MAPPING *CDC* MUTATIONS IN THE YEAST *S. Cerevisiae* BY *RAD52*-MEDIATED CHROMOSOME LOSS

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ABSTRACT

Using the chromosome loss-mapping method of Schild and Mortimer, I have mapped several new temperature-sensitive mutations that define five *CDC* genes. Modified procedures were used to facilitate mapping temperature-sensitive mutations in general, and these modifications are discussed. The mutations were assigned to specific chromosomes by chromosome loss procedures, and linkage relationships were determined subsequently by standard tetrad analysis. Four of the mutations define new loci. The fifth mutation, *cdc63-1*, is shown to be allelic to previously known mutations in the *PRT1* gene.

THE genetic map of *S. cerevisiae* is composed of 17 linkage groups (chromosomes) and several acentromeric fragments not yet assigned to particular chromosomes (MORTIMER and SCHILD 1980, 1981b, 1982). The large number of chromosomes and the generally high level of meiotic recombination often make classical linkage analysis an impractical procedure for assigning mutations to specific chromosomes (MORTIMER and SCHILD 1981a). Therefore, other methods have been devised for this task, including meiotic trisomic analysis (MORTIMER and HAWTHORNE 1973; WICKNER 1979), mitotic linkage analysis (NAKAI and MORTIMER 1969; MORTIMER and HAWTHORNE 1973), meiotic analysis of recombinationless strains (KLAPHOLZ and EASTON ESPOSITO 1982a) and chromosome loss procedures (KAWASAKI 1979; WOOD 1982). Here, I have used a new chromosome loss-mapping procedure, originally designed by SCHILD and MORTIMER (1985), to map temperature-sensitive *cdc* mutations. The method employs diploid strains homozygous for a *rad52* mutation and thereby defective in meiotic (GAME *et al.* 1980) and mitotic (GAME *et al.* 1980; PRAKASH *et al.* 1980; SAEKI, MACHIDA and NAKAI 1980) recombination and in repair of radiation-induced DNA damage (HAYNES and KUNZ 1981). MORTIMER, CONTOPOULOU and SCHILD (1981) have shown that *rad52/rad52* diploids also lose chromosomes spontaneously and that exposure to X rays increases the frequency of loss. Here, I show that the *rad52* chromosome loss method is an accurate and rapid method for mapping temperature-sensitive mutations and, unlike other chromosome loss-mapping procedures, requires screening relatively small number of colonies.

In this report I present mapping data, obtained by the *rad52* chromosome

loss method and by classical tetrad analysis, for several new temperature-sensitive *cdc* mutations that define five *CDC* genes (BEDARD, JOHNSTON and SINGER 1981). Strains bearing these mutations arrest at the nonpermissive temperature within one division at the cell cycle regulatory point referred to as "start." I present genetic evidence that one of these mutations, *cdc63-1*, is allelic to previously known *prt1* mutations.

MATERIALS AND METHODS

Strains: The genotypes and sources of the haploid yeast strains used in this work are presented in Table 1.

Media: The enriched liquid and solid media YM-1 and YEPD, respectively, have been described (HARTWELL 1967). Synthetic complete (SC) medium contained (per liter): Difco yeast nitrogen base without amino acids, 6.7 g; succinic acid, 10 g; sodium hydroxide, 6 g; glucose, 20 g; L-amino acids arginine, aspartic acid, histidine, isoleucine, leucine, methionine, phenylalanine, tryptophan, threonine, tyrosine, serine, and valine, 40 mg each; and adenine and uracil, 20 mg each. Auxotrophic mutations were scored on SC medium without one supplement ("dropout" media). Galactose fermentation mutations were scored on SC medium containing 2% galactose instead of glucose as the carbon source. The *pep4-3* mutation was indicated by the inability of mutant strains to cleave *N*-acetyl-DL-phenylalanine β-naphthyl ester contained in agar overlays, as described by JONES (1977).

Genetic analysis: Standard yeast genetic techniques were employed (MORTIMER and HAWTHORNE 1969).

Mapping protocol: The procedure for mapping by *rad52*-induced chromosome loss was suggested by D. SCHILD and R. K. MORTIMER and is described in the accompanying paper. However, several modifications were made here to facilitate mapping of temperature-sensitive mutations.

Diploid strains homozygous for the *rad52-1* mutation and heterozygous for both an unmapped temperature-sensitive *cdc* mutation and recessive auxotrophic mutations were induced to undergo chromosome loss by exposure to γ rays. Following irradiation, cells were incubated on YEPD medium at 23° for 7–9 days. Colonies were replica-plated to SC and to dropout media to screen for the expression of nutritional requirements; subclones that grew poorly on SC medium were excluded from further analysis. To determine which subclones had become temperature sensitive due to expression of the unmapped *cdc* mutation, complementation tests were performed; *MAT***a** and *MATα* haploid tester strains carrying a temperature-sensitive mutation other than the unmapped mutation were included to assess mating ability. Subclones that complemented control tester strains, but failed to complement tester strains of either mating type carrying the unmapped *cdc* mutation, were temperature sensitive due at least in part to expression of that *cdc* mutation.

The assessment of independent expression of two mutations was based on a chi square test of independence (2 × 2 contingency table; DANIEL 1978). Initially, 200–400 randomly chosen subclones from γ-irradiated diploids carrying an unmapped *cdc* mutation in repulsion to chromosome markers were analyzed. A particular chromosome was considered a possible location for an unmapped mutation if that mutation and a marker on that chromosome were expressed together significantly less often than expected on the basis of independence ($P < 0.05$). All but a few chromosomes could be eliminated from consideration using the markers-in-repulsion method. Then, 100–200 subclones from γ-irradiated diploids carrying the unmapped mutation in coupling with chromsomal markers were analyzed. The unmapped mutation was assigned to a particular chromosome if that mutation and a marker on that chromosome were expressed together significantly more often than expected ($P < 0.05$).

