A DELETION MAP OF *cyc1* MUTANTS AND ITS CORRESPONDENCE TO MUTATIONALLY ALTERED ISO-1-CYTOCHROMES *c* OF YEAST

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ABSTRACT

Mutants arising spontaneously from sporulated cultures of certain strains of yeast, *Saccharomyces cerevisiae*, contained deletions of the *CYC1* gene which controls the primary structure of iso-1-cytochrome *c*. At least 60 different kinds of deletions were uncovered among the 104 deletions examined and these ranged in length from those encompassing only two adjacent point mutants to those encompassing at least the entire *CYC1* gene. X-ray-induced recombination rates of crosses involving these deletions and *cyc1* point mutants resulted in the assignment of 211 point mutants to 47 mutational sites and made it possible to unambiguously order 40 of these 47 sites. Except for one mutant, *cyc1-15*, there was a strict colinear relationship between the deletion map and the positions of 13 sites that were previously determined by amino acid alterations in iso-1-cytochromes *c* from intragenic revertants.

RECENTLY we described 210 single-site mutants of the *CYC1* gene which determines the primary structure of iso-1-cytochrome *c* in *Saccharomyces cerevisiae* (SHERMAN *et al.* 1974). A consistent genetic map could not be constructed from X-ray-induced recombination rates of over 2000 allelic pairs of these *cyc1* mutants, even though this procedure is most often used for fine-structure mapping of yeast mutants. The difficulties in ordering mutational sites are understandable since the disparities between X-ray-induced recombination rates and physical distances were clearly revealed in a detailed investigation of four *cyc1* mutants (MOORE and SHERMAN 1974, 1975), although approximate relationships may exist for at least some combinations of mutants (PARKER and SHERMAN 1969; SHERMAN *et al.* 1970). The ordering of mutational sites was no more satisfactory with rates of mitotic and meiotic recombination or with rates of recombination induced by various agents (MOORE and SHERMAN 1975). Even three-point crosses could not be used to definitively deduce the correct order of mutational sites (SHERMAN and JACKSON, unpublished experiments).

Thus, it appeared to us that the only genetic means for establishing the order of mutational sites within the gene involves the use of deletion mutants. Unlike mapping with two-point crosses, deletion mapping is not strongly dependent on nucleotide sequences or extrinsic factors influencing recombination rates. If crosses of a deletion and a point mutant yield recombination values that are less

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than or equal to the back mutation values, the point mutants are assumed to fall within the deletion; recombination values greater than the back mutation values indicate that the point mutant is outside the deletion even though the absolute values may not be accurate measures of physical distance.

However, forward mutants of various loci in yeast rarely contain deletions, and no extended deletions were uncovered among the 210 *cyc1* mutants that arose spontaneously or that were induced by a variety of mutagens (SHERMAN *et al.* 1974). The only previously-known deletions of the *CYC1* locus were the *cyc1-1* (PARKER and SHERMAN 1969; SHERMAN *et al.* 1974) and similar deletions (LIEBMAN, SINGH and SHERMAN, manuscript in preparation; SINGH and SHERMAN, manuscript in preparation) that encompass at least the entire locus, and small deletions at one end of the gene that arise in revertants of *cyc1* frameshift mutants and in certain other *cyc1* revertants having functional iso-1-cytochromes *c* (SHERMAN and STEWART 1973; STEWART and SHERMAN 1974). Neither deletions that cover the entire gene, nor small deletions that yield functional proteins can be directly used for mapping.

In this paper we describe strains that spontaneously give rise to various lengths of deletions of the *CYC1* gene. These strains were uncovered during a systematic search to obtain deletions from mutants having functional but altered iso-1-cytochromes *c*. Diploid strains were constructed with extensive dissimilarities of sequence in homologous regions of the *CYC1* gene. These strains were sporulated and mutants having deficiencies of iso-1-cytochrome *c* were selected from the meiotic progeny. Over one-fourth of the mutants from some crosses contained deletions of various lengths, from those covering only two adjacent sites to those covering at least the entire *CYC1* gene. Although the genetic control and the mechanism responsible for the production of these deletions have not yet been clearly established, deletions were obtained that could be used for mapping single-site mutants.

In this investigation 104 deletions were isolated, characterized and used to unambiguously order 40 of the 47 single-site mutants in the *CYC1* gene. Except for one site, the positions of the mutational sites determined by deletion mapping are in agreement with the positions of 15 sites previously determined by amino acid replacements in revertant forms of iso-1-cytochrome *c*.

MATERIALS AND METHODS

Nomenclature for mutants of the CYC1 *locus*: The symbol *cyc1* denotes mutants of the structural gene of iso-1-cytochrome *c*; the mutants may have deficiency in either the absolute amount or activity of the protein, resulting in the lack of growth on medium having lactate as the sole carbon source and increased growth on chlorolactate medium (see SHERMAN *et al.* 1974). The second number, or allele number, designates mutants that were derived by independent events. These mutants were derived either by direct mutation of normal strains (*cyc1-2* through *cyc1-211*), or by recombination of frameshift revertants (*cyc1-239*), or after meiosis by unknown mechanisms (*cyc1-363* through *cyc1-466*). The wild-type gene is designated *CYC1* while intragenic revertants having at least partially active iso-1-cytochrome *c* are designated by *CYC1* with the allele number and additional letters to distinguish independent origin. Thus *CYC1-183-B*, *CYC1-183-G* and *CYC1-183-AD* designate three intragenic revertants of the *cyc1-183* mutant. *CYC1* followed by a number and lower case letter designates recombinants with functional iso-1-

cytochromes *c* that were derived from two *cyc1* mutants. For example, *CYC1-340b* and *CYC1-340c* denote two independent recombinants from the heteroallelic cross *cyc1-239* × *cyc1-242*. In summary, upper-case letters, *CYC1*, designate strains having iso-1-cytochrome *c* that is either normal or altered and that is functional to the exent of supporting growth on lactate medium. In contradistinction, lower-case letters, *cyc1*, designate strains either lacking iso-1-cytochrome *c* or containing altered iso-1-cytochromes *c* that are non-functional to the extent of not supporting growth on lactate medium.

CYC1 *mutant alleles*: For production of deletions in this investigation, we initially used pairs of alleles that were expected to produce the greatest degree of mispairing (see RESULTS). These mutants were chosen from the collection of revertants having alterations in the dispensable region of iso-1-cytochrome *c*. We have reported the structures of 304 iso-1-cytochromes *c* that were obtained from revertants of 23 *cyc1* mutants which had lesions within the first 33 nucleotides of the gene (SHERMAN and STEWART 1973; STEWART and SHERMAN 1974). The widely varied structures pointed out the unessential nature of the region preceding glycine 11. Amino acid residues in this amino terminal region can be widely varied and can be even deleted without impairing the function of iso-1-cytochrome *c*, at least to the extent that the revertant strains grow normally on lactate medium.

Mainly revertants of *cyc1-183* and *cyc1-31* were chosen for this study. The *cyc1-183* mutant is a frameshift mutant which has an insertion of an A·T base pair at the codon of lysine 10 and which reverts primarily by deletions of any one of the single base pairs in the dispensable region at the end of the gene and more rarely by multiple base-pair deletions and insertions and certain complex changes. While the exact nucleotide change in the *cyc1-31* mutant has not been established, it appears to contain a frameshift mutation that generates a nearby nonsense codon. Most reverse mutations of *cyc1-31* occur by short deletions that apparently correct the reading frame and exclude the nonsense codon. The structures of iso-1-cytochromes *c* from the three revertants, *CYC1-31-K*, *CYC1-183-AD* and *CYC1-183-B* that were used for most of the crosses are listed in Table 1. The *CYC1-31-K* revertant has a deletion of residues 2 through 7. The *CYC1-183-AD*

TABLE 1

Amino terminal sequences of iso-1-cytochromes c *from normal* (CYC1) *and revertant strains*

Genotype	Sequences
	−1 1 2 3 4 5 6 7 8 9 10 11 12 13 14
CYC1	(Met)Thr-Glu-Phe-Lys-Ala-Gly-Ser-Ala-Lys-Lys-Gly-Ala-Thr-Leu-
	AUG ACU GAA UUC AAG GCC GGU UCU GCU AAG AAA GGU GCU ACA CUN
CYC1-31-K	(Met)Thr——————————Ala-Lys-Lys-Gly-Ala-Thr-Leu-
CYC1-183-AD	(Met)Thr-Glu-Phe-Lys-Ala-Gly-Ser-——————————Thr-Leu-
CYC1-183-B	(Met)Thr-Glu-Phe-Lys-Ala-Gly-Ser-Ala-Lys-Lys-*Met-Leu-Arg-Lys*-Gly-Ala-Thr-Leu-
CYC1-183-G	(Met)Thr-Glu-Phe-Lys-Ala-Gly-Ser-*Val-Arg*-Lys-Gly-Ala-Thr-Leu-
CYC1-179-H	(Met)Thr-Glu-Phe-Lys-Ala-Gly-Ser-Ala-*Tyr*-Lys-Gly-Ala-Thr-Leu-
CYC1-340b	(Met)Thr-Glu-Phe-$\frac{\text{Lys}}{Met}$-Ala-Gly-Ser-Ala-Lys-Lys-Gly-Ala-Thr-Leu-
CYC1-340c	(Met)Thr-Glu-Phe-$\frac{\text{Lys}}{Met}$-Ala-Gly-Ser-Ala-Lys-Lys-Gly-Ala-Thr-Leu-

The normal mRNA sequence is from STEWART and SHERMAN (1974). The amino acid sequences of the *CYC1-31-K*, *CYC1-183-AD*, *CYC1-183-B*, *CYC1-183-G* are from SHERMAN and STEWART (1973) and STEWART and SHERMAN (1974); the *CYC1-179-H* is from STEWART and SHERMAN (1972). The *CYC1-340b* and *CYC1-340c* alleles were derived by recombination and they have been inferred to have sequences either that are normal or that contain methionine at position 4. Amino acid replacements and insertions are indicated in *italics*.

revertant has a deletion of residues 8 through 12, causing glycine 6 to be aligned at the invariant position 11. The *CYC1-183-B* revertant, containing an insertion of four amino acid residues, occurred by a tandem duplication of 11 base pairs. Strains *CYC1-340b* and *CYC1-340c*, having normal or near normal sequences of iso-1-cytochrome *c* (Table 1), are closely related to strains that were shown in other studies to produce high proportions of *cyc1* mutants among chlorolactate-resistant mutants, and these two strains were used when normal sequences were desired.

