DEPENDENCE ON MATING TYPE FOR THE OVERPRODUCTION OF ISO-2-CYTOCHROME *c* IN THE YEAST MUTANT *CYC7–H2*

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ABSTRACT

The *CYC7–H2* mutation causes an approximately 20-fold overproduction of iso–2–cytochromo *c* in **a** and α haploid strains of the yeast *Saccharomyces cerevisiae* due to an alteration in the nontranslated regulatory region that is presumably contiguous with the structural region. In this investigation, we demonstrated that heterozygosity at the mating type locus, **a**/α or **a**/**a**/α/α, prevents expression of the overproduction, while homozygosity, **a**/**a** and α/α, and hemizygosity, **a**/0 and α/0, allow full expression of the *CYC7–H2* mutation, equivalent to the expression observed in **a** and α haploid strains. There is no decrease in the overproduction of iso-2-cytochrome *c* in **a**/α diploid strains containing either of the other two similar mutations, *CYC7–H1* and *CYC7–H3*. It appears as if active expression of one or another of the mating-type alleles is required for the overproduction of iso-2-cytochrome *c* in *CYC7–H2* mutants.

ISO-1-CYTOCHROME *c* and iso-2-cytochrome *c* constitute, respectively, 95% and 5% of the total complement of cytochrome *c* in the yeast *Saccharomyces cerevisiae*. These iso-cytochromes *c* differ from each other by 21 amino acids (STEWART and PUTTERMAN, in preparation) and each are determined by separate loci located on different chromosomes. The primary structure of iso-2-cytochrome *c* is determined by the *CYC7* locus (DOWNIE *et al.* 1977), which is located on the left arm of chromosome *V* (SHERMAN *et al.* 1978). Mutations at the *CYC7* locus can result in deficiency, overproduction and amino acids changes of iso-2-cytochrome *c* (DOWNIE *et al.* 1977; DOWNIE, STEWART and SHERMAN 1977; SHERMAN *et al.* 1978). The *CYC7–H1*, *CYC7–H2* and *CYC7–H3* mutants contain especially high levels of iso-2-cytochrome *c*, approximately 20 to 40 times the normal level. The *CYC7–H1* mutation is a reciprocal translocation, with one of its breakpoints near the structural gene (SHERMAN and HELMS 1978); the *CYC7–H2* and *CYC7–H3* mutations map as single sites at the *CYC7* locus. The mutation causing overproduction of iso-2-cytochrome *c* in the *CYC7–H2* mutant and a mutation causing an amino acid change of iso-2-cytochrome *c* were shown to be genetically linked, and the two sites were observed to recombine only rarely by single-site gene conversion (SHERMAN *et al.* 1978). Since iso-2-cytochrome *c* from the *CYC7–H1* (DOWNIE *et al.* 1977) and *CYC7–H2* (SHERMAN *et al.* 1978)

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mutants are normal, it is concluded that the overproduction of iso-2-cytochrome *c* is caused by alterations in the nontranslated regulatory region that is presumably contiguous with the structural region.

In this paper, we describe an unusual property of the *CYC7–H2* allele; iso-2-cytochrome *c* is overproduced in **a** and α haploid strains, as well as in **a**/**a** and α/α diploid strains, but not in **a**/α diploid strains. In contrast to **a**/α *CYC7–H2/CYC7–H2* diploid strains, overproduction of iso-2-cytochrome *c* was observed in **a**/α *CYC7–H1/CYC7–H1* and **a**/α *CYC7–H3/CYC7–H3* strains similar to the overproduction observed in haploid strains.

MATERIALS AND METHODS

Genetic nomenclature: The symbols *cyc* and *CYC* denote, respectively, any of a number of recessive and dominant genes that affect the levels or activity of either or both of the iso-cytochromes *c*. The symbol *cyc1* denotes mutations at the structural locus that cause deficiency of iso-1-cytochrome *c*. The wild-type locus determining the primary structure of iso-2-cytochrome *c* is designated *CYC7+*. The mutant alleles at this locus that cause overproduction of iso-2-cytochrome *c* are denoted *CYC7–H1*, *CYC7–H2* and *CYC–H3*; they were previously denoted, respectively, *CYC7–1*, *CYC7–2* and *CYC7–3*. The *cyc8* and *cyc9* mutant genes, which are unlinked to the *CYC7* structural locus, cause slight overproduction of iso-2-cytochrome *c* (ROTHSTEIN and SHERMAN 1980).

