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REVERTANTS OF A TRANSCRIPTION TERMINATION MUTANT OF YEAST CONTAIN DIVERSE GENETIC ALTERATIONS

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ABSTRACT

Revertants of the *cyc1-512* transcription termination mutant of the yeast *Saccharomyces cerevisiae* were isolated and subjected to a detailed genetic analysis. The *cyc1-512* mutation previously was shown to be a 38-base pair deletion that causes only 10% of the normal steady-state levels of *CYC1* mRNA and of the *CYC1* gene product, iso-1-cytochrome c. Forty-one *cyc1-512* revertants were classified by their content of iso-1-cytochrome c and by their genetic properties in meiotic crosses. Many of the revertants contain local genetic changes that either partially or completely restore the level of iso-1-cytochrome c. One revertant was shown to contain an unlinked extragenic suppressor, designated *sut1*, that causes partial suppression of the transcription termination defect. Four revertants of *cyc1-512* contain chromosomal rearrangements with breakpoints that are tightly linked to the *CYC1* locus; these include one duplication, one possible inversion, and two reciprocal translocations. Detailed genetic mapping demonstrated that one of the reciprocal translocations is between the right arms of chromosomes X and XII, with a breakpoint mapping 3′ to the *CYC1* locus. These results indicate that the defect in transcription termination in *cyc1-512* can be restored in a variety of ways, including the translocation of different chromosomal regions to the 3′ end of the *CYC1* locus, local changes presumably at or near the original defect, and by mutation at another locus distinct from *CYC1*.

THE ability to selectively isolate mutations in genetic control regions provides a powerful tool to systematically analyze functional requirements adjacent to the translated portions of genes. This methodology has proved useful for studying the control of various genetic loci of the yeast *Saccharomyces cerevisiae*, mostly because of the relative ease with which this simple eukaryotic organism can be genetically manipulated (SHERMAN and LAWRENCE 1974). For example, *cis*-dominant mutations that affect the level of transcription of yeast protein coding genes such as *CYC7* (ERREDE *et al.* 1980; MCKNIGHT, CARDILLO and SHERMAN 1981), *HIS4* (CHALEFF and FINK 1980; ROEDER and FINK 1980), and *ADR2* (WILLIAMSON, YOUNG and CIRIACY 1981; YOUNG *et al.* 1982) have been isolated and shown to contain chromosomal alterations that are 5′ to the

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respective transcribed regions. A mutation at the 3′ end of the *CYC1* locus, *cyc1-512*, has been described by ZARET and SHERMAN (1982), and is the subject of this paper.

The *CYC1* and *CYC7* loci encode iso-1-cytochrome *c* and iso-2-cytochrome *c*, respectively, which comprise 95% and 5% respectively, of the total amount of cytochrome *c* in aerobically grown cells. Both iso-1-cytochrome *c* and iso-2-cytochrome *c* are encoded in the nucleus but are located in the mitochondria, where they function in the mitochondrial electron transport chain during respiratory growth. The *cyc1-512* mutation has been shown to be a 38-base pair deletion in the 3′ nontranslated region of *CYC1* mRNA (ZARET and SHERMAN 1982), occurring just before the normal site of poly(A) addition (Boss *et al.* 1981). This mutation prevents the normal transcription termination and polyadenylation of *CYC1* mRNA, causing the messages to be much longer and presumably more unstable than normal (ZARET and SHERMAN 1982). In addition, the *cyc1-512* deletion affects an adjacent gene in a similar way, causing overlapping transcription of *CYC1* and the adjacent gene. Because the *cyc1-512* mutant produces only about 5% to 10% of the normal amount of *CYC1* mRNA and iso-1-cytochrome *c*, it can barely grow on certain nonfermentable carbon sources such as lactate medium.

This paper describes the initial genetic characterization of the *cyc1-512* mutation and the genetic analysis of 41 *cyc1-512* revertants that were isolated on lactate medium. Each *cyc1-512* revertant was characterized with regard to its cytochrome *c* content and genetic behavior in standard test crosses. The genetic analysis indicates that a significant proportion of *cyc1-512* revertants contain chromosomal rearrangements with breakpoints at or near the *CYC1* locus. One revertant contains an extragenic suppressor that is allele-specific for the transcription termination defect. The widely varying levels of iso-1-cytochrome *c* and the various genetic structures observed in the revertants suggest that there may be a number of ways to affect transcription termination and polyadenylation of mRNA in yeast.

MATERIALS AND METHODS

Genetic procedures and strains: Conventional yeast genetic procedures of crossing, sporulation, tetrad analysis, and scoring of nutritional markers were used for constructing strains with desired markers and for meiotic analysis (SHERMAN and LAWRENCE 1974; MORTIMER and HAWTHORNE 1975). Genetic procedures used with the iso-cytochrome *c* system have been described in detail by SHERMAN *et al.* (1974). Deletion mapping of *cyc1-512* was performed by using the techniques and deletion mutants described by SHERMAN *et al.* (1975). Revertants of *cyc1-512* were induced with either 40, 60, or 80 Jm^{-2} of ultraviolet light at a dose rate of 1 $Jm^{-2}s^{-1}$, or with 15 kilorads of X rays at a dose rate of 12 kilorads per minute. Independent revertant colonies arising on lactate medium were subcloned and then retested for growth on lactate medium and for cytochrome *c* content. Each revertant was then further characterized by meiotic analysis in crosses to strains containing the marker *cyc1-1*, which is a deletion encompassing the *CYC1*, *OSM1* and *RAD7* loci (SINGH and SHERMAN 1978). The genetic symbols used to denote the various types of alterations at or adjacent to the *CYC1* locus are listed in Table 1.

Determination of cytochrome c content: Total amounts of cytochrome *c* were determined by spectroscopic examination of intact cells at −196° (SHERMAN and SLONIMSKI 1964) and by visually comparing the intensities of the c_α-bands to the c_α-bands of strains having known amounts of cytochrome *c*. Derepressed cells for spectral examination were prepared by growing strains as narrow lines on nutrient plates (SHERMAN *et al.* 1974). The relative levels of cytochrome *c* in the

TABLE 1

Designation of CYC1 mutations used in genetic analysis

Strain	Symbol	Chromosomal constitution	% Normal iso-1-cytochrome *c*
D311-3A	*CYC1*$^+$	Normal	100
Various	*cyc1-1*	Deletion of *CYC1*, *OSM1* and *RAD7* loci	0
B-4060	*cyc1-512*	38-base pair deletion 3′ to *CYC1*	5–10
B-4307	*CYC1-512-Tl1*	Reciprocal translocation	40
J184-7A-2	*cyc1-512-Tl1-2*	Reciprocal translocation	0
B-5407	*CYC1-512-Tl2*	Reciprocal translocation	100
Z-110	*cyc1-512-Tl2-1*	Reciprocal translocation	0
B-4308	*CYC1-512-In1*	Possible inversion	20
B-4304	*CYC1-512-Dp1*	Duplication of *CYC1*	70

strains described in this paper could be accurately assessed because all of the strains are in an isogenic background.

Chromosome loss mapping: Genetic mapping by chromosome loss was employed with *cdc14* strains as described by KAWASAKI (1979). Since *cdc14-1* is a temperature-sensitive mutation that causes cells to randomly lose chromosomes, the following constructions were performed at the permissive temperature of 23°. First, the *cyc1-512* revertants to be mapped were crossed to *cdc14* strains in order to construct haploid strains containing the revertant *cyc1* allele and *cdc14*. These strains were then crossed to different *cdc14 cyc1-1* strains, each containing a number of genetic markers on various yeast chromosomes. The resulting diploids were homozygous for *cdc14*, heteroallelic for *cyc1*, and heterozygous for a variety of nutritional markers. These diploid strains as well as other diploid strains used in genetic analysis are listed in Table 2. About 2000 diploid cells were spread onto plates containing nutrient medium (1% (w/v) Bacto-yeast extract, 2% (w/v) Bacto-peptone, 2% (w/v) dextrose, and 2% (w/v) agar), incubated at the restrictive temperature of 35° for 6 hr, then incubated at 23° for several days. The temporary shift to the restrictive temperature caused chromosome loss in these homozygous *cdc14* diploids. The resulting colonies were then replica-plated to synthetic minimal medium (0.67% (w/v) Bacto-yeast nitrogen base without amino acids, 2% (w/v) dextrose, and 2% (w/v) agar) in order to detect derivatives that had lost a chromosome and thereby exposed one of the auxotrophic markers. Chromosome loss derivatives were subsequently picked and tested on the appropriate synthetic media to determine which marker was lost. Approximately 10% of the diploids survived the temperature shift treatment and 2.5% of the survivors lost one or more chromosomes.