Media: General types of media and media used specifically with the iso-1-cytochrome *c* system have been described in detail by SHERMAN *et al.* (1974). Chlorolactate medium (1% Bacto-yeast extract, 2% Bacto-peptone, 3% [v/v] glycerol, 1% Ionagar [Wilson Diagnostic, Inc.], and 0.05% β-chlorolactic acid) is used to enrich for *cyc1* mutants or *cyc1* recombinants. Lactate medium (0.67% Bacto-yeast nitrogen base "without amino acids", 0.05% Difco-yeast extract, 1.5% Ionagar and 1% DL-lactate) is used to select for *CYC1* revertants or *CYC1* recombinants.

General genetic methods: Conventional yeast-genetic procedures of crossing, sporulation and tetrad analysis were used to construct strains with desired markers (see SHERMAN and LAWRENCE 1974). Segregations of *cyc1* genes were scored by the levels of cytochrome *c*, using low-temperature (−190°) spectroscopic examination of intact cells (SHERMAN and SLONIMSKI 1964).

Isolation of cyc1 *mutants*: Diploid strains containing the appropriate *CYC1* alleles and homozygous for the *arg4* marker (see RESULTS) were sporulated by first growing on presporulation medium for 1 or 2 days and then on sporulation medium for 3 to 5 days (see SHERMAN and LAWRENCE 1974). The sporulation was carried out either in liquid media or on solid media. The degree of sporulation of the strains used in this study was unusually low, and in most cases ranged from 1% to 10% asci. The sporulated cultures were suspended in sterile water and approximately 10^7 cells were spread on each chlorolactate plate. The plates were incubated for 5 to 7 days at 30°, and the chlorolactate-resistant colonies were crossed to an **a** *cyc1 lys2 his1 trp2* tester strain, and those that mated, i.e., the α haploids, were tested further. The presence of *cyc1* mutations in the chlorolactate-resistant mutants were determined by testing suspensions of the diploid crosses on lactate medium and chlorolactate medium as described by SHERMAN *et al.* (1974). A chlorolactate-resistant mutant was suspected to be a *cyc1* mutant when the diploid grew less on lactate medium and more on chlorolactate medium in comparison to a normal strain. The original haploid mutants derived from the chlorolactate plates were purified by subcloning and one subclone from each strain was crossed to a *cyc1* tester strain, and the diploid was retested for growth on lactate and chlorolactate media. In addition, the cytochrome *c* content of the presumptive *cyc1* haploids were determined by low-temperature spectroscopy.

Identification and characterization of the deletions: The *cyc1* mutants were tested for deletions first by crossing each of the mutants to either 15 or 32 *cyc1* point mutants which are described in the RESULTS, and then by determining the X-ray-induced mitotic recombination frequencies of the heteroallelic diploids. The preparation and transfer of cell suspensions for crossing, testing, etc. was facilitated by the use of inoculating rods previously described by SHERMAN *et al.* (1974). Cell suspensions were spotted on several lactate plates (see SHERMAN *et al.* 1974) and the surfaces of one or two sets of plates were irradiated with 5 kilorad (KR), using an OEG-60-T Machlett X-ray tube powered by a custom-made X-ray generator (Picker Corp.). The unit was operated at 50 KVP and 25 ma, with only inherent filtration and delivered 28 KR per min to the plates. The lactate plates were scored after five days of incubation at 30°. Recombination was indicated when increased numbers of colonies appeared over the spotted inocula that were irradiated in comparison to the unirradiated controls. Lack of colonies indicated that recombination did not occur or occurred at a low rate.

A more quantitative measure of recombination rates was undertaken with certain of the heteroallelic diploids that appeared to give no recombinants, as well as with the heteroallelic diploids that appeared to establish the ends of the suspected deletion. Cell suspensions were conveniently prepared free of growth medium and at a known titre by inoculating the surfaces of 0.5 ml of medium (0.4% Bacto-yeast extract, 0.8% Bacto-peptone, 0.8% glucose and 1% Ionagar) in one-dram vials and after three days of incubation, suspending the cells in 2 ml of sterile water (SHERMAN *et al.* 1974). Lactate plates were each spread with 0.2 ml of the suspensions, which contained approximately 1.2×10^7 cells, and two sets of plates were irradiated with 5 KR. The irradiated and unirradiated plates were scored after incubation as indicated above.

RESULTS

Strains giving rise to deletions: It was believed that extended deletions might arise in the meiotic progeny of heteroallelic crosses having grossly dissimilar sequences in homologous regions. While there was no precedence for the belief that regions with extensive mispairing may be deleted during meiosis, it was reasonable to speculate that spontaneous deletions arise as errors in recombination and that the frequencies may be enhanced in regions with abnormal pairing conditions. Selective techniques and certain mutants of the *CYC1* locus were available to examine this possibility. Briefly, the plan was to construct diploid crosses, each containing two dissimilar iso-1-cytochromes *c* that were functional. The diploid strains were sporulated and *cyc1* haploid mutants that lacked iso-1-cytochrome *c* were selected on chlorolactate medium. The resulting *cyc1* mutants were crossed to either 15 or 32 tester strains containing *cyc1* point mutants that will be described below. The crosses were X-irradiated, and plated on lactate medium, and the resulting recombination frequencies were estimated. A *cyc1* mutant was assumed to contain a deletion when recombination did not occur with at least two separate point mutants. A complete description of the procedure for assigning deletions is presented in the next section.

Since almost all of the *cyc1* point-mutants to be used in conjunction with the deletions were derived from the strain D311-3A (**a** *lys2 his1 trp2*) (SHERMAN *et al.* 1974), it was desirable to have the set of deletions with the mating type *α* and with markers complementary to *lys2 his1 trp2*, thus facilitating the selection of hybrid crosses. Therefore, the appropriate *CYC1* revertant alleles were coupled with the *arg4* marker, taking care to use strains that were previously shown to be capable of producing chlorolactate-resistant mutants with high proportions of *cyc1* mutations.

The first genetic analysis was performed on the meiotic progeny from the two strains D-746 and SL-382 (Table 2), each containing the heteroallelic pair

TABLE 2

The number of cyc1 *deletions and other mutants derived from each of the diploid strains*

Diploid crosses			Number of mutants		
Strain no.	Haploid strain no.	*CYC1* genotypes	Chlorolactate resistant	*α cyc1* total	*α cyc1* deletions
D-746	D179-4B × D743-15A	*CYC1-31-K* × *CYC1-183-AD*	6038	186	51
SL-382	D719-1B × D743-8C	*CYC1-31-K* × *CYC1-183-AD*	404	38	4
D-756	D719-4B × B-3181	*CYC1-31-K* × *CYC1-340b*	1300	89	32
D-755	D719-4B × B-3175	*CYC1-31-K* × *CYC1-340c*	149	6	1
D-757	B-1557 × D743-1B	*CYC1-183-B* × *CYC1-183-AD*	588	6	1
D-791	E761-6A × D743-1B	*CYC1-183-B* × *CYC1-183-AD*	90	3	1
D-792	B-3846 × D743-1B	*CYC1-183-B* × *CYC1-183-AD*	99	14	6
D-793	E762-4C × D743-1B	*CYC1-183-B* × *CYC1-183-AD*	275	1	1
D-753	SL216-3B × B-1597	*CYC1-183-G* × *CYC1-179-H*	402	2	2
D-754	SL216-3B × B-3175	*CYC1-183-G* × *CYC1-340c*	288	82	1
SL-383	D719-4B × D719-1B	*CYC1-31-K* × *CYC1-31-K*	439	27	4
			10567	454	104

CYC1-31-K/CYC1-183-AD. These two alleles determine functional iso-1-cytochromes *c* with two dissimilar deletions shown in Table 1 (also see MATERIALS AND METHODS). Approximately 27% and 11% of the *cyc1* mutants from the D-746 and SL-382 strains, respectively, were shown to contain deletions. Additional crosses containing other types of abnormal iso-1-cytochromes *c* were then examined. These included combinations of the *CYC1-31-K* deletion with normal or near-normal alleles *CYC1-340c* and *CYC1-340b* (D-755 and D-756) and combinations of the *CYC1-183-AD* deletion with the *CYC1-183-B* insertion (D-757, D-791, D-792 and D-793). These crosses also gave rise to *cyc1* deletions, and the proportions of deletions among the *cyc1* mutants appear comparable to the proportions observed with the first crosses D-746 and SL-382 (see Table 2).