In this report, aneuploid strains (2n–1) and deletion-containing diploid strains that have gained the ability to mate are denoted as either **a**/0 or α/0, where 0 designates the loss of all or part of chromosome *III* containing the mating-type locus.

Genetic techniques and media: The standard yeast genetic techniques (MORTIMER and HAWTHORNE 1969; SHERMAN and LAWRENCE 1974) and media (SHERMAN *et al.* 1974) used in this study have been previously described.

The prototrophic selection of a rare cross between an α *CYC7–H2* and α *CYC7+* strain was accomplished by incubating the two strains together on a nutrient plate for one day at 30° and then replica-plating the mixture onto minimal medium. The resulting prototrophic colonies were picked, subcloned and tested for the ability to mate and sporulate. A clone that mated and did not sporulate was presumed to be either an α/α or α/0 diploid strain.

Isolation of diploid maters: Mitotic crossing over in the right arm of chromosome *III*, resulting in homozygosity at the mating-type locus and therefore in the ability to mate, was detected by modification of the procedure described by HAEFNER (1966). From 1000 to 2000 cells of the diploid strain, W241, were plated on nutrient medium, and the plates were exposed to 5 krads of X rays and incubated at 30° for 2 days. The resulting colonies were replicated onto plates containing lawns of either **a** cells or α cells or that contained auxotrophic markers complementary to the markers in the W241 diploid strain. A colony that could mate was detected as a prototrophic colony after replica plating the mating mixtures to minimal medium. A clone that exhibited mating was picked from the original plate, subcloned and retested for its ability to mate.

These presumably diploid clones having mating ability were further tested by genetic analysis of crosses of these clones to either an **a**/**a** or α/α tester strain. A high frequency of spore viability suggests that the cross is a tetraploid strain and, therefore, that the mater is still a diploid strain derived from W241 by mitotic crossing over. By contrast, a low frequency of spore viability suggests that the cross is a triploid strain and, therefore, that the mater is probably a haploid strain derived from W241 by a rare meiotic event. Most of the crosses yielded high frequencies of viable spores, indicating that the mating strains were indeed diploid; several of these were further analyzed. Most of the analyzed crosses gave rise to tetrads having 0:4, 4:0 and 2:2 ratios of mating spores to nonmating spores, a result expected for a normal **a**/**a**/α/α tetraploid segregation (ROMAN, PHILLIPS and SANDS 1955; see RESULTS). However, some of the presumably tetraploid crosses did not give rise to the expected segregation pattern, and the ratio of mating spores to

nonmating spores of a predominant class was 3:1. This ratio suggests that the genetic configuration at the mating type locus is **a**/**a**/α/0 or α/α/**a**/0, where 0 represents the loss of chromosome *III* or at least a deletion of the mating-type locus.

Determination of iso-2-cytochrome c *levels*: Derepressed cells used for iso-2-cytochrome *c* determinations were prepared on nutrient plates (SHERMAN *et al.* 1974) or in liquid culture (STEWART *et al.* 1971) as previously described. The levels of iso-2-cytochrome *c* were conveniently estimated by spectroscopic examination of intact cells at low temperature (—190°) (SHERMAN and SLONIMSKI 1964). Because the strains used in this study have no iso-1-cytochrome *c* due to mutations at the *cyc1* locus, the intensity of the c_α band reflects the level of iso-2-cytochrome *c*. The intensities of the c_α band in the mutants were visually compared to the intensities in strains having known amounts of cytochrome *c*.

Quantitative determinations of total cytochrome *c* contents were made by spectrophotometric measurements of extracted cytochrome *c* from known amounts of yeast (SHERMAN, TABER and CAMPBELL 1965).

