ALTERED FIDELITY OF MITOTIC CHROMOSOME TRANSMISSION IN CELL CYCLE MUTANTS OF *S. CEREVISIAE*

LELAND H. HARTWELL AND DAVID SMITH

Department of Genetics SK-50, University of Washington, Seattle, Washington 98195

Manuscript received August 21, 1984
Revised copy accepted March 18, 1985

ABSTRACT

Thirteen of 14 temperature-sensitive mutants deficient in successive steps of mitotic chromosome transmission (*cdc2, 4, 5, 6, 7, 8, 9, 13, 14, 15, 16, 17* and *20*) from spindle pole body separation to a late stage of nuclear division exhibited a dramatic increase in the frequency of chromosome loss and/or mitotic recombination when they were grown at their maximum permissive temperatures. The increase in chromosome loss and/or recombination is likely to be due to the deficiency of functional gene product rather than to an aberrant function of the mutant gene product since the mutant alleles are, with one exception, recessive to the wild-type allele for this phenotype. The generality of this result suggests that a delay in almost any stage of chromosome replication or segregation leads to a decrease in the fidelity of mitotic chromosome transmission. In contrast, temperature-sensitive mutants defective in the control step of the cell cycle (*cdc28*), in cytokinesis (*cdc3*) or in protein synthesis (*ils1*) did not exhibit increased recombination or chromosome loss.—Based upon previous results with mutants and DNA-damaging agents in a variety of organisms, we suggest that the induction of mitotic recombination in certain mutants is due to the action of a repair pathway upon nicks or gaps left in the DNA. This interpretation is supported by the fact that the induced recombination is dependent upon the *RAD52* gene product, an essential component in the recombinogenic DNA repair pathway. Gene products whose deficiency leads to induced recombination are, therefore, strong candidates for proteins that function in DNA metabolism. Among the mutants that induce recombination are those known to be defective in some aspect of DNA replication (*cdc2, 6, 8, 9*) as well as some mutants defective in the G2 (*cdc13* and *17*) and M (*cdc5* and *14*) phases of the mitotic cycle. We suggest that special aspects of DNA metabolism may be occurring in G2 and M in order to prepare the chromosomes for proper segregation.

IN the yeast *Saccharomyces cerevisiae* conditional lethal mutants have been used to define many genes that control discrete steps in the cell division process (PRINGLE and HARTWELL 1981). The mutants selected for this analysis are nullomorphs (completely defective for cell division) at the restrictive temperature. Such mutants provide useful material for physiological, biochemical and cytological analyses because large populations of cells can be synchronously arrested at discrete developmental stages.

In *Drosophila melanogaster*, the genetic control of meiosis has been analyzed

using mutants, many of which are hypomorphs (partially functional) (BAKER *et al.* 1976). These mutants produce meiotic products, sperm or eggs, less efficiently and often with less fidelity than the wild type. Study of the products of these abnormal meioses has yielded useful information about the roles that these gene products play in chromosome transmission. In this way, genes responsible for meiotic recombination or disjunction at meiosis I or meiosis II have been identified.

Information obtained from hypomorphic defects would augment that from nullomorphic defects if both types of defect could be achieved for the same gene products. Hypomorphic defects might result in a lower fidelity of mitotic chromosome transmission. The prior work of Kawasaki, who observed chromosome loss in several *cdc* mutants after extended incubation at their restrictive temperature (KAWASAKI 1979), and the finding of increased recombination in *cdc9* mutants (MONTELONE, PRAKASH and PRAKASH 1981), encouraged us to examine the existing nullomorphic *cdc* mutants at intermediate temperatures for alterations in the fidelity of mitotic chromosome transmission. We found that nearly all *cdc* mutants implicated in mitotic chromosome transmission behave as hypomorphs when grown at their maximum permissive temperature. They complete the mitotic process with reduced fidelity as evidenced by high frequencies of mitotic recombination and/or chromosome loss. These findings provide new approaches for defining the roles of *cdc* gene products in chromosome replication or segregation.

MATERIALS AND METHODS

Strains: All of the *cdc* mutations used in this study were obtained by backcrossing the original *cdc* mutations isolated in A364A at least three times to strains congenic with the original A364A parent. The *cdc* alleles used were: *2-2, 3-1, 4-1, 6-1, 7-4, 8-1, 9-1, 13-1, 14-1, 15-2, 16-1, 17-1, 20-1, 23-1* and *28-4*. The *ade1, ade2, tyr1* and *lys2* alleles of the original A364A parent were reverted to prototrophy and confirmed to be intragenic revertants. All other markers were obtained either by mutation (*can1*) or by backcrossing the mutation (*hom3, rad52-1*) from another *S. cerevisiae* strain into the A364A congenic background by at least ten backcrosses. Strain 4051-1, used in the initial experiments, is closely related to the A364A background but not entirely congenic.

