JOURNAL OF BACTERIOLOGY, Sept. 1979, p. 1068-1071
0021-9193/79/09-1068/04$02.00/0

Vol. 139, No. 3

Extrachromosomal ψ^+ Determinant Suppresses Nonsense Mutations in Yeast†

SUSAN W. LIEBMAN[1] AND FRED SHERMAN[2]*

Department of Biological Sciences, University of Illinois, Chicago, Illinois 60680,[1] and Department of Radiation Biology and Biophysics, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642[2]

Received for publication 25 May 1979

The extrachromosomal ψ^+ determinant in the yeast *Saccharomyces cerevisiae* enhanced the expression of Mendelian UAA suppressors by 6- to 10-fold. The ψ^+ determinant by itself is a weak UAA suppressor that caused the production of approximately 1% of the normal level of iso-1-cytochrome *c* in a strain containing the UAA mutation *cyc1-72*.

The non-Mendelian factor ψ^+ was first identified by its ability to modify the expression of certain UAA nonsense suppressors in *Saccharomyces cerevisiae* (1, 2). Subsequent investigations established that some UAA and frameshift suppressors are expressed at higher levels in ψ^+ strains than in ψ^- strains (3, 7, 8). In addition, phenotypic suppression of UAA, UAG, and UGA nonsense mutations by paromomycin was found to be greater in ψ^+ strains than in ψ^- strains (11). Genetic analyses have shown that the ψ^+ factor is not coded by nuclear genes (1, 5), by mitochondrial DNA (20), or by the double-stranded RNA controlling the killer phenotype (9). High rates of $\psi^+ \rightarrow \psi^-$ mutations occur when cultures are incubated in hypertonic media (17), and it is believed that such growth conditions do not induce chromosomal mutations. Although the molecular identity of ψ^+ and its site of action is unknown, it appears to be a self-replicating extranuclear determinant that perturbates certain steps in protein synthesis. In this report we demonstrate that the ψ^+ factor can act as a weak UAA suppressor even in the absence of known Mendelian suppressors. The degree of suppression of the ψ^+ determinant and the degrees of stimulation of Mendelian suppressors by the ψ^+ determinant were quantitatively estimated from the amounts of iso-1-cytochrome *c* in *cyc1* mutants.

The levels of iso-1-cytochrome *c* were compared in ψ^+ and ψ^- strains having the *cyc1-72* marker, which contains a UAA codon corresponding to amino acid residue position 66 in iso-1-cytochrome *c* (J. W. Stewart and F. Sherman, unpublished data; see reference 14). Quantitative measurements (Table 1) shows that the ψ^+ factor acts as a UAA suppressor, since the level of iso-1-cytochrome *c* was elevated from an undetectable level in the ψ^- *cyc1-72* strain to 0.8 to 1.2% of the wild-type level in the ψ^+ *cyc1-72* strain. The iso-1-cytochrome *c* from the ψ^+ *cyc1-72* strain was identified by its characteristic chromatographic elution position from ion-exchange columns, and its identity was verified by its absorption maximum at 549.7 nm, which could be distinguished from the iso-2-cytochrome *c* maximum at 549.2 nm. Unfortunately, the lack of a sufficient quantity of a purified sample of iso-1-cytochrome *c* prevented determination of the amino acid residue at the site of the UAA mutation. The results of plating cells on appropriate media indicated that the iso-1-cytochrome *c* from the ψ^+ *cyc1-72* culture did not arise from rare intragenic revertants or from nuclear suppressors. No large colonies, characteristic of intragenic revertants, were observed after 5×10^4 cells were plated on lactate medium; less than 1 in 5×10^4 cells gave rise to chromosomal UAA suppressors capable of suppressing the *lys2-1* marker.

In addition, we observed partial growth of ψ^+ *trp5-48* strains and the lack of growth of ψ^- *trp5-48* strains on tryptophanless medium, suggesting that the UAA mutation *trp5-48* also is suppressed by the ψ^+ determinant. However, ψ^+ does not suppress most UAA markers or suppresses them at a level too low to be detected. The *cyc1-2* mutant, which has a UAA codon corresponding to the amino acid at position 21 in iso-1-cytochrome *c* (J. W. Stewart and F. Sherman, unpublished data; see reference 14) is not suppressed or is suppressed only at a very low level by the ψ^+ determinant. No iso-1-cytochrome *c* was detected in a ψ^+ *cyc1-2* strain by a procedure that would have revealed as low as 0.3% of the normal level (Table 1). Furthermore, there is no evidence with *cyc1* markers and

† Report no. UR-3490-1642.

