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Genetics

Chromosome mapping of the *CYC7* gene determining yeast iso-2-cytochrome *c*: Structural and regulatory regions

(regulatory mutation/amino acid sequence/*Saccharomyces cerevisiae*)

FRED SHERMAN, JOHN W. STEWART, CYNTHIA HELMS, AND J. ALLAN DOWNIE*

Department of Radiation Biology and Biophysics, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642

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ABSTRACT **The primary structures of iso-1-cytochrome *c* and iso-2-cytochrome *c* in the yeast *Saccharomyces cerevisiae* are determined by the genes *CYC1* and *CYC7*, respectively. The *CYC1* locus was previously shown to be on the right arm of chromosome X, and the *CYC7* locus is shown in this investigation to be on the left arm of chromosome V closely linked to the *min1* and *mak10* markers. The *CYC7* locus appears to be composed of a structural region and a regulatory region. Mutations in the structural region can cause a deficiency or alteration of iso-2-cytochrome *c*, whereas mutations in the regulatory region can cause increases in the amount of iso-2-cytochrome *c*. Single-site gene conversion, occurring at a relatively high frequency of approximately 4%, caused intragenic recombination of a mutational site in the structural region and a mutational site in the regulatory region, enabling us to suggest the order of the sites in relationship to other markers on the chromosome.**

Bakers' yeast, *Saccharomyces cerevisiae*, contains two cytochromes *c*, iso-1-cytochrome *c* and iso-2-cytochrome *c* which normally constitute 95 and 5%, respectively, of the total amount of cytochrome *c* in derepressed cells. Iso-1-cytochrome *c* is 108 amino acids long and iso-2-cytochrome *c* is 112 amino acids long; they differ from each other at a total of 21 residue positions (J. W. Stewart and G. J. Putterman, unpublished data). Genetic studies of strains containing mutationally altered proteins have established that the primary structure of iso-1-cytochrome *c* is determined by the *CYC1* gene (1), which is located on the right arm of chromosome X adjacent to the markers *rad7*, *SUP4*, and *cdc8* (2). Similarly, it was recently demonstrated that iso-2-cytochrome *c* is determined by the *CYC7* gene (3). A mutant, *CYC7-1*, was shown to contain an approximately 30-fold increase of iso-2-cytochrome *c* (3) due to a chromosomal translocation having one of its breakpoints adjacent to the structural locus, *CYC7*, for iso-2-cytochrome *c* (4). Mutants lacking or having decreased levels of iso-2-cytochrome *c* were selected by use of the benzidine staining procedure (3, 5). Altered iso-2-cytochromes *c* were observed in several of the partially deficient mutants having alterations at the *CYC7* locus, in intragenic revertants of some of these *cyc7* mutants, and in mutants suppressed by an amber suppressor (3, 5). Thus, it was established that the *CYC7* locus determines the primary structure of iso-2-cytochrome *c*. However, due to the translocation, the map position of the *CYC7* locus could not be unambiguously determined in relationship to the normal chromosomal arrangement because the *CYC7* locus could be assigned to either of the two breakpoints (4).

In this investigation we isolated *CYC7* mutants from strains having the normal chromosomal constitution. The finding of an altered iso-cytochrome *c* in several of these mutants confirms our earlier conclusion that the *CYC7* gene controls the structure of iso-2-cytochrome *c*. The normal chromosomal arrangement in the mutant strains allowed us to locate the *CYC7* gene on the left arm of chromosome V near the markers *min1* and *mak10*. We demonstrate that the *CYC7* locus consists of at least two regions, one region that controls the primary structure of the protein and a regulatory region that encompasses a mutational alteration which increases the level of iso-2-cytochrome *c*. Mutational sites in the two regions can be separated by recombination and their order has been assigned in relationship to other markers on the chromosome.

