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Genetics

The *cyc1-11* mutation in yeast reverts by recombination with a nonallelic gene: Composite genes determining the iso-cytochromes *c*

(cytochrome *c*/*Saccharomyces cerevisiae*/transposition)

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ABSTRACT **DNA sequence analysis of a cloned fragment directly established that the *cyc1-11* mutation of iso-1-cytochrome *c* in the yeast *Saccharomyces cerevisiae* is a two-base-pair substitution that changes the CCA proline codon at amino acid position 76 to a UAA nonsense codon. Analysis of 11 revertant proteins and one cloned revertant gene showed that reversion of the *cyc1-11* mutation can occur in three ways: a single base-pair substitution, which produces a serine replacement at position 76; recombination with the nonallelic *CYC7* gene of iso-2-cytochrome *c*, which causes replacement of a segment in the *cyc1-11* gene by the corresponding segment of the *CYC7* gene; and either a two-base-pair substitution or recombination with the *CYC7* gene, which causes the formation of the normal iso-1-cytochrome *c* sequence. These results demonstrate the occurrence of low frequencies of recombination between nonallelic genes having extensive but not complete homology. The formation of composite genes that share sequences from nonallelic genes may be an evolutionary mechanism for producing protein diversities and for maintaining identical sequences at different loci.**

Genetic changes that alter protein structure are usually base-pair mutations or chromosomal aberrations, deletions and insertions having break points within genes. Here we report another type of genetic process, recombination between nonallelic genes, that can alter the primary structures of proteins.

The yeast *Saccharomyces cerevisiae* contains two iso-cytochromes *c*, denoted iso-1-cytochrome *c* and iso-2-cytochrome *c*, that perform similar functions but differ in primary structure. Iso-1-cytochrome *c* and iso-2-cytochrome *c* constitute, respectively, ≈95% and ≈5% of the total cytochrome *c* complement and are encoded, respectively, by the *CYC1* locus (1) located on chromosome X (2) and the *CYC7* locus (3) located on chromosome V (4). The translated portions of the *CYC1* and *CYC7* loci (5) and the corresponding iso-1-cytochrome *c* and iso-2-cytochrome *c* proteins (unpublished results cited in ref. 6) are both ≈80% homologous.

Numerous forward and reverse mutations at the *CYC1* locus have been isolated and characterized (7). Mutants lacking iso-1-cytochrome *c* or its activity have been isolated by using a spectroscopic scanning procedure, a benzidine-staining procedure, and chlorolactate medium, whereas revertants containing at least partially functional iso-1-cytochrome *c* have been isolated by using lactate medium (7). Sequence analysis of large numbers of proteins and of a limited number of cloned DNA segments indicated that ≈90% of forward and reverse mutations involve single base-pair changes and that the remaining ≈10% involve multiple base-pair changes (8–11). A rare novel type of mutation has been identified by analyzing revertants from the *cyc1-11* mutant, which contains a UAA nonsense mutation corresponding to amino acid position 76. The analysis reported herein of 11 revertant proteins and a cloned revertant gene demonstrates that some of the revertants arose by single base-pair substitutions while others contained a segment equivalent to a portion of the iso-2-cytochrome *c* sequence. These findings suggest that the UAA lesion was corrected in certain revertants by recombination of the *cyc1-11* allele with the normal *CYC7* gene.

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MATERIALS AND METHODS

Genetic Procedures. Standard yeast genetic procedures of crossing, sporulation, and tetrad analysis were used to construct strains having the desired genotypes (12). The techniques and media specifically used with cytochrome *c* mutants have been described, including the procedures for mutagenic treatment, for reversion, for testing of revertants, and for estimating the amounts of cytochrome *c* (7).

Protein Analysis. Iso-1-cytochromes *c* were prepared (13) and subjected to amino acid analysis and to peptide mapping according to described procedures (14, 15). The procedures for cyanogen bromide cleavage, fractionation of cyanogen bromide digests on Sephadex G-50 (15, 16), and sequence analysis of isolated peptides (14, 15) also have been described.

