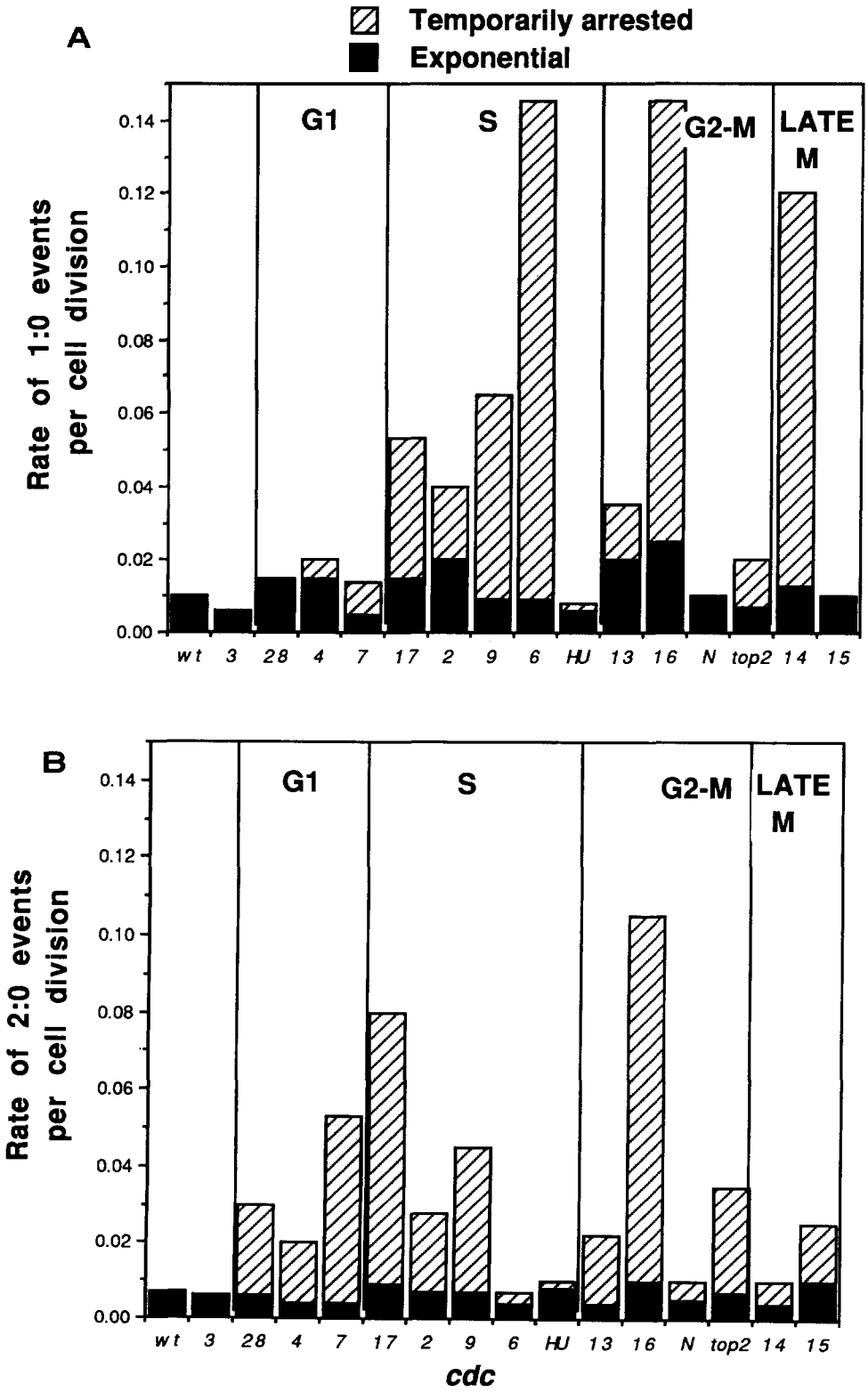


FIGURE 2.—Rate of 1:0 and 2:0 events for the minichromosome, pDK243, in *cdc* mutants. The rates of 1:0 and 2:0 events for the minichromosome, pDK243 (Figure 1A) were measured in wild-type cells and *cdc* mutants (see Table 1) growing exponentially at 23° (solid bar) and in wild-type and *cdc* mutants temporarily arrested (striped bar) by incubation for 3 hr at the nonpermissive condition (see text and MATERIALS AND METHODS). The rates in exponentially growing cells and temporarily arrested cells were the average of 2–4 independent trials in which 500–1000 colonies were scored. The absolute rates of 1:0 and 2:0 events measured in a given trial differed by less than 50% (data not shown) from the mean. When wild-type cells were subjected to conditions used to arrest the *cdc* mutants (3 hr at 36°), the rates of 1:0 and 2:0 events were identical to those rates observed in cells incubated only at 23°. Similarly, the lack of a striped area for a few *cdc* mutants indicates that the rates after temporary arrest and the rates in mutant cells growing exponentially at 23° were the same. The *cdc* mutants are grouped according to the stage in the cell cycle where they arrest. The *cdc3* mutant blocks cytokinesis but does not block the nuclear cycle. Though *top2* does not give a uniform arrest, its function is needed prior to anaphase (HOLM *et al.* 1985); therefore, it was placed in the G2-M class. N, nocodazole arrested wild-type cells. HU, hydroxyurea arrested wild-type cells.