RESULTS

Origin and properties of CYC7 *mutants overproducing iso-2-cytochrome c*: *Cyc1* strains lack iso-1-cytochrome *c* due to mutations in the structural gene and thus, are unable to utilize lactate as a sole carbon source. Revertants that can utilize lactate arise either by back mutations at the *cyc1* locus or by mutations at any of several loci that cause increased amounts of iso-2-cytochrome *c* (CLAVILIER, PÉRÉ and SLONIMSKI 1969; CLAVILIER *et al.* 1976; STEWART *et al.* 1972; SHERMAN *et al.* 1974). Intragenic revertants usually arise as large colonies on lactate medium and usually have normal levels of cytochrome *c*, while extragenic revertants usually appear as distinctly smaller colonies and usually have diminished levels of cytochrome *c* (SHERMAN *et al.* 1974). However, if intragenic reversion is infrequent or absent or if large numbers of revertant colonies are examined, one observes relatively rare extragenic revertants that form large colonies on lactate medium and that have near-normal levels of cytochrome *c*. These extragenic revertants containing exclusively iso-2-cytochrome *c* can be conveniently disinguished from intragenic revertants containing primarily iso-1-cytochrome *c* by the slightly different spectral properties of iso-2-cytochrome *c* and iso-1-cytochrome *c* and by simple genetic tests that are based on the resistance of *cyc1* mutants to chlorolactate (SHERMAN *et al.* 1974). The three mutants, *CYC7–H1*, *CYC7–H2* and *CYC7–H3*, containing an amount of iso-2-cytochrome *c* ap proximately equivalent to the amount of total cytochrome *c* found in normal cells were uncovered during the course of experiments designed for reverting *cyc1 strains*. The first mutant, *CYC7–H1* (DOWNIE *et al.* 1977), was induced by γ irradiation and was shown to contain a reciprocal translocation with the breakpoint near the structural gene (SHERMAN and HELMS 1978). The second mutant, *CYC7–H2*, was induced by nitrous acid and appeared to contain a single-site mutation near the structural gene, but outside the translated region (SHERMAN *et al.* 1978). We have analyzed a third mutant, *CYC7–H3* (strain number B–1589), that appeared among the revertants from a *cyc1–31* strain treated with diethyl sulfate. Normal spore viability of tetrads from crosses with this mutant, B–1589, indicated that the revertant did not contain any obvious chromosomal aberrations, and segregational analysis indicated that the overproduction was caused by

TABLE 1

Approximate levels of iso-2-cytochrome c *in* **a** *and* α *haploid strains and* **a**/α *diploid strains containing various* CYC7 *genes*

Genetic constitution	*CYC7+*	*CYC7–H1*	*CYC7–H2*	*CYC7–H3*
a *CYC7*	5–10	100–200	80–150	80–150
α *CYC7*	5–10	100–200	80–150	80–150
a *CYC7*/α *CYC7*	5–10	100–200	5–20	80–150

The range of levels of iso-2-cytochrome *c* are presented as the percent of total cytochrome *c* found in normal strains. The results are estimations from numerous strains.

a single mutation located on the left arm of chromosome *V* at the position assigned for the *CYC7* structural locus. Also, no *CYC7*$^{+}$ recombinants having the normal low-level amount of iso-2-cytochrome *c* were observed among the segregants from a *CYC7–H2/CYC7–H3* diploid cross. Thus, the *CYC7–H2* and *CYC7–H3* mutants appeared to be similar or identical in their genetic properties, as well as in their degree of overproduction of iso-2-cytochrome *c*. However, examination of diploid strains homozygous for the various *CYC7* mutations revealed that in contrast to **a** *CYC7–H1*/α *CYC7–H1* and **a** *CYC7–H3*/α *CYC7–H3* strains, the overproduction of iso-2-cytochrome *c* was not fully expressed in **a** *CYC7–H2*/α *CYC7–H2* strains (Table 1). Low-temperature spectroscopic examination of numerous **a** *CYC7–H2*/α *CYC7–H2* strains constructed from different haploid strains revealed a range of iso-2-cytochrome *c* levels from approximately the normal level found in *CYC7*$^{+}$ strains to approximately twice the normal iso-2-cytochrome *c* level. Quantitative determination of iso-2-cytochrome *c* in typical haploid and diploid strains, presented in Table 2, reflects the amount visually estimated by spectroscopic examination in intact cells.

TABLE 2

Amount of iso-2-cytochrome c *in various haploid and diploid strains*

Strain no.	Genotype	Iso-2-cytochrome *c* (mg/kg dry wt.)
Various*	**a** *cyc1 CYC7+* / α *cyc1 CYC7+*	18–50
W216–5A	α *cyc1 CYC7–H2*	511
D860–48A	**a** *cyc1 CYC7–H2*	299
W241	**a** *cyc1 CYC7–H2* / α *cyc1 CYC7–H2*	22
W244–1C	**a** *cyc1 CYC7–H2* / **a** *cyc1 CYC7–H2*	345

* From this investigation and from SHERMAN, TABER and CAMPBELL (1965), GILMORE *et al.* (1971), LIEBMAN *et al.* (1975) and DOWNIE *et al.* (1977).