Cloning by Integrative Transformation. The strains B-5643 (*α cyc1-11 ura3-52 can1-100 leu⁻*) and B-5644 (*α CYC1-11-C ura3-52 met3 can1-100*) were transformed with the plasmid pAB30 (11) according to described procedures (17) except that pAB30 was linearized by digestion with *Eco*RI, a condition that increased the transformation frequencies at least 6-fold. DNA from transformants was digested with *Bam*HI and ligated at 14°C for 16 hr as described (11). Competent *Escherichia coli* strain HB101 cells were transformed with ligated DNA, and transformants were selected on ampicillin medium (18). The resulting plasmids, pAB87 and pAB88, consist of a 4.6-kilobase (kb) *Eco*RI/*Bam*HI fragment of yeast carrying, respectively, the *cyc1-11* and *CYC1-11-C* loci inserted into a 4.0-kb *Eco*RI/*Bam*HI fragment of pAB30.

DNA Sequence Analysis. DNA sequence analyses of the *cyc1-11* and *CYC1-11-C* genes were performed by the strategy outlined in Fig. 1. Restriction endonucleases, exonuclease III, T4 polynucleotide kinase, and the large fragment of polymerase I were obtained from New England BioLabs and used according to the recommendation of the manufacturer. Bacterial alkaline phosphatase and 2′,3′-dideoxynucleotides were obtained from P-L Biochemicals; polymerase I was obtained from Boehringer Mannheim; the synthetic oligonucleotide dC-G-T-T-T-C-T-G-T-C was a custom-made gift from BIO LOGICALS (Toronto); [γ-^{32}P]dATP and [α-^{32}P]dATP were from Amersham.

The region within the *Eco*RI and *Taq* I site shown in Fig. 1 was sequenced in part according to the modification of the dideoxy-terminator method as described by Seif *et al.* (19). The

Abbreviation: kb, kilobase(s).

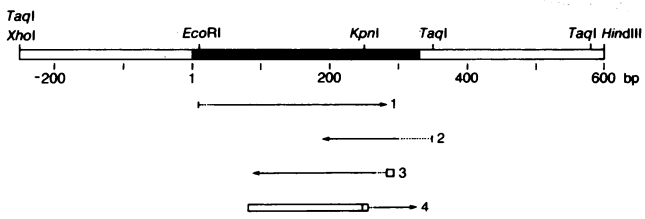

FIG. 1. Restriction map and strategy for DNA sequence analyses of the *cyc1-11* and *CYC1-11-C* genes. ■, Translated region of the *CYC1* locus. Distances (bp) are denoted below the map. ← and →, Sequence runs of the transcribed and nontranscribed strands, respectively; ——, DNA sequences determined; ----, sequences not determined. The modified dideoxy-terminator method described by Seif *et al.* (19) was used in sequence runs 1 and 2 after 5′ end labeling at, respectively, the *Eco*RI and *Taq* I sites. The dideoxy-terminator method (20) was used in sequence runs 3 and 4 with, respectively, dT-C-G-T-T-T-C-T-G-T-C and the 170-bp *Hae* III/*Hae* III fragment as primers. □, Primers; the primer in sequence run 4 was cleaved with *Kpn* I prior to separating the reaction products on the sequencing gels.