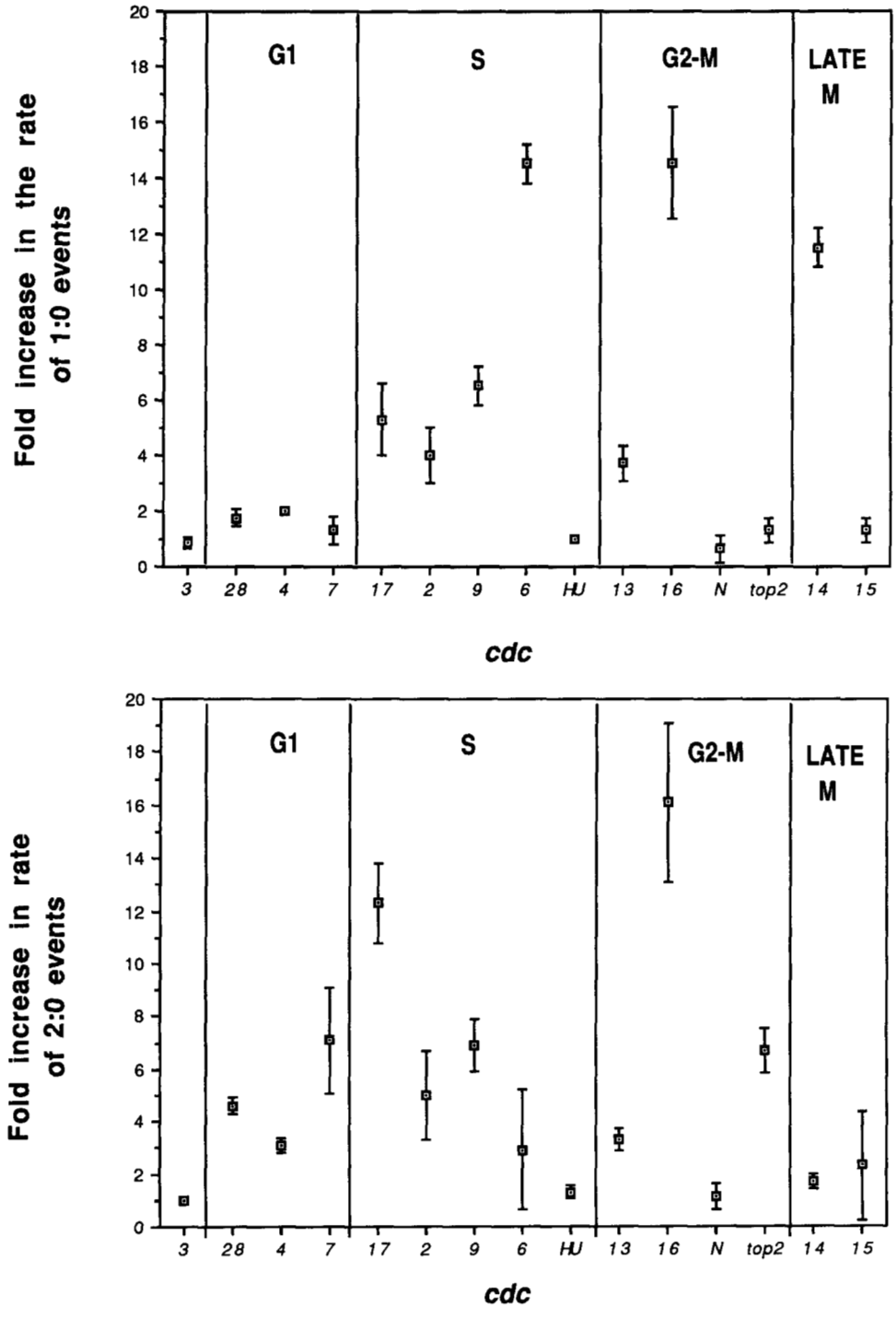


FIGURE 3.—The fold increase in 1:0 and 2:0 events for the minichromosome in cells temporarily arrested by different *cdc* mutations. The rate of 1:0 and 2:0 events was measured in *cdc* mutants that were temporarily arrested by incubation for 3 hr at the nonpermissive temperature (see Figure 2 and text). The fold increase in 1:0 and 2:0 events relative to wild-type cells was calculated by dividing the rate of these events in cells temporarily arrested by the rate observed in wild-type cells subjected to mock arrest conditions (see Figure 2). Points are the mean of two to four independent trials; the standard deviation for each value is indicated by the error bars.

those previously reported for this plasmid in other yeast strains (KOSHLAND, KENT and HARTWELL 1985). The rates of 1:0 and 2:0 events in mutants grown at the permissive temperature were similar to the rates observed in exponentially growing wild-type cells (Figure 1). Therefore, when these *cdc* mutants were grown at their permissive temperature, sufficient *CDC* function existed to ensure a fidelity of minichromosome transmission similar to wild-type cells.

The rates of 1:0 events and 2:0 events for the 155 kb linear chromosome fragment occurred at a rate of $4 \times 10^{-4}$ events per cell division in wild-type cells. These rates are in agreement with the published rate of loss for a nearly identical chromosome fragment in other yeast strains (Hegemann *et al.* 1988). Furthermore, they are 20-fold lower than those rates observed for pDK243 (Tables 2 and 3), which is consistent with previous observations that large linear chromosomes are more stable than small circular chromosomes (SUROSKY, NEWLON and TYE 1986; MURRAY, SCHULTES and SZOSTAK 1986; HIETER *et al.* 1985). In exponentially growing *cdc7*, *cdc17*, *cdc16* and *cdc14* cells, the

rates of 1:0 and 2:0 events for the linear fragment were similar to the rates of these events in exponentially growing wild-type cells. Therefore, sufficient *cdc* function apparently existed in these mutants at their permissive temperature to ensure a fidelity of chromosome transmission similar to wild type. However, the rate of 1:0 events for the chromosome fragment in the *cdc6* mutant strain was 10-fold higher than that observed in the wild-type strain apparently because *CDC6* function may be already partially reduced in this strain even at its permissive temperature. A similar increase in the absolute rate of 1:0 events for the minichromosome may have occurred in *cdc6* mutants growing at the permissive temperature, but this increase was obscured by the high basal rate of 1:0 events observed for the minichromosome in all strains.