The expression of CYC7–H2 *in strains homozygous at the mating-type locus*: In addition to examining **a** *CYC7–H2*/α *CYC7–H2* diploid strains, we also examined **a** *CYC7–H2*/**a** *CYC7–H2* and α *CYC7–H2*/α *CYC7–H2* diploid strains in order to determine if the lack of overproduction was due to diploidy itself or to heterozygosity at the mating-type locus. A diploid strain, W241 (**a** *cycl CYC7–H2 leu1 trp2 ura3* × α *cyc1 CYC7–H2 leu1 trp2 his1*), was irradiated with X rays to induce mitotic crossingover at the mating-type locus that was detected by the ability of the resulting clones to mate with either **a** or α tester strains. Since X rays can also induce chromosome loss, the presumptive **a**/**a** and α/α strains were crossed, respectively, to α/α and **a**/**a** tester strains, and the presumptive tetraploids were sporulated, dissected and analyzed for segregation of the mating-type locus. The segregational patterns (ROMAN, PHILLIPS and SANDS 1955) indicated that both **a**/**a** and α/α diploid strains were recovered, as well as aneuploid **a**/0 and α/0 strains, which had presumably lost one or another of the mating-type chromosomes *III*. Spectral examinations demonstrated that both the **a**/**a** and α/α diploid strains, as well as the **a**/0 and α/0 aneuploid strains, contained righ levels of iso-2-cytochrome *c* characteristic of *CYC7–H2* haploid strains. A tetraploid strain, constructed by crossing an **a** *CYC7–H2*/**a** *CYC7–H2* strain and an α *CYC7–H2*/α *CYC7–H2* strain, did not overproduce iso-2-cytochrome *c*. The tetraploid strain was sporulated, dissected, and the levels of iso-2-cytochrome *c* of the segregants were determined. As expected from a tetraploid strain (Table 3), diploid segregants with mating-type configuration **a**/**a**, α/α and **a**/α were observed (ROMAN, PHILLIPS and SAND 1955). As also expected, all segregants homozygous for the mating-type locus overproduced iso-2-cytochrome *c*, while the **a**/α segregants contained the lower level observed in typical **a** *CYC7–H2*/α *CYC7–H2* diploid strains (Table 3).

The importance of the mating-type configuration for expression of the *CYC7–H2* allele was confirmed by isolating overproducing strains from the W241 diploid strain. Approximately 10^8 cells were irradiated with 5 kilorad of X rays

TABLE 3

Segregants from a typical tetraploid ascus

Spore no.	Genotype	Ability to mate × a	Ability to mate × α	Ability to sporulate	Level of iso-2-cytochrome c
1A	**a** *cyc1 CYC7–H2* / **a** *cyc1 CYC7–H2*	—	+	—	100
1B	**a** *cyc1 CYC7–H2* / α *cyc1 CYC7–H2*	—	—	+	5
1C	**a** *cyc1 CYC7–H2* / α *cyc1 CYC7–H2*	—	—	+	5
1D	α *cyc1 CYC7–H2* / α *cyc1 CYC7–H2*	+	—	—	100

and plated on lactate medium, which does not permit the growth of the W241 strain because of its low amount of cytochrome *c*. Three revertants arose on the lactate plate and each of these contained a higher level of iso-2-cytochrome *c*. Two of the revertants exhibited mating ability with an α tester strain, while the third exhibited mating ability with an **a** tester strain. Although these revertants were not tested further, it is clear that the expression of the *CYC7–H2* gene in diploid strains is dependent on the acquisition of mating ability.

In order to test the dominance of the *CYC7–H2* allele, we constructed a *CYC7–H2/CYC7*$^+$ heterozygote homozygous at the mating-type locus. The diploid was constructed by selection of rare matings between α *CYC7–H2* and α *CYC7*$^+$ haploid strains; the α/α genotype was confirmed by the ability to mate with an **a** strain and by the inability to sporulate. Spectral examination of one such α *CYC7–H2*/α *CYC7*$^+$ strain revealed an intermediate level of iso-2-cytochrome *c*, similar to *CYC7–H1/CYC7*$^+$ and *CYC7–H3/CYC7*$^+$ diploid strains, indicating that the *CYC7–H2* allele is also dominant to the wild-type allele.