MATERIALS AND METHODS

Genetic Procedures. The designation of genes controlling the iso-cytochromes *c* has been described in detail (3,6,7), and pertinent examples are presented in Table 1. Also, the media and procedures used for selecting and testing cytochrome *c* mutants have been described (3,6) and are summarized in Table 1.

Conventional yeast genetic procedures of crossing, sporulation, and tetrad analysis were used for meiotic analysis and for construction of strains with desired markers (8).

Because all of the strains used for pedigree analyses contained *cyc1* genes and therefore lacked iso-1-cytochrome *c*, the *CYC7+*, *CYC7-2*, and *cyc7-2-1* alleles could be scored from the growth responses on glycerol medium and from the levels of iso-2-cytochrome *c* that were estimated by low-temperature (−190°) spectroscopic examinations of intact cells (9). The *CYC7-2* strains contain high levels of iso-2-cytochrome *c* and are easily distinguished from *CYC7+* and *cyc7-2-1* strains. In contrast to *CYC7-2* and *CYC7+* strains, the *cyc7-2-1* strains do not grow on glycerol medium and in certain genetic backgrounds they are also deficient in cytochromes a-a_3 due to the low level of total cytochrome *c* (3). Also, the *cyc7-2-1* strains are more deficient in iso-2-cytochrome *c* than are the *CYC7+* strains, although this distinction is not clearly seen in ρ^+ strains. Because all ρ^- strains do not grow on glycerol medium and because ρ^- strains occasionally arise in pedigrees, spectral examinations were used to identify ρ^- *CYC7+*, ρ^- *CYC7-2*, and ρ^- *cyc7-2-1* strains.

The canavanine-resistant marker, *can1* (10), located on the left arm of chromosome V (11), was scored on a synthetic medium containing canavanine sulfate at 60 mg/liter.

The *mak10* gene, which previously has been designated *m* (12), mmm1 (13), mak_1 (14), and *MAV1* (15), prevents the maintenance or replication of the killer plasmid [KIL-k] and is located on the left arm of chromosome V [Bevan & Theivendirarajah, cited by Wickner and Lebowitz (16)]. Strains carrying the *mak10* gene were scored by spotting suspensions

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* Present address: Department of Biochemistry, The John Curtin School of Medical Research, The Australian National University, Canberra City, A. C. T. 2601, Australia

Table 1. Lineage and series of steps used for obtaining a mutationally altered form of iso-2-cytochrome *c*

Strain	Genotype	Procedure	Iso-cytochromes *c*, % of total wild-type level
D311-3A	ρ^+ *CYC1+ CYC7+*		95% iso-1; 5% iso-2
	↓	ρ^- isolation	
D311-3A-1	ρ^- *CYC1+ CYC7+*		95% iso-1; 5% iso-2
	↓	Benzidine staining	
B-456	ρ^- *cyc1-9 CYC7+*		5% iso-2
	↓	Genetic cross, etc.	
JP109-3A	ρ^+ *cyc1-9 CYC7+*		5% iso-2
	↓	Lactate medium	
B-929	ρ^+ *cyc1-9 CYC7-2*		100% iso-2
	↓	Genetic cross, etc.	
B-4625	ρ^- *cyc1-1 CYC7-2*		100% iso-2
	↓	Benzidine staining	
B-4626	ρ^- *cyc1-1 cyc7-2-1*		Deficient
	↓	Genetic cross, etc.	
D872-2C	ρ^+ *cyc1-1 cyc7-2-1*		Deficient
	↓	Lactate medium	
B-4651	ρ^+ *cyc1-1 CYC7-2-1-A*		80% iso-2 (altered)

of cells onto a buffered nutrient medium containing methylene blue and a lawn of sensitive cells [KIL-o], similar to the procedure described by Somers and Bevan (12).