**Rates of 1:0 and 2:0 events for the minichromosome in cells reduced for *cdc* function:** The fidelity of minichromosome transmission was reduced in many different *cdc* mutants when they were temporarily arrested as evidenced by an increase in the rate of 1:0 events, 2:0 events, or both (Figures 2 and 3). Several observations support the conclusion that the increases in the rate of 1:0 and 2:0 events resulted from the temporary reduction in the function of specific *CDC* products. Exposure of yeast cells to 36° was not sufficient by itself to increase the rate of 1:0 or 2:0 events since wild-type cells shifted to 36° for 3 hours showed the same rate of 1:0 and 2:0 events as wild-type cells grown at 23° (Figure 2). Furthermore, different *cdc* mutants caused different changes in the rates of 1:0 and 2:0 events (Figures 2 and 3). No increase in the absolute rates of 1:0 or 2:0 events were observed in cells temporarily arrested by *cdc3* (cytokinesis), hydroxyurea (DNA synthesis), or nocodazole (mitosis). The rate of 1:0 and 2:0 events were induced to approximately equal values in *cdc16*, *cdc17*, *cdc2* and *cdc9* mutants. The rates of 1:0 events were significantly greater than the rate of 2:0 events in *cdc6* and *cdc14* mutants while the rates of 2:0 events were significantly greater than 1:0 events in *cdc7*, *cdc28* and *cdc4* mutants.

The induction of the 1:0 and 2:0 events by *cdc* mutants were analyzed as a function of the stage of the cell cycle where the mutants arrest. The induction of 1:0 and 2:0 events was not confined to mutants that act at a specific stage of the cell cycle (Figures 2 and 3). For example an induction of 1:0 events was observed in all S mutants (*cdc2*, *cdc6*, *cdc9* and *cdc17*), in both $G_2$-M mutants (*cdc13* and *cdc16*) as well as in one late M phase mutant (*cdc14*). Similarly, an increase in the rate of 2:0 events was observed in all the $G_1$ mutants (weakly for *cdc4* and *cdc28* but strongly for *cdc7*), in all S phase mutants except *cdc6*, and for both $G_2$ mutants. However, 1:0 and 2:0 events may not be inducible at all stages of the cell cycle since the

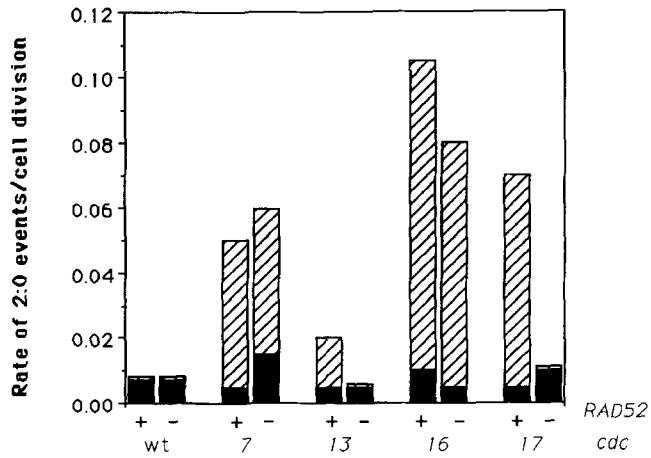


FIGURE 4.—*Rad52* dependent 2:0 events. Cells harboring a *cdc* mutation, the *rad52* mutation, and the minichromosome, pDK243, were constructed (see Table 1). The rate of 2:0 events was measured using the half-sector colony assay (see Figure 1) in exponentially growing cells and cells temporarily arrested as described in the text. The rate of 2:0 events in cells grown at the permissive temperature (solid bar) and after temporary arrest (striped bar) are shown. The rate of 2:0 events in the appropriate *RAD52 cdc* mutant (Figure 2) were regraphed here for ease of comparison.

induction of 1:0 events was not observed in any of the $G_1$ mutants, and the induction of 2:0 events was not observed in late M mutants. Additional mutants and additional alleles of these genes must be analyzed to confirm this preliminary pattern. Finally, mutants that arrest in the same phase of the cell cycle gave different responses. Among the S phase mutants, *cdc6* showed an increase in mostly 1:0 events, while the *cdc2*, *cdc9*, and *cdc17* showed a dramatic increase in both 1:0 and 2:0 events. Similarly, among the two late M phase mutants, the *cdc14* mutant showed a dramatic increase in the 1:0 events while the *cdc15* mutant did not.

**RAD52 dependent 2:0 events:** The *cdc2*, *cdc9*, *cdc17* and *cdc13* mutations had previously been shown to increase the rate of recombination between homologs as well as the rate of loss of endogenous chromosomes; the appearance of recombinant progeny in these mutants was blocked by a *rad52* mutation (HARTWELL and SMITH 1985). Therefore, it was possible that the *RAD52* dependent hyperrecombination in these *cdc* mutants might cause the observed increase in their rate of 2:0 events for the circular minichromosome. To address this hypothesis the rate of 2:0 events for the minichromosome was measured in different *cdc* strains (Table 1) which also carried a *rad52* mutation.

