Molecular and Cellular Biology, Jan. 1992, p. 402–412
0270-7306/92/010402-11$02.00/0

Vol. 12, No. 1

NAM9 Nuclear Suppressor of Mitochondrial Ochre Mutations in *Saccharomyces cerevisiae* Codes for a Protein Homologous to S4 Ribosomal Proteins from Chloroplasts, Bacteria, and Eucaryotes

MAGDALENA BOGUTA,[1*] ALEKSANDRA DMOCHOWSKA,[2] PIOTR BORSUK,[2] KATARZYNA WROBEL,[2] ALI GARGOURI,[3] JAGA LAZOWSKA,[3] PIOTR P. SLONIMSKI,[3] BARBARA SZCZESNIAK,[1] AND ANNA KRUSZEWSKA[1†]

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Rakowiecka 36, 02-532 Warsaw,[1] and Department of Genetics, Warsaw University, 00-478 Warsaw,[2] Poland; Centre de Genetique Moleculaire du Centre National de la Recherche Scientifique, 91198 Gif-sur-Yvette, France[3]

Received 26 July 1991/Accepted 24 October 1991

We report the genetic characterization, molecular cloning, and sequencing of a novel nuclear suppressor, the *NAM9* gene from *Saccharomyces cerevisiae*, which acts on mutations of mitochondrial DNA. The strain *NAM9-1* was isolated as a respiration-competent revertant of a mitochondrial *mit* mutant which carries the V25 ochre mutation in the *oxi1* gene. Genetic characterization of the *NAM9-1* mutation has shown that it is a nuclear dominant omnipotent suppressor alleviating several mutations in all four mitochondrial genes tested and has suggested its informational, and probably ribosomal, character. The *NAM9* gene was cloned by transformation of the recipient *oxi1*-V25 mutant to respiration competence by using a gene bank from the *NAM9-1 rho*^{0} strain. Orthogonal-field alternation gel electrophoresis analysis and genetic mapping localized the *NAM9* gene on the right arm of chromosome XIV. Sequence analysis of the *NAM9* gene showed that it encodes a basic protein of 485 amino acids with a presequence that could target the protein to the mitochondrial matrix. The N-terminal sequence of 200 amino acids of the deduced *NAM9* product strongly resembles the S4 ribosomal proteins from chloroplasts and bacteria. Significant although less extensive similarity was found with ribosomal cytoplasmic proteins from lower eucaryotes, including *S. cerevisiae*. Chromosomal inactivation of the *NAM9*$^+$ gene is not lethal to the cell but leads to respiration deficiency and loss of mitochondrial DNA integrity. We conclude that the *NAM9* gene product is a mitochondrial ribosomal counterpart of S4 ribosomal proteins found in other systems and that the suppressor acts through decreasing the fidelity of translation.

Mitochondria possess their own translation apparatus responsible for the synthesis of only a handful of the hundreds of mitochondrial proteins (for a review, see reference 59a). The biogenesis of this apparatus depends on the coordinate expression of both mitochondrial and nuclear genes (9). Although the whole set of tRNAs required for mitochondrial translation and the rRNAs of mitochondrial ribosomes are encoded by the mitochondrial genes, the mitochondrial ribosomal proteins, as well as other elements of the mitochondrial translation system, are encoded by nuclear genes and transported to mitochondria. The proteins of mitochondrial ribosomes differ from those of cytoplasmic ribosomes. Thus, two different sets of nuclear genes code for mitochondrial and cytoplasmic ribosomal proteins (r-proteins). During the last few years, genes for approximately half of the 70 to 80 yeast cytoplasmic r-proteins have been isolated and characterized (for a review, see reference 52). At present, however, very little is known about the structure and organization of genes for the mitochondrial r-proteins (*MRP* genes). So far, sequences for only 10 of 60 to 70 genes for mitochondrial r-proteins have been published (10, 10a, 14, 23, 33, 45–46, 51, 59). Thus, to gain more insight into the structure, chromosomal organization, and evolution of *MRP* genes, a more comprehensive set of genes should be studied.

The genes for mitochondrial r-proteins characterized so far were identified either by direct biochemical approaches or by screening *pet* respiration-deficient nuclear mutants (59). Our approach, called the NAM approach (for nuclear accommodation of mitochondria), for the identification of the nuclear genes involved in mitochondrial biogenesis consists of studying suppressors of mitochondrial respiration-deficient *mit* mutations (13). The suppressor approach can also reveal unexpected interactions between particular mitochondrial and nuclear genes.