Irradiation: The γ-ray source was ^{60}Co, with an initial dose rate of 21.9 rads/sec. The dose rate in each experiment was calibrated according to the half-life of the isotope. To induce chromosome loss, diploid cells growing logarithmically in YM-1 medium were transferred to YEPD solid medium and irradiated with a dose of 10 krad, to a survival value of 0.5–5% at 23°. Effective screening for the presence of the *rad52-1* mutation was achieved after colonies were replica-plated to YEPD medium and incubated for 12 hr at 23°; these replicas were then replica-plated to YEPD medium

TABLE 1

Haploid strains

Strain	Genotype	Source[a]
XD-1	*MAT***a** *cdc60-1*	DPB
1D-1	*MATα cdc60-1 ade1 leu1 gal2*	DPB
GD-2-1	*MAT***a** *cdc61-1 his6 ura1 gal*	DPB
S7	*MAT***a** *cdc62-1 leu1 ura*	DPB
SG44-1	*MAT***a** *cdc63-1 his6 ura1*	DPB
XS44	*MATα cdc63-1 his6 ura1*	DPB
S104	*MAT***a** *cdc64-1 leu1*	DPB
A4840A	*MATα ade2 ade his5-35*	CSH
Sc100	*MAT***a** *GAL80^S his trp MEL1*	JEH
A236-57B	*MAT***a** *leu2-3 trp1 met4 aro7 his3 lys11 SUC2 MAL3 can1*	MRC
A236-24C	*MATα leu2-3 trp1 met4 aro7 his3 lys11 SUC2 MAL3 can1 ts*(?)	MRC
A334-49B	*MAT***a** *prt1-1 leu2 petx pha2 arg8*	MRC
A334-27B	*MATα prt1-1 leu2-3 petx pha2 arg8 his ura*	MRC
A141-37C	*MATα leu2-3 met1 ade6 cdc11 pet17 lys11 his1*	MRC
171	*MAT***a** *prt1-2 gal1 ade1 ade2 ura1 his7 lys2 tyr1*	CSM
19-29	*MAT***a** *prt1-3 gal1 ade1 ade2 ura1 his7 lys2 tyr1*	CSM
R15	*MATα leu2-3*	MRC
N435-1A	*MAT***a** *gal4 lys7 met6 arg1 his7 MAL2 SUC*	YGSC
N442-4A	*MAT***a** *his6 ade2 lys9 ura1 trp5 met2 arg4 mal suc*	YGSC
ts136	*MAT***a** *rna1-1 ade1 ade2 ura1 his7 tyr1 gal1*	YGSC
20B-12	*MATα pep4-3 trp1*	YGSC
S1896D	*MAT***a** *met7 trp1 leu1 ade1 gal1 gal2 [rho$^-$]*	YGSC
F33	*MATα met7 gal2 [rho$^-$]*	YGSC
BH24-3	*MATα cdc60-1 lys11 met4 trp1 aro7 ade1 leu*	1D-1 × A236-57B
BH1-10	*MATα cdc61-1 ade2*	GD-2-1 × A4840A
BH12-3	*MATα cdc61-1 leu2*	GD-2-1 × R15
BH9-4	*MAT***a** *cdc62-1 his3 lys11 met4 aro7 trp1 ura*	S7 × A236-24C
BH32-3	*MAT***a** *cdc64-1 ade2 his6 lys9 met2 trp5*	BH4-7 × N442-4A
BH4-7	*MATα cdc64-1 ade2 ade*	S104 × A4840
XS122-57D	*MAT***a** *rad52-1 ura3*	DS
XS122-49C	*MATα rad52-1 leu2*	DS
XS195-23B	*MAT***a** *rad52-1 his7 leu2 trp1 ade5 arg4 ilv3 lys7*	DS
XS194-23C	*MAT***a** *rad52-1 ade1 trp1 ura3 his2 leu1 arg4 aro7*	DS
XS206-9B	*MAT***a** *rad52-1 his7 leu2 lys2 met6 ade2 arg1 ade4 ilv3*	DS
XS209-11C	*MAT***a** *rad52-1 leu2 trp1 met10 ura4 his3 ade4*	DS
XS214-1B	*MAT***a** *rad52-1 leu2 trp5 arg4 his6 ilv3 ura1 lys9 ade2 met2*	DS
HR15	*MATα rad52-1 cdc60-1*	1D-1 × XS122-57D
HR30	*MATα rad52-1 cdc61-1 ura3*	BH1-10 × XS122-57D

TABLE 1—Continued

Strain	Genotype	Source[a]
HR2	*MATα rad52-1 cdc62-1 leu1*	S7 × XS122-49C
HR7	*MATα rad52-1 cdc63-1*	SG44-1 × XS122-49C
HR21	*MATα rad52-1 cdc64-1*	S104 × XS122-49C
HR109-4	*MATa rad52-1 cdc60-1 lys11 met4 aro7 leu1 ade1*	BH24-3 × XS122-57D
HR203-6	*MATα rad52-1 cdc61-1 ade4 his3 leu2 ura4*	BH12-3 × XS209-11C
HR202-2	*MATa rad52-1 cdc62-1 lys11 met4 aro7 trp1 ura*	BH9-4 × XS122-49C
HR201	*MATα rad52-1 cdc62-1 lys7 leu1 met6*	HR2 × N435-1A
HR14-3	*MATa rad52-1 cdc63-1 met10 trp1 his3 ura*	XS44 × XS209-11C
HR106-2	*MATα rad52-1 cdc64-1 ade2 leu2 lys9 met2*	BH32-3 × XS122-49C