Media and growth conditions: The diploid *cdc* strains were isolated after conjugation of appropriate *MAT***a** and *MAT*α strains by cloning onto selective medium. Three clones were picked and grown to stationary phase in synthetic medium (WOOD 1982) supplemented with histidine, methionine and threonine at 21°. Aliquots from these stocks were diluted 10^5 into the same medium and grown at the designated temperature until they reached stationary phase. All *cdc* strains were incubated at temperatures from 25° to 34° in 2° increments to determine the maximum permissive temperature for growth. To determine the frequency of recombination and chromosome loss, cultures were diluted and plated onto complete plates (WOOD 1982) to determine viability and onto complete minus arginine plus canavanine sulfate (60 mg/liter) to select chromosome loss and recombination events at 21°. The plates were incubated for 4–6 days to allow time for the slower growing chromosome *V* monosomes to form colonies and then replica plated to complete medium lacking methionine. About 2 yr after most of the experiments reported here were completed we discovered that some *MAT***a**/*MAT*α strains would sporulate in our synthetic medium upon prolonged incubation in stationary phase. This could be prevented by increasing the ammonium sulfate concentration to 5 g/liter as originally recommended by WICHERHAM (1946). Sporulation must not have occurred in the original experiments since CDC^+/CDC^+ strains cultured at every temperature in each experiment displayed reproducible low frequencies of Can^r cells (Table 4). Nevertheless, we repeated the experiment of Table 4 for all *cdc/cdc* strains that had originally displayed elevated frequencies of mitotic recombination or chromosome loss in medium containing 5 g/liter

of ammonium sulfate; in all cases effects of similar magnitude to those recorded in Table 4 were observed.

RESULTS

The spontaneous rate of mitotic recombination and chromosome loss: Mitotic recombination and chromosome loss are rare events. It is essential, therefore, to be able to select cells that have experienced such an event in order to make meaningful comparisons between the *cdc* mutant and wild-type strains for the frequencies of such events. Cells that are recombinant for one arm of chromosome *V* or that are monosomic due to the loss of one chromosome *V* homologue can be selected from diploid cells that are heterozygous for the *can1* locus conferring resistance to canavanine. Recombinants can be distinguished from monosomes if the opposite arm of chromosome *V* is also marked by an appropriate recessive marker (*hom3*) (Figure 1).

These frequencies would be in error if selection for the *can1* marker was leaky (*i.e.*, recombination or loss events occurred after plating on selective medium) or if phenotypic lag occurred (*i.e.*, some events that occurred prior to plating on selective medium failed to be recovered). The possibility of leakiness or phenotypic lag was tested previously for the *can1* allele by examining the haploid progeny of transient heterokaryons containing one sensitive nucleus and one resistant nucleus on inhibitor-containing medium (DUTCHER 1980). Little or no leakiness or phenotypic lag was found.

The spontaneous rates of mitotic recombination and chromosome loss were determined by a clonal analysis of the frequency of *HOM3 can1* cells and *hom3 can1* cells (Table 1). The rates were determined for both *MAT***a**/*MATα* strain 4051 and for a *MATα*/*MATα* strain (4051-1) derived from 4050 by mitotic recombination; the former strain is a control for the *cdc* diploid *MAT***a**/*MATα* strains reported below and the latter was necessary to verify monosomy of the purported chromosome loss events. Mitotic recombination events between the centromere and *can1* were observed at a rate of 5.9×10^{-6} recombinations/division and, therefore, occurred at a rate of 1.2×10^{-5} (since only half should appear as canavanine resistant) and chromosome loss events occurred at a rate of 8.3×10^{-6} losses/division for the *MATα*/*MATα* strain; the corresponding rates for the *MAT***a**/*MATα* strain were nearly identical (1.2×10^{-5} recombinants/division and 7.8×10^{-6} losses/division). The rate of chromosome *V* loss from diploid cells is nearly the same as that found for chromosome *VII* loss from disomic haploid strains (ESPOSITO *et al.* 1982; HARTWELL *et al.* 1982).

Meiotic analysis was carried out in order to test whether or not the putative chromosome *V* monosomes were monosomic. The putative chromosome *V* monosomes from the *MAT***a**/*MATα* strain did not sporulate. To circumvent this problem *MATα*/*MATα* monosomes were analyzed by mating to a *MAT***a**/*MAT***a** strain, the resulting tetraploid was sporulated and the spore clones were analyzed. Fourteen independent clones from 4051-1 of presumptive genotype *MATα*/*MATα cyh2*/*cyh2 can1 hom3* were crossed to 4070 (*MAT***a**/*MAT***a** *CYH2*/*CYH2 CAN1 HOM3*/*CAN1 hom3*). Sporulation and dissection of four spored asci produced spores with high viability (94.6%). If the *MATα*/*MATα* parent

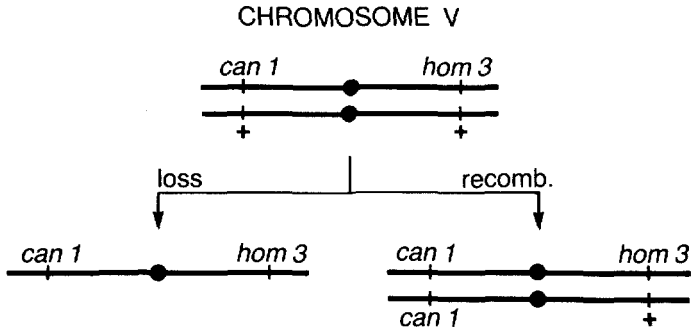

FIGURE 1.—Genetic consequences of chromosome *V* loss or recombination. Loss of one homologue of chromosome *V* or recombination on one arm followed by appropriate segregation yields canavanine-resistant clones. Loss clones are methionine requiring (and grow more slowly), whereas recombinants are methionine prototrophs.