Several *nam*-type suppressors were identified previously. *NAM1*, *NAM2*, *NAM7*, and *NAM8* suppressors are involved in both translation and splicing (2, 13, 26, 40). The recessive suppressor mutations *nam3* and ribosome series suppressors (5, 36, 37, 68) seem to be analogous to *Escherichia coli* ribosomal *ram* suppressors in the sense of their allele-specific, gene-nonspecific mode of action (18). Those suppressors presumably act by decreasing the fidelity of translation resulting from the changes in mitochondrial r-proteins. The final proof for that, however, is still lacking. This results from the failure to isolate and clone the appropriate genes, which is difficult because the suppressor mutations are recessive.

This report presents the genetic analysis, followed by molecular cloning and sequencing, of a novel nuclear gene, *NAM9*, which selectively suppresses certain mitochondrial ochre mutations. This is, to our knowledge, the first successful molecular analysis and sequencing of an informational nuclear suppressor of mitochondrial DNA mutations. Interestingly, the putative protein encoded by *NAM9* belongs to the superfamily of S4 r-proteins. The strongest similarity concerns several r-proteins encoded by the chloroplast genomes of higher plants and algae. Significant homology is

* Corresponding author.
† Deceased 18 March 1991.

TABLE 1. *S. cerevisiae* strains used in this study

Strain	Genotype		Reference or source
	Mitochondrial	Nuclear	
11D	*rho*$^+$*mit*$^+$	α *ura3 leu2 his3*	11
777-3A	*rho*$^+$*mit*$^+$	α *op1 ade1 met3 op1*	34
DP1-1B/7/50	*rho*^{0}	α *his1 trp1*	38
JC8/55	*rho*^{0}	**a** *kar1 leu1 can*^{r}	8
MB29-26A/50	*rho*^{0}	**a** *SNT1 his1 trp1*	Meiosis of MB23-73A × 11D
MB35-2B/50	*rho*^{0}	α *SNT1 ura3 leu2 his3 trp1*	Meiosis of MB29-26A/50 × 11D
MB35-21D/50	*rho*^{0}	α *ura3 leu2 his3 trp1*	Meiosis of MB29-26A/50 × 11D
2069-10A	*rho*$^+$	α *met4 ade5 ura4 lys2 leu4 his4 ile7 trp1/tyr1 + phe/tyr7*	D. C. Hawthorne
UM9	*rho*$^+$*mit*$^+$	**a** *his4C rad9*	57
UM9R	*rho*$^+$*oxi1*-V25	**a** *NAM9-1 SNT1 his4C rad9*	Revertant of UM9
MB19-2A	*rho*$^+$*oxi1*-V25	**a** *NAM9-1 SNT1 his4C*	Meiosis of DP1-1B/7/50 × UM9R
MB23-73A	*rho*$^+$*oxi1*-V25	**a** *NAM9-1 SNT1 his4 trp1*	Meiosis of DP1-1B/7/50 × MB19-2A
AB1-4D/V25	*rho*$^+$*oxi1*-V25	α *ade1 met3 op1*	39
CD111	*rho*$^+$*oxi1*-V25	**a** *kar1 leu1 can*^{r}	Cytoductant from JC8/55 × AB1-4D/V25
CD112	*rho*$^+$*oxi1*-V25	α *kar1 ade2 his4-15 can*^{r}	Cytoductant from JC25/60 × CD11
CD114	*rho*$^+$*oxi1*-V25	α *ura3 leu2 his3*	Cytoductant from 11D/50 × CD111
CD113	*rho*$^+$*oxi1*-V25	**a** *ura3 leu2 his3 his1*	Cytoductant from MB35-21D/50 × CD112
CD113/TA18	*rho*$^+$*oxi1*-V25	**a** *ura3 leu2 his3 his1*/pH13 (URA3 NAM9-1)	CD113 transformed with pH13
CD113/TA18/51	*rho*^{0}	**a** *ura3 leu2 his3 his1*/pH13 (URA3 NAM9-1)	EB treatment of CD113/TA18
BS501	*rho*$^+$*oxi1*-V25	**a** *SNT1 ura3 leu2 his3 his1 trp1* α + *ura3 leu2 his3* + +	Diploid MB35-2B/50 × CD114
BS502	*rho*$^+$*oxi1*-V25	**a** *ura3 leu2 his3 his1 trp1* α *ura3 leu2 his3* + +	Diploid MB35-21D/50 × CD114
BS504	*rho*$^+$*mit*$^+$	**a** *ura3 leu2 his3* + α *ura3 leu2* + *trp1*	Diploid 11D × 4a

found to the *Bacillus subtilis* and *E. coli* r-protein S4 and also to the S13 r-protein from *Saccharomyces cerevisiae* cytosol.