Meiotic mapping of chromosome XII: The genes encoding yeast ribosomal RNA are tandemly repeated at the *RDN1* locus on chromosome *XII* (PETES 1979a). By using recombinant DNA techniques and yeast transformation, it has been possible to insert the yeast *LEU2* gene into the *RDN1* locus (PETES 1980; SZOSTAK and WU 1980). A diploid that is homozygous for *leu2/leu2* at the normal *LEU2* locus on chromosome *III* and hemizygous for *LEU2/0* at the *RDN1* locus on chromosome *XII* segregates 2:2 for *LEU2*$^+$, with the Leu$^+$ phenotype mapping on chromosome *XII*. Therefore, in order to map *cycl* alleles to chromosome *XII*, a nonreverting *leu2-3,112* allele on chromosome *III* was first combined with the appropriate *cycl* marker. Next, a *LEU2* insertion into *RDN1* (obtained from T. PETES), designated *LEU2::RDN1* in Table 2, was crossed into the appropriate *cycl leu2-3,112* background and used for mapping purposes. The Leu$^+$ phenotype was unlinked to mating type in the mapping crosses, indicating that the *LEU2::RDN1* gene was not at the site of the normal *LEU2* locus on chromosome *III*.

RESULTS

The cyc1-512 mutation is leaky and maps outside the translated region of the CYC1 locus: *Cyc1-512* is a spontaneous mutant derived from the *CYC1*$^+$ strain D311-3A by the chlorolactate enrichment procedure (SHERMAN *et al.* 1974). The

TABLE 2

Diploid strains used for genetic analysis

Diploid strains used for chromosome loss mapping	
J491	*MAT**a** CYC1-512-Tl1 + + cdc14 his2 his6 lys2 + + + +* *MATα cyc1-1 ilv3 asp5 cdc14 his2 + + leu2 lys10 aro7 arg4*
J524	*MATα CYC1-512-Tl2 + cdc14 his2 his5 trp2 leu2 + + + + +* *MAT**a** cyc1-1 ilv3 cdc14 his2 + + + his6 ade6 trp1 rad52 arg*
J529	*MATα CYC1-512-Tl2 + cdc14 his2 his5 leu2 trp2 + + + + +* *MAT**a** cyc1-1 ilv3 cdc14 his2 his5 leu2 + ura3 trp1 met14 rad52 ade*
CYC1 homozygous normal crosses	
KZ-241	*MAT**a** cyc1-1 met3 ilv3 cdc11 leu1 trp5 ura1 + + +* *MATα cyc1-1 + + + + + + mak9 trp3 aro7*
KZ-242	*MAT**a** cyc1-1 met3 ilv3 cdc11 leu1 trp5 ura1 + + + +* *MATα cyc1-1 + + + + + + mak9 trp3 aro7 arg4*
KZ-243	*MAT**a** cyc1-1 met3 ilv3 cdc11 leu1 trp5 ura1 +* *MATα cyc1-1 + + + + + + mak11*
CYC1-512-Tl1 heterozygous translocation crosses	
KZ-228	*MAT**a** CYC1-512-Tl1 + + [+] his1 lys2 trp2 + + + +* *MATα cyc1-1 met3 ilv3 cdc11 + + + leu1 ura1 can1 his5*
KZ-231	*MAT**a** CYC1-512-Tl1 + + [+] his1 lys2 trp2 + +* *MATα cyc1-1 met3 ilv3 cdc11 + + + ura1 his5*
KZ-236	*MATα ura4 CYC1-512-Tl1 + lys2 + +* *MAT**a** [+] cyc1-1 ilv3 + leu2 can1*
KZ-170	*MATα + cyc1-512-Tl1-1 + trp1 his1 trp2 lys2 + + +* *MAT**a** [ura4] CYC1+ asp5 trp1 + + + arg8 his8 ade*
KZ-171	*MATα + cyc1-512-Tl1-2 + trp1 his1 lys2 trp2 + + +* *MAT**a** [ura4] CYC1+ asp5 trp1 + + + arg8 his8 ade*
KZ-235	*MATα ura4 cyc1-512-Tl1-2 + lys2 + +* *MAT**a** [+] cyc1-1 ilv3 + can1 leu2*
KZ-245	*MATα ura4 cyc1-512-Tl1-2 ilv3 [+] leu2 can1 lys2 +* *MATα [+] cyc1-1 + LEU2::RDN1 leu2 can1 + his4*

TABLE 2—*Continued*

KZ-246	*MATa ura4 cyc1-512-Tl1-2 [+] leu2 can1 +* *MATα [+] cyc1-1 LEU2::RDN1 leu2 can1 his4*
CYC1-512-Tl1 Homozygous translocation crosses	
KZ-179	*MATa + CYC1-512-Tl1 his1 lys2 trp2 +* *MATα ura4 cyc1-512-Tl1-2 + + + trp1*
KZ-247	*MATa + CYC1-512-Tl1 met3 ilv3 cdc11 lys2 his1 + +* *MATα ura4 cyc1-512-Tl1-2 + ilv3 + + + can1 leu2*
KZ-255	*MATa + CYC1-512-Tl1 met3 ilv3 cdc11 + leu2 his1 + + +* *MATα ura4 cyc1-512-Tl1-2 + + + LEU2::RDN1 leu2 + his4 lys2 can1*
KZ-256	*MATa + CYC1-512-Tl1 met3 ilv3 cdc11 + leu2 his1 + + +* *MATα ura4 cyc1-512-Tl1-2 + + + LEU2::RDN1 leu2 + lys2 can1 his4*
KZ-257	*MATa + CYC1-512-Tl1 met3 ilv3 cdc11 + leu2 can1 + +* *MATα ura4 cyc1-512-Tl1-2 + + + LEU2::RDN1 leu2 can1 his4 lys2*
CYC1-512-Tl2 Heterozygous translocation crosses	
KZ-158	*MATα CYC1-512-Tl2 lys1 his5 trp1 + + + +* *MATa cyc1-1 + + + his2 ade6 leu1 cdc14*
KZ-225	*MATα CYC1-512-Tl2 his2 cdc14 his5 + + +* *MATa cyc1-1 his2 cdc14 + lys9 ade6 trp1*
KZ-175	*MATa cyc1-512-Tl2-1 lys2 his1 trp2 +* *MATα CYC1 + + + lys9*
KZ-191	*MATa cyc1-512-Tl2-1 [+] his1 lys9 + + +* *MATα CYC1 cdc8 + + his6 met1 ura1*
CYC1-512-Tl2 Homozygous translocation crosses	
KZ-174	*MATα CYC1-512-Tl2 his5 + + +* *MATa cyc1-512-Tl2-1 + his1 lys2 trp1*
KZ-190	*MATα CYC1-512-Tl2 his5 + +* *MATa cyc1-512-Tl2-1 + his1 lys9*
CYC1-512-In1 Heterozygous inversion crosses	
KZ-157	*MATa CYC1-512-In1 his1 lys2 trp2 + + + + +* *MATα cyc1-1 + + + trp1 cdc14 ura3 his2 his6*
KZ-230	*MATa CYC1-512-In1 + + + his1 lys2 trp2 + + + +* *MATα cyc1-1 met3 ilv3 cdc11 + + + leu1 ura1 can1 his5*