was monosomic for chromosome *V*, the tetraploid would segregate two euploid diploid cells: two diploid cells monosomic for chromosome *V*. There should only be one *can1* allele segregating in the tetraploid. Most of the time the *can1* allele should be present either in a monosomic spore, which would have the canavanine-resistant phenotype (Can^r), or in a diploid spore containing the *CAN1* allele as well; the latter would produce Can^r progeny at high frequency due to mitotic recombination (Can^P for papillation). The progeny that do not contain a *can1* allele will segregate only sensitive progeny (Can^s) whether they are monosomic or disomic for chromosome *V*. In tetraploids from 12 of the 14 Can^r Met^- clones (Can^r + Can^P):Can^s segregated predominantly 2:2 as expected if the primary *can1 hom3* clones were monosomic for chromosome *V* (Table 2). In the two exceptions (clones 4 and 6) we presume that a mitotic nondisjunction event had restored disomy before the mating was performed; reconstituted euploids have a considerable growth advantage over monosomes and would be strongly selected. The sporadic occurrence of 4:0 and 3:1 segregations in the other clones probably derive from the same explanation; the clones, except 4 and 6, were probably a mixture of tetraploids derived from matings between monosomes and reconstituted diploids.

Two controls were carried out. The purported monosomes were homozygous *cyh2/cyh2* on chromosome *VII*, and thus, another recessive resistance marker present in two copies could be analyzed in the same tetrads, providing an example of how the Can^P + Can^r should have segregated if the *can1* allele were present in two copies in the tetraploid. The 14 tetraploids segregated predominantly 3 (Cyh^r + Cyh^P): 1 Cyh^s progeny as anticipated and in contrast to the segregation for *can1* (Table 2). Furthermore, four presumed mitotic recombinants for chromosome *V* (Can^r, Met^+) were also analyzed. They demonstrated predominantly 3 (Can^r + Can^P): 1 Can^s and 3 (Cyh^r + Cyh^P): 1 Cyh^s segregations from the tetraploid as expected (Table 3).

Many cdc *mutations decrease the fidelity of mitotic chromosome transmission*: Our goal was to study the fidelity of mitotic chromosome transmission under con-

TABLE 1

Determination of the rate of chromosome loss and recombination for chromosome V

	4051-1	
	Met$^-$ (loss)	Met$^+$ (recombination)
	63	271
	58	63
	57	36
	46	36
	35	30
	35	27
	32	22
	30	22
	30	20
	27	17
	25	16
	25	16
	21	12
	20	11
	17	10
	17	10
	16	7
	15	7
	13	6
	0	6
r_o	26	16.5
M	8.3×10^{-6}	5.9×10^{-6}

Twenty clones of strain 4051-1 (*MATα/MATα can1 hom3/+ + cyh2/cyh2*) were grown at 30° on complete medium to an average colony of about 10^6 cells. Individual colonies were cut from the Petri dish on agar slabs, resuspended in 3 ml and sonicated. A sample of 0.1 ml was removed from each sample, and the 0.1-ml aliquots were pooled and plated on C medium to determine the average number of viable cells per colony. The samples were centrifuged and the pellets were resuspended in 0.1 ml and plated on canavanine-containing medium. The colonies that grew up were replica plated to medium lacking methionine to determine the number that were *hom3*. Columns list the rank order of Met$^-$ and Met$^+$ clones. r_o is the median number of resistant clones of given phenotype and M is their rate of production calculated by the method of the median (LEA and COULSON 1948).

ditions in which a particular *cdc* gene product was rate limiting for cell division. Congenic strains were constructed to examine recombination and chromosome loss in *MAT***a**/*MATα* diploid cells homozygous for different *cdc* mutations. The strains were heterozygous for the *can1* and *hom3* markers in coupling on opposite arms of chromosome *V* (Figure 1). In preliminary experiments the *cdc* mutants were grown at a variety of temperatures in order to determine the maximum temperature at which the strains would grow to stationary phase culture from a light inoculum. For each mutant strain three clones were then

TABLE 2

Meiotic segregation of purported chromosome V monosomes (Can^r, Met$^-$)

	(Can^r + Can^P):Can^s					(Cyh^r + Cyh^P):Cyh^s				
Clone	4:0	3:1	2:2	1:3	0:4	4:0	3:1	2:2	1:3	0:4
1	0	0	8	0	0	1	5	2	0	0
2	0	0	8	1	0	3	4	2	0	0
3	1	0	7	0	0	4	3	1	0	0
4	3	3	0	0	0	0	6	0	0	0
5	1	1	4	1	0	0	3	4	0	0
6	4	0	4	0	0	2	3	3	0	0
9	1	0	6	2	0	2	5	2	0	0
10	0	2	3	0	0	2	2	1	0	0
11	2	1	5	0	0	4	2	2	0	0
12	1	0	6	1	0	3	4	1	0	0
13	0	0	7	1	0	2	3	3	0	0
14	0	0	8	0	0	3	2	3	0	0
Total	13	7	66	6	0	26	42	24	0	0