TABLE 1. *Levels of suppression in ψ^+ and ψ^- strains*

Genotype	Strain no.	Cytochrome *c* concn (mg of cytochrome *c*/kg [dry wt])[a]			Iso-1-cytochrome *c* (% of normal)
		Total	Iso-1	Iso-2	
ψ^+ *cyc1-72*	B-577	36, 39	5	33	0.8–1.2
ψ^- *cyc1-72*	SL381-7C	9, 10	<0.2	8.8	<0.06
ψ^+ *cyc1-2*[b]	D604-2A	17, 21	<1.1	17.9	<0.3
ψ^+ *cyc1-179*	SL82-1D	21, 21	<0.4	20.6	<0.1
ψ^+ *cyc1-2 SUP16-o*[b]	D604-7D	94, 111	78.6	23.4	12–18
ψ^- *cyc1-2 SUP16-o*	SL106-9A	19, 23, 26, 38, 51	6.8	26.2	1.0–1.6
$\psi^+ \frac{cyc1\text{-}2\ SUP11\text{-}o}{cyc1\text{-}2\ +}$	SL-286	114, 130	68.6	53.4	11–16
$\psi^- \frac{cyc1\text{-}2\ SUP11\text{-}o}{cyc1\text{-}2\ +}$	SL-285	44, 50	11.2	36.8	1.7–2.6
CYC1 + normal strains[c]			430–650	6–50	100

[a] Total contents were determined by spectrophotometric measurements of quantitatively extracted cytochrome *c* from a known amount of yeast as described by Sherman et al. (16). The values listed under "Total" are from independent determinations. The relative amounts of iso-1-cytochrome *c* (Iso-1) and iso-2-cytochrome *c* (Iso-2) were determined spectrophotometrically after chromatographic separation (12).

[b] From Liebman et al. (8).

[c] From Sherman et al. (16), Gilmore et al. (6), Stewart et al. (19), Liebman et al. (8), and Downie et al. (4).