sequence of the nontranscribed strand (sequence run 1) was obtained after 5′-end labeling at the *Eco*RI site (Fig. 1) by using [γ-^{32}P]ATP and T4 polynucleotide kinase (21). Plasmid DNA (50 μg) was cut with *Eco*RI, end labeled, and digested with *Hin*dIII. The 0.6-kb *Eco*RI/*Hin*dIII fragment was isolated by electrophoresis in a 0.7% agarose gel and purified on a DE52 column (20). To determine the sequence of the transcribed strand (sequence run 2), 150 μg of plasmid DNA was digested with *Xho*I/*Hin*dIII; the 850-bp *Xho*I/*Hin*dIII fragment was isolated by electrophoresis in a 0.7% agarose gel (21). This fragment was treated with *Taq* I and end labeled (21). The fragment mixture was digested with *Eco*RI, and the 340-bp *Eco*RI/*Taq* I fragment was isolated on a 5% acrylamide gel (21). The sequence analyses were carried out according to the procedures described by Seif *et al.* (19) and S. Zain (personal communication). "Forward" assays (e. g., A reaction) used 3000–10,000 cpm of 5′-end-labeled fragment in 6.6 mM Tris·HCl, pH 7.5/6.6 mM $MgCl_2$/2 mM dithiothreitol/2 mM NaCl/40 μM dCTP/40 μM dGTP/40 μM dTTP/20 μM ddATP (final vol, 5 μl). "Backward" assays used 5′-end-labeled fragment in 6.6 mM Tris·HCl, pH 7.5/6.6 mM $MgCl_2$/2 mM dithiothreitol/2 mM NaCl/200 μM in any of the four dideoxynucleosidetriphosphates (final vol, 5 μl.) Reactions were started by the addition of 0.5 μl (2.5 units) of DNA polymerase I (holoenzyme) and incubated for 30 min at 37°C. Further processing of samples was as described (20).

Sequences obtained in sequence runs 1 and 2 were confirmed by the dideoxy-terminator method (20). To obtain the sequence of the transcribed strand, 50 ng of the synthetic oligodeoxyribonucleotide dT-C-G-T-T-T-C-T-G-T-C was used as primer and 1 μg of plasmid pAB87 or pAB88 cut with *Hin*dIII was used as template (sequence run 3). Part of the sequence of the nontranscribed strand (sequence run 4) was obtained by extension (20) of a central 170-bp *Hae* III/*Hae* III fragment (5) on 1 μg of a template prepared by cleaving the plasmid DNA with *Hin*dIII and treating with exonuclease III (20); this chain was cleaved with *Kpn* I (Fig. 1). The products of all sequence analysis reactions were separated on 8% and 10% urea/acrylamide gels (21) and autoradiographed using Kodak XS-1 film with or without Cronex Lightning Plus intensifying screens (Du Pont).

RESULTS

The *cyc1-11* Mutation. *cyc1-11* is a UV-induced mutation that was detected with the benzidine staining procedure (13). Low-temperature (−196°C) spectroscopic examination of intact cells and chromotographic analysis of cell homogenates showed that the *cyc1-11* mutant lacks iso-1-cytochrome *c* but contains the normal amount of iso-2-cytochrome *c*. Deletion mapping indicated that the *cyc1-11* mutation is located at or near a site corresponding to amino acid position 76 (22). Genetic analysis established that the *cyc1-11* mutation is weakly suppressible by suppressors that specifically act on UAA nonsense mutations. Several *cyc1-11* strains were crossed to strains containing either the UAA suppressor *SUP4*, which causes insertion of tyrosine residues (23), or the UAA suppressor *SUP16*, which causes insertion of serine residues (16, 24). Low-temperature spectroscopic examination of the meiotic progeny from these crosses showed that every *cyc1-11 SUP4* and *cyc1-11 SUP16* segregant contained a higher amount of total cytochrome *c* compared with unsuppressed *cyc1-11* segregants. Estimations of the levels of total cytochrome *c* indicated that the *SUP4* and *SUP16* suppressors caused the formation of ≈5% of the normal amount of iso-1-cytochrome *c*, a level slightly below the level observed in most other suppressed *cyc1* UAA mutants. Although the *cyc1-11 SUP4* strains contained low amounts of iso-1-cytochrome *c*, presumably with tyrosine residues at position 76, the lack of growth of these strains on lactate medium suggested that the iso-1-cytochrome *c* was not functional (see below).

DNA sequence analysis of the appropriate region established that *cyc1-11* is a UAA mutation at the site corresponding to amino acid position 76. Strain B-5643 (*α cyc1-11 ura3-52 can1-100 leu*$^-$) was transformed with plasmid pAB30, which integrates adjacent to the *CYC1* locus because of the presence of a homologous segment (11). DNA was extracted from one of the transformants, digested with restriction endonuclease *Bam*HI, ligated, and then used to transform *Escherichia coli* for ampicillin resistance. Restriction mapping indicated that the plasmid pAB87, isolated from *E. coli*, contained the region encompassing the *CYC1* locus. The appropriate fragment was isolated, and a sequencing gel is shown in Fig. 2. The results establish that the *cyc1-11* mutation arose by base-pair substitutions of two adjacent nucleotides, changing the proline codon at position 76 from CCA to UAA, as shown in Fig. 3.