TABLE 3

Meiotic segregation of purported chromosome V mitotic recombinants (Can^r, Met$^+$)

	Can^r + Can^P:Can^s					Cyh^r + Cyh^P:Cyh^s				
Clone	4:0	3:1	2:2	1:3	0:4	4:0	3:1	2:2	1:3	0:4
21	3	4	3	0	0	5	4	1	0	0
22	2	2	1	0	0	3	2	0	0	0
23	2	4	1	0	0	2	3	2	0	0
24	5	2	0	0	0	3	3	1	0	0
Total	12	12	5	0	0	13	12	4	0	0

grown at this maximal permissive temperature and the cultures were titered for the frequency of viable cells and for the frequency of cells that had become canavanine resistant (Table 4). In all experiments three wild-type clones were grown as controls. The data in Figure 2 present the increase in recombination or chromosome loss exhibited by a particular mutant strain in comparison to the wild type. Most mutants exhibit a frequency of recombination or loss that is either within a factor of 3 of the wild-type value or is increased by a factor of 10 or more. Values that are more than three-fold above wild type are considered significant. Most *cdc* mutants that are defective in some aspect of the nuclear cycle exhibit significantly elevated levels of genetic recombination or chromosome loss or both when they are grown at their maximal permissive temperatures. However, elevated recombination or loss was not found for strains homozygous for the *ils1* mutation (a temperature-sensitive defect in the isoleucine tRNA synthetase gene, HARTWELL and MCLAUGHLIN 1968), the *cdc3* mutation (a defect in cytokinesis, HARTWELL 1971), the *cdc28* mutation (a defect in the start event of cell division, HARTWELL *et al.* 1974) or the *cdc23*

TABLE 4

Frequency of mitotic chromosome loss and recombination in cdc/cdc *strains*

Mutation	Temperature	Frequency in culture	
		Recombinants ($\times 10^4$)	Loss ($\times 10^5$)
cdc2/2	30°	85	520
cdc3/3	25°	3.7	7.4
cdc4/4	30°	8.6	5.0
cdc5/5	30°	23	570
cdc6/6	30°	190	2500
cdc7/7	28°	6.3	4.7
cdc8/8	28°	31	10
cdc9/9	25°	360	56
cdc13/13	25°	110	88
cdc14/14	29°	260	760
cdc15/15	31°	6.8	37
cdc16/16	32°	6.2	41
cdc17/17	32°	95	330
cdc20/20	28°	4.4	370
cdc23/23	25°	5.5	35
cdc28/28	28°	2.7	8.2
ils1/1	30°	3.1	5.3
CDC+/+	21°–25°	2.66 ± 0.34	13.6 ± 5.6
	28°–32°	2.66 ± 0.34	3.8 ± 1.4

mutation (a defect in the G2 portion of the cell cycle, CULOTTI and HARTWELL 1971; WOOD and HARTWELL 1982). Elevated recombination and/or loss is not, therefore, a consequence of a nonspecific repair system provoked by temperature-induced growth limitation. The responses differ among the mutants, with some exhibiting primarily elevated recombination, others primarily chromosome loss and still others recombination and loss. These individual differences result from the different *cdc* mutations and presumably reflect the individual roles of the gene products since the strains are otherwise isogenic (see DISCUSSION).

The apparent recombination events are not the result of mutation: Since we do not have a marker on the chromosome *V* distal to *can1*, it is formally possible that the *can1 HOM3* colonies we observe are due to mutations of the *CAN1* allele to *can1*. This is unlikely because the frequencies of the purported recombination events observed in these experiments are of the order of 10^{-3} to 10^{-2} events per viable cell which would be an elevation of the spontaneous mutation frequency by three to four orders of magnitude. Nevertheless, we tested this possibility for the *cdc* mutants exhibiting induced recombination by exposing haploid *cdc CAN1* strains to the same growth conditions used for the diploid strains and measuring the frequency of mutation to *can1*. The results (Table 5) demonstrate that the mutation frequencies observed are insufficient to account for the purported recombination events recorded in Figure 2. It is