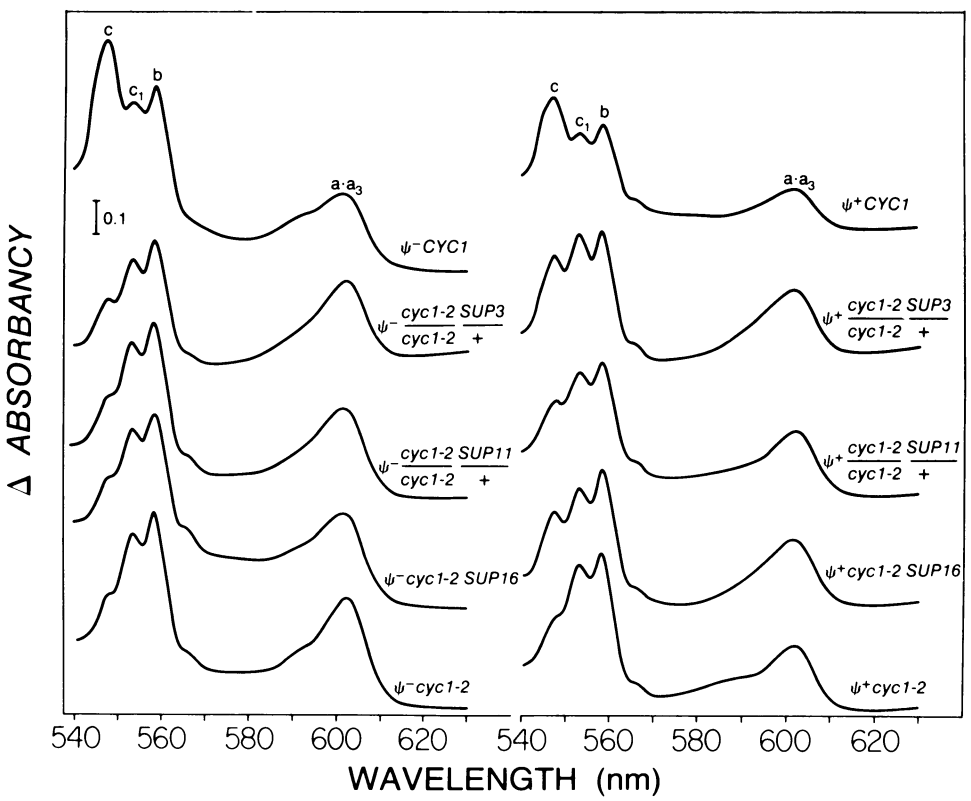

FIG. 1. *Low-temperature (−190°C) spectrophotometric recordings of intact cells of the following strains, demonstrating suppression of cyc1-2 by various UAA suppressors in ψ^+ and ψ^- strains: ψ^- CYC1, normal strain D311-3A; ψ^- cyc1-2 SUP3-o/cyc1-2 sup$^+$, strain SL-221; ψ^- cyc1-2 SUP11-o/cyc1-2 sup$^+$, strain SL-285; ψ^- cyc1-2 SUP16-o, strain SL106-9A; ψ^- cyc1-2, nonsuppressed mutant strain D609-1B; ψ^+ CYC1, normal strain SL111-11C; ψ^+ cyc1-2 SUP3-o/cyc1-2 sup$^+$, strain L-45; ψ^+ cyc1-2 SUP11-o/cyc1-2 sup$^+$, strain SL-286; ψ^+ cyc1-2 SUP16-o, strain D604-7D; and ψ^+ cyc1-2, nonsuppressed mutant strain D604-2A. The absorption maxima of cytochromes a·a$_3$, b, c$_1$, and c are found at 603, 559, 554, and 547 nm, respectively. The efficiencies of suppression can be estimated from the heights of the cytochrome c peaks at 547 nm.*

nutritional markers that ψ^+ suppresses UAG mutations. The *cyc1-179* mutant, which has a UAG codon corresponding to position 9 in iso-1-cytochrome *c* (18), was not suppressed or was suppressed at less than 0.1% of the normal level (Table 1).

The interaction between the ψ^+ determinant and chromosomal suppressors was investigated by measuring the levels of iso-1-cytochrome *c* in ψ^+ and ψ^- strains that contained the UAA mutation *cyc1-2* and one or another of the UAA suppressors *SUP3*-o, *SUP11*-o, and *SUP16*-o. Haploid strains were used to examine the *SUP16*-o suppressor, previously denoted *SUQ5*, which causes insertion of serine residues at UAA sites (8, 10). Because ψ^+ haploid strains grow poorly or are inviable when they contain the *SUP3*-o or *SUP11*-o suppressors, which cause insertion of tyrosine residues at UAA sites (6), iso-1-cytochrome *c* levels were determined in diploid strains that were heterozygous for the suppressors. The amounts of iso-1-cytochrome *c* were determined either by spectrophotometric measurements of quantitatively extracted cytochrome *c* (16) or by low-temperature spectrophotometric recordings of intact cells (15). The UAA suppressors *SUP3*-o, *SUP11*-o and *SUP16*-o acted on the *cyc1-2* mutation approximately 10-fold more efficiently in ψ^+ strains than in ψ^- strains (Fig. 1; Table 1). In contrast, low-temperature spectroscopic examination of the cytochrome *c* level in intact cells indicated that there was no detectable difference between ψ^+ and ψ^- strains containing the UAG mutation *cyc1-179* and the UAG suppressor *SUP7*-a.

The levels of suppression in the ψ^+ *SUP*-UAA strains were considerably greater than the sum of the levels in the individual ψ^- *SUP*-UAA strains and ψ^+ strains (Table 1), indicating a synergistic interaction that intensifies each other's levels of suppression. Because *SUP16*-o inserts serine in both ψ^+ and ψ^- strains (8), it appears that the ψ^+ determinant enhances the level of suppression of the *SUP16*-o suppressor rather than vice versa.

The ψ^+ determinant influences a variety of processes and yet exhibits remarkable specificities. Investigations reported here and elsewhere with nutritional markers and *cyc1* mutants indicate that the ψ^+ factor enhances the expression of UAA and certain frameshift suppressors (3) but not UAG suppressors; that it enhances the phenotypic suppression of all nonsense mutations (UAA, UAG, and UGA) (11); and that it suppresses the UAA mutations *cyc1-72* and *trp5-48* but not other UAA mutations or UAG mutations.