Although this first successful procedure for producing *cyc1* deletions appeared to be predicted from our assumptions concerning meiotic mispairing, deletions were also obtained from crosses of alleles whose combination would not be expected to produce extensive mispairing. In an unrelated study, the cross D-753 was observed to give rise to a *cyc1* mutant that proved to be a deletion. The two alleles in the cross, *CYC1-183-G* and *CYC1-179-H*, contain substitutions in a small region of the gene (see Table 1), and this combination would not be expected to produce extensive mispairing. Only two *cyc1* mutants were found after examining a total of 402 chlorolactate-resistant mutants from D-753, but both mutants contained deletions. Thus it appears as if a high proportion of the *cyc1* mutants from the strain D-753 contained deletions, although there was a low proportion of total *cyc1* mutants among the chlorolactate-resistant colonies. However, another cross, D-754 (*CYC1-183-G* × *CYC1-340c*), that also would not be expected to produce extensive mispairing (see Table 1), gave rise to only one deletion among the 82 *cyc1* mutants examined. In contrast, the SL-383 cross, in which mispairing should be absent since it is homoallelic for *CYC1-31-K*, yielded deletions at frequencies observed for heteroallelic crosses (Table 2). While these results appear to indicate that mispairing of heteroallelic genes is not a necessary condition for the production of deletions, the nature of the deletions obtained from the homoallelic strain SL-383 suggests that they may have arisen by a different mechanism than the deletions obtained from the heteroallelic strains (see DISCUSSION). Whatever mechanisms may be involved, sporulated cultures of 11 different strains gave rise to 104 deletions that could be used for mapping.

Tests for deletions: All of the 454 α *cyc1* mutants listed in Table 2 were tested for the presence of deletions by crossing each mutant to a set of either 15 or 32 tester strains whose sites and allele numbers are indicated, respectively, in the first and third columns of Table 3. These tester strains contain single-site mutations distributed throughout the gene. The positions of the mutational sites in some of the tester strains have been inferred from the alterations of revertant iso-1-cytochromes *c* that are listed in Table 4. The positions of the mutational sites in the other tester strains, as well as the positions of all 47 mutational sites that were used to establish and characterize deletions, are discussed in detail below. Suspensions of the heteroallelic crosses of the deletions with the point

TABLE 3

Assignments of the cyc1-2 *through* cyc1-211 *mutants to the 46 mutational sites*

Site*	Residue position†	Allele number and inducing mutagen‡
1	−1	*74*UV 13NIL 51UV 85UV 100SPO 131UV 133UV 163SPO 181UV
2	2	91UV 9UV 147UV 160SPO 172UV
3	3	31UV
4	9	135UV 138UV 179UV
5	10	134UV 183UV 49UV
6	12	*6*NA *4*NA *5*NA *22*UV **25**UV *30*UV *37*UV *46*UV *54*SPO *55*SPO *66*UV *112*UV *114*UV *116*SPO **164**SPO
7	13	120UV 10UV 29UV 65UV *88*UV *115*UV 139UV 142UV 143UV *152*SPO 178UV
8	(16)	210ICR *75*UV
9	(18)	36UV 14NIL 38UV 86UV *87*UV 128UV 130UV 193ICR
10	21	17SPO 2NA 69SPO 93UV 159SPO 202ICR
11	(23)	19UV 7UV 12UV 20UV 23UV 34NIL 53UV 81SPO 89UV 99SPO 101SPO 165SPO 194ICR 207ICR
12	(26)	156SPO
13	(27)	200ICR 126UV 145UV 196ICR
14	(28)	137UV
15	(29)	39ICR 15ICR 16ICR 191ICR 195ICR 201ICR 203ICR 204ICR 208ICR 209ICR
16	(30)	67UV
17	(31)	48UV
18	(32)	*177*UV 118UV
19	(32.5)	205ICR
20	(33)	*73*UV **155**SPO
21	(34)	*8*UV *3*NA *27*NIL *56*SPO *174*UV **188**UV
22	(35)	32UV 192ICR 198ICR
23	(35.5)	**21**UV **50**UV
24	(36)	**59**UV **61**UV **64**SPO **103**UV **108**UV **113**UV **186**UV
25	(36.5)	119UV
26	(37.5)	33UV *63*UV
27	(38)	**190**ICR
28	(41)	40ICR 41ICR

TABLE 3 (continued)

Site*	Residue position†	Allele number and inducing mutagen‡
29	(45)	*95*UV
30	(49)	*77*UV *90*UV 175UV
31	(53)	117UV
32	(59)	44UV 96SPO?
33	(61)	71SPO 158SPO
34	64	**166**SPO 28NIL 84SPO **132**UV 136UV 149UV 151SPO 153SPO 154SPO 182UV **189**ICR
35	66	72UV
36	71	76UV 47UV 70SPO 79SPO 82SPO 83SPO 97SPO 98SPO 125UV 146UV 150SPO 161SPO 162SPO 173UV 197ICR 199ICR
37	(73)	35UV **57**UV **60**UV
38	(76)	211ICR 11UV **24**UV **58**UV **104**UV **110**UV **169**UV **185**UV
39	(79)	94UV 18SPO 127UV 144UV
40	(83)	176UV 26UV *42*ICR 121UV 141UV 206ICR
41	(87)	**187**UV
42	(92)	80SPO 78SPO 122UV 157SPO
43	93	140UV
44	(95)	*92*UV
45	(98)	**107**UV 43ICR **52**UV **102**UV **106**UV **109**UV 129UV 148UV *168*UV 180UV
46	(102)	45UV **62**UV 68SPO **105**UV **111**UV 123UV 124UV **167**UV **170**UV **171**UV **184**UV

* The first column, numbers 1 through 46, correspond to the 46 mutational sites in the deletion map, starting from the initiation mutants *cyc1-74* etc., but excluding the *cyc1-239* site. Mutants at the 15 sites which are indicated with **boldface** type were used for initial tests of deletions. In later experiments deletions were initially tested with these mutants as well as the mutants at the 17 sites indicated in *italics*.

† The numbers refer to the amino acid positions of the corresponding mutational sites that were determined from altered iso-1-cytochromes *c* (Table 3) except for the mutants *cyc1-8* (site 21) and *cyc1-15* (site 15). Estimates of positions are presented in parentheses. Sites having ambiguous orders are grouped with brackets.

‡ The *cyc1* allele numbers, 2 through 211, are followed by abbreviations of the mutagens used to induce them (UV, ultraviolet light; NIL, 1-nitrosoimidazolidone-2; NA, nitrous acid; ICR, 2-methoxy-6-chloro-9-[3-N-ethyl-N-1-chloroethyl)-aminopropyl]-aminoacridine dihydrochloride; SPO, none). Mutants that are believed to completely or almost completely lack iso-1-cytochrome *c* appear in Roman numbers; those having a detectable increase to almost one-half the normal level appear in *italic* numbers; those having approximately one-half to almost the normal level appear in **boldface**. Ochre and amber mutants are designated by lines and dots, respectively, under the allele numbers. The first mutant listed at each mutational site was used as the primary tester strain for the deletion map.

TABLE 4

Correspondence of the tester alleles to amino acid positions in iso-1-cytochromes c

Tester allele	Identification from altered iso-1-cytochromes *c* in revertants				
	Residue position	Normal residue	Reverted alleles	*cyc1* lesions	References
*cyc*1-74	−1	Met	*cyc*1-13 etc.	initiator	STEWART *et al.* 1971
*cyc*1-91	2	Glu	*cyc*1-9 etc.	ochre	STEWART *et al.* 1972; SHERMAN and STEWART 1973
*cyc*1-31	3	Phe	*cyc*1-31	frameshift?	SHERMAN and STEWART 1973; STEWART and SHERMAN 1974
*cyc*1-239	4	Lys	*cyc*1-239	frameshift	STEWART and SHERMAN 1974
*cyc*1-135	9	Lys	*cyc*1-179 etc.	amber	STEWART and SHERMAN 1972; SHERMAN and STEWART 1973
*cyc*1-134	10	Lys	*cyc*1-183 etc.	frameshift	SHERMAN and STEWART 1973; STEWART and SHERMAN 1974
*cyc*1-6	12	Ala	*cyc*1-6	missense	PUTTERMAN, MARGOLIASH and SHERMAN 1974
*cyc*1-120	13	Thr	*cyc*1-10	missense?	PUTTERMAN, MARGOLIASH and SHERMAN 1974
*cyc*1-39	17	Thr	*cyc*1-15	?	PUTTERMAN, MARGOLIASH and SHERMAN 1974
*cyc*1-17	21	Gln	*cyc*1-2	ochre	STEWART *et al.* unpublished; see SHERMAN and STEWART 1974
*cyc*1-8	31	His	*cyc*1-8	?	SHERMAN *et al.* 1970
*cyc*1-166	64	Trp	*cyc*1-166 etc.	missense etc.	SCHWEINGRUBER, STEWART and SHERMAN 1974; SCHWEINGRUBER *et al.* unpublished
*cyc*1-72	66	Glu	*cyc*1-72	ochre	STEWART *et al.* unpublished; see SHERMAN and STEWART 1974
*cyc*1-76	71	Glu	*cyc*1-76	amber	STEWART and SHERMAN 1973
*cyc*1-140	93	Glu	*cyc*1-140	ochre	STEWART *et al.* unpublished

mutants were spotted on lactate plates and X-irradiated. The frequencies of the resulting induced recombination were used in attempts to estimate the position and length of the *cyc1* lesions. If the *cyc1* mutant did not recombine with at least two tester strains, more precise X-ray-induced recombination frequencies were determined with two or more tester mutants whose lesions appeared to be included within the presumptive deletion.