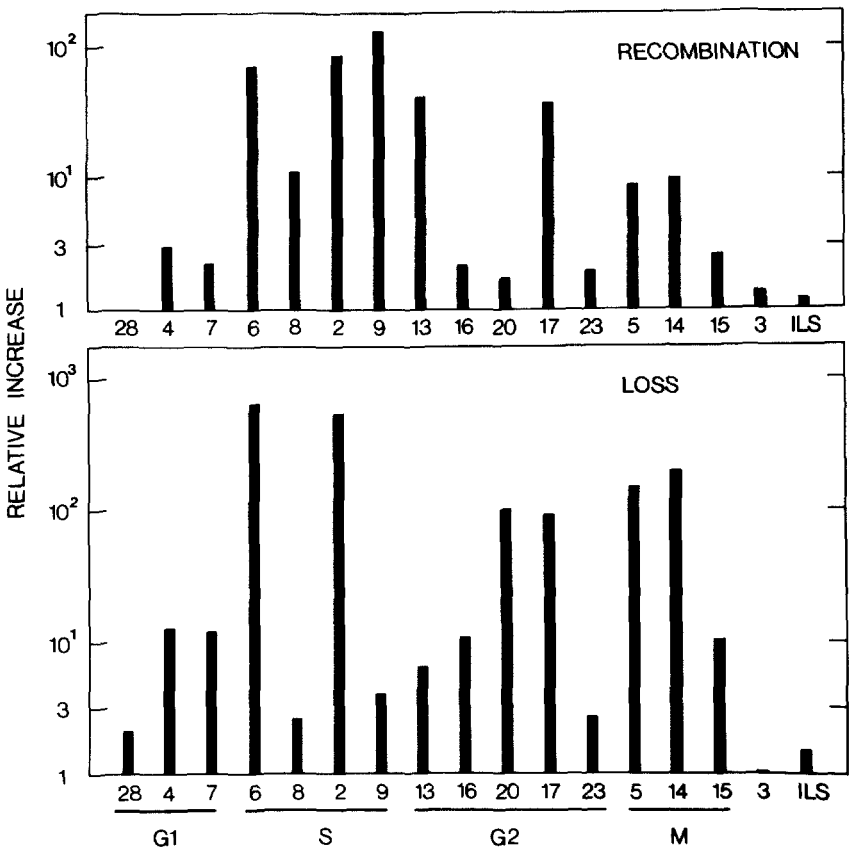

FIGURE 2.—Mitotic chromosome loss and recombination in *cdc/cdc* diploid strains grown at their maximum permissive temperature. Cells were grown and plated as described in MATERIALS AND METHODS. Numbers along the abscissa designate the *cdc* mutation; *ils1* designates the mutant deficient in isoleucyl-tRNA synthase. The ordinate is the frequency of loss or recombination events per viable cell normalized to the value obtained in a wild-type culture (Table 3).

noteworthy that some mutants exhibit an elevated frequency of mutation when stressed at the maximal permissive temperature. We interpret this observation to indicate that DNA damage resulting from deficient *cdc* gene product is being repaired by an error-prone repair pathway.

The induction of recombination or chromosome loss is recessive for most cdc *mutations*: To test whether the induction of recombination or chromosome loss in homozygous *cdc* mutant strains was dominant or recessive, strains were constructed in which the *cdc* mutation was heterozygous to its wild-type allele and chromosome *V* was marked with *can1* and *hom3* as before. These strains were grown at the same temperature as the homozygous *cdc/cdc* diploids reported in Table 3 and at 36°, the restrictive temperature for these *cdc* mutants, in order to provide maximum stress. Only one (*cdc5/+*) of the heterozygous *cdc* strains exhibited a recombination or chromosome loss frequency greater than three-fold above that of the parent strain when grown at 36° (Table 6).

TABLE 5

Mutation to canavanine resistance in cdc *strains*

Mutant	Temperature	Frequency ($\times 10^5$)	Mutant/ control
cdc2	30°	5.67 ± 2.62	9.76
cdc6	30°	2.50 ± 0.67	4.30
cdc8	28°	1.03 ± 0.08	1.77
cdc9	25°	6.13 ± 2.03	10.6
cdc13	25°	4.10 ± 0.84	7.06
cdc17	32°	6.15 ± 2.94	10.6
rad52	21°	4.85 ± 2.29	8.3
+	21°–32°	0.58 ± 0.25	1.0

Haploid strains of genotype *MAT*a *cdc his7 ura1* were grown and analyzed in the same way as the diploid strains in the experiment of Figure 2 except that the medium was supplemented with uracil. Between three and six independent cultures were monitored for each mutant.

TABLE 6

Frequency of mitotic chromosome loss and recombination in cdc/CDC *strains*

	Grown at 36°		Grown at maximum permissive temperature[a]	
CDC	Recombinants ($\times 10^4$)	Loss ($\times 10^5$)	Recombinants ($\times 10^4$)	Loss ($\times 10^5$)
2/+	6.3	4.5	2.6	1.6
4/+	5.9	9.3	3.6	0.76
5/+	17.0	9.8	3.5	4.1
6/+	7.4	4.7	3.1	2.1
8/+	7.9	4.3	2.1	1.9
9/+	7.2	9.4	1.7	2.6
13/+	5.5	5.5	2.1	3.0
14/+	7.2	8.0	2.4	1.2
15/+	5.2	6.0	4.0	8.2
16/+	7.1	9.2	3.6	1.8
17/+	7.6	6.2	2.0	3.4
20/+	2.5	3.1	1.7	2.3
+/+	3.7	2.9	2.8	2.8

[a] Same temperature as corresponding mutant in Table 3.

We conclude that the effect of the *cdc* mutation on recombination or chromosome loss is recessive to the wild-type allele with the exception of the *cdc5* mutation. The loss of fidelity in chromosome transmission observed with the homozygous *cdc* diploid strains is, therefore, unlikely to be the result of an aberrant function of the mutant gene product but rather can be attributed to a deficiency of gene product.