The *cyc1-11* Revertants. Quantitative reversion studies using UV, x-ray, nitrous acid, and 1-nitrosoimidazolidone-2

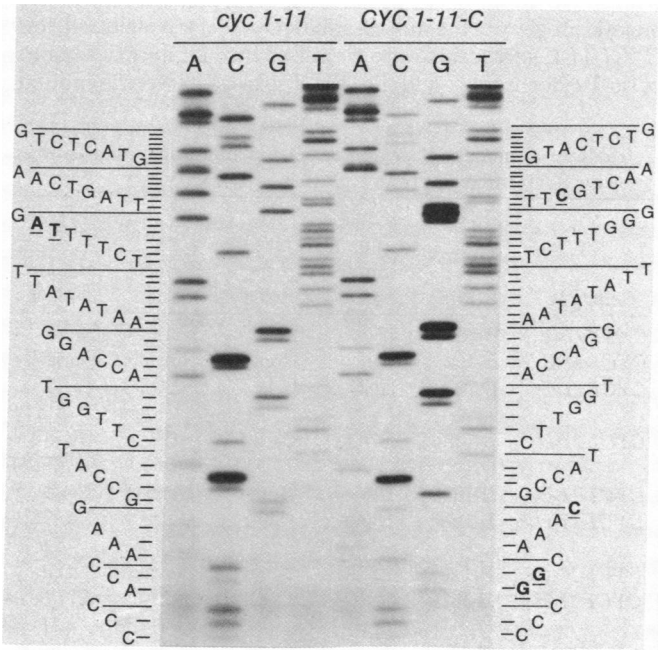

FIG. 2. Autoradiogram of the gel used in sequence run 3 (Fig. 1) showing partial DNA sequences of the transcribed strands of *cyc1-11* and *CYC1-11-C*. Underlined boldface letters indicate deviations from the *CYC1*$^+$ wild-type sequence.

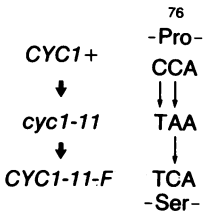

FIG. 3. Mutational pathways leading to the formation of the *cyc1-11* mutation and the *CYC1-11-F* revertant. The triplets of the DNA-nontranscribed strand corresponding to amino acid position 76 are presented along with the amino acids that they specify.

showed that the *cyc1-11* strains had the lowest intragenic reversion rate among *cyc1* mutants containing UAA mutations and other *cyc1* mutations that revert by single base-pair substitutions (ref. 25; unpublished results). For example, the UV-induced reversion rate of the *cyc1-11* mutant was ≈1% of the rate of the *cyc1-2* mutant, which contains a UAA mutation at position 21, and only 5% of that of the *cyc1-13* mutant, which contains an AUA codon instead of the normal AUG initiator codon (25). It should be noted that reversion due to nonsense suppression usually is not detected with lactate medium (7), although such revertants can be seen with certain strains as pinpoint colonies.

Rare spontaneous *cyc1-11* revertants or revertants induced with either nitrous acid, UV, ethyl methanesulfonate, or diethyl sulfate were selected on lactate medium; the selection was carried out either by using a large number of plates or by an initial selection step in liquid medium. The frequencies of revertants induced by some of these mutagens have been reported (25). The revertants were subcloned, and the amounts of total cytochrome *c* were estimated by low-temperature spectroscopic examination of intact cells. The revertants containing normal or near normal amounts of cytochrome *c* were analyzed genetically for the cause of the reversion (7). A total of 11 revertants were shown to be intragenic—i.e., the restoration of iso-1-cytochrome *c* in the *cyc1-11* mutant was due to events that produced changes at the *CYC1* locus (Table 1). For example, the *CYC1-11-C* strain B-703 was crossed to a *cyc1-1* strain and the meiotic progeny of the diploid strain (D-1161) was analyzed; the *CYC1-11-C* segregants were identified by their normal amount of iso-1-cytochrome *c* and the *cyc1-1* segregants were identified