[a] DPB, DENNIS P. BEDARD (Dalhousie University), see also BEDARD, JOHNSTON and SINGER (1981); JEH, JAMES E. HOPPER (Pennsylvania State University); CSH, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; MRC, MICHAEL R. CULBERTSON (University of Wisconsin), see also GABER *et al.* (1983); CSM, CALVIN S. MCLAUGHLIN (University of California, Irvine); YGSC, Yeast Genetic Stock Center (Berkeley); DS, DAVID SCHILD (University of California, Berkeley).

and the second replicas were immediately irradiated with a dose of 50 krad. After incubation for 2 days at 23°, radiation sensitivity of the second replicas was scored.

RESULTS

Chromosome loss by rad52/rad52 *diploids*

Diploid strains homozygous for *rad52* and heterozygous for recessive chromosomal mutations conferring nutritional requirements were irradiated, and surviving colonies were screened for nutritional requirements. The expression of heterozygous recessive traits in irradiated *rad52/rad52* diploids has been shown to result almost exclusively from loss of entire chromosomes (MORTIMER, CONTOPOULOU and SCHILD 1981). The extent of recessive marker expression, in each case indicating loss of a particular chromosome, is summarized in Table 2 for several diploids studied here. On this basis chromosome loss events for chromosomes *I–XVI* were found with proportions of 0.05–0.20 per colony; chromosome *XVII* was not followed since it has no easily scorable markers (WICKNER, BOUTELET and HILGER 1983). Chromosomes lost most often were chromosomes *IV*, *VI*, *VII*, *XII* and *XV*.

Previous studies indicated that chromosome loss in irradiated *rad52/rad52* diploids is an ongoing process (MORTIMER, CONTOPOULOU and SCHILD 1981). My observations confirm this finding. Table 2 also shows the extent of chromosome loss in diploids that were first irradiated and then subjected to subculturing and incubation at 23° and 4° over an 8-month period. Loss of certain chromosomes increased, with proportions of colonies indicating loss up to 0.3 for chromosomes *VII* and *XV*.

In most cases, each *rad52/rad52* diploid strain constructed for these experiments carried only one auxotrophic marker on each marked chromosome. Often the markers for a chromosome differed in individual strains, and in

TABLE 2

Expression of recessive markers after irradiation of rad52/rad52 *homozygous diploid strains*

		Time after γ-irradiation			
		2 wk[a]		8 mo[b]	
Chromosome	Marker	No. of subclones tested	Fraction of subclones expressing marker	No. of subclones tested	Fraction of subclones expressing marker
I	*ade1*	1464	0.119	139	0.094
II	*his7*	727	0.091		
III	*leu2*	2553	0.078	100	0.040
IV	*trp1*	2846	0.222	239	0.159
V	*ura3*	786	0.108	139	0.180
VI	*met10*[c]	1152	0.197	100	0.270
	his2	1464	0.166	98	0.265
VII	*leu1*	1464	0.194	139	0.288
	trp5	674	0.134		
	ade5	230	0.039		
VIII	*arg4*	2368	0.063	139	0.151
IX	*lys1*	497	0.050		
	his6	674	0.150		
X	*ilv3*	1401	0.084		
XI	*ura1*	451	0.104		
XII	*ura4*	1152	0.220	100	0.200
XIII	*lys7*	230	0.074		
XIII	*ade4*	1649	0.109	100	0.020
XIV	*met2*[d]	674	0.136		
	lys9[d]	674	0.076		
XV	*arg1*	497	0.107		
	his3	1152	0.253	100	0.310
	ade2	674	0.147		
XVI	*aro7*	1464	0.100	139	0.180
F12	*met6*	394	0.086		

[a] Subclones derived by irradiation of various *rad52/rad52* homozygous diploid strains heterozygous for one of the *cdc* mutations and some chromosomal markers listed were assessed for nutritional requirements within 2 wk of irradiation. The data are the sum of many different experiments.

[b] Subclones derived by irradiation of two *rad52/rad52* homozygous diploid strains heterozygous for *cdc63-1* and chromosome markers were assessed for nutritional requirements after as many as 8 months of serial transfer and storage.

[c] Different markers were used to indicate loss of the same chromosome in different strains.

[d] Although these chromosome *XIV* markers were in coupling they were expressed at different frequencies (see text).

these cases the proportions of colonies showing expression of each of the various markers are listed in Table 2. Several diploids carried two auxotrophic markers in coupling on opposite arms of chromosome *XIV*. These markers, *lys9* and *met2*, also showed different frequencies of expression (Table 2). This behavior is addressed in the DISCUSSION.

Temperature sensitivity in rad52/rad52 *diploids*

Diploids homozygous for *rad52* and heterozygous for an unmapped *cdc* mutation, as well as for recessive chromosome markers conferring auxotrophies, were irradiated to induce chromosome loss. Survivors were screened for the expression of recessive nutritional requirements. Since aneuploid strains of Saccharomyces usually retain viability (PARRY and COX 1970), cells that had lost a single homolog of one or more chromosomes would still be expected to form colonies. In diploids heterozygous for recessive mutations loss of one homolog could lead to expression of an auxotrophic mutation. However, because loss of both homologs of a particular chromosome would be lethal, an unmapped mutation and an auxotrophic marker in repulsion on the same chromosome pair should never be expressed together. Conversely, an unmapped mutation and an auxotrophic marker in coupling on the same chromosome should always be expressed together.