This investigation was supported in part by Public Health Service research grant GM12702 from the National Institutes of Health, in part by American Cancer Society grant PF959, and in part by the U.S. Department of Energy at the University of Rochester, Department of Radiation Biology and Biophysics.

LITERATURE CITED

1. **Cox, B. S.** 1965. A cytoplasmic suppressor of super-suppressor in yeast. Heredity **20**:505–521.
2. **Cox, B. S.** 1971. A recessive lethal super-suppressor mutation in yeast and other ψ phenomena. Heredity **26**: 211–232.
3. **Culbertson, M. R., L. Charmas, M. T. Johnson, and G. R. Fink.** 1977. Frameshifts and frameshift suppressors in *Saccharomyces cerevisiae*. Genetics **86**:745–764.
4. **Downie, J. A., J. W. Stewart, N. Brockman, A. M. Schweingruber, and Fred Sherman.** 1977. Structural gene for yeast iso-2-cytochrome *c*. J. Mol. Biol. **113**:369–384.
5. **Fink, G. R., and J. Conde.** 1977. Studies on *KAR1*, a gene required for nuclear fusion in yeast, p. 414–419. *In* B. R. Brinkley and K. R. Porter (ed.), International cell biology, 1976–1977. First Intern. Congress on Cell Biology, Boston, Mass. Rockefeller University Press, New York.
6. **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.
7. **Liebman, S. W., J. W. Stewart, and F. Sherman.** 1975. The effect of the non-Mendelian factor ψ^+ on the efficiency of nonsense suppression in *Saccharomyces cerevisiae*. Genetics **80**:253.
8. **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.
9. **McCready, S. J., B. S. Cox, and C. S. McLaughlin.** 1977. The extrachromosomal control of nonsense suppression in yeast: an analysis of the elimination of [*psi*$^+$] in the presence of a nuclear gene *PNM*. Mol. Gen. Genet. **150**:265–270.
10. **Ono, B., J. W. Stewart, and F. Sherman.** 1979. Yeast UAA suppressors effective in ψ^+ strains. Serine-inserting suppressors. J. Mol. Biol. **128**:81–100.
11. **Palmer, E., J. M. Wilhelm, and F. Sherman.** 1979. Variation of phenotypic suppression due to the ψ^+ and ψ^- extrachromosomal determinants in yeast. J. Mol. Biol. **128**:107–110.
12. **Sherman, F., S. W. Liebman, J. W. Stewart, and M. Jackson.** 1973. Tyrosine substitution resulting from suppression of amber mutants of iso-1-cytochrome *c* in yeast. J. Mol. Biol. **78**:157–168.
13. **Sherman, F., and J. W. Stewart.** 1971. Genetics and biosynthesis of cytochrome *c*. Annu. Rev. Genet. **5**:257–296.
14. **Sherman, F., and J. W. Stewart.** 1974. Variation of mutagenic action of nonsense mutants at different sites in the iso-1-cytochrome *c* gene of yeast. Genetics **78**: 97–113.
15. **Sherman, F., J. W. Stewart, J. H. Parker, E. Inhaber, N. A. Shipman, G. J. Putterman, R. L. Gardisky, and E. Margoliash.** 1968. The mutational alteration of the primary structure of yeast iso-1-cytochrome *c*. J. Biol. Chem. **243**:5446–5456.
16. **Sherman, F., H. Taber, and W. Campbell.** 1965. Genetic determination of iso-cytochromes *c* in yeast. J. Mol. Biol. **13**:21–39.
17. **Singh, A., C. Helms, and F. Sherman.** 1979. Mutation of the non-Mendelian suppressor, ψ^+, in yeast by hyper-

tonic media. Proc. Natl. Acad. Sci. U.S.A. **76**:1952–1956.
18. **Stewart, J. W., and F. Sherman.** 1972. Demonstration of UAG as a nonsense codon in bakers' yeast by amino acid replacements in iso-1-cytochrome *c*. J. Mol. Biol. **68**:429–448.
19. **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.
20. **Young, C. S. H., and B. S. Cox.** 1972. Extrachromosomal elements in a supersuppression system of yeast. II. Relations with other extrachromosomal elements. Heredity **28**:189–199.