TABLE 2—*Continued*

KZ-233	*MATa*	*CYC1-512-In1*	+	+	+	*his1*	*lys2*	*trp2*	+	+	
	MATα	*cyc1-1*	*met3*	*ilv3*	*cdc11*	+	+	+	*ura1*	*his5*	
CYC1-512-In1 Homozygous inversion cross											
KZ-293	*MATα*	*CYC1-512-In1*	*met3*	*ilv3*	*cdc11*	*his1*	*trp2*	+			
	MATa	*CYC1-512-In1*	+	+	+	*his1*	*trp2*	*lys2*			

Solid lines indicate markers on normal chromosomes; dashed lines indicate markers on rearranged chromosomes.

cyc1-512 mutation is leaky; in different genetic backgrounds *cyc1-512* strains produce from 5% to 10% of the normal amount of iso-1-cytochrome *c*. *Cyc1* mutants that are completely deficient in iso-1-cytochrome *c* are capable of growth on glycerol as a sole energy source due to the presence of the normal low level of iso-2-cytochrome c; however, such *cyc1* mutants are incapable of growth on lactate. Since *cyc1-512* strains generally exhibit slow but positive growth on lactate, it suggests that the iso-1-cytochrome *c* produced by *cyc1-512* can function normally in electron transport, but is present in insufficient quantities to permit the normal growth on lactate seen in $CYC1^+$ strains. Diploid strains such as *cyc1/cyc1-512* grow very weakly on lactate medium.

Point mutations that alter the primary structure of iso-1-cytochrome *c* at known amino acid positions have been used to determine the endpoints of a series of *CYC1* deletions that encompass various portions of the structural gene (SHERMAN *et al.* 1975). The deletions have subsequently been used to map other *cyc1* mutations to the translated and nontranslated regions of the *CYC1* locus (SHERMAN *et al.* 1975; STILES *et al.* 1981; F. SHERMAN and S. CONSAUL, unpublished results). Only 2 of the 464 *cyc1* mutations that were tested map outside of the translated region of *CYC1*, *cyc1-362* and *cyc1-512*. The *cyc1-362* mutation contains two single base pair substitutions in the leader region of *CYC1* mRNA that cause improper initiation of translation 5′ to the normal initiation codon (STILES *et al.* 1981). The *cyc1-512* mutation recombines with the *cyc1-362* mutation as well as the deletions *cyc1-369* through *cyc1-379*, all of which extend from upstream of the *cyc1-362* site and together encompass the region up to amino acid position 79 in the protein-coding sequence (Figure 1). *Cyc1-512* also recombines with the deletions *cyc1-428*, *cyc1-458* and *cyc1-462*, which collectively span the region from amino acid position 18 to beyond amino acid position 102. No recombinants are detected between *cyc1-512* and the following: the *cyc1-363* and *cyc1-364* deletions that encompass the *CYC1* protein-coding sequence; the *cyc1-366* and *cyc1-368* deletions that encompass *CYC1* as well as a neighboring, centromere-distal locus *OSM1* (SINGH and SHERMAN 1978); and the *cyc1-1*, *cyc1-364* and *cyc1-365* deletions that encompass *CYC1*, OSM1, and another closely linked, centromere-distal locus *RAD7*. Because *cyc1-512* recombines with all of the partial deletions of *CYC1* that together cover the entire protein-coding sequence, the *cyc1-512* mutation maps in the nontranslated portion of the *CYC1* locus. Because the deletions that encompass *cyc1-512* have no endpoints within the protein-coding region, the deletion mapping could not

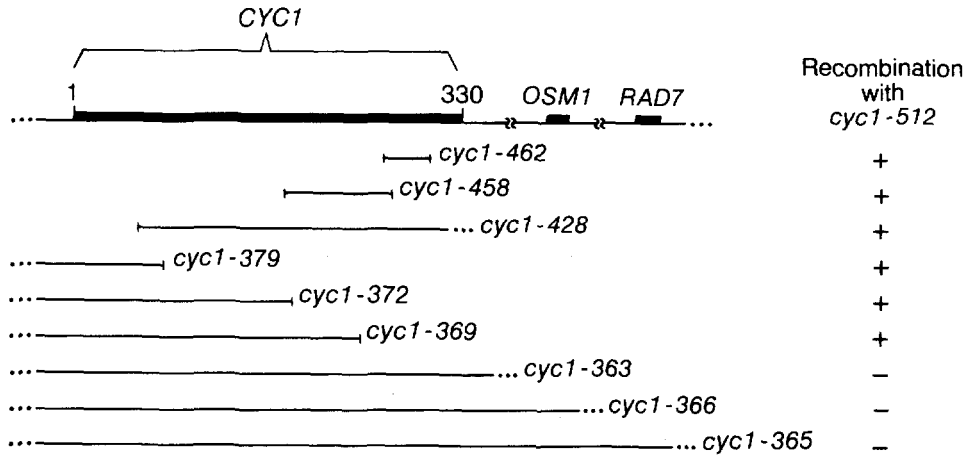


FIGURE 1.—Deletion mapping of the *cyc1-512* mutation. The genes *CYC1*, *OSM1*, and *RAD7* on chromosome X are depicted. The *CYC1* region is expanded to show the translated region which extends from nucleotide position 1 to 330. The deletions, which are designated by their respective *cyc1* allele number, do not recombine with the point mutations in the *CYC1* locus and encompass the *OSM1* and *RAD7* loci as indicated by the horizontal lines (SHERMAN *et al.* 1975; SINGH and SHERMAN 1978). The dots extending from the horizontal lines indicate that the exact deletion endpoint is unknown. The deletions shown are representative of those that were used to test for recombination with *cyc1-512*. The results indicate that the *cyc1-512* mutation maps outside the translated portion of the *CYC1* locus.

establish whether the *cyc1-512* mutation is located 5′ or 3′ to the iso-1-cytochrome *c*-coding sequence. The deletion mapping results do indicate that the *cyc1-512* mutation is very closely linked to the *CYC1* protein-coding sequence; in addition, meiotic mapping studies have shown that the *cyc1-512* mutation is located at the normal *CYC1* position on chromosome X. DNA blot hybridization studies and DNA sequence analysis of cloned DNA have subsequently shown that the *cyc1-512* mutation consists of a 38-base pair deletion in the 3′ nontranslated region of *CYC1* (ZARET and SHERMAN 1982). The results from deletion mapping not only corroborate the results from the DNA analysis but also indicate that there are no other lesions causing defects in the *cyc1-512* mutant.

Cyc1-512 reverts spontaneously at a low rate and is stimulated to revert by mutagenic treatment with ultraviolet light or X rays. Forty-one independent revertants of *cyc1-512* were isolated on lactate medium and characterized by their spore viability patterns in genetic crosses in order to detect gross chromosomal aberrations. In addition, 25 revertants were further characterized for linkage of the reverting mutation to the *CYC1* locus. All of the revertants were directly derived from the original *cyc1-512* strain B-4060, which was originally obtained from the normal $CYC1^+$ strain D311-3A. The revertants fall into three distinct classes based upon their genetic properties in tetrad analyses. A summary of the mutations causing reversion of *cyc1-512* and the frequency of occurrence of the revertants in each class is presented in Table 3.

Class I: Strictly local mutations: When crossed to the deletion mutant, *cyc1-1*, the majority of revertants of *cyc1-512* exhibit the normal 2:2 (Cyc^+:Cyc^-)

TABLE 3

Numbers of cyc1-512 revertants in each genetically defined class

		Treatment			% Normal iso-1-cytochrome *c*
		None	UV	X rays	
Class I:	Strictly local mutations	0	11	9	15–100
Class II:	Extragenic suppressor	0	1	0	20
Class I or II[a]		1	6	9	15–100
Class III:	Chromosomal aberrations				
	Reciprocal translocations	0	0	2	40–100
	Possible inversion	0	0	1	20
	Duplication	0	0	1	70
	Totals:	1	18	22	

[a] Revertants in this group gave rise to normal patterns of spore viability but were not tested for linkage of the reverting mutation to the *CYC1* locus.

meiotic segregation pattern for producing iso-1-cytochrome *c*. At the genetic level, these revertants could be considered "intragenic" because the reverse mutation is tightly linked to the *CYC1* locus. However, certain revertants in this class contain relatively small DNA rearrangements in a locus adjacent to *CYC1*, which causes the reversion of *cyc1-512* (ZARET and SHERMAN, unpublished results). Since the *CYC1* locus is not directly altered by these particular genomic alterations, whereas other reversion events of this genetic class do alter the *CYC1* locus, the reverse mutations of this class will be designated "strictly local" because they only alter the yeast genome in the local vicinity of the *CYC1* locus.