The deletions which failed to recombine with two or more point mutants were used to establish the order of the sites in some of the original tester strains, as well as the order of sites in additional tester strains that are described below. In turn, the knowledge of the order of sites could be used for more critical tests of the deletions. If a *cyc1* mutant recombined with all but one *cyc1* tester in the original set, further tests of recombination with the potential deletion were carried out with this tester and with testers having adjacent mutational lesions. Ultimately point mutants at 47 sites were used in crosses with the deletions. The *cyc1* mutants that recombined with all but one of the 47 testers were not characterized further; these *cyc1* mutants contain either single-site lesions, or short deletions that would be of little value for mapping. Also of little value were the mutants that recombined with all tester strains, since they too were believed to contain single-site lesions or short deletions that did not encompass any of the 47 tester sites. Thus, all of the mutants were classified into two categories: those having deletions that included at least two sites; and those having single-site lesions or short deletions that included only one or none of the previously known sites.

Cytochrome c *content of deletion mutants*: The levels of total cytochrome *c* in all 104 deletion mutants were estimated by low-temperature spectroscopic examination of intact cells. All but two of the deletion mutants appeared to contain an amount of cytochrome *c* that is characteristic of mutants completely lacking iso-1-cytochrome *c* but containing the normal low amount of iso-2-cytochrome *c*. In contrast, the intensities of the c_α-bands in the two mutants, *cyc1-449* and *cyc1-453*, were judged to correspond, respectively, to approximately 10% and 20% of the normal amount of total cytochrome *c*. Since deletions were expected to cause total deficiencies, an appropriate genetic analysis was undertaken to test whether the levels of cytochrome *c* in the two exceptional mutants were controlled by these *cyc1* alleles or by unrelated genes that may have increased the amount of iso-2-cytochrome *c*. The *cyc1-449* and *cyc1-453* mutants were each crossed to a normal strain and, more importantly, to a *cyc1-1* mutant that contains a deletion of the entire *CYC1* locus and of adjacent genes (SINGH and SHERMAN, manuscript in preparation). Examination of the cytochrome *c* content of the meiotic progeny from these crosses strongly suggested that the two deletion mutants contain a low amount of nonfunctional iso-1-cytochrome *c*. A 2:2 segregation of the parental concentrations of cytochrome *c* was observed in tetrad analysis of the crosses. However, the cytochrome *c* level in some of the *cyc1-449* segregants could not be easily distinguished from *cyc1* mutants that completely lack iso-1-cytochrome *c*. This type of result is not uncommon in pedigrees of mutants almost completely deficient in iso-1-cytochrome *c*, since different segregants containing the same *cyc1* allele have been shown to have

slightly different levels of cytochrome *c* (see SHERMAN *et al.* 1974). Nevertheless, it is concluded that the slight increase above the iso-2-cytochrome *c* level is determined by the *cyc1-449* and *cyc1-453* alleles since this increase was not observed with the *cyc1-1* segregants from these crosses, but was always observed with the *cyc1-453* segregants and usually observed with the *cyc1-449* segregants. The genetic analysis has not excluded the unlikely possibility that a mutant gene closely linked to the *CYC1* locus causes an increase of the level of iso-2-cytochrome *c*.

Characterization of the deletions and the ordering of point mutants: In a previous study, over 35 different mutational sites were revealed from the frequencies of X-ray-induced recombination of various pairwise crosses involving 210 point mutants (SHERMAN *et al.* 1974). One or more mutants at each of these sites were used in the initial attempts to characterize each of the 104 deletions. Almost all crosses of a deletion with a point mutant yielded X-ray-induced values either that were low, characteristic of X-ray-induced reversion rates, or that were obviously high, characteristic of X-ray-induced recombination rates. The values derived with two typical deletions, *cyc1-424* and *cyc1-438*, that were each crossed to various point mutants, are presented in Figure 1, where the unambiguity is clearly illustrated.

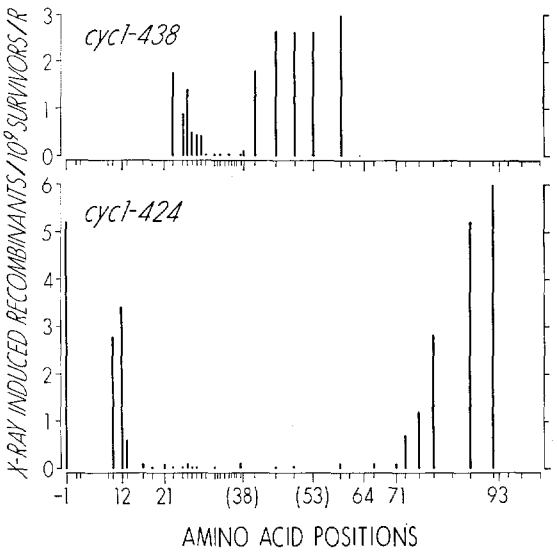


FIGURE 1.—X-ray-induced rates of recombination of crosses of each of the deletion mutants, *cyc1-424* and *cyc1-438*, to various point mutants that correspond to the amino acid position shown at the bottom of the figure. These typical results indicate that the deletions *cyc1-424* and *cyc1-438* encompass mutational sites at, respectively, positions 16 through 71 and positions 31 through 38 (see Table 5).

Since the deletions were overlapping, the order of the point mutant could be inferred by the analysis classically used for deletion mapping. The order deduced from the first deletion-mapping experiments could then be used to re-

assess the assignments of the 210 *cyc1* mutants to the various mutational sites that were previously deduced from the two-point crosses. While the assignments of the vast majority of the mutants into groups at each of the sites were consistent with both the two-point crosses and the deletion map, there were several changes and improvements of the earlier findings. A correction of our previously published results (SHERMAN *et al.* 1974) is that the mutant *cyc1-155* mapped at the *cyc1-73* site instead of at a separate site. Deletion mapping indicated that both of these mutations were located within the same segment and the lack of recombination of the two-point cross, *cyc1-73* × *cyc1-155*, established the identity of their mutational sites.

An increased resolving power was attained with deletion mapping since crosses with certain deletions proved to be a more sensitive means of grouping some of the clusters of mutants. For example, by two-point crosses, 18 mutants were previously assigned to the site of *cyc1-8* and three mutants to the neighboring site *cyc1-32*. Crosses of these 21 point mutants and the *cyc1-155* mutant to deletions having endpoints within the cluster led to grouping of the point mutants into seven close sites. Similarly the cluster of mutants previously assigned to the *cyc1-15* site were resolved into three sites by deletion mapping. These and additional subdivisions led to grouping the 210 point mutants into 46 mutational sites that are listed in Table 3. The mutational sites included in each of the 104 deletions are presented in Table 5. The genetic mapping indi-

TABLE 5

The 104 cyc1 *deletions*

No.	Type	Allele no.	Parental diploid	Inclusive length	
				Tester alleles	Amino acid positions*
1	1	*cyc1-363*	D-746	*cyc1-74* → *cyc1-45*	−1 → (102)
2	1	*cyc1-364*	D-746	*cyc1-74* → *cyc1-45*	−1 → (102)
3	1	*cyc1-365*	D-746	*cyc1-74* → *cyc1-45*	−1 → (102)
4	1	*cyc1-366*	D-746	*cyc1-74* → *cyc1-45*	−1 → (102)
5	1	*cyc1-367*	SL-383	*cyc1-74* → *cyc1-45*	−1 → (102)
6	1	*cyc1-368*	SL-383	*cyc1-74* → *cyc1-45*	−1 → (102)
7	2	*cyc1-369*	D-746	*cyc1-74* → *cyc1-94*	−1 → (79)
8	3	*cyc1-370*	D-746	*cyc1-74* → *cyc1-72*	−1 → 66
9	4	*cyc1-371*	D-746	*cyc1-74* → *cyc1-71*	−1 → (61)
10	5	*cyc1-372*	D-746	*cyc1-74* → *cyc1-44*	−1 → (59)
11	5	*cyc1-373*	D-746	*cyc1-74* → *cyc1-44*	−1 → (59)
12	6	*cyc1-374*	SL-382	*cyc1-74* → *cyc1-190*	−1 → (38)
13	7	*cyc1-375*	D-746	*cyc1-74* → *cyc1-119*	−1 → (36.5)
14	7	*cyc1-376*	D-746	*cyc1-74* → *cyc1-119*	−1 → (36.5)
15	8	*cyc1-377*	D-746	*cyc1-74* → *cyc1-177*	−1 → (32)
16	9	*cyc1-378*	D-756	*cyc1-74* → *cyc1-200*	−1 → (27)
17	10	*cyc1-379*	D-756	*cyc1-74* → *cyc1-19*	−1 → (23)
18	11	*cyc1-380*	D-753	*cyc1-74* → *cyc1-17*	−1 → 21
19	11	*cyc1-381*	D-756	*cyc1-74* → *cyc1-17*	−1 → 21
20	11	*cyc1-382*	D-792	*cyc1-74* → *cyc1-17*	−1 → 21
21	12	*cyc1-383*	D-746	*cyc1-74* → *cyc1-210*	−1 → (16)