The presence of the *rad52* mutation had no effect on the basal rate of 2:0 events in wild-type cells or mutant cells grown at their permissive condition (Figure 4). While *rad52* mutations are known to cause loss of endogenous chromosomes (MORTIMER, CONTOPOULOU and SCHILD 1981), the effect of this *rad52* mutation on the transmission of the minichromosome was apparently obscured by the high basal rate of

minichromosome loss. The *rad52* mutation did significantly reduce the rate of 2:0 events in cells limited for *CDC13* and *CDC17* function but not in cells limited for *CDC7* and *CDC16* function, indicating that the induction of 2:0 events in some *cdc* mutants was *RAD52* dependent, and in others *RAD52* independent. Furthermore, the *RAD52* dependent 2:0 events were observed in the two *cdc* mutants (*cdc13* and *cdc17*) which exhibit elevated levels of *RAD52* dependent recombination (HARTWELL and SMITH 1985) while the *RAD52* independent 2:0 events were observed in the two *cdc* mutants (*cdc7* and *cdc16*) which exhibit no increase in recombination (HARTWELL and SMITH 1985). These results are consistent with the hypothesis that elevated levels of *RAD52* dependent recombination were responsible for the induction of 2:0 events in *cdc13* and *cdc17* cells and probably in the *cdc2* and *cdc9* mutants as well since these mutants also show elevated levels of *RAD52* dependent recombination.

**Rates of 1:0 and 2:0 events for a linear chromosome fragment in cells reduced for *cdc* function:** To address whether small circular minichromosomes and large linear chromosomes responded similarly to the reduction in functional *CDC* product, we examined the effect of temporarily reducing *CDC* function on the transmission of the 155-kb linear chromosome fragment (Figure 1A). The transmission of this chromosome fragment was analyzed in a subset of *cdc* mutations. These mutations were chosen either because they acted at different stages of the cell cycle or because their effects on minichromosome transmission were different. For technical reasons, the analysis of the transmission of the chromosome fragment was performed in homozygous *cdc* diploids rather than in haploid strains that were used to analyze the minichromosome (see MATERIALS AND METHODS). However, the rates of 1:0 and 2:0 events observed for the minichromosome in wild-type and *cdc16* diploid strains (both exponential and temporarily arrested) were identical to those observed in haploid cells (data not shown). Therefore, since the overall ploidy of the cell apparently had no affect on the transmission of the minichromosome, it was valid to compare the rates of 1:0 and 2:0 events for the minichromosome in haploid cells with those observed for the chromosome fragment in diploid cells.

The pattern of 1:0 events and 2:0 events for the minichromosome was compared with the pattern of 1:0 and 2:0 events for the chromosome fragment in the different *cdc* strains (Table 2). The following *cdc* mutations induced the same patterns of loss for both chromosomes: the *cdc14* mutation induced only 1:0 events; *cdc16* induced equally 1:0 and 2:0 events; and finally, *cdc6* caused a small induction of 2:0 events and a very large induction of 1:0 events. Therefore, in *cdc6*, *cdc14* and *cdc16* mutants the pattern of 1:0 events and 2:0 events was independent of the structure (the topology, length or nucleotide content) of the test chromosome. In contrast, the *cdc17* mutation caused an equal induction of 1:0 and 2:0 events for the minichromosome but a much greater increase in 1:0 events relative to 2:0 events for the chromosome fragment. For the minichromosome, the *cdc7* mutation caused an increase in the rate of 2:0 events only while for the chromosome fragment this mutation induced more 1:0 events than 2:0 events. Therefore, in both the *cdc7* and *cdc17* mutants the fold induction of 1:0 events relative to 2:0 events was dependent on the structure of the test chromosome.

TABLE 2

**Rate of 1:0 and 2:0 events per cell division**

| | Minichromosome | | Chromosome fragment | |
|---|---|---|---|---|
| Strain | 1:0 | 2:0 | 1:0 | 2:0 |
| wt | 0.010 | 0.006 | 0.0004 | 0.0004 |
| *cdc7* | 0.015 (1.5) | 0.050 (8) | 0.0030 (7) | 0.0010 (3) |
| *cdc6* | 0.140 (14) | 0.020 (3) | 0.0200 (50) | 0.0030 (9) |
| *cdc17* | 0.050 (5) | 0.070 (11) | 0.0700 (175) | 0.0040 (5) |
| *cdc16* | 0.140 (14) | 0.100 (16) | 0.0050 (12) | 0.0040 (10) |
| *cdc14* | 0.120 (12) | 0.010 (1.6) | 0.0400 (120) | 0.0005 (1) |

Comparison of the absolute rate of 1:0 and 2:0 events and the fold increase in 1:0 and 2:0 events for the minichromosome and chromosome fragment in *cdc* mutants. The rate of 1:0 and 2:0 events for the chromosome fragment were measured in homozygous *cdc* diploids (Table 1) that were growing exponentially or temporarily arrested. Absolute rates of 1:0 and 2:0 events are the mean of 2 to 4 independent trials, approximately 5000 colonies were scored. The data for the absolute rate of 1:0 and 2:0 events for the minichromosome are taken from Figures 2 and 3 and represented here to facilitate a qualitative comparison of the effect of reducing different *CDC* functions on the transmission of the minichromosome and chromosome fragment. The fold increase in the rates of 1:0 and 2:0 events observed in *cdc* mutants relative to the rates observed in wild-type cells is shown in parentheses. It was calculated by dividing the rate of these events in cells temporarily arrested by the rate observed in wild-type (wt) cells subjected to mock arrest conditions.