Strains carrying the *min1* gene, previously designated *met-4*$_1$, are inhibited by relatively low concentrations of methionine, histidine, and possibly other nutrients (17, 18). The *min1* allele used in this investigation, *min1-3*, was derived independently of *min1-1* and *min1-2* (17) during the course of mutation of the *CYC7* locus (see *Results*). In contrast to the other *min1* strains (17), *min1-3* strains were not inhibited by adenine or leucine and were only slightly inhibited by histidine. In addition, the *min1* strains were inhibited by casamino acids and Bacto-yeast extract but not by Bacto-peptone which, in fact, alleviated the inhibition caused by yeast extract. Therefore, the *min1* strains were conveniently grown and stored on our standard YPD nutrient medium (8). The *min1* strains were routinely scored on synthetic complete media either containing 1% casamino acids or containing 11 nutrilites (8) including L-methionine (20 mg/liter).

Characterization of Revertant Iso-2-cytochromes *c*. Iso-2-cytochromes *c* were prepared by the procedure used to prepare iso-1-cytochromes *c* (10, 19), and the structural changes were identified from the amino acid compositions and the tryptic and chymotryptic peptide maps as described (19). Some of the altered iso-2-cytochromes *c* were further purified by isoelectric focusing (20).

RESULTS

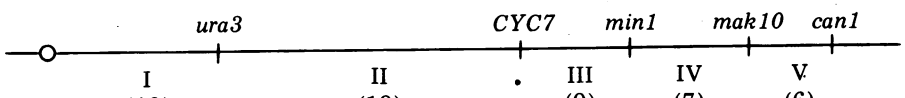
Mutants of the *CYC7* Locus. In our initial investigations of mutationally altered iso-2-cytochromes *c* (3, 5), we used the mutant *CYC7-1* which contains an abnormally high amount of iso-2-cytochrome *c* due to a chromosomal translocation having one of its breakpoints adjacent to the structural locus *CYC7* (4). As described above, mutants with altered iso-2-cytochromes *c* were obtained from the *CYC7-1* mutant but the presence of the translocation made the assignment of the structural locus to a chromosome ambiguous (4). Therefore, using the same procedures used in our previous studies, we isolated *CYC7* mutants from strains having the normal-chromosomal constitution.

The steps used for obtaining *CYC7* mutants with mutationally altered iso-2-cytochromes *c* are outlined in Table 1. A mutant, B-929, containing an abnormally high level of iso-2-cytochrome *c* was obtained in an experiment designed for reverting *cyc1-9* strains (21). This mutant was derived from nitrous acid treatment of strain JP109-3A (*cyc1-9 CYC7+*) and contained an amount of iso-2-cytochrome *c* approximately equivalent to the amount of total cytochrome *c* found in normal cells. Nevertheless, the revertant, *cyc1-9 CYC7-2*, containing iso-2-cytochrome *c* was conveniently distinguished from the intragenic revertants of *cyc1-9* by the characteristic spectrum of iso-2-cytochrome *c* which has an unusually narrow C_α band slightly shifted toward the blue (21). Amino acid compositional analysis and peptide mapping indicated that the iso-2-cytochrome *c* from the strain B-929 was normal, similar to the results previously obtained with iso-2-cytochrome *c* from a *CYC7-1* strain (3). In contrast to crosses with *CYC7-1* strains, normal spore viability was observed from crosses with the B-929 strains, suggesting that the high level of iso-2-cytochrome *c* was not caused by a chromosomal rearrangement. In addition, the high level of iso-2-cytochrome *c* segregated as a simple Mendelian gene in numerous crosses that are described below. This mutant gene was designated *CYC7-2*. No *CYC7+* strains were recovered among the 24 meiotic segregants from a *CYC7-1* × *CYC7-2* cross, indicating that the *CYC7-2* gene is located at or near either of the two breakpoints of the *CYC7-1* translocation and probably at the structural locus *CYC7*.