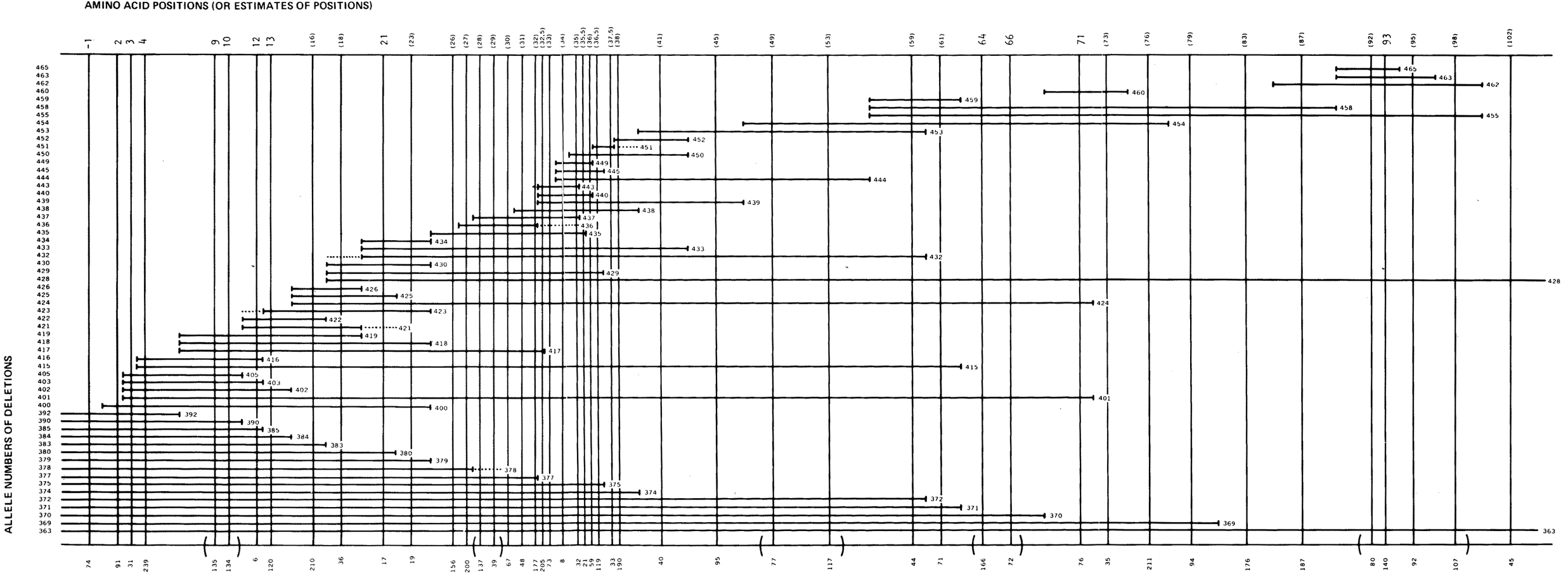
No.	Type	Allele no.	Parental diploid	Inclusive length	
				Tester alleles	Amino acid positions*
22	13	*cyc1-384*	D-756	*cyc1-74* → *cyc1-120*	−1 → 13
23	14	*cyc1-385*	D-754	*cyc1-74* → *cyc1-6*	−1 → 12
24	14	*cyc1-386*	D-746	*cyc1-74* → *cyc1-6*	−1 → 12
25	14	*cyc1-387*	D-756	*cyc1-74* → *cyc1-6*	−1 → 12
26	14	*cyc1-388*	D-746	*cyc1-74* → *cyc1-6*	−1 → 12
27	14	*cyc1-389*	D-756	*cyc1-74* → *cyc1-6*	−1 → 12
28	15	*cyc1-390*	D-756	*cyc1-74* → *cyc1-134*	−1 → 10
29	15	*cyc1-391*	D-756	*cyc1-74* → *cyc1-134*	−1 → 10
30	16	*cyc1-392*	D-746	*cyc1-74* → *cyc1-239*	−1 → 4
31	16	*cyc1-393*	D-756	*cyc1-74* → *cyc1-239*	−1 → 4
32	16	*cyc1-394*	D-756	*cyc1-74* → *cyc1-239*	−1 → 4
33	16	*cyc1-395*	D-756	*cyc1-74* → *cyc1-239*	−1 → 4
34	16	*cyc1-396*	D-756	*cyc1-74* → *cyc1-239*	−1 → 4
35	16	*cyc1-397*	D-756	*cyc1-74* → *cyc1-239*	−1 → 4
36	16	*cyc1-398*	D-746	*cyc1-74* → *cyc1-239*	−1 → 4
37	16	*cyc1-399*	SL-382	*cyc1-74* → *cyc1-239*	−1 → 4
38	17	*cyc1-400*	D-756	*cyc1-91* → *cyc1-19*	2 → (23)
39	18	*cyc1-401*	D-746	*cyc1-31* → *cyc1-76*	3 → 71
40	19	*cyc1-402*	D-746	*cyc1-31* → *cyc1-120*	3 → 13
41	20	*cyc1-403*	D-746	*cyc1-31* → *cyc1-6*	3 → 12
42	20	*cyc1-404*	D-746	*cyc1-31* → *cyc1-6*	3 → 12
43	21	*cyc1-405*	D-756	*cyc1-31* → *cyc1-134*	3 → 10
44	21	*cyc1-406*	D-756	*cyc1-31* → *cyc1-134*	3 → 10
45	21	*cyc1-407*	D-756	*cyc1-31* → *cyc1-134*	3 → 10
46	21	*cyc1-408*	D-756	*cyc1-31* → *cyc1-134*	3 → 10
47	21	*cyc1-409*	D-756	*cyc1-31* → *cyc1-134*	3 → 10
48	21	*cyc1-410*	D-756	*cyc1-31* → *cyc1-134*	3 → 10
49	21	*cyc1-411*	D-756	*cyc1-31* → *cyc1-134*	3 → 10
50	21	*cyc1-412*	D-756	*cyc1-31* → *cyc1-134*	3 → 10
51	21	*cyc1-413*	D-756	*cyc1-31* → *cyc1-134*	3 → 10
52	21	*cyc1-414*	D-756	*cyc1-31* → *cyc1-134*	3 → 10
53	22	*cyc1-415*	D-756	*cyc1-239* → *cyc1-71*	4 → (61)
54	23	*cyc1-416*	D-756	*cyc1-239* → *cyc1-6*	4 → 12
55	24	*cyc1-417*	D-757	*cyc1-135* → *cyc1-205*	9 → (32.5)
56	25	*cyc1-418*	D-756	*cyc1-135* → *cyc1-19*	9 → (23)
57	26	*cyc1-419*	D-746	*cyc1-135* → *cyc1-36*	9 → (18)
58	26	*cyc1-420*	D-792	*cyc1-135* → *cyc1-36*	9 → (18)
59	27	*cyc1-421*	D-793	*cyc1-6* → *cyc1-36*	12 → (18)
60	28	*cyc1-422*	D-792	*cyc1-6* → *cyc1-210*	12 → (16)
61	29	*cyc1-423*	D-792	*cyc1-120* → *cyc1-19*	13 → (23)
62	30	*cyc1-424*	D-753	*cyc1-210* → *cyc1-76*	(16) → 71
63	31	*cyc1-425*	D-746	*cyc1-210* → *cyc1-17*	(16) → 21
64	32	*cyc1-426*	D-791	*cyc1-210* → *cyc1-36*	(16) → (18)
65	32	*cyc1-427*	D-792	*cyc1-210* → *cyc1-36*	(16) → (18)
66	33	*cyc1-428*	D-746	*cyc1-36* → *cyc1-45*	(18) → (102)
67	34	*cyc1-429*	D-746	*cyc1-36* → *cyc1-119*	(18) → (36.5)
68	35	*cyc1-430*	D-746	*cyc1-36* → *cyc1-19*	(18) → (23)
69	35	*cyc1-431*	D-746	*cyc1-36* → *cyc1-19*	(18) → (23)
70	36	*cyc1-432*	D-756	*cyc1-17* → *cyc1-44*	21 → (59)
71	37	*cyc1-433*	D-756	*cyc1-17* → *cyc1-40*	21 → (41)
72	38	*cyc1-434*	D-746	*cyc1-17* → *cyc1-19*	21 → (23)