The absolute rate of loss (rate of 1:0 events plus 2:0 events) of the minichromosome was compared with the absolute rate of loss of the chromosome fragment in the different *cdc* mutants. The absolute rate of loss of the circular minichromosome and linear chromosome fragment were within 3 fold of each other for cells limited for *CDC17*, *CDC14* and *CDC20* functions (Table 3). Therefore, the absolute rate of chromosome loss induced by these mutants appears to be independent of chromosome size or structure. In contrast, the absolute rate of minichromosome loss observed in cells limited for *CDC6*, *CDC7* and *CDC16* functions were respectively 8, 20 and 26 fold greater than the rates observed for the linear chromosome fragment (Table 3). Therefore, the absolute rate of chromosome loss in cells limiting *CDC6*, *CDC7* and *CDC16* function appeared to be sensitive to chromosome size and or structure.

TABLE 3

**Quantitative comparison of the rates of minichromosome loss and chromosome fragment loss in *cdc* mutants**

| Strain | Rate of chromosome loss per cell division for: Minichromosome | Chromosome fragment | Ratio of minichromosome to chromosome fragment |
|---|---|---|---|
| wt | 0.016 | 0.0008 | 20 |
| *cdc7* | 0.065 | 0.0040 | 16 |
| *cdc6* | 0.160 | 0.0230 | 8 |
| *cdc17* | 0.120 | 0.0740 | 1.7 |
| *cdc16* | 0.240 | 0.0090 | 26 |
| *cdc14* | 0.130 | 0.0400 | 3 |
| *cdc20* | 0.035 | 0.0280 | 1.3 |

The rates of 1:0 and 2:0 events for the minichromosome and the chromosome fragment were measured in wild-type (wt) cells and in temporarily arrested *cdc* mutants (Table 2) and were summed to give the total rate of chromosome loss.

## DISCUSSION

**A comparison of the rate of loss of circular minichromosomes and large linear chromosomes in cells with reduced *CDC* function:** All of the *cdc* mutants that induced loss of endogenous linear chromosomes in the study of HARTWELL and SMITH (1985) also induced loss of circular minichromosomes in our study (either 1:0 or 2:0 events). The similar response of minichromosomes and endogenous chromosomes to the many different *cdc* mutants suggests that all of these *CDC* gene products are important in the transmission of both minichromosomes and endogenous chromosomes. Since the cell division cycle mutants appear to identify gene products that participate in the major pathway of replication and segregation of endogenous chromosomes in yeast, the minichromosome appears to be transmitted by this major pathway. Therefore, the high basal instability of minichromosomes apparently is not a consequence of minichromosomes being transmitted by some secondary pathway, but rather because they may be poor substrates for one or more steps in the major pathway (see below). Furthermore, since all of *cdc* mutants tested induce loss of both endogenous chromosomes and circular minichromosomes, the *CDC* products must act directly or indirectly on structural features (nucleotides or proteins) shared by minichromosomes and endogenous chromosomes. Therefore, none of these *CDC* gene products appear to interact with proteins or sequences that are present exclusively on endogenous linear chromosomes such as telomere proteins or telomere sequences.

The absolute rate of minichromosome loss was significantly greater than the rate of loss of the linear chromosome fragment for *cdc6*, *cdc7* and *cdc16* mutants suggesting that the minichromosome is hypersensitive to the level of function of these *CDC* gene products. In fact, the high basal level of minichromosome loss observed in all cells may be a consequence of the hypersensitivity of the minichromosome to the level of activity of these gene products in wild-type cells. This hypersensitivity of the circular minichromosome could result from its small size, circular topology, or nucleotide content. Understanding which of these factors is responsible for the hypersensitivity of minichromosome to *CDC6*, *CDC7* and *CDC16* products may help to elucidate the functions of these products as well as provide insight into the role of chromosome structure in chromosome transmission.

The *cdc28* mutant caused minichromosome loss to increase to an absolute rate of 3% per cell division, while in the experiments of HARTWELL and SMITH (1985), this mutant exhibited no detectable increase in loss of a linear endogenous chromosome. The failure of linear chromosome loss to increase to an absolute rate of 3% per cell division may reflect some hypersensitivity of the minichromosome to reductions in *CDC28* function like that observed for *CDC6*, *CDC7* and *CDC16*. However, the apparent difference between minichromosome loss and endogenous chromosome loss may simply reflect that *CDC28* function was more significantly reduced in this study than in the experiments of HARTWELL and SMITH, perhaps because different alleles of *CDC28* and different methods for reducing *CDC* function were used.

**The induction of 1:0 and 2:0 events in different *cdc* mutants:** The induction of 1:0 and 2:0 events in the different *cdc* mutants provided new insights into the function of their gene products. In the following discussion our interpretations of 1:0 and 2:0 events are based upon the following assumptions. A 1:0 event for a test chromosome is diagnostic of the failure of that chromosome to replicate or the loss of one of the two sister chromatids subsequent to replication. A 2:0 event is diagnostic of a failure in chromosome segregation since the two sister chromatids end up in the same progeny cell. However, it should be kept in mind that improper chromosome segregation might be caused by defects in replication machinery as well as in the segregation machinery. For example, a failure to complete DNA replication presumably would prohibit sister chromatid separation. The partially replicated molecule might be pulled to one pole, and then its replication might be completed thereafter or within the next cell cycle giving rise to the 2:0 event.