An appropriate strain, B-4625 (Table 1), was constructed and was used for obtaining mutants deficient in iso-2-cytochrome *c*. The benzidine staining procedure (22) was used to examine approximately 20,400 colonies derived from ethyl methanesulfonate-treated cells and approximately 4000 colonies derived from UV-irradiated cells. Twenty-five mutants having 30% or less of the parental level of iso-2-cytochrome *c* were recovered and were subjected to genetic complementation tests as described (5). Similar to the results with the *CYC7-1* strain (5), the most deficient mutants could be assigned to the *cyc2*, *cyc3*, or *cyc7* locus, there being three *cyc3* mutants, four *cyc2* mutants, and two *cyc7* mutants. The assignment of the mutants *cyc7-2-1* and *cyc7-2-2* was confirmed by pedigree analysis of *CYC7+* × *cyc7-2-1* and *CYC7+* × *cyc7-2-2* crosses.

The extent of the deficiencies of iso-2-cytochrome *c* was estimated in numerous ρ^+ segregants containing either the *cyc7-2-1* or *cyc7-2-2* mutant genes. In contrast to ρ^+ *cyc1 CYC7+* segregants, all ρ^+ *cyc1 cyc7-2-1* and ρ^+ *cyc1 cyc7-2-2* segregants failed to grow on glycerol medium, and spectral examinations indicated that all of these segregants appeared to contain less than the *CYC7+* level of iso-2-cytochrome *c* which corresponds to approximately 5% of the *CYC7-2* amount. We have reported that the content of cytochromes a-a_3 is an indicator of the absence or low activity of total cytochrome *c*. When ρ^+ strains contain *cyc7* mutant genes causing deficiency of iso-2-cytochrome *c* and *cyc1* mutant genes causing deficiencies of iso-1-cytochrome *c*, the level of total cytochrome *c* is below a crucial level, which results in the deficiency of

Table 2. Tetrad analysis and the genetic map of the markers on the left arm of chromosome V



	I*			II				III				IV				V				III + IV + V				IV + V		
	PD +																									
Strains	NPD	T	cM	PD	NPD	T	cM	PD	NPD	T	cM	PD	NPD	T	cM	PD	NPD	T	cM	PD	NPD	T	cM	PD	NPD	T
D-879	—	—		—	—	—		10	0	0		—	—	—		—	—	—		—	—	—		—	—	—
D-876	4	1		5	0	0		—	—	—		—	—	—		—	—	—		—	—	—		—	—	—
D-872	5	0		3	0	1		4	0	0		—	—	—		—	—	—		—	—	—		—	—	—
D-885	15	5		14	0	8		18	0	2		—	—	—		—	—	—		—	—	—		10	1	9
D-886	12	2		12	0	3		13	0	1		—	—	—		—	—	—		—	—	—		10	0	4
D-855	5	3		6	0	2		—	—	—		—	—	—		—	—	—		6	0	3		—	—	—
D-860	100	26	10	83	0	42	17	—	—	—		—	—	—		—	—	—		68	1	55	25	—	—	—
D-892	—	—		4	0	2		5	0	1		4	0	0		3	0	1		—	—	—		—	—	—
D-897	—	—		5	0	13		16	0	4		17	0	1		18	0	1		—	—	—		—	—	—
D-893	94	20	8	66	1	42	22	81	1	16	11	93	1	10	8	98	0	14	6	—	—	—		—	—	—
Totals	235	57	10	198	1	113	19	147	1	24	9	114	1	11	7	119	0	16	6	74	1	58	24	20	1	13

The order of the markers was unambiguously deduced from the exchange patterns in the individual tetrads and the configurations of the flanking markers. Abbreviations: PD, number of parental ditype tetrads; NPD, number of nonparental ditype tetrads; T, number of tetratype tetrads; cM, map distance in centimorgans (cM), calculated from 50(T + 6NPD)/(PD + NPD + T).