No.	Type	Allele no.	Parental diploid	Inclusive length	
				Tester alleles	Amino acid positions*
73	39	*cyc1-435*	D-746	*cyc1-156* → *cyc1-21*	(26) → (35.5)
74	40	*cyc1-436*	SL-383	*cyc1-200* → *cyc1-177*	(27) → (32)
75	41	*cyc1-437*	D-746	*cyc1-137* → *cyc1-32*	(28) → (35)
76	42	*cyc1-438*	D-746	*cyc1-48* → *cyc1-190*	(31) → (38)
77	43	*cyc1-439*	SL-382	*cyc1-205* → *cyc1-95*	(32.5) → (45)
78	44	*cyc1-440*	D-746	*cyc1-205* → *cyc1-59*	(32.5) → (36)
79	44	*cyc1-441*	D-746	*cyc1-205* → *cyc1-59*	(32.5) → (36)
80	44	*cyc1-442*	D-746	*cyc1-205* → *cyc1-59*	(32.5) → (36)
81	45	*cyc1-443*	D-746	*cyc1-205* → *cyc1-32*	(32.5) → (35)
82	46	*cyc1-444*	D-746	*cyc1-8* → *cyc1-117*	(34) → (53)
83	47	*cyc1-445*	D-746	*cyc1-8* → *cyc1-119*	(34) → (36.5)
84	47	*cyc1-446*	D-746	*cyc1-8* → *cyc1-119*	(34) → (36.5)
85	47	*cyc1-447*	D-756	*cyc1-8* → *cyc1-119*	(34) → (36.5)
86	47	*cyc1-448*	D-746	*cyc1-8* → *cyc1-119*	(34) → (36.5)
87	48	*cyc1-449*	D-746	*cyc1-8* → *cyc1-59*	(34) → (36)
88	49	*cyc1-450*	D-756	*cyc1-32* → *cyc1-40*	(35) → (41)
89	50	*cyc1-451*	SL-383	*cyc1-119* → *cyc1-33*	(36.5) → (37.5)
90	51	*cyc1-452*	D-746	*cyc1-190* → *cyc1-40*	(38) → (41)
91	52	*cyc1-453*	D-746	*cyc1-40* → *cyc1-44*	(41) → (59)
92	53	*cyc1-454*	D-746	*cyc1-77* → *cyc1-211*	(49) → (76)
93	54	*cyc1-455*	D-746	*cyc1-44* → *cyc1-107*	(59) → (98)
94	54	*cyc1-456*	D-746	*cyc1-44* → *cyc1-107*	(59) → (98)
95	54	*cyc1-457*	D-746	*cyc1-44* → *cyc1-107*	(59) → (98)
96	55	*cyc1-458*	D-755	*cyc1-44* → *cyc1-187*	(59) → (87)
97	56	*cyc1-459*	D-746	*cyc1-44* → *cyc1-71*	(59) → (61)
98	57	*cyc1-460*	D-746	*cyc1-76* → *cyc1-35*	71 → (73)
99	57	*cyc1-461*	D-792	*cyc1-76* → *cyc1-35*	71 → (73)
100	58	*cyc1-462*	D-746	*cyc1-187* → *cyc1-107*	(87) → (98)
101	59	*cyc1-463*	SL-382	*cyc1-80* → *cyc1-92*	(92) → (95)
102	59	*cyc1-464*	D-756	*cyc1-80* → *cyc1-92*	(92) → (95)
103	60	*cyc1-465*	D-746	*cyc1-80* → *cyc1-140*	(92) → (93)
104	60	*cyc1-466*	D-746	*cyc1-80* → *1-140*	(92) → (93)

* Estimates of amino acid position are indicated in parentheses.

cated that these deletions consisted of at least 60 different kinds. The complete deletion map is presented in Figure 2, which includes the 60 different deletions and the 46 single sites as well as the *cyc1-239* site. While sharp demarcations were generally observed, a few crosses gave rise to very low frequencies of recombination that were still above the basal level, and these are indicated by dotted lines in Figure 2.

FIGURE 2.—The deletion map of the *cyc1* mutants and the correspondence to iso-1-cytochrome *c*. The primary tester alleles are presented at the bottom of the figure and the corresponding amino acid positions are presented at the top. Tentative amino acid positions, shown in parentheses, are estimated from the deletion map and from preliminary protein analyses. Point mutants within parentheses at the bottom of the figure were not ordered by the deletions although some of these were ordered from the alterations in iso-1-cytochromes *c*. The ends of the deletions showing particularly low rates of recombination are indicated by dotted lines.


AMINO ACID POSITIONS (OR ESTIMATES OF POSITIONS)
ALLELE NUMBERS OF DELETIONS
ALLELE NUMBERS OF POINT MUTANTS

Two sites cannot be oriented unless they are separated by the endpoint of a deletion. Since the five groups of mutants which are bracketed on the map by parentheses (Figure 2) could not be ordered, only 40 of the 47 sites could be unambiguously ordered relative to each other by deletion mapping. In one instance three sites (*cyc1-140*, *cyc1-92* and *cyc1-107*) could be ordered relative to each other but not to the overall map. Two of the groups, the *cyc1-135* and *cyc1-134* pair and the *cyc1-166* and *cyc1-72* pair, could not be ordered by deletion mapping, although their order was established from the amino acid changes in revertant iso-1-cytochromes *c*.

While almost all of the 210 point mutants could be unambiguously assigned to one of the 47 mutational sites, the exact position of the *cyc1-96* mutant is in question. As previously reported (SHERMAN *et al.* 1974), the *cyc1-96* mutant reverts at such a high rate that the X-ray-induced rates of heteroallelic crosses are difficult to measure. The map position was estimated by independent deter-

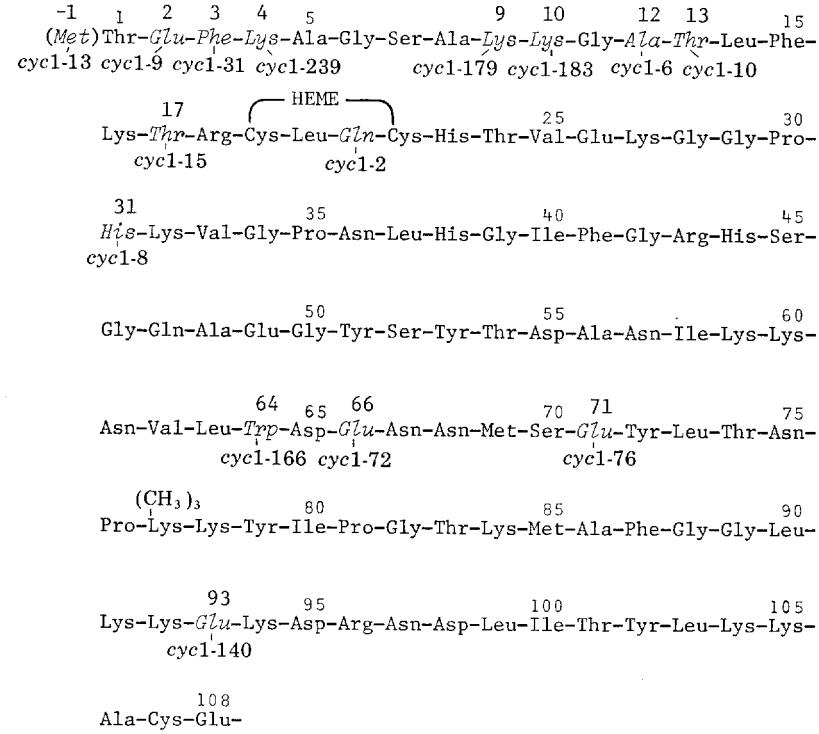


FIGURE 3.—The amino acid sequence of iso-1-cytochrome *c* (NARITA and TITANI 1969; LEDERER, SIMON and VERDIÈRE 1972; STEWART and SHERMAN, manuscript in preparation). The residues related to the mutational lesions that were deduced from revertant iso-1-cytochromes *c* are shown in italics, directly above the corresponding *cyc1* mutant designations (Table 4). However, the site of the *cyc1-15* and *cyc1-8* mutation may not be at the corresponding site of the amino acid replacement (see RESULTS). Also shown are the sites of heme attachment at positions 19 and 22, the ϵ-N-trimethyllysine at position 77 (DELANGE, GLAZER and SMITH 1970) and the amino terminal residue of methionine that is excised from the normal protein (STEWART, *et al.* 1971).

minations of X-ray-induced rates of numerous *cyc1-96* crosses and choosing the induced values with the lowest spontaneous rate.

Relationships between the deletion map and altered iso-1-cytochromes c: Presented in Table 4 and Figure 3 are the positions and types of mutational lesions that have been deduced from the amino acid changes in revertant iso-1-cytochromes *c*. The internal consistencies of the alterations of numerous independent revertants firmly established the deduced positions of the mutational sites in most of these *cyc1* mutants. However, only one revertant with an altered iso-1-cytochrome *c* was uncovered from each of the three mutants, *cyc1-8*, *cyc1-10* and *cyc1-15*. Since multiple base-pair changes have been occasionally observed in revertants (STEWART and SHERMAN 1972, 1974; SHERMAN and STEWART 1973), the type and position of the mutational lesion in these three mutants are less certain. Excluding these less-defined *cyc1* mutants, there is complete agreement in the relationship between the amino acid positions of the deduced alterations and the positions of the mutational sites in the deletion map (Figure 2). Thus, the deletion map can be confidently used to evaluate the suggested assignments of the uncertain sites.