**$G_1$ mutants:** The *cdc28* and *cdc4* mutations induce only 2:0 events for the circular minichromosomes. Given the observation that these mutants seem to arrest the cell cycle before DNA synthesis is even initiated (PRINGLE and HARTWELL 1981), it may seem surprising that these mutants should cause a defect in chromosome segregation. However, the earliest events of the cell cycle in budding yeast involve morphogenesis of the spindle pole bodies, the microtubule

organizing centers, that are key to the formation and function of the spindle. In fact, early steps in spindle pole body morphogenesis are blocked in *cdc28* and *cdc4* arrested cells (BYERS and GOETSCH 1974; BYERS 1981). Therefore, it is not surprising that temporarily reducing the function of these two gene products may perturb the normal assembly of the spindle pole bodies. When *cdc28* and *cdc4* cells reenter the cell cycle, aberrant spindles may be formed and subsequently malfunction during mitosis leading to chromosome nondisjunction.

The *cdc7* mutants have extremely complex cell cycle phenotypes. The initial characterization of this mutant indicated that it arrest cells with both a G1 phenotype (failure to initiate DNA synthesis) (HARTWELL 1973) and a G2-M, phenotype (formation of a bipolar spindle) (BYERS and GOETSCH 1974). The function of this protein became more unclear when subsequent experiments demonstrated that a small amount non-random DNA synthesis does occur in mitotically arrested *cdc7* cells and that *cdc7* mutations block meiosis after the completion of premeiotic DNA synthesis (SCHILD and BYERS 1978; SIMCHEN 1974). In this study we document another perplexing phenotype of this mutant. The transmission of the minichromosome and large linear chromosome fragment differ from each other both qualitatively (the fold induction of 1:0 relative to 2:0 events) and quantitatively (the absolute rate of loss). Recently, the *CDC7* product has been shown to be a protein kinase (PATTERSON *et al.* 1986). Perhaps the identification of its substrates will help to elucidate the relationship between these unusual phenotypes.

**S phase mutants and RAD52 dependent 2:0 events:** The product of the *cdc17* genes is required for progression through S phase because it encodes a DNA polymerase essential for DNA replication (CARSON 1987). Impairing its function apparently leaves lesions in the DNA which stimulate *RAD52* dependent recombination between homologs (HARTWELL and SMITH 1985). In this study, we show that the *cdc17* mutation also increase the rate of 1:0 and 2:0 events for the minichromosome. This increase in the rate of 2:0 events was observed only in the *RAD52* background and only with the circular minichromosome, but not the linear chromosome fragment.

To explain these observations, we propose that the *cdc17* mutation stimulates *RAD52* dependent recombination between sister chromatids as well as homologs (HARTWELL and SMITH 1985). Sister chromatid exchange between linear sister chromatids would simply cause the exchange of sequences distal to the cross over, while exchange between circular chromatids would cause the formation of a dicentric dimer circle. Dicentric minichromosomes often survive mitosis in yeast and segregate to one of the two progeny cells (KOSHLAND *et al.* 1987). The subsequent resolution of the dimer into two monomers before the next mitosis would give rise to a 2:0 event. A *rad52* mutation would block the induction of 2:0 events by blocking sister chromatid exchange between the sister minichromatids.

If sister chromatid exchange in *cdc17* mutants can induce 2:0 events for the circular minichromosome, then other *cdc* mutations which have elevated levels of sister chromatid exchange should also induce 2:0 events for the minichromosome. Limiting cells for *CDC2*, *CDC9* or *CDC13* function stimulates recombination between homologs (HARTWELL and SMITH 1985). Since mutations in these genes arrest cells after the initiation of DNA synthesis but prior to chromosome separation (PRINGLE and HARTWELL 1981), that is within the window of the cell cycle when sister chromatid exchange can occur, it is reasonable to suggest that these mutations may induce sister chromatid exchange as well as homolog exchange. Interestingly, mutations in *CDC2*, *CDC9* and *CDC13* all induced 2:0 events. While mutations in *CDC14* and *CDC6* also stimulate homolog exchange, they apparently arrest cells at stages of the cell cycle where sister chromatid exchange may not be possible because sister chromatids have already been separated (*cdc14*) (BYERS and GOETSCH 1947) or because sister chromatids have not yet been formed (*cdc6*) (HARTWELL 1976). Interestingly, these two mutants induce 1:0 but not 2:0 events for the minichromosome.

**$G_2$-M:** The *cdc16* mutant induces 1:0 and 2:0 events for both the minichromosome and the chromosome fragment. Unlike the S phase mutants the 2:0 events were not dependent upon the *RAD52* gene product or on the topology of the chromosome. In addition, the *cdc16* mutant does not induce recombination between homologs (HARTWELL and SMITH 1985). Therefore, *cdc16* mutants do not appear to induce 2:0 events as a consequence of aberrant DNA metabolism. These phenotypes coupled with its arrest at $G_2$-M boundary suggest that the *CDC16* product is a good candidate for a protein directly required for proper initiation or completion of chromosome segregation. The increase in 1:0 events in *cdc16* mutants is not inconsistent with its putative role in chromosome segregation since improper chromosome segregation could occasionally lead to destruction of one of the two sister chromatids.