An unusual feature about the levels of iso-1-cytochrome *c* in these strains is that different revertants contain from normal levels to as low as 10% of the normal level of iso-1-cytochrome *c* (Figure 2). This result is in contrast to the revertants of *cyc1* mutations that fall in the translated portion of the gene, which usually produce normal or near normal levels of iso-1-cytochrome *c*. It is concluded that there are a variety of degrees to which the defect in *cyc1-512* can be restored.

Class II: Extragenic suppressor: One *cyc1-512* revertant, when crossed to the *cyc1-1* deletion, exhibits 2:2, 1:3 and 0:4 segregation for producing higher levels of iso-1-cytochrome *c*. When this revertant is crossed to *cyc1-512*, the revertant phenotype segregates 2:2. Because the original *cyc1-512* mutation can be recovered from the revertant strain by a simple genetic cross, these results indicate that the revertant contains an unlinked extragenic suppressor that is allele-specific for *cyc1-512*. The suppressor was tested for its ability to act on any of a number of *lys2* alleles that are suppressed by translational suppressors (CHATTOO *et al.* 1979). The suppressor of *cyc1-512* is unable to suppress various UAA, UAG, UGA, and missense *lys2* mutations in haploid segregants. The *cyc1-512* suppressor, which is recessive, is altered at a genetic locus designated *SUT1*, for Suppressor of Termination mutant. The *sut1* mutation elevates the level of iso-1-cytochrome *c* in *cyc1-512* strains to 20% of the normal amount.

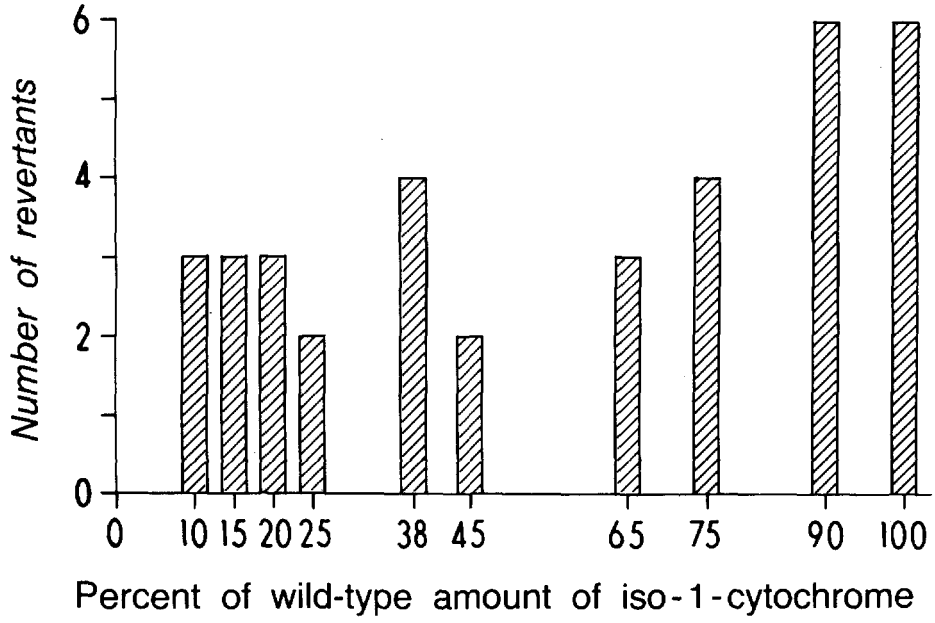


FIGURE 2.—The % normal amount of iso-1-cytochrome *c* produced by independent revertants of *cyc1-512* are plotted against the number of revertants isolated that produce cytochrome *c* at the designated level. The amount of cytochrome *c* in each revertant was determined by low-temperature absorption spectroscopy of intact cells (SHERMAN and SLONIMSKI 1964).

The *sut1* suppressor can be lost under nonselective conditions, suggesting that the *sut1* mutation reduces the rate of growth.

Class III: Chromosomal aberrations, reciprocal translocations: Four revertants of *cyc1-512*, when crossed to *cyc1-1*, exhibit either digenic transmission for producing iso-1-cytochrome *c* or unusual spore viability patterns that are associated with the *CYC1* locus. These results, which are described in detail below, are indicative of genomic rearrangements in the revertant strains. The tight linkage of the breakpoints of the rearrangements to the *CYC1* locus implies that the new chromosomal regions restore the mutant function of *cyc1-512*. It should be noted that although the aberrations were induced with 15 kilorads of X rays, such events are extremely rare in yeast and have never been observed among revertants of translational *cyc1* mutants.

As shown in Figure 3, from a cross between a strain containing a reciprocal translocation and a strain containing a normal chromosomal constitution, complete spore viability (4/4) is expected when the chromosomes involved in the translocation segregate together and when the normal chromosomes segregate together at the first meiotic division (alternate segregation). As also shown in Figure 3, when the centromere of one translocation chromosome segregates with the centromere of one of the normal chromosomes at the first meiotic division, the other translocation chromosome must segregate with the other normal chromosome, and each spore will contain a duplication of a region on the normal chromosome and a deficiency of a region on the translocation

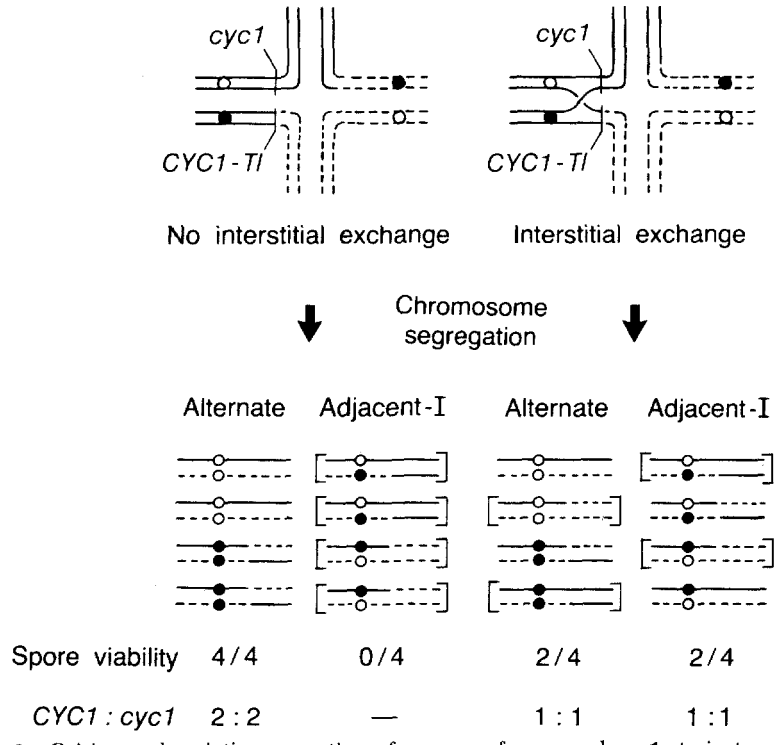


FIGURE 3.—Pairing and meiotic segregation of a cross of a normal *cyc1* strain to a *CYC1-Tl* mutant, which has a reciprocal translocation. The tight linkage of the aberrant spore viability pattern with *cyc1* segregation indicates that one of the translocation breakpoints is closely linked to the *CYC1* locus. Solid lines indicate genetic material that is normally associated with chromosome X; dashed lines indicate material that is normally associated with another chromosome. Open circles indicate centromeres of chromosomes initially of normal constitution; closed circles indicate centromeres of chromosomes initially involved in a reciprocal translocation. The designated modes of disjunction assume that homologous centromeres go to opposite poles at anaphase I. Higher frequencies of two viable spores per tetrad are expected with a longer interstitial region between a translocation breakpoint and its respective centromere.