The assignment of the mutational site of the *cyc1-10* mutant to amino acid position 13 is in complete agreement with the results of genetic mapping. Fine-structure mapping of two-point crosses previously suggested that the *cyc1-10* site was close to the *cyc1-6* site (PARKER and SHERMAN 1969) which corresponds to amino acid position 12 (PUTTERMAN, MARGOLIASH and SHERMAN 1974). Deletion mapping confirmed this finding and established an order that was consistent with the replacement data (Figure 2). However inconsistencies appeared, with the other two *cyc1* mutants having only a single altered revertant.

A clear disparity between the deletion map and the position of alterations in iso-1-cytochromes *c* exists for the mutant *cyc1-15*. PUTTERMAN, MARGOLIASH and SHERMAN (1974) recently reported that the normal residue of threonine at position 17 was replaced by a residue of isoleucine in iso-1-cytochrome *c* from the intragenic revertant *CYC1-15-C*. Two other revertant iso-1-cytochromes *c* from the *cyc1-15* mutant were normal. These first results appeared to indicate that the *cyc1-15* mutant, which completely lacks iso-1-cytochrome *c*, contains a lesion corresponding to amino acid position 17 and that this lesion is the direct cause of the deficiency. However, deletion mapping unequivocally established that the *cyc1-15* mutational site (at tester site *cyc1-39*) is to the right of the *cyc1-2* mutational site (at tester site *cyc1-17*) (Figure 2), which has been clearly shown to correspond to amino acid position 21 (Table 4). This discrepancy appears partially resolved by preliminary results of PUTTERMAN (unpublished results), who has recently examined additional revertants of *cyc1-15*. A heme peptide. encompassing residues 16 through 31, was isolated from chymotryptic digests of two particular revertant proteins. Amino acid compositions of acid hydrolysates of both peptides were identical and differed from analyses of the normal peptide only by lacking two residues of glycine and by having extra single residues of alanine and valine. Sequential Edman degradation of one of the two peptides revealed amino terminal Lys-Thr-. These results indicate that

threonine 17 was retained and that glycines 28 and 29, the sole glycyl residues in the normal heme peptide, were lost and probably replaced by the extra residues of alanine and valine. Replacement of these two adjacent glycyl residues is compatible with frameshift mutations where there is an insertion and a deletion of base pairs spanning the two codons corresponding to positions 28 and 29. Since the deletion map is consistent with the correspondence of the *cyc1-15* sites to residue positions 28 or 29, it appears as if the *CYC1-15-C* revertant arose by a mutational event that corrected the *cyc1-15* lesion and concomitantly induced a G·C → A·T transition at position 17, causing threonine to be replaced by isoleucine. This tentative suggestion concerning the nature of the mutational lesion in the *cyc1-15* mutant may be ultimately resolved by sequencing the altered region of the revertant proteins and by examining additional revertants.

In addition we have some reservation in concluding that the *cyc1-8* site corresponds to amino acid position 31. A *cyc1-8* revertant contained iso-1-cytochrome *c* with a residue of asparagine replacing the normal residue of histidine at position 31; six other revertants contained normal iso-1-cytochrome *c* (SHERMAN *et al.* 1970). Although the deletion map initially appears to be consistent with the position of the amino acid replacement in the revertant protein, there is tentative evidence that the site of the *cyc1-177* amber mutant, which maps to the left of *cyc1-8*, may correspond to amino acid position 32. Peptide mapping and amino acid analysis of iso-1-cytochromes *c* suggested that *cyc1-177* revertants may have replacements at position 32 (STEWART and SHERMAN, unpublished results). Possibly the replacement of histidine 31 by asparagine in the *cyc1-8* revertant may have arisen concomitantly with the correction of the primary lesion. The uncertainty of the *cyc1-8* site may be resolved after the positions of amino acid replacements in *cyc1-177* revertants are firmly established by sequencing the altered regions of the protein and after examining additional revertant proteins from *cyc1* mutants having neighboring sites.

We have attempted to estimate the corresponding amino acid position for the mutational sites that have not been definitively deduced from altered iso-1-cytochromes *c*. Some sites have not yet been examined for altered revertant proteins, other sites have yielded only normal revertant proteins and still other sites have yielded altered proteins that have been examined so far only by peptide mapping and in most cases also by amino acid composition analysis. While peptide mapping and amino acid analysis are usually too limited for precisely determining amino acid changes, and while definitive determinations require the use of sequencing techniques, some of these preliminary structural data have been useful for assigning tentative positions to several of the mutational sites, including the *cyc1-24* (tester site *cyc1-211*), *cyc1-45*, *cyc1-156*, *cyc1-177*, and *cyc1-190* sites that correspond, respectively, to residues at the tentative positions 76, 102, 26, 32 and 38 (STEWART *et al.*, unpublished results). In addition, the remaining sites have been assigned arbitrary positions on the basis of their genetic location relative to the position of the sites determined definitively or tentatively from altered iso-1-cytochromes *c*. For example, the *cyc1-210* and *cyc1-36* sites have estimated to correspond to positions 16 and 18, respectively, since they fall in the

region enclosed by the defined sites at positions 13 and 21. In another example, a cluster of 16 mutational sites are restricted by the three mutational sites that were tentatively assigned to amino acid positions 26, 32 and 38. Since eight sites appeared to fall within positions 32 through 38, it was necessary to assign some of the sites to half-integral positions. Although the tentative and arbitrary assignments were mainly made for a convenient means of presentation, and not for representations of the true positions, it is clear that some of these estimates must be very near or at the actual values. All of the tentative and arbitrary assignments, which are in parentheses, and the definitive assignments, are presented in Table 3 and Figure 2. The mutational sites and the corresponding amino acid positions included in each of the deletions are presented in Table 5 and Figure 2.

Phenotypic characterizations of the point mutants: The assignments of the 210 point mutants, *cyc1-2* through *cyc1-211*, to each of the 46 mutational sites are presented in Table 3, along with the following other properties previously reported for each of the mutants (SHERMAN *et al.* 1974): the cytochrome *c* content, suppressibility and the inducing mutagens. One can refer to the earlier publication (SHERMAN *et al.* 1974) for additional properties previously investigated but not presented here, including growth on lactate medium, ability to revert, and in some instances whether intragenic revertants contained altered iso-1-cytochrome *c*. After completion of the previous investigation, it was determined that the *cyc1-11* and *cyc1-123* mutants are slightly suppressible by ochre-specific suppressors. These mutant alleles were shown to be suppressible in haploid strains containing suppressors but not in diploid strains that were heterozygous for the suppressor genes and hemizygous for the *cyc1* alleles (see SHERMAN *et al.* 1974). The low efficiency of suppression thus distinguishes the *cyc1-123* ochre mutant from three other ochre mutants that map at this site, *cyc1-45*, *cyc1-68* and *cyc1-124*. Since distances of four base pairs are usually resolved by recombination frequencies of two-point crosses, it appears as if the *cyc1-123* mutant and the *cyc1-45* mutant may contain adjacent ochre condons.

DISCUSSION

In this investigation 104 deletions of the *CYC1* gene were obtained among the mutants arising spontaneously from sporulated cultures of certain strains of yeast. The lengths of the deletions ranged from the six encompassing all of the sites (deletions *cyc1-363* through *cyc1-368*) to the seven encompassing only two adjacent sites (deletions *cyc1-426*, *cyc1-434*, *cyc1-451*, *cyc1-452*, *cyc1-459*, *cyc1-460* and *cyc1-465*). The extension of some the longer deletions into neighboring genes will be described in a future publication (SINGH and SHERMAN, manuscript in preparation). It has not been excluded that some of the mutants covering two adjacent sites or possibly other "short deletions" may contain particular point mutations that undergo low rates of recombination with close markers. In fact such a point mutant showing an irreconcilable pattern of recombination was previously reported (SHERMAN *et al.* 1974); the point mutant *cyc1-132* does not significantly recombine with either the *cyc1-71* or *cyc1-166* point mutants

which are located at separate sites. Apparently the *cyc1-132* mutant, which is at the *cyc1-166* site, recombines at a particularly low rate with the *cyc1-71* mutant which is clearly at a distinct site (MOORE and SHERMAN, unpublished result). However, since such gross disparities of distant sites were never observed with the 210 point mutants, it must be concluded that at least the longer deletions are correctly classified. In addition it has not been excluded that some of these multiple-site mutants may contain gross abnormalities other than deletions which lead to the lack of recombination with point mutants at distinct sites. Nevertheless, the internal consistency of the deletion map irrefutably establishes the order of the mutational sites.