DISCUSSION

In this investigation, we have examined the unanticipated influence of the mating-type constitution on the *CYC7–H2* mutation that causes an approximately 20-fold overproduction of iso-2-cytochrome *c* in **a** and α haploid strains. Heterozygosity at the mating-type locus, **a**/α or **a**/**a**/α/α, prevents expression of the overproduction; on the other hand, homozygosity, **a**/**a** and α/α, and hemizygosity, **a**/0 and α/0, allows full expression of the *CYC7–H2* mutation equivalent to the expression observed in **a** and α haploid strains. Thus, the overproduction appears to require active expression of one or another of the mating-type alleles.

The mating-type dependence exhibited by the *CYC7–H2* allele is not a general property of strains that overproduce iso-2-cytochrome *c*. There is no decrease in the overproduction of iso-2-cytochrome *c* in **a**/α diploid strains containing either of the other two mutations, *CYC7–H1* and *CYC7–H3*, at the structural locus. In addition, there is no decrease of iso-2-cytochrome *c* levels in **a**/α diploid strains homozygous for either *cyc8* or *cyc9* mutants that cause overproduction of iso-2-cytochrome *c* and that are unlinked to the *CYC7* structural locus (ROTHSTEIN and SHERMAN 1980). The *cyc9* mutation, however, exhibits a mating defect; α *cyc9* strains are sterile, while **a** *cyc9* strains mate normally. We believe that the sterility of α *cyc9* strains is not related to the requirement of mating functions for the expression of the *CYC7–H2* mutation. The overproduction of iso-2-cytochrome *c* occurs not only in **a**/α *cyc9/cyc9* diploid strains, but also in α *cyc9* and α *cyc9 CYC7–H2* strains.

The *CYC7–H2* mutation maps close to (but outside) the structural region of the iso-2-cytochrome *c* gene (SHERMAN *et al.* 1978). Finding the *CYC7–H2* mutation *cis*-dominant in the α *CYC7–H2*/α *CYC7*$^+$ diploid strain and finding the *CYC7–H2* level much higher than the extreme derepressed *CYC7*$^+$ level suggest that the overproduction may be due to an alteration in a promoter-like region of the gene. While the overproduction of iso-2-cytochrome *c* in *CYC7–H1* and

CYC7–H3 mutants was not diminished by **a**/α heterozygosity, the *CYC7–H2* type of mutation may not be rare; the *CYP3–4* mutation reported by CLAVILIER *et al.* (1976) appears in retrospect similar to the *CYC7–H2* mutation. Also, the **a**/α hindrance of overproduction is not restricted to *CYC7* mutants; similar **a**/α effects have been observed with *cargA*$^{+}$*O*^{h}, *cargB*$^{+}$*O*^{h} and *durO*^{h}, which cause constitutive overproduction of, respectively, arginase (DUBOIS *et al.* 1978), ornithine transaminase (DESCHAMPS and WIAME 1979) and urea amidolyase (LEMOINE, DUBOIS and WIAME 1978). Similar to the *CYC7–H2* mutation, each of these mutations is located in the regulatory region adjacent to the structural region. While the mechanism for the lowered expression in **a**/α diploid strains is unknown, it appears as if a certain type of alteration, which can occur at different loci, causes the regulatory regions to respond to signals normally directed towards genes controlling mating functions or diploid functions.

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LITERATURE CITED

CLAVILIER, L., G. PÉRÉ and P. P. SLONIMSKI, 1969 Mise en évidence de plusieurs loci indépendents impliques dans la synthèse de l'iso-2-cytochrome *c* chez la levure. Mol. Gen. Genet. **104**: 195–218.

CLAVILIER, L., G. PÉRÉ-AUBERT, M. SOMLO and P. P. SLONIMSKI, 1976 Réseau d'interactions entre des gènes non liés: regulation synergique ou anatogoniste de la synthese de l'iso-1-cytochrome *c*, de l'iso-2-cytochrome *c* et due cytochrome b_2. Biochimie **58**: 155–172.