* The PD + NPD and T refer to either of the gene pairs *ura3-trp1* or *ura3-leu1*. The centromere-*ura3* distances were calculated by assuming *trp1* and *leu1* are, respectively, 1 and 2 cM from their centromeres and by the relationship $f(T_{ab}) = x + y - (1.5)\,xy$, in which $f(T_{ab})$ is the fraction of tetratype tetrads and x and y are fractions of tetrads having second-division segregation for the a and b centromere-linked markers, respectively.

cytochromes a·a_3 as previously described for certain ρ^+ *cyc1 cyc7-1-x* strains (3, 5) and other types of single and double mutants (23, 24). All of the ρ^+ *cyc1 cyc7-2-2* segregants and approximately one-half of the ρ^+ *cyc1 cyc7-2-1* segregants contained cytochromes a·a_3, thus suggesting that a low residual activity of iso-2-cytochrome *c* remains in the *cyc7-2-2* and some *cyc7-2-1* segregants. There were no differences in the absorption spectra of numerous ρ^+ *cyc1 cyc7-2-1* segregants that were grown at 22° in comparison to those grown at the normal incubation temperature of 30°, thus indicating that the iso-2-cytochrome *c* is not thermal labile like iso-2-cytochromes *c* in numerous *cyc7-1-x* mutants (5). Spectroscopic examination of the meiotic progeny from crosses of the *cyc7-2-1* mutant with strains containing the UAA suppressor *SUP4-o*, the UAG suppressor *SUP3-a*, or the UAG suppressor *SUP8-a*, all of which cause insertion of tyrosine at the UAA or UAG site (25, 26), showed that the *cyc7-2-1* mutant was not suppressible by any of these suppressors.

Independently derived spontaneous revertants were isolated and two *cyc7-2-1* intragenic revertants and three *cyc7-2-2* intragenic revertants were subjected to genetic and biochemical analysis. Low-temperature spectra revealed that the *cyc7-2-1* and *cyc7-2-2* revertants contained approximately 80 and 100%, respectively, of typical *CYC7-2* levels of iso-2-cytochrome *c*. Preparative isolation revealed only iso-2-cytochrome *c* which, in the revertants of *cyc7-2-1*, was slightly unstable. Amino acid compositions and peptide maps of iso-2-cytochromes *c* from the three revertants of *cyc7-2-2* appeared normal. The compositions and peptide maps of the iso-2-cytochromes *c* from the two revertants of *cyc7-2-1* appeared to be identical and altered. The sole change observed in amino acid composition was the loss of the single tryptophanyl residue. The peptide maps confirmed this complete loss of tryptophan by failing to exhibit a positive Ehrlich reaction for tryptophan, which is normally observed at a single position with normal iso-2-cytochrome *c*. By revealing neither loss nor gain of spots but only a diminution of intensity of a single normal ninhydrin-positive peptide that normally does not contain tryptophan, the peptide maps of both tryptic and chymotryptic digests suggest but do not require that there may be one or more structural changes other than at the tryptophan residue in the altered proteins from the two *cyc7-2-1* revertants. An altered peptide from iso-2-cytochrome *c* of the *CYC7-2-1-A* revertant was isolated, partially sequenced, and found to contain a tyrosine replacement of the normal residue of tryptophan at position 68 (details to be reported elsewhere).

These results suggest that the *cyc7-2-1* mutant contains two lesions affecting iso-2-cytochrome *c*. The first, *CYC7+* → *CYC7-2*, caused a 20-fold increase of the amount of iso-2-cytochrome *c* without affecting its structure. The second, *CYC7-2* → *cyc7-2-1*, essentially abolished iso-2-cytochrome *c*. The third mutation, *cyc7-2-1* → *CYC7-2-1-A*, caused nearly a total restoration of the *CYC7-2* level of a structurally altered iso-2- cytochrome *c*. These mutational events are summarized in Table 1.