All but two of the deletion mutants appeared to completely lack iso-1-cytochrome *c* and it is believed that these two exceptional mutants, *cyc1-449* and *cyc1-453*, contain nonfunctional iso-1-cytochromes *c* at, respectively, approximately 10% and 20% of the normal level. Such a result with the *cyc1-449* mutant is not surprising since this mutant only contains a relatively short deletion and since one might expect to find at least some degree of heme attachment to iso-1-cytochrome *c* with small deletions of uncritical regions. However, the *cyc1-453* deletion encompasses a region that is estimated to correspond approximately to residues 40 through 60. Apparently this extensive deletion does not distort the protein sufficiently to totally prevent heme attachment. Interestingly, it has been suggested that Pseudomonas cytochrome *c* arose from an ancestral eukaryotic type of cytochrome *c* by a similar deletion of residues 31 through 46 in horse cytochrome *c* (or residues 36 through 51 in iso-1-cytochrome *c*) which removes one hairpin bend with little alteration of the overall folding (DICKERSON 1971).

The crosses producing the deletions were made with haploid strains having mutant forms of functional iso-1-cytochromes *c* that had alterations in the dispensable amino-terminal region of the protein (Table 1). It was speculated that mispairing due to extensive difference in homologous regions of the *cyc1* gene might lead to deletions during meiosis. Relatively high proportions of deletions were obtained from heteroallelic crosses that would be expected to have some degree of mispairing. The lack of the deletions in any of the 210 mutants previously selected from a haploid strain (SHERMAN *et al.* 1974) indicates that deletions do not readily arise in haploid strains and suggests that meiosis may be required. Surprisingly, one homoallelic cross (SL-383) gave rise to four deletions out of total of 27 *cyc1* mutants. However, the four deletions (*cyc1-367*, *cyc1-368*, *cyc1-436* and *cyc1-451*) derived from the homoallelic cross are not typical of the deletions derived from heteroallelic crosses. In fact it has not been excluded that the two short "deletions," *cyc1-436* and especially *cyc1-451*, may be merely point mutants recombining at low rates with nearby sites. Also, the long deletions *cyc1-367* and *cyc1-368*, which encompass the entire gene, belong to a class that has been observed occasionally in apparently normal haploid strains and that has been observed at high frequencies in certain strains having a mutable gene associated with the *CYC1* locus (LIEBMAN, SINGH and SHERMAN, manuscript in preparation). Surprisingly, the heteroallelic regions are not

necessarily deleted, since approximately 40% of the deletions did not encompass the altered segment of the parent strains. We believe that our results do not clearly establish nor refute that the majority of the deletions arose as the result of heteroallelic mispairing. Further experiments designed to answer this question are in progress and will be reported in a future publication (LIEBMAN, SINGH and SHERMAN, manuscript in preparation).

Whatever mechanism may be involved, these deletions provided a definitive means for ordering 40 of the 47 mutational sites (Figure 2). The corresponding amino acid positions of 15 of these sites were previously deduced from altered iso-1-cytochromes *c* in intragenic revertants (Table 4). The order of the mutational sites that was determined by deletion mapping is in agreement with the order deduced from altered iso-1-cytochrome *c* except for the amino acid replacement in one *cyc1-15* revertant. However, additional revertant proteins from the *cyc1-15* mutant suggest that this inconsistency may be due to a secondary mutation (see RESULTS). In addition to the definitive correspondences, the deletion map and preliminary protein analyses have been used for assigning tentative amino acid positions to all of the mutational sites.

The distribution of the 210 point mutants and some of their characteristics are presented in Figure 4, where it is evident that mutants mapping at a particular site may share common properties and that some mutagens are more active at certain sites. Some aspects of the distribution pattern were previously discussed (SHERMAN *et al.* 1974) before the order of the mutational sites was determined and before the mutants were clearly assigned to each of the sites. While distinct types of mutants appear at most sites that include a sufficient number necessary for evaluating homogeneity, these new results reveal a more or less uniform cluster of 15 mutants at the *cyc1-6* site (position 12) and 7 mutants at the *cyc1-59* site (tentative position 36) that all appear to contain nonfunctional iso-1-cytochrome *c*. Similarly, all 15 mutants at the *cyc1-19* site (tentative position 23) and all 10 mutants at the *cyc1-39* site (tentative position 29) lack iso-1-cytochrome *c* and are not suppressible. The distribution pattern also reveals that mutations at some sites occur with several mutagens while mutations at other sites are restricted. Relatively high rates of mutations at the *cyc1-19* site (tentative position 23), the *cyc1-166* site (position 64) and the *cyc1-76* site (position 71) occurred spontaneously and were induced with most mutagens. The *cyc1-120* site (position 13), *cyc1-107* site (tentative position 98) and the *cyc1-45* site (tentative position 102) appear particularly mutable with UV. While only five mutants were induced with nitrous acid, it appears as if this mutagen is particularly active on the *cyc1-6* site. Another "hotspot" or mutable site is suggested from the finding of 22% of the spontaneous mutants at the *cyc1-76* site (position 71). Thet highest degree of specificity occurred with the mutations at the *cyc1-39* site (tentative position 29) that were induced exclusively by ICR-170 and that constituted 30% of the ICR-170-induced mutations. More extensive distribution patterns are currently being determined with increased numbers of *cyc1* mutants that were induced by these and other mutagens.

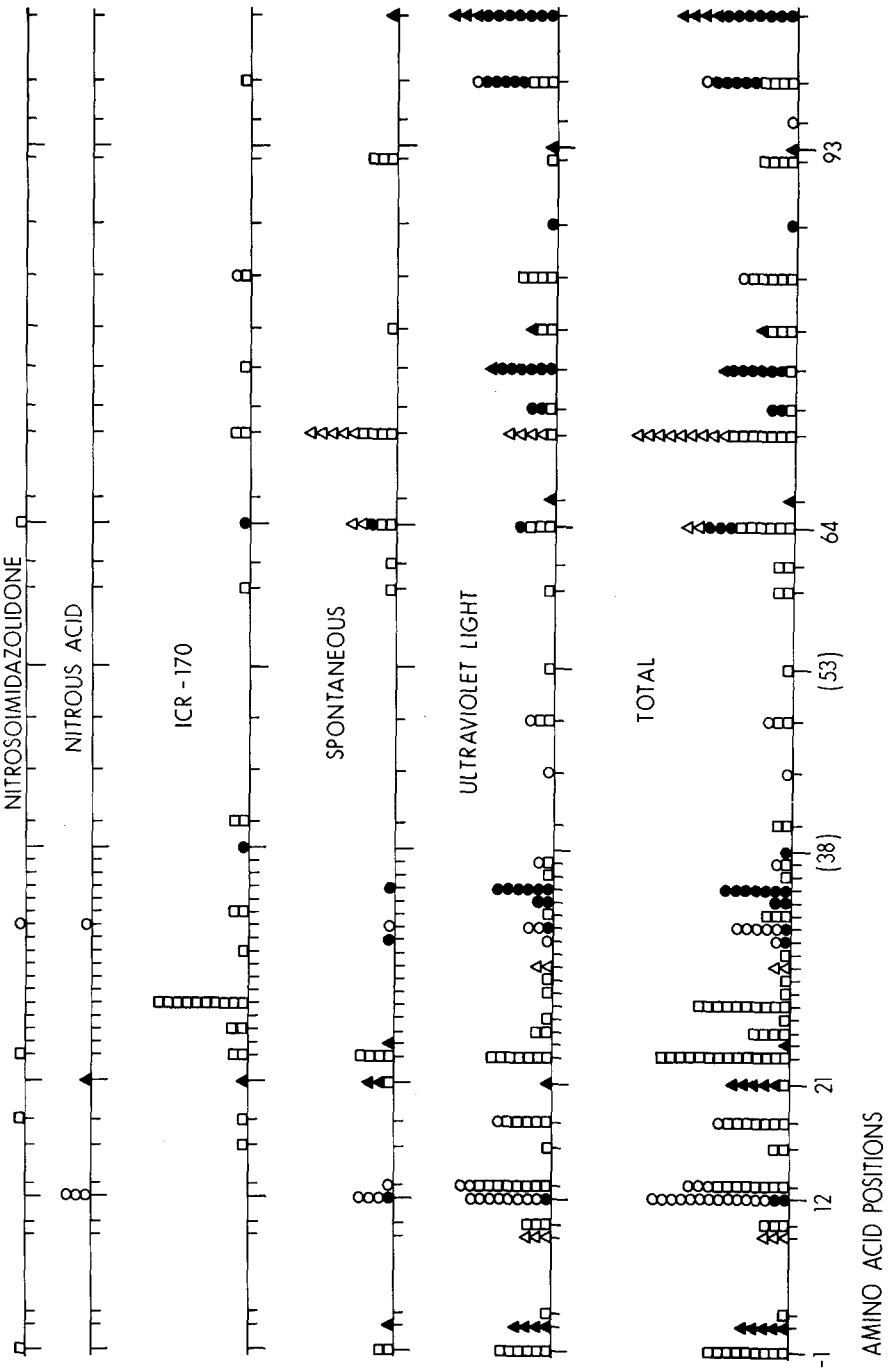


FIGURE 4.—The assignments of the *cyc1* mutants to each of the mutational sites (from Table 3). Nonsuppressible mutants that are believed to completely or almost lack iso-1-cytochrome *c* are indicated by □; those having a detectable increase to almost one-half the normal level are indicated by ○, while those having approximately one-half to almost the normal level are indicated by ●. Ochre and amber mutants are designated, respectively by ▲ and △.

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