Table 1. Summary of peptide maps and amino acid compositions of iso-1-cytochromes *c* from *cyc1-11* revertants

Genotype	Strain	Inducing mutagen	Altered peptides	Amino acid	
				Composition	Replacement
CYC1-11-A	B-701	UV	None	Normal	None
CYC1-11-B	B-702	UV	None	Normal	None
CYC1-11-C	B-703	HNO_2	C-12, T-11	−Gly, +Ala	Gly-88 → Ala-88
CYC1-11-D	B-1054	HNO_2	C-12, T-11	−Gly, +Ala	Gly-88 → Ala-88
CYC1-11-E	B-1055	EtMes	None	Normal	None
CYC1-11-F	B-1268	None	C-9, C-9′	−Pro, + Ser	Pro-76 → Ser-76
CYC1-11-G	B-1269	Et_2SO_4	None	Normal	None
CYC1-11-H	B-1529	HNO_2	C-12, T-11	−Gly, + Ala	Gly-88 → Ala-88
CYC1-11-I	B-3422	None	None	Normal	None
CYC1-11-J	B-3423	None	C-9, C-9′	−Pro, +Ser	Pro-76 → Ser-76
CYC1-11-K	B-3424	None	C-9, C-9′	−Pro, +Ser	Pro-76 → Ser-76

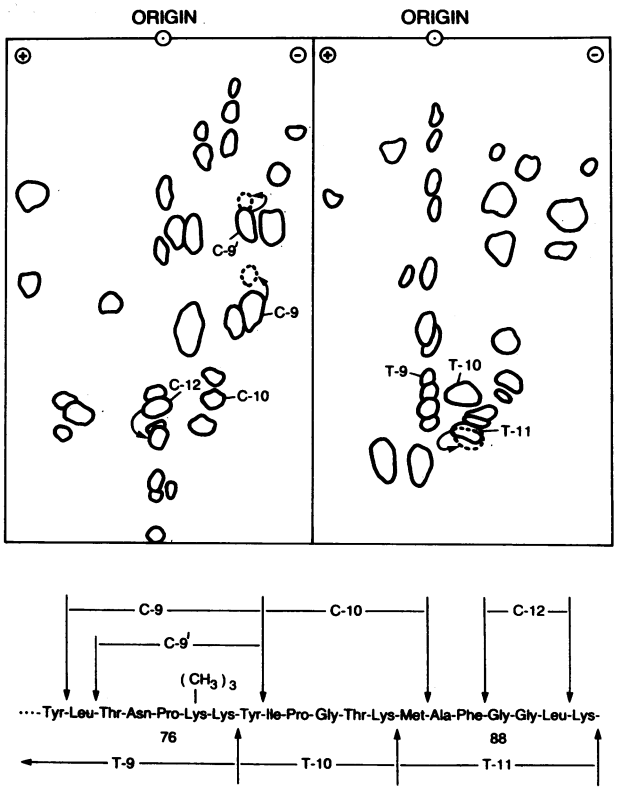

FIG. 4. Cumulative peptide maps (horizontal, electrophoresis; vertical, chromatography) of iso-1-cytochromes *c* from 11 strains (Table 1) and sequences of the normal peptides that contain the sites of amino acid replacements. (*Left*) Chymotryptic digest. (*Right*) Tryptic digest. Continuous outlined areas denote positions of ninhydrin-stained peptides of normal iso-1-cytochrome *c*; areas outlined by dashed lines denote positions of abnormal peptides (the altered position of the C-12 peptide was determined by the intensified color at the coincident position of a normal peptide). On the basis of ninhydrin, Pauly, and Ehrlich staining of the maps, alterations were observed for either the C-12 and T-11 peptides or the C-9 and C-9′ peptides.

by their deficiency of iso-1-cytochrome *c* and by their UV sensitivity. The 2:2 segregation of the *CYC1-11-C*:*cyc1-1* alleles in five tetrads indicates that the *CYC1-11-C* mutational change producing normal amounts of iso-1-cytochrome *c* is located at the *CYC1* locus.