chromosome (adjacent-I segregation). The deficiencies can cause all four spores to be inviable (0/4). Assuming that homologous centromeres assort randomly with respect to other homologous centromeres in meiosis, the frequency of both types of segregation described above should be equal and thus the number of 4/4 and 0/4 tetrads should be equal as well. Recombination in the interstitial region between a centromere and a translocation breakpoint will cause each of the two homologous centromeres to possess a normal and a translocation chromatid. At the second meiotic division, the translocation chromatid will produce a viable spore when it segregates with the other translocation chromatid and will produce an inviable spore when it segregates with the normal chromatid. The result is that a single crossover in the interstitial region will generate tetrads that are 2/4 for spore viability. If one or both interstitial regions are large, crossovers should be frequent and 2/4 tetrads will be the predominant class. A final prediction is that in a cross between two strains containing the

TABLE 4

Number of asci having various viable spores per tetrad

Chromosomal constitution	CYC1 genotype		Viable spores per tetrad 4	3	2	1	0	Total
Homozygous normal	*cyc1/cyc1*		22	11	3	0	0	36
		Percentage	61%	31%	8%	0%	0%	
Heterozygous translocation	*CYC1-512-Tl1/cyc1-1*		4	6	24	7	16	57
	cyc1-512-Tl1-1/CYC1$^+$		0	3	9	4	4	20
	cyc1-512-Tl1-2/CYC1$^+$		1	2	11	1	5	20
	cyc1-512-Tl1-2/cyc1-1		11	6	63	13	18	111
		Total	16	17	107	25	43	208
		Percentage	8%	8%	51%	12%	21%	
Homozygous translocation	*CYC1-512-Tl1/ CYC1-512-Tl1*		17	2	0	0	0	19
	CYC1-512-Tl1/ cyc1-512-Tl1-2		120	64	31	10	8	233
		Total	137	66	31	10	8	252
		Percentage	54%	26%	12%	4%	3%	
Heterozygous translocation	*CYC1-512-Tl2/cyc1-1*		9	0	25	3	7	44
	cyc1-512-Tl2-1/cyc1-1		5	3	21	11	13	53
		Total	14	3	46	14	20	97
		Percentage	14%	3%	47%	14%	21%	
Homozygous translocation	*CYC1-512-Tl2/ cyc1-512-Tl2-1*		6	15	12	7	7	47
		Percentage	13%	32%	26%	15%	15%	
Heterozygous inversion	*CYC1-512-In1/cyc1-1*		23	23	63	13	8	130
		Percentage	18%	18%	48%	10%	6%	
Homozygous inversion	*CYC1-512-In1/ CYC1-512-In1*		40	25	3	2	0	70
		Percentage	57%	36%	4%	3%	0%	

The diploid strains used for the above analysis are listed in Table 2; the chromosomal aberrations and their respective *CYC1* designations are listed in Table 1.

same reciprocal translocation, recombination and disjunction will not produce deficiencies, and the normal 4/4 spore viability pattern should result.

When two of the *cyc1-512* revertants, B-4307 and B-5407, are crossed to *cyc1-1* strains of normal chromosomal constitution they produce tetrads that predominantly give rise to only two viable spores (Table 4). Ninety-nine percent (98 out of 99) of the two spore survivor tetrads from the B-4307 crosses segregate Cyc^+:Cyc^- and 90% (32 out of 36) of the two spore survivor tetrads from the B-5407 crosses segregate Cyc^+:Cyc^-. In addition, the frequencies of tetrads having four viable spores per ascus (4/4) are roughly equal to the frequencies of tetrads having zero viable spores per ascus (0/4). Although these ratios are not exact, it should be noted that the spore viability patterns are biased in the direction of lower viability. This generally reduced viability has been observed in control crosses with strains that lack any detectable chromosomal rearrangements

(Table 4) and is probably caused by random factors causing inviability between strains. When all four progeny of a 4/4 tetrad are crossed to a normal strain, it can be shown that the ability to produce abnormal spore viability patterns segregated 2:2 in the original cross. These results suggest that B-4307 and B-5407 each contain a reciprocal translocation with a breakpoint closely linked to the *CYC1* locus. These revertants containing translocations have been designated *CYC1-512-Tl1* and *CYC1-512-Tl2*, respectively.

Mutant derivatives of *CYC1-512-Tl1* and *CYC1-512-Tl2* that lack iso-1-cytochrome *c* were isolated in order to map the aberrations in B-4307 and B-5407. These mutants are designated *cyc1-512-Tl1-2* and *cyc1-512-Tl2-1*, respectively, and presumably contain point mutations in the translated portion of the *CYC1* gene. As can be seen in Table 4, these mutants exhibit the same abnormal spore viability patterns as their parents that produce iso-1-cytochrome *c*, and therefore contain the same aberration as in the parent strains. When these mutant derivatives are used to make crosses with strains harboring the original respective aberrations, relatively normal spore viabilities and 2:2 segregation patterns for producing iso-1-cytochrome *c* are observed for *CYC1-512-Tl1*. Because in a homozygous translocation cross one would expect no duplications and deficiencies from meiosis and therefore normal spore viability, these data further suggest that B-4307 contains a reciprocal translocation. A limited amount of data on homozygous *CYC1-512-Tl2* crosses demonstrates better spore viability than in heterozygous *CYC1-512-Tl2* crosses, but not the expected normal pattern; more crosses will be required to determine whether the difference is significant. When *CYC1-512-Tl1* is crossed to *CYC1-512-Tl2*, extremely poor spore viability is observed (data not shown), suggesting that these two translocations are distinct from one another. Mapping of these translocations should reveal abnormal linkage relationships between the *CYC1* locus and other loci in the yeast genome.

The *cdc14* mapping method (KAWASAKI 1979) was employed to determine both the position of the translocation breakpoint on chromosome X and the presence of new chromosomal regions linked to chromosome X. Diploids that are homozygous for *cdc14* randomly lose chromosomes when they are incubated at 35° for 6 hr. By constructing a strain that is homozygous for *cdc14* and heterozygous for a number of other chromosomal markers, including the marker to be mapped, one can determine which chromosome the unmapped marker is on by monitoring for comcomitant loss of the unmapped marker with a marker from a known chromosome. Table 5 presents the chromosome losses resulting from homozygous *cdc14* diploids that were heterozygous for various chromosome markers and that contained the *cyc1-1* deletion (which spans *CYC1*, *OSM1* and *RAD7*) with either *CYC1-512-Tl1* or *CYC1-512-Tl2*. The data for *CYC1-512-Tl1* indicates that when the *CYC1-512-Tl1* allele of *CYC1* is lost, the chromosome X marker *ilv3* is uncovered. This suggests that the *CYC1* locus is linked to chromosome X in *CYC1-512-Tl1*. Since these strains that have lost the *CYC1-512-Tl1* homolog of chromosome X still remain *RAD7*$^+$, it suggests that *RAD7* is not linked to the *CYC1* locus in *CYC1-512-Tl1*. Correspondingly, the data show that when the *RAD7*$^+$ homolog is lost, exposing the *rad7* marker in

TABLE 5

Summary of cdc14-mediated chromosome losses in heterozygous translocation diploids

Diploid genotypes	Phenotypes of chromosome loss isolates				Total number of isolates
	Ilv$^+$ Cyc$^+$ Rad$^+$ Asp$^+$	Ilv$^-$ Cyc$^-$ Rad$^+$ Asp$^+$	Ilv$^+$ Cyc$^+$ Rad$^-$ Asp$^-$	Ilv$^-$ Cyc$^-$ Rad$^-$ Asp$^-$	
+ CYC1-512-Tl1 [+] / *ilv3* *cyc1-1* *rad7*	22[a]	4	4	1[b]	31
	Ilv$^+$ Cyc$^+$ Rad$^+$	Ilv$^-$ Cyc$^-$ Rad$^+$	Ilv$^+$ Cyc$^+$ Rad$^-$	Ilv$^-$ Cyc$^-$ Rad$^-$	
+ CYC1-512-Tl2 [+] / *ilv3* *cyc1-1* *rad7*	39[a]	1	10	0	50

The results for *CYC1-512-Tl1* are from diploid J491; the results for *CYC1-512-Tl2* represent totals from J524 and J529 (see Table 2 for complete genotypes). Although the *cyc1-1* deletion encompasses the *RAD7* locus, they are shown as separate alleles to clarify the complementation analysis in the table.