MATERIALS AND METHODS

Media. Yeast YPGA, YP10, YPGALA, N3, NOEB40, WO, and WO10 media were those described by Dujardin et al. (13). When necessary, WO minimal medium was supplemented with the appropriate amino acids. For sporulation, SP1 medium containing 0.25% yeast extract, 0.1% glucose, and 0.98% potassium acetate was used. *E. coli* cells were grown on Luria-Bertani media.

Strains. A list of most of the strains of *S. cerevisiae* used is shown in Table 1. Three hundred and thirty-four *mit* mutants used for testing specificity of the suppressor were derived from several laboratories. Most of them were isolated in the 777-3A background. The *cob-box*, *oxi1*, and *oxi2* mutants tested were, with a few exceptions, those listed by Kruszewska and Słonimski in Table 4 of reference 37. The complete list of the *mit* mutants tested can be obtained upon request.

For the transformation with the gene bank, diploid recipient strain BS501 was used. For the transformations with *NAM9* which carried plasmids derived from pAD1, the haploid recipient CD113 strain and the diploid BS502 strain were used. The BS504 diploid strain was used for the integrative transformation with the disrupted copy of the *NAM9* gene. The genotypes and origins of strains are given in Table 1.

For *E. coli* transformation, the strain XL1 *endA1 hsdR17 supE44 thi-1* λ *recA1 gyrA96 relA1* Δ*lac IF proAB lac9* Δ*M15* Tn*10 tetR* was used.

Plasmids. For the yeast vector, YCp50 centromeric plasmid was used (53). The source of the *URA3* gene was pFL44, which was provided by F. Lacroute. *E. coli* pUC18 plasmid was used for disruption experiments (49).

Testing the specificity of the suppressor. Specificity of the suppressor was tested as described by Kruszewska and Słonimski (37). Three hundred and thirty-four *mit* mutants were crossed on complete glucose medium (YPGA) by a replica-cross technique to three different *NAM9-1 rho*^{0} strains, UM9R/50, MB19-2A/50, and CD113/TA18/51. The first two strains carried the *NAM9-1* allele in the chromosome as well as the *SNT1* mutation very closely linked to *NAM9-1*. The last strain carried the *NAM9-1* allele on a plasmid and was devoid of the *SNT1* mutation. All the *mit* mutants were also crossed in parallel to three different control wild-type *rho*^{0} strains with the same genetic background except the *NAM9-1* and *SNT1* mutations: UM9/50, JC8/55, and CD113/TA18/53. The plates with replica crosses, after 1 day of incubation at 28°C, were replicated on N3 glycerol complete medium, and the growth of diploids on this medium was scored after 5 days of incubation at 28°C. The growth on glycerol medium of a diploid from a cross of a given mutant in the first set of crosses including the suppressor and the lack of growth in the case of the diploid with the same mutant in the second set of crosses with the wild-type control strain indicated suppression of the mutant.

Other genetic procedures. Synchronous crosses, induction of the *rho*^{0} mutations, and cytoduction experiments were performed as described by Dujardin et al. (13).

For cytochrome spectra, the cells were grown for 2 days at 28°C on YPGA plates. The spectra were recorded with a Cary 128 spectrophotometer as described by Claisse et al. (6).

Transformation and plasmid isolation. Yeast strains were transformed by the lithium acetate method (31). Yeast DNA was isolated by the method of Nasmyth and Reed (48). *E. coli* transformation was done by the $CaCl_2$ method (43). Plasmid DNAs from *E. coli* were isolated by the alkaline lysis procedure (4) or by lysis with Triton X-100 (7).

DNA manipulations. Standard recombinant DNA techniques were used (43). Specific DNA fragments were recovered from the gel slices by electroelution. Probes were radiolabelled by using the random primer system (15). Hybridization of the DNA bound to nitrocellulose membranes was done as recommended by Maniatis et al. (43). Blots with yeast chromosomes separated by orthogonal-field-alternation gel electrophoresis were kindly provided by R. Maleszka (University of Canberra, Australia). Sequencing was done by using M13 vectors and the dideoxy-chain termination method (56).

Construction of the yeast gene bank. The gene bank was constructed from the DNA of the yeast strain MB19-2A/50 (Table 1). Total DNA was isolated by sodium dodecyl sulfate lysis of the yeast spheroplasts, followed by overnight proteinase K treatment, phenol-chloroform extraction, and ethanol precipitation. After RNase digestion, the DNA was phenol extracted again and ethanol precipitated. DNA was partially digested with endonuclease *Sau*3A and fractionated on a 10 to 40% (wt/wt) sucrose gradient. DNA was recovered from sucrose by ethanol precipitation. Fractions containing 10- to 20-kb fragments were combined and used for ligation with YCp50 vector linearized with *Bam*HI and treated with alkaline phosphatase (60). The ligation mixture was used to transform *E. coli*, yielding 7,700 single colonies. The frequency of the recombinant plasmids in the bank was 65%. The average insert size was estimated to be 14 kb.