DESCHAMPS, J. and J. M. WIAME, 1979 Mating-type effect on *cis* mutations leading to constitutivity of ornithine transaminase in diploid cells of *Saccharomyces cerevisiae*. Genetics **92**: 749–758.

DOWNIE, J. A., J. W. STEWART, N. BROCKMAN, A. M. SCHWEINGRUBER and F. SHERMAN, 1977 The structural gene for yeast iso-2-cytochrome *c*. J. Mol. Biol. **113**: 369–384.

DOWNIE, J. A., J. W. STEWART and F. SHERMAN, 1977 Yeast mutants defective in iso-2-cytochrome *c*. J. Mol. Biol. **117**: 369–386.

DUBOIS, E., D. HIERNAUX, M. GENSON and J. M. WIAME, 1978 Specific induction of catabolism and its relation to repression of biosynthesis in arginine metabolism of *Saccharomyces cerevisiae*. J. Mol. Biol. **122**: 383–406.

GILMORE, R. A., J. W. STEWART and F. SHERMAN, 1971 Amino acid replacements resulting from super-suppression of nonsense mutants of iso-1-cytochrome *c* from yeast. J. Mol. Biol. **61**: 157–173.

HAEFNER, K., 1966 A technique to establish systems of haploid to polyploid *Saccharomyces* strains which are most identical with respect to their chromosome sets and most homozygous. Zeitschr. f. allg. Mikrob. **6**: 86–88.

LEMOINE, Y., E. DUBOIS and J. M. WIAME, 1978 The regulation of urea amidolyase of *Saccharomyces cerevisiae*. Mating type influence on a constitutivity mutation acting in cis. Molec. Gen. Genet. **166**: 251–258.

LIEBMAN, S. W., J. W. STEWART and F. SHERMAN, 1975 Serine substitutions caused by an ochre suppressor in yeast. J. Mol. Biol. **94**: 595–610.

MORTIMER, R. K. and D. C. HAWTHORNE, 1969 Yeast genetics. pp. 385–460. In: *The yeasts*, Vol. 1. Edited by A. H. ROSE and J. S. HARRISON. Academic Press, New York.

ROMAN, H., M. M. PHILLIPS and S. M. SANDS, 1955 Studies of polyploid Saccharomyeces. I. Tetraploid segregation. Genetics **40**: 546–561.

ROTHSTEIN, R. J. and F. SHERMAN, 1980 Genes affecting the expression of cytochrome *c* in yeast: genetic mapping and genetic interactions. Genetics **94**: 871–889.

SHERMAN, F. and C. HELMS, 1978 A chromosomal translocation causing overproduction of iso-2-cytochromo *c* in yeast. Genetics **88**: 689–707.

SHERMAN, F. and C. W. LAWRENCE, 1974 *Saccharomyces*. pp. 359–393. In: *Handbook of Genetics*. Edited by R. C. KING. Plenum Press, New York.

SHERMAN, F. and P. P. SLONIMSKI, 1964 Respiration-deficient mutants of yeast. II. Biochemistry. Biochim. Biophys. Acta **90**: 1–15.

SHERMAN, F., J. W. STEWART, C. HELMS and J. A. DOWNIE, 1978 Chromosome mapping of the *CYC7* gene determining yeast iso-2-cytochrome *c*: Structural and regulatory regions. Proc. Natl. Acad. Sci. U. S. **75**: 1437–1441.

SHERMAN, F., J. W. STEWART, M. JACKSON, R. A. GILMORE and J. H. PARKER, 1974 Mutants of yeast defective in iso-1-cytochrome *c*. Genetics **77**: 255–284.

SHERMAN, F., H. TABER and W. CAMPBELL, 1965 Genetic determination of iso-1-cytochrome *c* in yeast. J. Mol. Biol. **13**: 21–39.

STEWART, J. W., F. SHERMAN, M. JACKSON, F. L. X. THOMAS and N. SHIPMAN, 1972 Demonstration of the UAA ochre codon in bakers' yeast by amino acid replacements in iso-1-cytochrome *c*. J. Mol. Biol. **68**: 83–96.

STEWART, J. W., F. SHERMAN, N. A. SHIPMAN and M. JACKSON, 1971 Identification and mutational relocation of the AUG codon initiating translation of iso-1-cytochrome *c* in yeast. J. Biol Chem. **246**: 7429–7445.

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