[a] These chromosome loss isolates lost markers other than those shown in the table.

[b] Numerous other chromosome loss events were detected in this isolate.

cyc1-1, the *CYC1-512-Tl1* allele is usually retained. Thus, the translocation breakpoint has occurred between the *CYC1* and *RAD7* loci in the *CYC1-512-Tl1* revertant. The data in Table 5 suggest a similar structure for *CYC1-512-Tl2*. The mapping analysis shown in Table 5 also demonstrates that the loss of *RAD7*$^+$ is accompanied by the loss of the chromosome *XII* marker *ASP5*$^+$, suggesting that the *RAD7* locus is linked to chromosome *XII* in *CYC1-512-Tl1* strains.

A sensitive meiotic mapping technique was used to detect linkage of various chromosome *XII* markers to the translocation breakpoint in *CYC1-512-Tl1*. We constructed diploids that were heterozygous for the chromosome *XII* markers to be tested and heterozygous for the translocation and the closely linked genes *CYC1* and *RAD7*, which serve as markers for the translocation breakpoint. As discussed above, when there is a single crossover in the interstitial region in a heterozygous translocation cross, only two spores from a tetrad will survive. Heterozygous markers that are tightly linked to the translocation breakpoint segregate +:− (1:1) in the surviving spores. The mapping technique considers the tetrads with two surviving spores and examines the marker configurations when both the heterozygous marker to be tested segregates 1:1 and a marker that is tightly linked to the breakpoint segregates 1:1. If the unknown marker maps in the interstitial region, the two spores will contain the unknown marker in the parental configuration with respect to the breakpoint marker if the interstitial crossover occurred between the centromere and the unknown marker. The markers can be in the recombinant configuration if the interstitial crossover occurred between the marker and the breakpoint. If the unknown

marker maps beyond the interstitial region, recombinant configurations can arise when single exchanges occur in both the interstitial region and the distal region. If the unknown marker is unlinked to the marker at the breakpoint, the number of parental configurations should equal the recombinant types. Thus, the ratio of parental to recombinant configurations in 1:1 segregants can provide a sensitive measure of the distance between a marker and a translocation breakpoint.

As shown in Table 6, the configurations of markers in the 1:1 segregants from tetrads with two surviving spores clearly show linkage of various chromosome X and *XII* markers to the *CYC1-512-Tl1* translocation breakpoint. For example, 30 of the two-spore survivor tetrads that segregate $ILV3^{+}$:$ilv3^{-}$ are in the parental configuration with respect to *CYC1*, whereas 6 of the tetrads that segregate $ILV3^{+}$:$ilv3^{-}$ are in the recombinant configuration. Using a χ^2 test at the 5% level, the 30:6 ratio is significantly different from equality. Therefore, *ILV3* is linked to the translocation breakpoint. Similar analysis demonstrates linkage of *CDC11* to the breakpoint, as well as the chromosome *XII* marker *URA4*. However, by using a *LEU2* insertion in the ribosomal DNA as a marker for *RDN1* (see the Materials and Methods), the chromosome *XII* marker *RDN1* is not significantly linked to the breakpoint, nor is the chromosome *XII* marker *ASP5*. As controls, the chromosome *III* marker *HIS4* and the chromosome *II* marker *LYS2* appear unlinked to the breakpoint. Thus, certain of the markers on the right arm of chromosome *XII* are linked to the *CYC1-512-Tl1* breakpoint, whereas other markers on the same chromosome arm appear unlinked.

Tetrad analysis of homozygous translocation crosses provided definitive evidence that *CYC1-512-Tl1* involves a translocation between chromosome X and *XII*. The data in Table 7 indicate that in *CYC1-512-Tl1* the *CYC1* locus

TABLE 6

Marker configurations in tetrads with two surviving spores from CYC1-512-Tl1 *heterozygous translocation crosses*

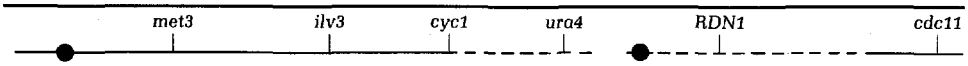
Genetic locus	Configurations of marker with respect to translocation breakpoint		χ^2	Linkage to translocation breakpoint
	Parental	Recombinant		
ILV3	30	6	16	Linked
URA4	80	3	71.4	Linked
CDC11	8	1	5.4	Linked
RDN1	17	17	0	Not significant
ASP5	4	6	0.4	Not significant
HIS4	15	24	2.1	Not significant
LYS2	21	26	0.53	Not significant

The +:− configurations of heterozygous markers for each genetic locus were compared with the +:− configurations of the heterozygous translocation breakpoint markers *CYC1* or *RAD7*. Each number represents the total number of tetrads with only two surviving spores that were scored. Linkage to the translocation breakpoint was determined by a χ^2 test of the departure of the parental:recombinant configuration from a 1:1 ratio at the 5% level of significance ($\chi^2 = 3.84$). The *CYC1-512-Tl1* heterozygous translocation diploids used in this analysis are listed in Table 2.

maintains its normal position with respect to the chromosome X centromere and that *CYC1* is also genetically linked to the *URA4* locus that is normally on chromosome XII. The *CDC11* locus is normally centromere-distal to *CYC1* on chromosome X and is unlinked to *CYC1* in *CYC1-512-Tl1* (data not shown). Although the results in Table 6 demonstrate that *CDC11* is linked to the translocation breakpoint, the homozygous translocation cross in Table 7 does not indicate linkage between *CDC11* and the *RDN1* locus on chromosome XII. This finding is expected since the data in Table 6 failed to show significant linkage between *RDN1* and the breakpoint. In fact, other studies have failed to demonstrate meiotic linkage of *RDN1* to *URA4* (PETES 1979b), whereas trisomic

TABLE 7

Tetrad analysis of CYC1-512-Tl1 *homozygous translocation crosses and the resulting genetic map*



Region		KZ-247	KZ-256	KZ-179	Total	Region		KZ-256
I [a]	PDP + NPD	15			15	A[a]	PD + NPD	25
	T	2			2		T	52
	cM	1			1		cM	N.S.
II	PD		56		56	B	PD	13
	NPD		0		0		NPD	11
	T		17		17		T	52
	cM		11.6		11.6		cM	N.S.
III	PD		43		43	A + B	PD + NPD	25
	NPD		0		0		T	51
	T		28		28		cM	N.S.
	cM		19.7		19.7			
IV	PD		49	8	57			
	NPD		2	1	3			
	T		27	8	35			
	cM		27	41	27.9			
II + III + IV	PD	3	13		16			
	NPD	0	6		6			
	T	14	60		74			
	cM	41	59.2		57.3			

The order of the markers was unambiguously deduced from the exchange patterns in the individual tetrads and the configurations of the flanking markers. The complete genotype of the strains are listed in Table 2. Only markers having 2:2 segregations were used in the calculations. N.S. stands for not significant linkage.

Abbreviations: PD, NPD, and T: number of, respectively, parental ditype, nonparental tetrads, and tetratype tetrads; cM, map distance in centimorgans which is calculated from 50(T+6NPD)/(PD+NPD+T) as described by MORTIMER and HAWTHORNE (1975).