RAD52 *is required for the hyperrecombination phenotype*: We interpret the

TABLE 7

Mitotic chromosome loss and recombination in rad52/rad52 cdc/cdc *strains*

		Frequency in culture		
		Recombination		
cdc	Temperature	$\times 10^4$	Ratio of frequencies of *rad52/RAD52*	Loss ($\times 10^2$)
2	28°	1.8 ± 0.1	0.008	3.2 ± 0.2
6	30°	3.0 ± 0.5	0.016	3.6 ± 0.2
8	28°	0.62 ± 0.5	0.019	0.37 ± 0.06
13	25°	2.3 ± 0.2	0.021	1.8 ± 0.3
17	28°	2.4 ± 0.6	0.025	2.4 ± 0.5
+	25°–30°	1.1 ± 0.2	0.41	0.8 ± 0.11

foregoing results as follows. A deficiency for certain *cdc* gene products results in an accumulation of lesions in DNA. These lesions are repaired by an error-prone and a recombinogenic repair pathway. The recombinogenic repair pathway in *S. cerevisiae* is known to require the *RAD52* gene product, since the recombinogenic stimulation produced by all DNA-damaging agents is essentially eliminated by mutation in the *RAD52* gene (RESNICK 1979). If our interpretation is correct, then a *rad52* mutation should eliminate the recombination that is induced by these *cdc* mutations. To test this possibility strains were constructed with the same genotype as those used in the experiments of Figure 2 except that the strains were homozygous for the *rad52-1* mutation. These strains were stressed by growth at elevated temperatures, following which the cultures were monitored for chromosome loss and recombination (Table 7). The recombination values obtained in this experiment are compared to those in Table 4. In two cases (*cdc2* and *17*) the temperatures used for the *rad52* strains (Table 6) were slightly lower than those used for the *RAD52* strains (Figure 2), since the *rad52* strains were more temperature sensitive than the *RAD52* strains. In both experiments the temperatures used were within 2° of the lethal temperature. The *rad52* mutation virtually eliminated the recombination events observed in the *RAD52* strains. All of the *rad52* strains exhibited high frequencies of chromosome loss as expected (MORTIMER, CONTOPOULOU and SCHILD 1981).

DISCUSSION

The fidelity of mitotic chromosome transmission in *S. cerevisiae* is high. We find that the spontaneous rate of recombination between the centromere and the *can1* locus on chromosome *V* is 1.2×10^{-5} events/cell division and that the spontaneous rate of loss of chromosome *V* is 8.3×10^{-6} events/cell division. This high fidelity is not peculiar to chromosome *V* since similar values have been obtained for the spontaneous frequency of recombination and chromosome loss for chromosome *VII* in haploid strains disomic for this chromosome (HARTWELL *et al.* 1982; ESPOSITO *et al.* 1982).

The fidelity of mitotic chromosome transmission was dramatically reduced by 13 of 14 *cdc* mutations when these mutants were grown at their maximal permissive temperatures. Each of the products of these genes is known to be essential for a discrete step in the nuclear cycle of mitotic chromosome replication and segregation. This result was not obtained for either of the two mutations in genes that function outside of this pathway of nuclear events, *cdc3* and *ils1*, or for a mutation in a gene essential at the control point in cell division, *cdc28*. That the effects we observe are due to a deficiency in the function of the *cdc* gene product rather than to an aberrant function is suggested by two facts. First, nearly all mutants examined in the nuclear pathway exhibit decreased fidelity; the acquisition of aberrant function by proteins as a result of mutation is rare. Second, all but one of the mutant alleles are recessive to their wild-type allele for the decrease in fidelity.

We interpret these observations as follows. The high fidelity of mitotic chromosome transmission requires that nearly all steps proceed without delay. When delays at particular steps occur due to deficient *cdc* gene product, errors are incurred. This view implies that the chromosome is in a metastable configuration at many, if not all, stages in its replication and segregation. Our interpretation suggests an explanation for a heretofore puzzling observation. BYERS and SOWDER (1980) have observed that nearly all of the *cdc* gene products are present in great excess in the cytoplasm over than needed for one cell division. Perhaps the excess quantity of gene product is provided to ensure a maximal rate of chromosome maturation by mass action in order to avoid the errors that are incurred when maturation is delayed by deficiency of function. Furthermore, our interpretation suggests an explanation for why most cells slow or arrest their division cycles at a control point in G1 prior to the initiation of DNA synthesis (PRESCOTT 1976); in *S. cerevisiae* this control point is at the step executed by *CDC28* product (HARTWELL *et al.* 1974). This control point may have been selected because it is the only stage at which the chromosome is in a stable configuration.

We assume that the induction of recombination in some *cdc* mutants is the consequence of a recombinational DNA repair pathway, that the induction of mutation in these same mutants is the result of an error-prone DNA repair process and that each pathway acts upon lesions left in DNA as a result of the delay in *cdc* gene product activity. This assumption is supported by the fact that all of the induced recombination is dependent upon the *RAD52* function. Furthermore, an extensive body of previous data supports our interpretation. All agents that cause DNA damage also induce genetic recombination (RESNICK 1979). In bacteriophage T4, blocks or delays in DNA replication imposed by mutations in any one of many genes whose products are required for DNA replication result in induced recombination (BROKER and DOERMANN 1975); nucleases act on nicks or gaps left in DNA at the growing fork to generate single-stranded regions which in turn promote strand invasion and recombination (BROKER and DOERMANN 1975). In *E. coli*, lesions in the genes coding for DNA polymerase I, DNA ligase, deoxyuridine triphosphatase (KONRAD 1977) and adenine methylase (TYE *et al.* 1977) are all recombinogenic and all increase the frequency of nicks or gaps in DNA. The observation that all of