Impairing topoisomerase II function or microtubule assembly might be expected to increase the rate of 2:0 events. Topoisomerase II functions at mitosis to allow chromosome segregation by removing tangles between sister chromatids caused by catenation of the sister DNA molecules (HOLM *et al.* 1985). Indeed, in this study and in a study of linear chromosome fragments (HOLM, STEARNS and BOTSTEIN 1989), mutants

in topoisomerase II did show and increase in the rate of 2:0 events. Similarly, microtubules are essential to the movement of chromosomes during all phases of mitosis. However, impairing microtubule assembly for 3 hr did not show an increase in 2:0 events for the minichromosome. Given the role of microtubules in chromosome segregation this result is surprising. However, the ability of cells to transmit linear endogenous chromosomes also seemed to be unaffected by microtubule depolymerizing reagents unless cells are incubated with these drugs for periods as long as 24 hr (WOOD 1982).

**Late M:** Cells arrested by *cdc14* and *cdc15* mutations block in late M phase (PRINGLE and HARTWELL 1981; BYERS 1981). Therefore, the fact that the *cdc14* mutant induced a much greater number of 1:0 events than 2:0 events suggests that a defect in terminal events in mitosis can lead to loss of one of the replicated sister chromatids from one of the two segregated genomes. Since the *cdc15* mutant fails to induce 1:0 events, arresting cells at this terminal stage of the cell cycle apparently is not sufficient to induce 1:0 events. The *CDC14* gene product may represent a class of proteins that are required to ensure that segregated chromosomes are packaged into the two daughter nuclei when the nucleus divides at the end of mitosis. A defect in these proteins may cause chromosomes to leech into the cytoplasm and be lost. Alternatively, DNA metabolism may occur in this late stage of mitosis, which is important for replication or segregation in the next cell division. A failure to properly perform this metabolism may lead to chromosome damage and subsequent destruction. The fact that the *cdc14* mutant does induce homolog recombination is consistent with its product playing some role in DNA metabolism (HARTWELL and SMITH 1985).

**Conclusion:** In this study we have analyzed the transmission of artificial chromosomes in *cdc* mutants. An understanding of the chromosome loss events (1:0 or 2:0 events) that are induced by mutations affecting defined stages of the cell cycle provides a framework for analyzing changes in chromosome ploidy that occur in mutants of other less well-characterized genes. This study also provides a framework for thinking about the mode of action of environmental toxins that reduce the fidelity of chromosome transmission. In this study, we showed that the reduction in activity of most *CDC* gene products during a single cell cycle is sufficient to induce chromosome loss. Therefore, chromosome loss in yeast potentially could be stimulated by a very brief (one cell cycle) exposure to any environmental toxin that reduces the function of one of the *CDC* gene products.

This work benefited greatly from the comments of LELAND HARTWELL. We thank GLEN BLUSTEIN for technical assistance, and thank PHIL HIETER, GARY KARPEN, KAREN LAMARCO and FORREST SPENCER for their comments on the manuscript. CHRISTINE NORMAN assisted with the preparation of the manuscript. R. E. P. was supported by National Institutes of Health Training Grant HD 07276 awarded to The Johns Hopkins School of Hygiene and Public Health, Department of Population Dynamics, Division of Reproductive Biology, and a Howard Hughes predoctoral grant awarded to the Carnegie Institution of Washington. D. K. is a Lucille P. Markey Scholar and this work was supported in part by a grant from the Lucille P. Markey Charitable Trust.

## LITERATURE CITED

ARION, D., L. MEIJER, L. BRIZUELA and D. BEACH, 1988 *cdc 2* is a component of the M phase-specific histone H1 kinase: evidence for identity with MPF. Cell **55:** 371–378.

BARKER, D. G., and L. H. JOHNSTON, 1983 *Saccharomyces cerevisiae cdc9*, a structural gene for yeast DNA ligase which complements *Schizosaccharomyces pombe cdc17*. Eur. J. Biochem. **134:** 315–319.

BEACH, D. H., B. DURKACZ and P. M. NURSE, 1982 Functionally homologous cell cycle control genes in budding and fission yeast. Nature **300:** 706–709.

BOULET, A., M. SIMON, G. FAYE, G. A. BAUER, P. M. BURGERS, 1989 Structure and function of the *Saccharomyces cerevisiae CDC2* gene encoding the large subunit of DNA polymerase III. EMBO J. **8:** 1849–1854.

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

BYERS, B., and L. GOETSCH, 1974 Duplication of spindle plaques and integration of the yeast cell cycle. Cold Spring Harbor Symp. Quan. Biol. **38:** 123–131.

CARSON, M. J., 1987 *CDC17*, the structural gene for DNA polymerase I of yeast: mitotic hyperrecombination and effects on telomere metabolism. Ph.D. thesis, University of Washington, Seattle.

CLARKE, L., and J. CARBON, 1980 Isolation of a yeast centromere and construction of functional small circular chromosomes. Nature **287:** 504–509.

ESPOSITO, M. S., D. T. MALEAS, K. A. BJORNSTAD and C. V. BRUSCHI, 1982 Simultaneous detection of changes in chromosome number, gene conversion and intergenic recombination during mitosis of *Saccharomyces cerevisiae* spontaneous and ultraviolet light induced events. Curr. Genet. **6:** 5–11.

FITZGERALD-HAYES, M., J. M. BUHLER, T. G. COOPER and J. CARBON, 1982 Isolation and subcloning analysis of functional centromere DNA (*CEN11*) from *Saccharomyces cerevisiae* chromosome XI. Mol. Cell. Biol. **2:** 82–87.