[a] The PD + NPD and T refer to the gene pairs of one of the markers *met3*, *ilv3*, or *RDN1*, and *leu2* or *met3*. The distance of a marker to its centromere was calculated from the fraction of tetrads having second-division segregation, assuming that *leu2* and *met3* are, respectively, 5 and 1 cM from their centromeres and by the relationship $f(T) = x + y - (1.5)xy$, where $f(T)$ is the fraction of tetratype tetrads and x and y are fractions of tetrads having second-division segregation for, respectively, the two-centromere-linked markers (see MORTIMER and HAWTHORNE, 1975).

and mitotic analyses do place *URA4* on the right arm of chromosome XII (MORTIMER and HAWTHORNE 1973). Because the chromosome loss results described above suggest linkage between *RAD7* and the chromosome *XII* marker *ASP5* in *CYC1-512-Tl1*, it is concluded that *CYC1-512-Tl1* is indeed a reciprocal translocation between the right arms of chromosomes X and *XII*, as depicted in Figure 4.

Class III: Chromosomal aberrations, duplication of CYC1: When crossed to *cyc1-1* tester strains, the *cyc1-512* revertant B-4304 segregates 4:0, 3:1 and 2:2 for Cyc^+:Cyc^-. B-4304 is not disomic for chromosome X because the chromosome X markers *ilv3* and *met3* segregate 2:2 in these crosses. However, in B-4304, the genes *OSM1* and *RAD7*, which are normally tightly linked to *CYC1*, also segregate 4:0, 3:1 and 2:2 along with *CYC1*. The digenic segregation of the *CYC1*, *OSM1* and *RAD7* loci indicates that a duplication of the *CYC1* region has occurred in B-4304, which is denoted *CYC1-512-Dp1*. The duplicated copy of *CYC1* segregates independently of chromosome X markers and is not centromere linked. Although the duplicated copy of *CYC1* has not been mapped, recent studies have shown that B-4304 contains several rearrangements in addition to a simple duplication (K. ZARET, unpublished results).

Class III: Chromosomal aberrations, inversion: When the B-4308 revertant of *cyc1-512* is crossed to normal *cyc1-1* strains it produces a high proportion of tetrads that give rise to only two viable spores, as shown in Table 4. In a cross between a strain that contains a inversion and strain of normal chromosomal constitution, one would expect complete spore viability when there is no crossing over in the inverted region or when there are two-strand double crossovers in the inverted region. A single crossover or a three strand double

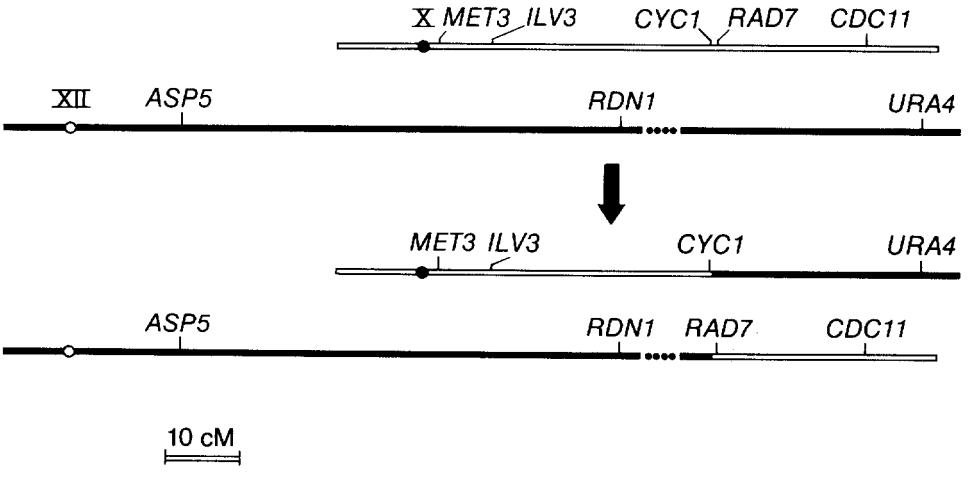


FIGURE 4.—Genetic maps of the normal chromosomes X and *XII* (top) and of the translocated chromosomes (bottom). The map of chromosome X is from the results of LAWRENCE *et al.* (1975), chromosome *XII* from the results of PETES (1979b) and the translocated chromosomes from the results presented in Table 7. The dotted lines indicate mitotic linkage between the designated genes.

crossover in the inverted region will create dicentric and acentric chromatids as well as a duplication and deficiency for each of the two chromatids involved in the crossover. These genetic aberrations will cause two of the four meiotic products to be inviable (2/4 for viability), as seen in the cross with B-4308. The larger the inverted region, the greater the number of 2/4 tetrads is expected. In a heterozygous inversion cross, genes that are tightly linked to a breakpoint should segregate +:− in the 2-spore survivor tetrads. As with certain other chromosomal aberrations, a cross between two strains containing the same inversion should not produce lethalities and the normal 4/4 spore viability pattern should result.

The results from the cross of the *cyc1-512* revertant B-4308 with *cyc1-1* is presented in Table 4. The tetrads predominantly have only two viable spores, whereas the frequency of tetrads having four viable spores per ascus (4/4) is significantly greater than the frequency of tetrads having zero viable spores per ascus (0/4). This result is in contrast to a heterozygous translocation cross, where the frequency of 4/4 asci are approximately equal to the frequency of 0/4 asci. The data in Table 4 show that a cross that is homozygous for the aberration in B-4308 gives rise to a relatively normal pattern of spore viability. Thus, B-4308 causes spore viability patterns that are consistent with the presence of an inversion in the strain. The aberration in B-4308 is denoted *CYC1-512-In1*.

The +:− configurations of markers in the two-spore survivor tetrads should reveal linkage of markers to the *CYC1-512-In1* aberration breakpoints. Table 8

TABLE 8

Marker configurations in two-spore survivor tetrads from heterozygous aberration crosses

		+:− marker configurations		
Marker	Aberration	1:1	2:0 or 0:2	χ^2
CYC1:cyc1	*CYC1-512-In1*	49	1	22.2
	CYC-512-Tl1	54	0	27
ILV3:ilv3	*CYC1-512-In1*	47	3	16.8
	CYC1-512-Tl1	36	18	0
CDC11:cdc11	*CYC1-512-In1*	38	12	1.96
	CYC1-512-Tl1	10	1	2.97
LYS2:lys2	*CYC1-512-In1*	32	18	0.15
	CYC-512-Tl1	32	22	1.33
TRP2:trp2	*CYC1-512-In1*	31	19	0.48

CYC1-512-In1 designates the aberration in B-4308; *CYC1-512-Tl1* designates the reciprocal translocation. The data are from strains listed in Table 2. The χ^2 values represent the deviations of the (1:1):(2:0 and 0:2) marker configurations from the random (unlinked) value of a 2:1 ratio. χ^2 values greater than 10.83 show a deviation from a 2:1 ratio at the 0.1% level of significance. Values greater than 10.83 therefore represent linkage of a marker to an aberration breakpoint.

shows the two-spore marker configurations in heterozygous *CYC1-512-In1* crosses; in addition, the results of crosses with the reciprocal translocation revertant *CYC1-512-Tl1* are presented for comparison. If a heterozygous marker was unlinked to a breakpoint, it should segregate randomly in the two-spore survivor tetrads. The predicted frequencies of +:− segregation would be in the ratio of 4:1:1 for (1+:1−):(2+:0−):(0+:2−), or more simply, the ratio of 2:1 for [1+:1−]:[(2+:0−) + (0+:2−)]. For example, consider the *CYC1-512-Tl1* translocation between chromosomes X and *XII*, and the *LYS2* locus on chromosome *II*. In heterozygous translocation crosses, the *lys2* marker segregates 1:1 32 times and 2:0 or 0:2 22 times. 32:22 is not statistically different from a ratio of 2:1, so as expected, the *LYS2* locus is unlinked to either of the translocation breakpoints. Similarly, the *lys2* and *trp2* markers are unlinked to a breakpoint in *CYC1-512-In1*. However, in Table 8 both *CYC1-512-In1* and *CYC1-512-Tl1* show highly significant linkage of the *CYC1* locus to a breakpoint. This is expected because the breakpoints presumably introduce new chromosomal regions that restore the *cyc1-512* defect. This sort of analysis, which is less sensitive than the technique previously described for *CYC1-512-Tl1* (Table 6), shows that the chromosome X marker *cdc11*, which is normally centromere-distal and 20 cM from *CYC1*, is not significantly linked to a breakpoint in either *CYC1-512-In1* or *CYC1-512-Tl1*. The chromosome X marker *ilv3* is between the centromere and *CYC1*, usually about 30 cM from the *CYC1* locus (LAWRENCE *et al.* 1975 and K. ZARET, unpublished results). The results in Table 8 shows that *ilv3* is not significantly linked to a breakpoint in *CYC1-512-Tl1*, but is significantly linked to a breakpoint in *CYC1-512-In1*. These results are consistent with the possibility that *CYC1-512-In1* contains an inversion on chromosome X, with one breakpoint near the *CYC1* locus and another breakpoint near or beyond the *ILV3* locus. These results do not distinguish whether an aberration breakpoint actually maps at or near *ILV3* or whether *ILV3* is contained entirely within the aberration. Although these results do not rule out other type of chromosomal rearrangements, it is clear that the aberration in *CYC1-512-In1* is distinct from a reciprocal translocation and that the creation of an aberration breakpoint at *CYC1* is associated with the reversion of the *cyc1-512* mutation.