Based on this rationale, chromosome loss mapping was used to localize the temperature-sensitive *cdc64-1* mutation. Following irradiation of diploids with *cdc64-1* in repulsion to other chromosomal markers, 0.33 of surviving subclones were temperature sensitive. Surprisingly, this proportion of temperature-sensitive subclones was at least two-fold higher than the proportion of subclones expressing any one of the recessive nutritional requirements and suggested that temperature sensitivity was occurring in unexpected ways. Therefore, complementation tests were performed to determine which subclones were temperature sensitive because of expression of the *cdc64-1* mutation.

Complementation tests are possible in this situation because a population of (initially) diploid cells undergoing chromosome loss contains some mating-competent cells. Loss of one chromosome *III* homolog by an otherwise diploid cell produces a cell expressing only *MAT***a** or *MAT*α information from the remaining chromosome *III* copy; cells of this type can mate with haploid cells of opposite mating type (LIRAS *et al.* 1978). Since production of such mating-competent cells via loss of chromosome *III* occurs at a reasonable frequency under these conditions (see Table 2), subclones that have already lost a chromosome undoubtedly will contain some cells that have also lost chromosome *III* and thus can mate.

Of the temperature-sensitive subclones, 54% (17.9% of all subclones) did not complement either *Mat***a** or *Mat*α *cdc64-1* tester strains but did complement either or both *MAT***a** and *MAT*α *cdc11* tester strains, indicating that these subclones were temperature sensitive at least in part because of expression of the *cdc64-1* mutation. The remaining 46% of the temperature-sensitive subclones complemented either or both *MAT***a** and *MAT*α *cdc64-1* tester strains as well as control *cdc11* tester strains, indicating that these colonies were not expressing the *cdc64-1* mutation but were temperature sensitive for other reasons.

Expression of this sort of uncharacterized temperature-sensitive phenotype was not specific to diploids heterozygous for *cdc64-1*. All *rad52/rad52* diploid

strains constructed for this study gave rise, in significant numbers following irradiation, to uncharacterized temperature-sensitive subclones. For instance, from a diploid strain constructed by mating strains XS122-49C and XS194-23C (which could each form colonies at 37°), 25.7% of the 116 subclones examined after irradiation had become temperature sensitive. Therefore, in all mapping studies reported here complementation tests were performed to determine which temperature-sensitive subclones were expressing the *cdc* mutation of interest.

Localization of cdc *mutation by chromosome loss mapping*

cdc64-1: The proportion of subclones that expressed both *cdc64-1* and a recessive marker on chromosome *XV* was significantly less than expected for markers in repulsion ($P < 0.05$) (Table 3). Moreover, no colonies were observed that expressed the *cdc64-1* mutation and a chromosome *XIV* marker. Although this latter finding was not statistically significant, since the expected value for expression of the *cdc64-1* mutation and loss of chromosome *XIV* was less than 1%, I did not wish to eliminate chromosome *XIV* from consideration on this basis. To determine whether *cdc64-1* is located on chromosome *XV* or *XIV*, a *rad52/rad52* homozygous diploid strain heterozygous for *cdc64-1* in coupling with chromosome markers was irradiated. The proportion of subclones expressing both *cdc64-1* and *ade2* was the only one significantly greater than expected, indicating that *cdc64-1* is located on chromosome *XV* (Tables 4 and 5). Meiotic mapping confirmed this assignment (see below).

cdc60-1: To localize the unmapped mutation *cdc60-1*, diploids with *cdc60-1* in repulsion to chromosome markers were irradiated. The proportions of subclones that expressed both the *cdc60-1* mutation and markers on chromosomes *XII* or *XVI* were significantly less than expected (Table 3). Results for a diploid carrying *cdc60-1* in coupling with chromosome markers indicated that *cdc60-1* is located on chromosome *XVI* (Table 4). This assignment was confirmed by tetrad analysis (see below).

cdc63-1: Mapping of the *cdc63-1* mutation by both the repulsion method (Table 3) and the coupling method (Table 4) indicated that *cdc63-1* is located on chromosome *XV*. Again, this assignment was confirmed by tetrad analysis (see below).

cdc61-1: Results for mapping of *cdc61-1* by the repulsion method (Table 3) suggested that *cdc61-1* is located either on chromosome *XV* or on chromosome *XIII* carrying the *ade4* marker (SCHILD and MORTIMER 1985). Mapping by the coupling method was used to distinguish between these two possibilities and indicated that *cdc61-1* is located on the same chromosome as *ade4* (Table 4). This finding was confirmed by tetrad analysis (see below). Note that the mapping results I obtained using the *lys7* marker on chromosome *XIII* in repulsion to *cdc61* (Table 3) did not indicate that *cdc61* is on chromosome *XIII* (see DISCUSSION).

cdc62-1: As indicated by the above results, the markers-in-repulsion method generally served to eliminate all but one or two chromosomes from further consideration. However, results obtained using this method to map the *cdc62-*