The *min1* Marker. While testing the *cyc7-2-1* mutant, it was noted that strain B-4626 contained at least two additional mutant genes not originally present in the parental strain B-4625. One was a *pet* gene that caused deficiencies of cytochromes a·a_3 and *b* and that prevented growth on glycerol medium. This *pet* marker segregated as a single Mendelian gene and was not linked to either the *CYC1* or *CYC7* locus. The second mutant gene, *min1*, caused sensitivity to methionine and did not complement or readily recombine with the *min1* mutant originally described by Meuris *et al.* (17). As described below, the *min1* and *cyc7-2-1* mutations are closely linked. Apparently, ethyl methanesulfonate induced mutations at three independent sites, causing formation of the *cyc7-2-1*, *min1*, and *pet* mutant genes. Ethyl methanesulfonate has been reported to induce multiple mutations at frequencies higher than those expected to occur by the coincidence of separate events (27). However, B-4626 is the only strain uncovered in our studies that acquired any observable additional mutations, including the 37 iso-2-cytochrome *c*-deficient mutants induced by ethyl

methanesulfonate (5). Because the sites of the *min1* and *cyc7-2-1* mutations are closely linked, it can be suggested that the mutated cell was at a particular physiological state in which certain regions of the chromosomes were more susceptible to the mutagenic action of ethyl methanesulfonate.

The *CYC7* locus is on Chromosome V. Pedigree analysis with the *CYC7-1* translocation suggested that the *CYC7* locus is on either the left arm of chromosome V or the right arm of chromosome XVI (4). Therefore, crosses were constructed with heterozygous markers, especially with the *can1*, *ura3* (11), and *mak10* (Bevan & Theivendirarajah, cited in ref. 16) markers which were previously shown to be on the left arm of chromosome V and with various *CYC7* markers and with the *min1* marker. The analysis of over 200 complete tetrads from these various crosses verified the previous genetic map assignment of the *ura3*, *mak10*, and *can1* markers and demonstrated that these markers are linked to the *CYC7* and *min1* markers. The sequence *ura3-CYC7-min1-mak10-can1* was unambiguously determined from the examination of the exchange patterns in individual tetrads and the configurations of the flanking markers as shown in Table 2.

The vast majority of the tetrads exhibited a normal 2:2 segregation for all of the heterozygous markers. The low frequencies of aberrant segregation of *CYC7* markers attributed to gene conversion, described below, allowed the ordering of the regulatory and structural regions of the gene with respect to the centromere.

Separation of Sites in the Structural and Regulatory Regions by Gene Conversion. Rare *CYC7-2* recombinants were observed among the meiotic progeny of *CYC7+* × *cyc7-2-1* crosses. Such recombinants could be anticipated because the *cyc7-2-1* mutant gene arose by two mutational steps from the normal gene *CYC7+*, first the *CYC7+* → *CYC7-2* mutation in the presumably regulatory region that produced an increase of iso-2-cytochrome *c* and second the *CYC7-2* → *cyc7-2-1* mutation in the structural region that produced a deficiency in iso-2-cytochrome *c*. Thus, the *cyc7-2-1* mutant can be considered to harbor two mutational lesions, a^- and b^- corresponding to the normal sites a^+ and b^+, and consequently the three *CYC7* alleles can be designated as follows: a^+b^+ for *CYC7+*; a^-b^+ for *CYC7-2*; and a^-b^- for *cyc7-2-1*. The cross $a^+b^+ \times a^-b^-$ (or *CYC7+* × *cyc7-2-1*) could yield two types of recombinants, a^-b^+ (or *CYC7-2*) and a^+b^-, a new mutant gene that would be expected to cause a deficiency similar to the *cyc7-2-1* allele but that could be distinguished from *cyc7-2-1* by genetic tests; the test cross $a^+b^- \times a^+b^+$ would not be expected to yield a^-b^+ (or *CYC7-2*) recombinants, whereas the test cross $a^-b^- \times a^+b^+$ yields a^-b^+ recombinants that can be selected on lactate medium.