Iso-1-cytochrome *c* was prepared from the 11 revertants and examined by peptide mapping and by amino acid analysis. Peptide maps of chymotryptic and tryptic digests (Fig. 4) indicated that there were three types of revertant iso-1-cytochrome *c*. One type of peptide map appeared normal, another was altered only in the chymotryptic peptides C-9 and C-9′, which had been replaced by chromatographically slower counterparts, and, in the third, chymotryptic peptide C-12 and tryptic peptide T-11 had been replaced by chromatographically faster peptides. Amino acid analyses of acid hydrolysates (data not shown) showed three types of compositions of the corresponding iso-1-cytochrome *c* (Table 1): one type appeared normal (26); in one, a proline had been replaced by a serine; and in a third, a glycine had been replaced by an alanine. As described below, these single amino acid replacements fully explain the chromatographic changes observed in the peptide maps.

The identities of the mutationally altered peptides and the corresponding normal peptides were deduced by sequence analyses and from their chromatographic, electrophoretic, and staining properties. A glycine-88 → alanine-88 change in the T-11 and C-12 peptides of the *CYC1-11-C* iso-1-cytochrome *c* was demonstrated by isolation and sequence analysis of the

Table 2. Types of *cyc1-11* revertants

Iso-1-cytochrome *c*	Type	Mutagen None	HNO_2	UV	EtMes	Et_2SO_4	Total
Serine-76	A-T→C-G transversion	3	0	0	0	0	3
Alanine-88	*CYC7* recombination	0	3	0	0	0	3
Normal	2-base-pair change or *CYC7* recombination	1	0	2	1	1	5

cyanogen bromide peptide commencing with alanine-86: the sequence for *CYC1-11-C* was *Ala-Phe-Ala-Gly-Leu-Lys-Lys-Glu-Lys-Asp*-Arg (italics indicate amino acids identified as phenylthiohydantoin derivatives and overbars indicate amino acids identified as dansyl derivatives), whereas the normal partial sequence in *CYC1-11-F* was Ala-*Phe-Gly*-Gly-*Leu*-. Neutral spot C-12 could be associated with the Gly-Gly-Leu sequence by the permanent yellow color that it gave with ninhydrin, while spot T-11 (and spot C-10) was assigned to the region of methionine-85 on the basis of chromatographic and electrophoretic positions and of methionine content revealed by chloroplatinate reagent; the peptides encompassing the other methionine-69 had been located previously (15). The chromatographically slower altered spots C-9 and C-9′ accompanied a net proline substitution by serine. Identical composition and peptide map properties were previously observed with iso-1-cytochrome *c* in revertant *CYC1-24-I*, for which amino acid sequence analysis has established a proline-76 replacement by serine-76 (unpublished).

These results, summarized in Tables 1 and 2, indicate that the revertants contained three kinds of iso-1-cytochrome *c*; one type was normal iso-1-cytochrome *c*, the second was one in which a serine residue has replaced the normal proline-76, and the third was one in which an alanine has replaced the normal glycine-88. The *CYC1-11-C* gene from one of the revertants having a glycine-88 → alanine-88 replacement was cloned by using the procedure described above for the *cyc1-11* mutation. The sequence data (Figs. 1 and 2) indicate that this *CYC1-11-C* revertant contains a contiguous segment that differs from the *cyc1-11* sequence by 6 base pairs and that this replacement is equivalent to the corresponding segment of the normal *CYC7*$^+$ gene. Because portions of the *CYC7*$^+$ and *CYC1*$^+$ (or *cyc1-11*) genes are completely equivalent, the precise end points of the replacement could be assigned only to regions spanned by differences shown in Fig. 5; thus, the *CYC1-11-C* revertant was formed by replacing a contiguous segment of 43–71 base pairs from the *cyc1-11* gene with the corresponding segment from the nonallelic gene *CYC7*$^+$.