TABLE 3

Coexpression of cdc *and chromosome markers in repulsion*

	cdc60			*cdc61*			*cdc62*			*cdc63*			*cdc64*		
Chromosome[a]	Obs.[b]	Exp.[c]	*P*[d]	Obs.	Exp.	*P*	Obs.	Exp.	*P*	Obs.	Exp.	*P*	Obs.	Exp.	*P*
I	1.5	1.2		2.8	2.8		2.5	1.5		5.8	5.5		5.8	3.4	
II	5.0	1.4		4.3	2.1		1.0	0.7		7.7	7.4		2.3	2.2	
III	1.6	1.1		4.2	2.1		ND			6.5	6.4		0.9	0.8	
IV	3.8	3.2		6.9	5.7		3.4	2.3		9.6	8.4		3.7	3.2	
V	1.1	1.2	<0.80	ND			2.0	1.5		11.5	10.5		2.9	2.7	
VI	3.1	3.3	<0.70	3.6	2.7		1.7	1.2		12.6	11.7		5.2	4.3	
VII	4.0	2.5		3.2	3.6	<0.40	0.9	1.6	<0.50	11.5	8.9		4.4	2.7	
VIII	1.0	0.8		1.2	1.3	<0.80	1.3	0.8		6.4	5.4		2.4	1.5	
IX	4.5	3.2		1.3	1.0		1.2	0.6		5.1	4.8		1.6	1.2	
X	2.4	2.0		2.1	1.9		0.2	0.4	<0.60	9.1	7.9		0.6	0.6	
XI	4.0	2.3		ND			0.9	1.2	<0.80	2.1	2.0		0.7	0.5	
XII	1.4	4.0	<0.005	2.2	2.4	<0.90	0.0	1.2	<0.10	11.0	9.0		4.5	5.3	<0.40
XIII lys7	ND			3.9	2.0		ND			ND			ND		
XIII ade4[e]	2.2	2.3	<0.90	0.0	1.1	<0.01	0.9	0.6		5.7	5.1		1.5	2.7	<0.10
XIV met2	4.3	3.8		0.9	0.7		0.0	0.3	<0.60	6.8	4.8		0.0	1.0	<0.20
XIV lys9	3.3	2.3		0.9	0.5		0.0	0.7	<0.40	7.3	5.4		0.0	0.3	<0.50
XV	4.2	3.6		1.1	2.4	<0.01	1.4	0.7		1.9	6.6	<0.0005	0.6	3.9	<0.0005
XVI	0.0	1.7	<0.005	2.5	2.6	<0.90	0.5	2.2	<0.10	13.7	10.5		0.6	0.6	
F12	ND			3.6	2.1		ND			ND			ND		

Observed (Obs.) and expected (Exp.) coexpression frequencies, in percent, are from analyses of subclones after irradiation of four different diploid strains for each *cdc* mutation (five diploids for *cdc61-1*). Diploid strains were constructed so that *cdc* mutations were located in repulsion to other chromosomal markers; coexpression of the *cdc* mutation and another marker at frequencies less than expected is evidence that the *cdc* mutation and the marker are on the same chromosome. For each *cdc* mutation the minimum number of subclones tested for each chromosome marker, and the total numbers tested, were *cdc60-1*, 161 and 1372; *cdc61-1*, 164 and 1655; *cdc62-1*, 115 and 847; *cdc63-1*, 100 and 613; *cdc64-1*, 147 and 990. ND = not done.

[a] Chromosomes were identified by the markers listed in Table 2.

[b] Observed frequencies of coexpression were the percentage of subclones that expressed both a particular chromosome marker and the *cdc* mutation of interest.

[c] Expected frequencies of coexpression of *cdc* and chromosome markers were the products of the frequencies of expression of each marker and the *cdc* mutation.

[d] *P* values were calculated as described in MATERIALS AND METHODS, and are shown only for cases for which observed coexpression was less than expected.

[e] See SCHILD and MORTIMER (1985).

TABLE 4

Coexpression of cdc *and chromosome markers in coupling*

	cdc60			*cdc61*			*cdc62*			*cdc63*			*cdc64*		
Chromosome[a]	Obs.[b]	Exp.	*P*[c]	Obs.	Exp.	*P*	Obs.	Exp.	*P*	Obs.	Exp.	*P*	Obs.	Exp.	*P*
I	5.5	5.2	<0.90												
III				0.6	0.8								2.0	0.9	<0.20
IV							6.3	2.8	<0.025	1.0	2.6				
VI										11.9	6.9	<0.01			
VII							16.4	4.8	<0.0005						
IX	2.4	4.7					0.0	0.5							
XI										0.0	1.0				
XII										8.9	7.7	<0.60			
XIII ade4[d]				11.0	1.5	<0.0005									
XIV met4	10.2	7.2	<0.10				12.6	3.6	<0.0005						
XIV met2													2.7	1.5	<0.20
XIV lys9													2.0	1.4	<0.50
XV				1.0	2.4					24.8	6.6	<0.0005	6.8	0.6	<0.0005
XVI	26.0	8.2	<0.0005				5.3	3.1	<0.20						

Observed (Obs.) and expected (Exp.) coexpression frequencies, in percent, are from analysis of subclones after irradiation of one diploid strain for each *cdc* mutation. Diploid strains were constructed so that *cdc* mutations were located in coupling to other chromosomal markers; coexpression of the *cdc* mutation and a marker at frequencies greater than expected is evidence that the *cdc* mutation and the marker are on the same chromosome. For each *cdc* mutation the numbers of subclones tested were *cdc60-1*, 127; *cdc61-1*, 309; *cdc62-1*, 95; *cdc63-1*, 101; *cdc64-1*, 148.

[a] Chromosomes were identified by markers listed in Table 2.

[b] Observed and expected frequencies were calculated as in Table 3.

[c] *P* values calculated as described in MATERIALS AND METHODS are shown only for cases for which observed coexpression was greater than expected.

[d] See SCHILD and MORTIMER (1985).