the gene products implicated in the mitotic S phase show induced recombination (*cdc2, 6, 8, 9*) is consistent with our interpretation. *cdc2* mutants replicate about two-thirds of their genome, apparently in a random fashion, and cease DNA synthesis before the completion of the S phase (CONRAD and NEWLON 1983). *cdc6* mutants appear to be defective in some aspect of the initiation of DNA synthesis and replicate much or all of their DNA following an aberrant initiation event (HARTWELL 1976). *cdc8* mutants are "fast stop" defects in DNA elongation (HARTWELL 1971a); *cdc8* is defective in thymidylate kinase (R. SCLAFANI and W. FANGMAN, personal communication). *CDC9* is the structural gene for DNA ligase (BARKER and JOHNSTON 1983) and the DNA that is replicated in *cdc9* mutant cells at the restrictive temperature contains a high frequency of single strand breaks (JOHNSTON and NASMYTH 1978). We anticipate, therefore, that all *cdc* mutants that exhibit induced recombination identify genes whose products perform essential functions in DNA metabolism. Although our experiments do not address the question of whether or not these gene products act directly on DNA, we suggest that their deficiency leads to the accumulation of nicks or gaps in DNA. We cannot rule out the possibility that some mutations induce recombination by an alternative mechanism; for example, that deficiency for the gene product might lead to the induction of recombination enzymes that act on undamaged DNA. However, we consider this model unlikely in view of the strong association of mitotic recombination (unlike meiotic recombination) with DNA damage.

Four mutants whose time of function is located outside of the S phase (HARTWELL 1971b; CULOTTI and HARTWELL 1971; WOOD and HARTWELL 1982) also induce high frequencies of genetic recombination (*cdc13, 17, 5* and *14*). If our assumption is correct, that recombination is evidence for DNA lesions that are being repaired by a recombinational repair pathway, this observation is highly significant. It indicates that not all aspects of DNA metabolism are confined to the S phase. We suggest that the *CDC13* and *17* gene products are essential for DNA metabolism during G2, whereas the *CDC5* and *14* gene products are essential for DNA metabolism occurring during the mitotic phase. Since the bulk of DNA replication is completed at the arrest point for these four mutants (HARTWELL 1976; CULOTTI and HARTWELL 1971; BYERS and GOETSCH 1974; WOOD and HARTWELL 1982) it is likely that the recombinogenic lesions that occur as a result of deficiency for the relevant gene products are associated with terminal aspects of chromosome replication and, hence, may be occurring in particular domains. The recombination events provoked by these mutations provide a signal for locating the domain of the chromosome upon which these genes function since the recombination events may occur in the vicinity of the domain.

The pattern of recombination and/or chromosome loss is different for different *cdc* mutants and must reflect the role of these gene products in mitotic chromosome transmission. Some mutants located both early and late in the cell cycle produce significant increases in chromosome loss with little or no associated recombination (*cdc4, 7, 16, 20* and *15*). Since there is little or no recombination induced by these mutants, there is no evidence for DNA dam-

age. Perhaps these gene products constitute elements of the mitotic spindle or segregational apparatus. *CDC4* is known to be necessary for the separation of the duplicated spindle pole bodies to establish the poles of the mitotic spindle (BYERS and GOETSCH 1974). *CDC16* is necessary for spindle elongation (BYERS and GOETSCH 1974) and *CDC15* for the division of the nuclei after the establishment of the elongated spindle (BYERS and GOETSCH 1974). In this context *CDC7* is of particular interest since it is thought to be involved in the initiation of DNA synthesis (HARTWELL 1971a). Two other genes, *CDC28* and *4*, control aspects of spindle pole morphogenesis and are essential for the initiation of DNA synthesis (HARTWELL 1971a). *CDC7* may, therefore, represent the latest event in spindle morphogenesis prior to DNA replication. We must also consider the possibility that mutants deficient in some aspect of DNA metabolism may fail to produce a recombination signal. Two possibilities occur to us. First, our methods detect only recombination events between homologues. Recombination events occurring between sister chromatids are undetected in our experiments and could be occurring in *cdc16*, *20* and *15*. Second, one might anticipate that the hyperrecombination phenotype expected for a mutant deficient in DNA synthesis would not occur if the mutant were also deficient in recombination. However, this possibility does not appear to be a limitation under the conditions of our experiments utilizing the maximum permissive temperature, since the *CDC9* product is known to be essential for recombination at the restrictive temperature (FABRE and ROMAN 1979) yet the *cdc9* mutant gives the strongest hyperrecombination phenotype at the maximum permissive temperature (Figure 2).

Some mutants that exhibit high frequencies of recombination also display high frequencies of chromosome loss (*cdc 2, 6, 17, 5, 14*), whereas others are elevated for recombination but not as dramatically for chromosome loss (*cdc8, 9, 13*). Chromosome loss in mutants that also exhibit elevated recombination is likely to be caused by DNA lesions that were not repaired. Presumably, the degree to which chromosome loss is associated with recombination events is a consequence of the particular types of lesions being left in the DNA in the different mutants and the efficacy of the various repair pathways that deal with these lesions.