Among 160 tetrads analyzed from *CYC7+* × *cyc7-2-1* crosses, 7 tetrads contained a *CYC7-2* recombinant that arose by recombination between the a and b sites. All seven of these tetrads can be explained by a gene conversion of b^- to b^+ as schematically represented in Fig. 1. Assuming the order shown in the figure, two of the converted tetrads had the parental configuration relative to the outside marker (top of Fig. 1) and the remaining five had a crossover between the b site and the *min1* marker (middle of Fig. 1). The five tetrads that contained a conversion and a crossover can be used to suggest the order of the a and b sites in relationship to the *min1* marker. The patterns of tetrads derived from crosses heteroallelic for three or four mutant sites within the *arg4* locus have revealed that the majority of crossovers associated with conversion are in an adjacent region and very close to the site of the conversion (28, 29). If this relationship of crossing over is also true for conversion

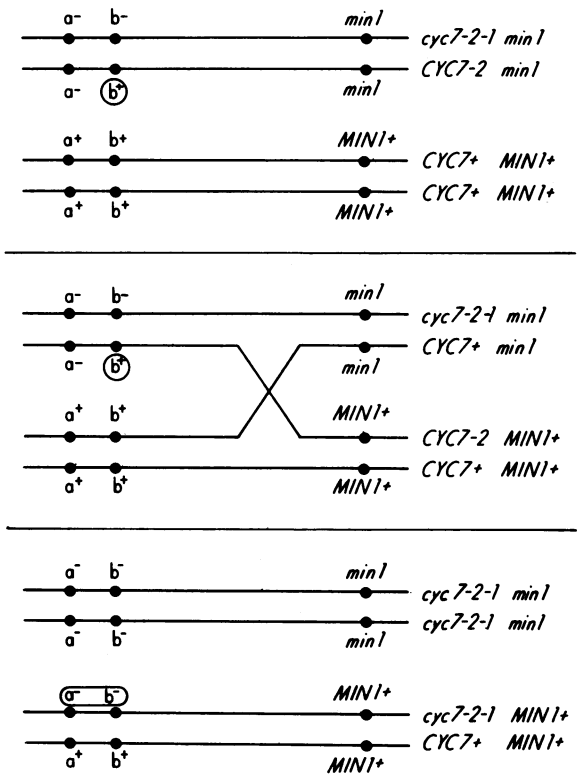


FIG. 1. Tetrads from *CYC7+ MIN1+* × *cyc7-2-1 min1* crosses that contain *CYC7-2* recombinants and convertants. Of 160 tetrads analyzed from various crosses, 2 had the genotypic pattern shown in the upper right of the figure, 5 had the pattern shown in the middle right, and 1 had the pattern shown in the lower right. The single-site conversion is denoted by the circled b^+, and the double-site coconversion is denoted by the circled a^-b^-. Assuming that crossovers associated with conversion occur immediately adjacent to the site of the conversion, one can deduce the order of the two mutational sites, a^- in the regulatory region and b^- in the structural region of the *CYC7* locus shown in the figure.

in the *CYC7* locus, then the order is a—b—*min1*, as shown in Fig. 1. If the order were b—a—*min1*, the *CYC7+* alleles would have been expected to remain in a parental configuration with the *min1* marker. However, this order cannot be considered definitive because the extensive data from *arg4* crosses indicate that approximately 10–20% of crossovers associated with conversion occur in regions not immediately adjacent to the site of the conversion (29).

In a total of 160 tetrads from *CYC7+* × *cyc7-2-1* crosses and 12 tetrads from a *CYC7+* × *cyc7-2-2* cross, the 7 tetrads containing the $b^- \rightarrow b^+$ conversion were the only ones having recombinants of the a and b sites. Reciprocal recombinants, $b^+ \rightarrow b^-$ convertants, and $a^+ \rightarrow a^-$ convertants were not observed. However, $a^- \rightarrow a^+$ convertants would remain undetected because the a^+b^- recombinants would be phenotypically identical to the a^-b^- (or *cyc7-2-1*) strains and the resulting tetrad would still exhibit a 2:2 segregation that is observed for the vast majority of the tetrads. Whereas the $b^- \rightarrow b^+$ conversion occurred at the relatively high frequency of approximately 4%, there was only one occurrence of an $a^+b^+ \rightarrow a^-b^-$ coconversion and none of an $a^-b^- \rightarrow a^+b^+$ coconversion (Fig. 1).