GAUTIER, J., C. NORBURY, M. LOHKA, P. NURSE and J. MALLER, 1988 Purified maturation-promoting factor contains the product of a *Xenopus* homolog of the fission yeast cell cycle control gene *cdc2*$^+$. Cell **54:** 433–439.

GERRING, S. L., C. CONNELLY and P. HIETER, 1989 Positional mapping of genes by chromosome blotting and chromosome fragmentation. Methods Enzymol. (in press).

HARTWELL, L. H., 1973 Three additional genes required for deoxyribonucleic acid synthesis in *Saccharomyces cerevisiae*. J. Bacteriol. **115:** 966–974.

HARTWELL, L. H., 1976 Sequential function of gene products relative to DNA synthesis in the yeast cell cycle. J. Mol. Biol. **104:** 803–817.

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

HARTWELL, L. H., S. K. DUTCHER, J. S. WOOD and B. GARVIK,

1982 The fidelity of mitotic chromosome reproduction in *S. cerevisiae*. Rec. Adv. Yeast Mol. Biol. **1:** 28–38.

HEGEMANN, J. H., J. H. SHERO, G. COTTAREL, P. PHILIPPSEN and P. HIETER, 1988 Mutational analysis of the centromere DNA from chromosome VI of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8:** 2523–2528.

HIETER, P., C. MANN, M. SNYDER and R. W. DAVIS, 1985 Mitotic stability of yeast chromosomes: A colony color assay that measures nondisjunction and chromosome loss. Cell **40:** 381–392.

HOLM, C., T. GOTO, J. C. WANG and D. BOTSTEIN, 1985 DNA topoisomerase II is required at the time of mitosis in yeast. Cell **41:** 553–563.

HOLM, C., T. STEARNS and D. BOTSTEIN, 1989 DNA topoisomerase II must act at mitosis to prevent nondisjunction and chromosome breakage. Mol. Cell. Biol. **9:** 159–168.

HSIAO, C.-L., and J. CARBON, 1979 High-frequency transformation of yeast by plasmids containing the cloned yeast *Arg4* gene. Proc. Natl. Acad. Sci. **76:** 3829–3833.

ITO, H., Y. FUKUDA, K. MURATA and A. KIMURA, 1983 Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153:** 163–168.

KOSHLAND, D., and P. HIETER, 1987 Visual assay for chromosome ploidy. Methods Enzymol. **155:** 351–372.

KOSHLAND, D., J. C. KENT and L. H. HARTWELL, 1985 Genetic analysis of the mitotic transmission of minichromosomes. Cell **40:** 393–403.

KOSHLAND, D., L. RUTLEDGE, M. FITZGERALD-HAYES and L. H. HARTWELL, 1987 A genetic analysis of dicentric minichromosomes in *Saccharomyces cerevisiae*. Cell **48:** 801–812.

LORINCZ, A. T., and S. I. REED, 1984 Primary structural homology between the product of yeast cell division control gene *CDC28* and vertebrate oncogenes. Nature **307:** 183–185.

MORTIMER, R. K., R. CONTOPOULOU and D. SCHILD, 1981 Mitotic chromosome loss in a radiation-sensitive strain of the yeast *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. **78:** 5778–5782.

MURRAY, A. W., N. P. SCHULTES and J. W. SZOSTAK, 1986 Chromosome length controls mitotic chromosome segregation in yeast. Cell **45:** 529–536.

PATTERSON, M., R. S. SCLAFANI, W. L. FANGMAN and J. ROSAMOND, 1986 Molecular characterization of cell cycle gene *CDC7* from *Saccharomyces cerevisiae*. Mol. Cell. Biol. **6:** 1590–1598.

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. N. STRATHERN, E. W. JONES and J. R. BROACH. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

SCHILD, D., and B. BYERS, 1978 Meiotic effects of DNA-defective cell division cycle mutations of *Saccharomyces cerevisiae*. Chromosoma **70:** 109–130.

SHERMAN, F., G. R. FINK, and J. B. HICKS, 1986 *Methods in Yeast Genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

SIMCHEN, G., 1974 Are mitotic functions required in meiosis? Genetics **76:** 745–753.

SITNEY, K. C., M. E. BUDD and J. L. CAMPBELL, 1989 DNA polymerase III, a second essential DNA polymerase, is encoded by the *S. cerevisiae CDC2* gene. Cell **56:** 599–605.

STINCHCOMB, D. T., C. MANN and R. W. DAVIS, 1982 Centromeric DNA from *Saccharomyces cerevisiae*. J. Mol. Biol. **158:** 157–179.

STINCHCOMB, D. T., K. STRUHL and R. W. DAVIS, 1979 Isolation and characterization of a yeast chromosomal replicator. Nature **282:** 39–43.

SUROSKY, R. T., C. S. NEWLON and B-K. TYE, 1986 The mitotic stability of deletion derivatives of chromosome III in yeast. Proc. Natl. Acad. Sci. **83:** 414–418.

VOLLRATH, D., R. W. DAVIS, C. CONNELLY and P. HIETER, 1988 Physical mapping of large DNA by chromosome fragmentation. Proc. Natl. Acad. Sci. **85:** 6027–6031.

WOOD, J. S., 1982 Genetic effects of methyl benzimidazole-2-yl carbamate on *Saccharomyces cerevisiae*. Mol. Cell. Biol. **2:** 1064–1079.

YOCHEM, J., and B. BYERS, 1987 Structural comparison of the yeast cell division cycle gene *CDC4* and a related pseudogene. J. Mol. Biol. **195:** 233–245.

Communicating editor: M. CARLSON