TABLE 5

Expression of recessive markers in coupling

Chromosome	Genotype	No. of colonies	Chromosome	Genotype	No. of colonies
XVI	*aro7 cdc60*	33	*VII*	*leu1 cdc62*	9
	aro7 CDC60	0		*leu1 CDC62*	0
	ARO7 cdc60	7		*LEU1 cdc62*	7
	ARO7 CDC60	87		*LEU1 CDC62*	39
XIII	*ade4 cdc61*	34	*XV*	*his3 cdc63*	25
	ade4 CDC61	5		*his3 CDC63*	1
	ADE4 cdc61	2		*HIS3 cdc63*	1
	ADE4 CDC61	268		*HIS3 CDC63*	74
XIV	*met4 cdc62*	12	*XV*	*ade2 cdc64*	10
	met4 CDC62	2		*ade2 CDC64*	0
	MET4 cdc62	11		*ADE2 cdc64*	2
	MET4 CDC62	70		*ADE2 CDC64*	136

Analysis was performed as described in Table 4. Detailed results are shown for chromosomes considered as likely locations for the unmapped *cdc* mutations. These data were used to calculate coexpression frequencies (Table 4).

TABLE 6

Tetrad analysis of linkage

		Ascus type			
Chromosome	Marker pair	PD	NPD	TT	X_e (cM)[a]
VII	*cdc62-1—ade6*	27	1	12	22.5
	cdc62-1—leu1	5	0	32	45.9
XIII	*cdc61-1—ade4*	15	0	26	32.4
	cdc61-1—rna1-1	18	1	21	34.7
	cdc61-1—GAL80^S	3	7	20	Not linked
XIV	*cdc62-1—pha2*	7	5	25	Not linked
	cdc62-1—met2	7	10	29	Not linked
	cdc62-1—met4	1	5	13	Not linked
	cdc62-1—lys9	4	9	31	Not linked
XV	*cdc63-1—prt1-1*	372	0	0	<0.13
	cdc63-1—his3	6	5	15	Not linked
	cdc64-1—prt1-1	46	1	67	32.7
	cdc64-1—met7	9	0	20	35.5
	cdc64-1—his3	8	10	45	Not linked
XVI	*cdc60-1—gal4*	37	6	98	51.4
	cdc60-1—pep4-3	36	0	3	3.7

Abbreviations: PD, parental ditype; NPD, nonparental ditype; TT, tetratype.

[a] Map distances in cM were calculated as described in MA and MORTIMER (1983).

1 mutation were less definitive (Table 3). Of the 15 chromosomes considered in this analysis, none appeared to be likely locations for the *cdc62-1* mutation. Results from a markers-in-coupling analysis were also ambiguous for those chromosomes examined but indicated that the *cdc62-1* mutation might be located on either chromosome *VII* or *XIV* (Table 4). Tetrad analysis was then used to distinguish between these two possibilities and unambiguously assigned the *cdc62-1* mutation to chromosome *VII* (see below).

Linkage relationships for cdc *mutations by tetrad analysis*

cdc64-1: Asci from sporulated diploids heterozygous for *cdc64-1* and markers on chromosome *XV* were dissected, and genotypes of the resulting spores were determined to establish meiotic linkage (Table 6). The *cdc64-1* locus was found to be linked both to *met7* and to *prt1-1*, at genetic distances that indicated that *cdc64-1* is located between these two loci. This same genetic region also contains two other markers, *cpa1* (MORTIMER and SCHILD 1980) and *suf13* (CULBERTSON, GABER and CUMMINS 1982). Compared to these two markers, *cdc64-1* maps farther from *met7* but closer to *prt1* and, thus, may be inferred to be centromere distal to the *cpa1—suf13* marker pair.

cdc63-1: During construction of diploid strains for *cdc63-1* meiotic linkage analysis, it was noted that a diploid heterozygous for *cdc63-1* and for the *prt1-1* mutation grew well at 23° but failed to grow at 37°. The failure of the *cdc63-1* mutation to complement the *prt1-1* mutation suggested that *cdc63-1* and *prt1-1* are mutations in the same gene. This diploid was sporulated, and more than 300 asci were dissected. All tetrads gave a 4−:0+ segregation of the temperature-sensitive phenotype, indicating that the two mutations are separated by <0.13 cM. In addition, *cdc63-1* did not complement the *prt1-2* or *prt1-3* alleles in heterozygous diploid strains. Taken together, these data lead to the conclusion that *cdc63-1* and *prt1-1* are allelic.

cdc60-1: Close linkage was found between *cdc60-1* and *pep4-3* on chromosome *XVI* (Table 6). The map distance between *cdc60-1* and *gal4* (Table 6), which exceeded that found between *pep4-3* and *gal4* (40 cM; E. JONES, personal communication), orients *cdc60-1* 3.7 cM centromere proximal to *pep4-3*. The *cdc60-1* marker is not linked to the centromere-proximal marker *rad1* (data not shown).

cdc61-1: The mutation *cdc61-1* was found to be linked to the chromosome *XIII* markers *ade4* and *rna1* by 32.4 and 34.7 cM, respectively (Table 6). No meiotic linkage was detected between *cdc61-1* and $GAL80^S$, a dominant mutation showing mitotic linkage with *ade4* and meiotic linkage with *rad52* on chromosome *XIII* (SCHILD and MORTIMER 1985).

cdc62-1: Chromosome loss-mapping results indicated that the *cdc62-1* mutation is located on chromosome *VII* or *XIV*; therefore, diploids heterozygous for *cdc62-1* and various mutations marking both of these chromosomes were constructed and sporulated. Tetrad analysis results (Table 6) showed that *cdc62-1* is not linked with markers on chromosome *XIV*; linkage was established with markers on chromosome *VII*. The *cdc62-1* mutation was found to be 22.5 cM from *ade6*. In the same cross, linkage for the *cdc62-1—leu1* marker pair

(45.9 cM; Table 6) and for the *leu1—ade6* marker pair (PD:NPD:TT = 12:0:25, X_e = 34.7 cM) indicated that *cdc62-1* is centromere distal to *ade6*.