DISCUSSION

In this investigation we have confirmed that the *CYC7* locus determines the primary structure of iso-2-cytochrome *c*. Similar to the series of *CYC7* mutants previously described (3), the

following series of *CYC7* mutants were prepared from *cyc1* strains by using lactate medium to select for mutants having increased amounts of iso-2-cytochrome *c* and the benzidine staining procedure to select for mutants deficient in iso-2-cytochrome *c* (Table 1): *CYC7+* → *CYC7-2* → *cyc7-2-1* → *CYC7-2-1-A*. The lack or low frequencies of recombinants from various heterozygous and heteroallelic crosses established that the sites of all of the mutations were at the same locus. Peptide mapping, amino acid compositional analysis, and partial sequencing of an altered peptide demonstrated that iso-2-cytochrome *c* from the *CYC7-2-1-A* strain is altered, thus confirming our previous conclusion that *CYC7* is the structural gene for iso-2-cytochrome *c* (3, 5). In contrast to the series of mutants generated in strains with the *CYC7-1* translocation, the present series of *CYC7* mutants were in strains having the normal chromosomal constitution, making it feasible to show that the *CYC7* gene is located on the left arm of chromosome V (Table 2). Thus, *CYC7* is distinctly remote from *CYC1*, which determines the primary structure of iso-1-cytochrome *c* (1) and which is located on the right arm of chromosome X (2). Because the *CYP3* gene, suggested by Verdière and Petrochilo (30) to be the structural gene for iso-2-cytochrome *c*, was reported to be on chromosome X, it does not appear to be identical to the *CYC7* gene.

Because iso-2-cytochrome *c* from the *CYC7-2* mutant appeared to be completely normal, the mutation that increased the level of iso-2-cytochrome *c* most likely occurred in a region of the *CYC7* gene that is not translated but that is probably involved in controlling the amount of the translated product. An investigation of the levels of iso-2-cytochrome *c* in the heterozygrous diploids *CYC7+/CYC7-2* and *CYC7+/cyc7-2-1*, which will be described elsewhere, indicated that the alteration in the *CYC7-2* mutant is *cis* dominant and *trans* recessive, a result that would be expected for a regulatory mutant. This result with *CYC7-2* and similar results with heterozygous *CYC7-1* mutants suggest that certain alterations in a controlling region that is contiguous with a structural gene can lead to overproduction of iso-2-cytochrome *c*. An approximately 20-fold increase was observed for the *CYC7-2* mutant which appears to contain a simple point-mutation, whereas an approximately 30-fold increase was observed for the *CYC7-1* mutant which appears to have acquired an abnormal segment due to the chromosomal translocation. However, these mutational changes did not lead to any obvious impairment of normal regulatory function because *CYC7-1* and *CYC7-2* strains, just as *CYC7+* strains, contained lower levels of iso-2-cytochrome *c* after growth in anaerobic conditions or glucose-repressed conditions.

The mutant site in the structural region had a high tendency to undergo gene conversion that was usually associated with reciprocal recombination of the flanking marker *min1*. Assuming that conversions and their associate crossovers occur immediately adjacent to each other, we have oriented the regulatory and structural regions of the *CYC7* gene in relationship to other markers on the chromosome as follows: centromere–*ura3*–regulatory region–structural region–*min1*–*mak10*–*can1*. This order of having the regulatory region proximal to the structural region is consistent with the finding that the *CYC7-1* translocation arose from a breakpoint adjacent to the proximal end of the structural gene and that this chromosomal fragment containing the structural gene was fused to a fragment of chromosome XVI (4). Thus, the sites of the *CYC7-2* mutation and the abnormal regulatory region in the *CYC7-1* translocation are both on the same side of the structural region of the *CYC7* locus.

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