DISCUSSION

We have shown by DNA sequence analyses that the *cyc1-11* mutation arose by substitution of 2 adjacent base pairs in the proline codon CCA at amino acid position 76, resulting in the formation of the nonsense codon UAA. The UAA mutation in the *cyc1-11* mutant is consistent with finding a complete deficiency of iso-1-cytochrome *c*, the action of specific UAA suppressors, and the serine replacement at position 76 in certain *cyc1-11* revertants. Also, 2 concomitant base-pair substitutions have been observed at low frequencies in revertants of *cyc1* mutants induced by UV, x-rays, α particles, and nitrous acid (8, 27) and in the UV-induction of *lac* I mutants in *E. coli* (28). In fact, an identical C-G/C-G → T-A/A-T double base-pair mutation that occurred in the *cyc1-11* mutant was observed in a UV-induced *lac* I mutant (28).

Unlike the pattern of amino acid replacements observed in functional revertants of other UAA mutants and of UAG mutants (9, 29), the replacements at position 76 in *cyc1-11* revertants appear to be restricted to proline and serine residues. The lack of single base-pair revertants having replacements of leucine, tyrosine, glutamine, glutamic acid, and lysine at position 76 may be due to the lack of function of iso-1-cytochrome *c* with replacements of these residues. Thus, the low reversion frequencies of the *cyc1-11* mutant are related to the limited number of mutational changes that restore function of iso-1-cytochrome *c*. Furthermore, the A-T → C-G transversion producing the serine replacement (see Fig. 3) is rarely observed among revertants of other UAA mutants (9). In an extreme example, no serine replacements were uncovered among the 62 revertants from the *cyc1-72* mutant, which contains a UAA codon corresponding to amino acid position 66, even though studies with serine-inserting suppressors indicated that serine at this site is compatible with function (9, 16, 24).

The low reversion frequency of the *cyc1-11* mutant allowed recovery of a rare type of genetic alteration that corrected the UAA lesion. Sequence analysis of a DNA segment from a representative mutant containing an alanine-88 replacement as well as the proline-76 indicated that the revertant contained 4 base-pair differences in addition to the 2 base-pair differences at position 76; the altered segment in the *cyc1-11* revertant is equivalent to the corresponding segment of the *CYC7*$^+$ gene that determines iso-2-cytochrome *c*. As shown in Fig. 5, the revertants containing alanine at position 88 appear to have arisen by a genetic exchange that resulted in the replacement of a segment that is minimally 47 and maximally 71 base pairs long. The exact homology between *CYC1* and *CYC7* genes prevents a precise determination of the size of the replacement.

The third type of *cyc1-11* revertants containing the normal iso-1-cytochrome *c* protein could have arisen either by a two-base-pair mutation or by genetic exchange of an even shorter segment of the *CYC7*$^+$ gene. Although rare, a double base-pair