DISCUSSION

Chromosome loss procedures have been used previously to map mutations in *S. cerevisiae*. Methods to induce chromosome loss have included use of recessive mutations in the *CHL1* (LIRAS *et al.* 1978), *CDC6* and *CDC14* (KAWASAKI 1979) genes and application of the chemical methyl benzimidazole-2-yl-carbamate (WOOD 1982). The results presented here show that the new procedure of *rad52*-induced chromosome loss mapping, originally suggested by D. SCHILD and R. K. MORTIMER (personal communication), is an accurate and rapid method for assigning mutations to specific chromosomes. Using a modified *rad52*-mediated mapping method I have assigned five temperature-sensitive *cdc* mutations to specific chromosomes; the results of tetrad analyses have confirmed the assignments.

The chromosome loss-mapping method used here is based on the finding that diploids homozygous for *rad52* lose chromosomes spontaneously and that the frequency of loss can be increased by exposure to ionizing radiation (MORTIMER, CONTOPOULOU and SCHILD 1981). Mapping by this method involves irradiation of *rad52/rad52* diploids heterozygous both for the unmapped mutation and for recessive chromosome mutations. Loss of chromosomes results in the expression of recessive mutations, and correlations observed between the expression of two mutations can indicate whether the two mutations are on the same chromosome.

The *rad52*-induced chromosome loss-mapping procedure has several advantages. Since *rad52/rad52* diploids are deficient in spontaneous (GAME *et al.* 1980; PRAKASH *et al.* 1980) and γ-ray-induced (PRAKASH *et al.* 1980; SAEKI, MACHIDA and NAKAI 1980) mitotic recombination, physical linkage relationships are not altered by recombinational events. Whereas the *chl1* mutation causes loss of only certain chromosomes (*I*, *III* and less often *XVI* and *VIII*; LIRAS *et al.* 1978), *rad52*-induced chromosome loss was without significant bias for all 16 chromosomes studied here (Table 2). The proportions of colonies showing chromosome loss following irradiation were high (5–20%) for all chromosomes; multiple losses occurred often (data not shown). Other methods previously used to induce chromsome loss in Saccharomyces result in lower frequencies of chromosome loss (KAWASAKI 1979; WOOD 1982). The frequent occurrence of chromosome loss obtained here by the *rad52* method allows assignment of mutations to specific chromosomes by screening relatively small numbers of subclones.

A problem with this procedure became evident during the mapping of *cdc* mutations and exists only when mapping temperature-sensitive mutations. Following irradiation, all *rad52/rad52* diploid strains studied here gave rise, as expected, to subclones that failed to grow at 37°. As the incubation period following irradiation was increased, the proportions of temperature-sensitive subclones also increased (data not shown). However, complementation tests showed that many of the temperature-sensitive subclones did not express the

unmapped temperature-sensitive *cdc* mutation. The unidentified temperature-sensitive defects in these subclones could be complemented in crosses with haploid strains carrying known temperature-sensitive *cdc* mutations such as *cdc11*. This feature enabled me to determine, by complementation testing, which of the temperature-sensitive subclones expressed the *cdc* mutation of interest.

For cells that did not express the *cdc* mutation present in the diploid, but were unable to grow at 37° following irradiation, the nature and origin of this temperature sensitivity have remained obscure. Many of the *rad52/rad52* diploid strains used here were derived from *rad52* haploid strains obtained from D. SCHILD (Table 1). Those haploid strains grew at 37°; nevertheless, when each of the five multiply marked strains from Schild was mated to a nontemperature-sensitive *RAD52* haploid strain and sporulated, some of the haploid spore products were temperature sensitive (data not shown). To examine the generality of this phenomenon, new multiply marked *rad52* haploid strains were constructed in this laboratory by crossing multiply marked *RAD52* strains, obtained from M. CULBERTSON (Table 1), with singly marked *rad52* strains obtained from D. SCHILD (Table 1). None of these parental haploid strains segregated temperature-sensitive meiotic products in various testcrosses. Nevertheless, when these new multiply marked *rad52* strains were used in *rad52/rad52* chromosome loss experiments, they too produced temperature-sensitive subclones (data not shown). Correlation between temperature sensitivity and loss of particular chromosomes could not be detected. These results suggest that suppressed temperature-sensitive mutations may reside in these multiply marked *rad52* haploid strains or that the temperature sensitivity may be a multigenic trait, conferred by certain combinations of parental alleles present in recombinant spore products.

Regardless of the source of these new temperature-sensitive phenotypes, it is unlikely that the failure of colonies to grow at 37° resulted from the induction of new temperature-sensitive mutations. Previous studies showed that, although the spontaneous mutability in *rad52* strains is increased compared to *RAD52* strains (VON BORSTEL, CAIN and STEINBERG 1971; MALONE and EASTON ESPOSITO 1980; PRAKASH *et al.* 1980), those increases are at most 20-fold (PRAKASH *et al.* 1980), leading to mutation frequencies of 10^{-6} to 10^{-7}. Moreover, γ-ray-induced mutability is also low (10^{-6} to 10^{-7}) and is unaffected by a *rad52* mutation (MCKEE and LAWRENCE 1979). Indeed, in this study there were no subclones from an irradiated *rad52/rad52 leu1/leu1* diploid which had mutated to a leu$^+$ phenotype (data not shown). Therefore, the frequency of induction of new temperature-sensitive mutations would be too low to account for the significant proportion of uncharacterized temperature-sensitive subclones.