DISCUSSION

This paper describes genetic revertants of the mutant, *cyc1-512*, which is defective in transcription termination and polyadenylation of the yeast *CYC1* gene. The revertants fall into three classes based upon their genetic properties in crosses to tester strains.

The first class of *cyc1-512* revertants is caused by small changes in chromosome structure at or near the *CYC1* locus, termed "strictly local" mutations. Genetically these mutations appear to be intragenic; they segregate 2:2 and exhibit normal patterns of spore viability. However, in a future paper (K. ZARET and F. SHERMAN, unpublished results), it is shown that some of the mutants in this class contain local aberrations or affect the adjacent gene that transcribes convergently into the *CYC1* locus in *cyc1-512* strains. The strictly local muta-

tions exhibit a great variation in the extent to which they restore the *cyc1-512* deficiency. Some of these revertants produce only slightly greater amounts of iso-1-cytochrome *c* than *cyc1-512*; others produce as much cytochrome *c* as the wild type. No revertants of *cyc1-512* produce more cytochrome *c* than do the wild type. Revertants producing cytochrome *c* at lower levels and at higher levels are found in approximately equal proportions, indicating that there is no preferred type of mutation that can be induced to revert *cyc1-512*. Clearly, there are a number of different levels to which the transcription termination defect can be restored.

The second class of *cyc1-512* revertants consists of an unlinked extragenic suppressor of the transcription termination mutant, *sut1*. In *Escherichia coli*, a variety of extragenic mutations are known to affect specific transcription terminators. *E. coli* mutants defective in rho protein (DAS, COURT and ADHYA 1976; GUARENTE *et al.* 1977), NusA protein (WARD and GOTTESMAN 1981), or ribosomal protein S10 (FRIEDMAN *et al.* 1981) can decrease transcription termination at certain sites *in vivo*, whereas mutants of RNA polymerase (GUARENTE and BECKWITH 1978) or the bacteriophage lambda N protein (SKALKA, BUTLER and ECHOLS 1967) can increase transcription termination. Many of these proteins have been shown to interact with one another and are involved in the regulation of lambda gene expression (WARD and GOTTESMAN 1982) or the attenuation of amino acid biosynthetic operons (YANOFSKY 1981). Recent studies have implicated transcription termination as a point of control of higher eukaryotic genes, such as in adenovirus (SHAW and ZIFF 1980; NEVINS and WILSON 1981) and SV40 (HAY, SKOLNIK-DAVID and ALONI 1982). It is also possible that *sut1* acts by increasing the rate of transcription of *CYC1* or by increasing *CYC1* mRNA stability. In either case, mutants such as *sut1* will be useful for the study of mRNA metabolism in yeast.

The third class of revertants of *cyc1-512* are strains containing gross chromosomal rearrangements. One type of revertant in this class consists of a duplication of *CYC1* and other closely linked, centromere-distal genes on chromosome X. A duplication of the *CYC1*, *OSM1* and *RAD7* loci has been described previously (STILES *et al.* 1981). However, in the present case, the duplication revertant of *cyc1-512* contains a more extended duplicated region and is accompanied by other chromosomal rearrangements (K. ZARET, unpublished results). The other type of revertants in this class are strains that contain reciprocal translocations and possibly an inversion, each with a breakpoint that is tightly linked to *CYC1*. A detailed analysis of the *CYC1-512-Tl1* translocation demonstrates that the right arms of chromosomes X and *XII* have exchanged with one another (Figure 4). This exchange results in meiotic linkage between the *CYC1* locus, which is retained on chromosome X, and the *URA4* locus, which is normally on chromosome *XII*. Mitotic chromosome loss experiments demonstrated that the translocation breakpoint is located centromere-distal to the *CYC1* locus, between *CYC1* and *RAD7*. Since it is known that the *CYC1* locus is oriented centromere-(5′-*CYC1*-3′)-*RAD7* (LAWRENCE *et al.* 1975), the results presented in this paper suggest that one of the *CYC1-512-Tl1* translocation breakpoints is located on the 3′ side of the *CYC1* locus. Apparently the

transcription termination defect of the *cyc1-512* mutant can be restored by translocating new chromosomal regions, presumably containing terminators, to the 3′ end of the *CYC1* locus.

Although a sensitive mapping technique was employed, meiotic linkage could not be established between the *CYC1-512-Tl1* breakpoint-distal marker *CDC11* and the breakpoint-proximal marker *RDN1* on chromosome *XII*. The mitotic chromosome loss method did show linkage of the breakpoint-distal marker *RAD7* to the breakpoint-proximal marker *ASP5* on chromosome *XII*. At the physical level, there might be an excess of genetic material between the *RDN1* locus and the translocation breakpoint, so that meiotic mapping would fail to show linkage. However, PETES (1980) has shown that meiotic recombination between nonsister chromatids is suppressed in the lengthy *RDN1* locus. If this local suppression was compensated by unusually high nonsister chromatid exchanges beyond the boundaries of *RDN1*, then markers that are distal to the *RDN1* locus would appear relatively further by genetic analysis than they are at the physical level. This could be a possible explanation for the inability to detect meiotic linkage between *RDN1* and the translocation breakpoint in this investigation, and *RDN1* and *URA4* in the normal crosses of a previous investigation (PETES 1979b).

Of 464 *cyc1* mutations that have been isolated and characterized (SHERMAN *et al.* 1974; F. SHERMAN and S. CONSAUL, unpublished results), only the *cyc1-362* and *cyc1-512* mutations map outside the translated portion of *CYC1*. As discussed in the introduction, the rare mutants that affect the rate of transcription of yeast protein coding genes are known to involve gross chromosomal alterations. Similarly, the *cyc1-512* mutant that affects transcription termination is a 38-base pair deletion. Because multiple base pair changes or gross rearrangements are required to alter function, these results suggest that the genetic signals that govern the rate of eukaryotic transcription initiation and termination are complex and cannot be readily destroyed by single base pair changes. In contrast, the genetic control signals in prokaryotes appear to be simpler, since single base pair changes can affect either transcription initiation or termination (reviewed by ROSENBERG and COURT 1979). Although the restitution of the *cyc1-512* deletion occurred by strictly local mutation, there were a significant frequency of chromosomal aberrations (4 out of 41; Table 3); in addition, some of the strictly local mutations appear to involve multiple base pair changes (K. ZARET and F. SHERMAN, unpublished results). Because gross chromosomal changes and multiple base pair changes are rare in yeast, the formation of transcription termination signals may not readily occur by single base pair changes even though large regions are available for their potential information. In conclusion, there are a variety of ways to alleviate the transcription termination defect in *cyc1-512* and a number of degrees to which the termination defect can be restored. An analysis of the DNA sequences along with the transcripts in the various *cyc1-512* revertants may reveal the mechanisms by which the restorations are occurring.

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