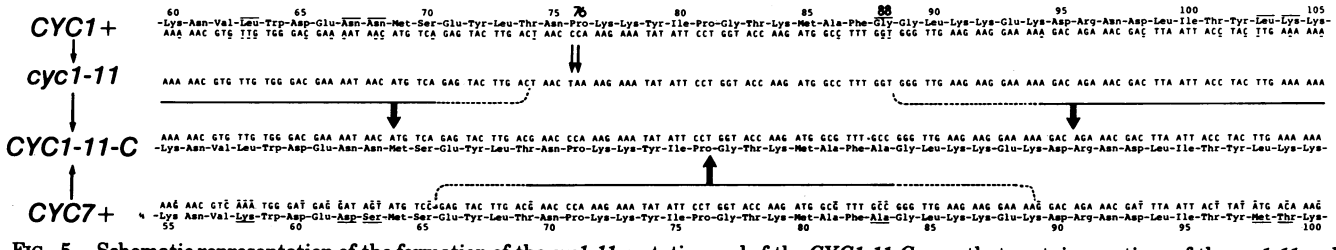

FIG. 5. Schematic representation of the formation of the *cyc1-11* mutation and of the *CYC1-11-C* gene that contains portions of the *cyc1-11* and *CYC7*$^+$ genes. Shown are protein sequences of regions of normal iso-1-cytochrome *c* (*CYC1*$^+$), normal iso-2-cytochrome *c* (*CYC7*$^+$), and the composite cytochrome *c* (*CYC1-11-C*), along with the corresponding nucleotide sequences of the DNA nontranscribed strand. The nucleotides and amino acid residues that are different in the iso-1-cytochrome *c* and iso-2-cytochrome *c* genes and proteins, respectively, are indicated by underlining or overbars in the normal sequences. The portions of the *CYC1-11-C* gene that were unambiguously derived from the *cyc1-11* and *CYC7*$^+$ genes are indicated by solid lines connected to arrows pointed, respectively, down and up; the ambiguous portions that were derived from either of the *cyc1-11* and *CYC7*$^+$ genes are indicated by dashed lines. The ambiguous segments have sequences identical to those in both the *cyc1-11* and *CYC7*$^+$ genes.

mutation, also resulting in a UAA → CCA change, was previously observed in a *cyc1-9* revertant (27). The numbers of the three types of *cyc1-11* revertants and the inducing mutagens are summarized in Table 2. These results show a striking relationship between the mutagen and the type of revertant. However, in studies with other *cyc1* mutants, mutagens other than nitrous acid were shown to induce revertants by recombination with the *CYC7*$^+$ gene (unpublished results).

The recombination between the *cyc1-11* and *CYC7*$^+$ alleles appeared to be a conversion-like event and not a reciprocal event. If recombination occurred by a reciprocal double crossover at the two-strand stage and if the chromatides from the two nonhomologous chromosomes segregated randomly, then one-half of the *CYC7* genes should harbor the UAA mutations at the equivalent position 76. No iso-2-cytochrome *c*-deficient segregants were observed among the meiotic segregants from (*CYC1-11-C* × *cyc1*) and (*CYC1-11-D* × *cyc1*) crosses; *cyc1 cyc7* mutants lacking both iso-1-cytochrome *c* and iso-2-cytochrome *c* are easily detected by their inability to grow on media containing nonfermentable substrates as the sole carbon source. Furthermore, a systematic study of numerous other *cyc1 CYC7* recombinational revertants fails to reveal iso-2-cytochrome *c* deficiencies (unpublished results).

The genetic exchange observed between the *cyc1-11* and *CYC7*$^+$ nonallelic genes may be similar to the genetic exchanges between yeast genes at their normal position and homologous genes that were artificially inserted at nonhomologous sites (30, 31). In addition, Munz and Leupold (32) have presented genetic evidence for occurrence of recombination between nonallelic tRNA genes in the yeast *Schizosaccharomyces pombe*; however, unlike the sequences of the *CYC1* and *CYC7* genes, the sequences of the nonallelic tRNA genes could be entirely equivalent. Also, the exchanges between the *CYC1* and *CYC7* loci that occur at low frequencies after mutagenic treatment are reminiscent of the exchanges of segments at the *MAT* locus with segments of the *HML* and *HMR* loci that occur spontaneously at high frequencies in homothallic yeast and at low frequencies in heterothallic yeast (33).

Genetic exchanges between nonallelic genes result in consequences that can be looked on with two points of view. On one hand, the new composite genes have sequences that are in common with one or another of the original genes but differ overall from each of them. Depending on the degree of difference among the nonallelic genes, the number of different types of composite genes could be large. Such recombination between nonallelic genes may play a role in generating protein diversity during evolution. On the other hand, recombination between similar genes at nonhomologous loci results in the formation of genes and proteins having identical segments. DNA sequences of mammalian globin genes and ribosomal genes suggest that nonallelic genes in a common family recombine and undergo concerted evolution (34–36). In fact, the 35-base-pair stretch of perfect homology between the *CYC1* and *CYC7* genes, encompassing amino acid residues 75–85 of iso-1-cytochrome *c* (Fig. 5), suggests that a conversion-like recombinational event took place in recent evolution.

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