In general, the results of chromosome loss mapping showed that correlations between the expression of two mutations on a particular chromosome were not absolute. In every case in which markers were in repulsion, some apparent coexpression of both markers was obtained (Table 3), and coexpression of markers in coupling also was not complete (Tables 4 and 5). Moreover, results

of several experiments reported here indicate that mechanisms other than simple loss of whole chromosomes played a role in expression of certain recessive markers. For example, *cdc62-1* and *lys7* are both on chromosome *XIII* (Table 6), but when present in repulsion both markers were expressed together *more* frequently than expected (Table 3). In this case both marker assignments were unambiguously determined by complementation tests. Another particularly interesting situation was found for several *rad52/rad52* diploids with potential lysine and methionine auxotrophies due to *lys9* and *met2* mutations in coupling on opposite arms of chromosome *XIV* (KLAPHOLZ and EASTON ESPOSITO 1982b). In 13.2% of subclones from irradiated diploids coexpression of both of these markers indicated that one homolog of chromosome *XIV* was lost from these cells (Table 2); chi square analysis confirmed that the expression of the two mutations was not independent ($P < 0.05$). However, in another 7.7% of subclones cells required methionine but not lysine. It is unlikely that these frequently found met^- lys^+ colonies resulted from mitotic recombination events, since significant recombination is lacking in *rad52* homozygous diploids (GAME *et al.* 1980; PRAKASH *et al.* 1980; SAEKI, MICHIDA and NAKAI 1980). It is also unlikely that many of these met^- subclones were produced by induced mutation in other *MET* genes, since as pointed out above, mutant cells with a leu^+ phenotype were not produced from *leu1/leu1* diploids. Although previous experiments have indicated that loss of chromosome fragments is rare in *rad52/rad52* diploids (MORTIMER, CONTOPOULOU and SCHILD 1981), my results suggest that loss of chromosome fragments is a formal possibility in *rad52/rad52* diploids and must be taken into account when assigning a chromosomal location to an unmapped mutation.

For the five mutations mapped here, the results of chromosome loss analysis are generally unambiguous, with the exception of the situation for *cdc62-1*. Chromosome loss data indicated that expression of the *cdc62-1* mutation was related to expression of markers located on two chromosomes, *VII* and *XIV*. Tetrad analysis was used to distinguish between these two possibilities; results showed that *cdc62-1* is linked to markers on chromosome *VII*, and linkage was not detected between *cdc62-1* and markers distributed along chromosome *XIV* (Table 5). The ambiguity in the chromosome loss data could be explained if there were coordinate loss of chromosomes *VII* and *XIV*. However, this explanation is ruled out by results of a markers-in-coupling experiment involving the *lys9* and *met2* markers on chromosome *XIV* and *trp5* on chromosome *VII*, which showed that these chromosomes are lost independently (data not shown). Another possible explanation is that expression of certain combinations of alleles as a result of chromosome loss results in a decreased or increased survival of those aneuploids, compared to aneuploids expressing other combinations of alleles. Perhaps cells that retained that homolog of chromosome *VII* carrying the *cdc62-1* mutation and that homolog of chromosome *XIV* carrying the *met4* mutation were at a selective advantage over those retaining other combinations of these homologs. This phenomenon could lead to preferential survival of certain subclones and, thus, a high frequency of colonies showing coexpression of the *cdc62-1* and *met4* mutations. Indeed, in haploidization stud-

ies an artifactual mapping result due to an interaction between alleles has been observed by KLAPOLZ and ESPOSITO (1982a).

In summary, mapping by *rad52*-induced chromosome loss procedures was used to assign several previously unmapped *cdc* mutations to linkage groups. Four of the mutations define new loci: *CDC60* on chromosome *XVI*, *CDC61* on chromosome *XIII*, *CDC62* on chromosome *XIV* and *CDC64* on chromosome *XV*. Tetrad analysis was used to establish the position of these mutations relative to other markers. Another mutation, *cdc63-1*, was shown to be allelic to mutations in the *PRT1* gene on chromosome *XV*. The mutations *cdc63-1*, *cdc64-1* and a new mutation *cdc66-1* (D. CARRUTHERS, unpublished results) were found to be linked to each other on chromosome *XV*. These three complementing mutations all cause cell cycle arrest at the regulatory step "start," and define "start" genes.

The finding that *prt1* and *cdc63* mutations are allelic is interesting with respect to cell cycle regulation. Cells bearing the *prt1-1* mutation when shifted to nonpermissive conditions arrest randomly in the cell cycle and exhibit an immediate cessation of protein synthesis (HARTWELL and MCLAUGHLIN 1969). The *prt1-1* mutation has been shown to cause a defect in the interaction of the ternary initiation complex with the 40 S ribosomal subunit during initiation of translation (FEINBURG, MCLAUGHLIN and MOLDAVE 1982). In contrast to this behavior, when cells bearing the *cdc63-1* mutation are shifted to the nonpermissive temperature they arrest as unbudded cells at the cell cycle regulatory step "start," and protein synthesis continues (BEDARD, JOHNSTON and SINGER 1981). Study of these alleles may provide understanding of the role of the *PRT1* gene product in metabolism and the cell cycle.

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Note added in proof: Results of a three-factor cross involving the *ade6*, *cdc62-1*, and *cly8* mutations indicate that the order of these mutations is *ade6-cdc62-1-cly8* (*ade6-cly8* PD:NPD:TT = 21:0:25, x_e = 27.5 cM; *cdc62-1-cly8* PD:NPD:TT = 39:0:8, x_e = 8.4 cM; and *cdc62-1-ade6* PD:NPD:TT = 27:0:19, x_e = 20.6 cM).

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