We thank LARRY SANDLER for many helpful discussions and DOUG KOSHLAND, DAN BURKE, WALT FANGMAN and NANCY HOLLINGSWORTH for their comments on this manuscript. We also thank BARBARA GARVIK for performing some of the experiments reported in Table 5. This work was supported by grant GM17709 from the National Institutes of Health, Division of General Medical Sciences.

LITERATURE CITED

BAKER, B. S., A. T. C. CARPENTER, M. S. ESPOSITO, R. E. ESPOSITO and L. SANDLER, 1976 The genetic control of meiosis. Annu. Rev. Genet. **10:** 53–134.

BARKER, D. G. and L. H. JOHNSTON, 1983 *Saccharomyces cerevisiae cdc*9, a structural gene for yeast DNA ligase which complements *Schizosaccharomyces pombe cdc*17. Eur. J. Biochem. **134:** 315–319.

BROKER, T. and A. H. DOERMANN, 1975 Molecular and genetic recombination of bacteriophage T4. Annu. Rev. Genet. **9:** 213–244.

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

BYERS, B. and L. SOWDER, 1980 Gene expression in the yeast cell cycle (Abstr.). J. Cell Biol. **87:** 6a.

CONRAD, M. N. and C. S. NEWLON, 1983 *Saccharomyces cerevisiae cdc*2 mutants fail to replicate approximately one-third of their nuclear genome. Mol. Cell. Biol. **3:** 1000–1012.

CULOTTI, J. and L. H. HARTWELL, 1971 Genetic control of the cell division cycle in yeast. III. Seven genes controlling nuclear division. Exp. Cell Res. **67:** 389–401.

DUTCHER, S. K., 1980 Genetic control of karyogamy in *Saccharomyces cerevisiae.* Ph.D. Thesis, University of Washington, Seattle.

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:** 5–12.

FABRE, F. and AND H. ROMAN, 1979 Evidence that a single DNA ligase is involved in replication and recombination in yeast. Proc. Natl. Acad. Sci. USA **76:** 4586–4588.

HARTWELL, L. H., 1971a Genetic control of the cell division cycle in yeast. II. Genes controlling DNA replication and its initiation. J. Mol. Biol. **59:** 183–194.

HARTWELL, L. H., 1971b Genetic control of the cell division cycle in yeast. IV. Genes controlling bud emergence and cytokinesis. Exp. Cell Res. **69:** 265–276.

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., J. CULOTTI, J. R. PRINGLE and B. J. REID, 1974 Genetic control of the cell division cycle in yeast. Science **183:** 46–51.

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.

HARTWELL, L. H. and C. S. MCLAUGHLIN, 1968 Mutants of yeast with temperature-sensitive isoleucyl-tRNA synthetases. Proc. Natl. Acad. Sci. USA **59:** 422–428.

JOHNSTON, L. H. and K. A. NASMYTH, 1978 *Saccharomyces cerevisiae* cell cycle mutant *cdc*9 is defective in DNA ligase. Nature **274:** 891–893.

KAWASAKI, G., 1979 Karyotypic instability and carbon source effects in cell cycle mutants of *Saccharomyces cerevisiae.* Ph.D. Thesis, University of Washington, Seattle.

KONRAD, E. B., 1977 Method for the isolation of *Escherichia coli* mutants with enhanced recombination between chromosomal duplications. J. Bacteriol. **130:** 167–172.

LEA, D. E. and C. A. COULSON, 1948 The distribution of numbers of mutants in bacterial populations. J. Genet. **49:** 264–284.

MONTELONE, B. A., S. PRAKASH and L. PRAKASH, 1981 Spontaneous mitotic recombination in *mms*8-1, an allele of the CDC9 gene of *Saccharomyces cerevisiae.* J. Bacteriol. **147:** 517–525.

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. USA **78:** 5778–5782.

PRESCOTT, D. M., 1976 pp. 1–177. In: *Reproduction of Eukaryotic Cells.* Academic Press, New York.

PRINGLE, J. R. and L. H. HARTWELL, 1981 The *Saccharomyces cerevisiae* cell cycle. pp. 97–142. In: *Molecular Biology of the Yeast Saccharomyces,* Vol. I, Edited by J. N. STRATHERN, E. W. JONES, and J. R. BROACH. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

RESNICK, M. A., 1979 The induction of molecular and genetic recombination in eukaryotic cells. Adv. Radiat. Biol. **8:** 175–217.

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

TYE, B.-K., P.-O. NYMAN, I. R. LEHMAN, S. HOCHHAUSER and B. WEISS, 1977 Transient accumulation of Okazaki fragments as a result of uracil incorporation into nascent DNA. Proc. Natl. Acad. Sci. USA **74:** 154–157.

WICKERHAM, L. J., 1946 A critical evaluation of the nitrogen assimilation tests commonly used in the classification of yeasts. J. Bacteriol. **52:** 293–301.

WOOD, J. S., 1982 Mitotic chromosome loss induced by methyl-benzimida-zole-2-ylcarbamate as a rapid mapping method in *Saccharomyces cerevisiae.* Mol. Cell Biol. **2:** 1080–1087.

WOOD, J. S. and L. H. HARTWELL, 1982 A dependent pathway of gene functions leading to chromosome segregation in *S. cerevisiae.* J. Cell. Biol. **94:** 718–726.

Communicating editor: E. JONES