RESULTS

Isolation and genetic analysis of the *NAM9-1* mutant. The suppressor *NAM9-1* was isolated by Smolinska (57) after strong ethyl methanesulfonate treatment of the *mit* mutant which carries the V25 ochre mutation (sequenced by Fox and Staempfli [16]) in the *oxil* gene coding for subunit II of cytochrome oxidase. The *NAM9-1* mutant strain was selected as a glycerol-positive colony on glycerol medium at 28°C. The mutant displayed a temperature-sensitive phenotype. It did not grow at all at 36°C on three different types of media containing a nonfermentable substrate (glycerol, ethanol, and lactate) and grew very poorly on the same types of media at 18°C. The *NAM9-1* mutation restored cytochrome *c* oxidase activity, cytochrome aa_3 in the spectrum, and the occurrence of the coxII polypeptide band among the mitochondrial translation products (data not shown).

The nuclear dominant character of the *NAM9-1* mutation was established by genetic methods (13). Analysis of tetrads resulting from respiration-competent diploids issued from the cross of *NAM9-1 rho*^{0} × *NAM9*$^+$ *rho*$^+$ *oxil*-V25 (UM9R/50 × AB1-4D/V25) showed monogenic segregation of the *NAM9-1* mutation. The 2:2 segregation of the *NAM9-1* mutation was confirmed by the analysis of tetrads from the cross of a *NAM9-1 rho*$^+$ *oxil*-V25 strain devoid of the *rad9* mutation (MB19-2A) with the wild-type *NAM9*$^+$ *rho*^{0} strain (DP1-1B/7/50). All 90 tetrads analyzed in this cross showed 2^+:2^- (respiration) segregation, which reflected 2:2 segregation of the *NAM9-1* suppressor.

The nuclear dominant character of the *NAM9-1* suppressor was also confirmed by quantitative analysis of the diploids from the above-mentioned cross. Of 480 diploid colonies tested, only 3.5% were glycerol negative. The latter ones proved to be spontaneous *rho*$^-$ mutants. Thus, the suppressor did not show mitotic segregation characteristic of mitochondrial mutations.

Specificity of the *NAM9-1* suppressor action. The first approach to establish the mechanism of suppressor action was testing its specificity. The action of the *NAM9-1* suppressor on 334 different mitochondrial mutations in the *oxil*, *oxi2*, and *oxi3* genes was tested as described in Materials and Methods. The results are summarized in Table 2. The specificity of the chromosomal *NAM9-1* suppressor was tested with three different suppressor strains. In spite of differences in genetic background, all of them showed identical action spectra.

Inspection of Table 2 shows that the *NAM9-1* action is allele specific and gene nonspecific, suppressing some mutations in all four mitochondrial genes tested. Of 334 mutations tested, 16 were suppressed by *NAM9-1*. Its action spectrum resembled that of the *nam3* suppressor (37). Like the latter suppressor, *NAM9* suppressed some mutations in the nonsplit *oxil* and *oxi2* genes, as well as mutations in the mosaic genes *oxi3* and *cob-box*. In the latter genes, *NAM9* acted exclusively on intron mutations in maturase-encoding regions, like ribosomal recessive suppressors. However, the action spectrum of *NAM9-1* was significantly narrower than the spectrum of ribosomal recessive suppressors. All mutations suppressed by *NAM9-1* were those suppressed by *nam3*, but the latter suppressor acted on a total of 35 mutations from the same collection. The *NAM9* action spectrum differed markedly from the spectrum of the *NAM1* suppressor, which is the only previously isolated gene-nonspecific, allele-specific nuclear dominant suppressor of *mit* mutations. *NAM1* acts exclusively on mutations localized in mitochondrial introns, which suggested direct involvement in splicing (2, 24). The *NAM9* action spectrum refuted the direct involvement in splicing of *NAM9* suppressor and suggested its purely informational, probably ribosomal, character. Table 3 shows that only ochre mutations, but not all of them, are suppressed. This will be analyzed in the Discussion.

Cloning of the *NAM9* gene. To get a better insight into the nature of the *NAM9-1* suppressor and the mechanism of its action, we cloned the *NAM9* mutant gene. A recombinant plasmid bank from the *NAM9-1 rho*^{0} strain MB19-2A/50 was constructed in the shuttle centromeric YCp50 plasmid as described in Materials and Methods. The bank was screened by transforming the recipient yeast diploid strain BS501 which carries the target *oxil*-V25 mutation to respiration competence (Table 1). This strain was particularly suitable because its level of reversion in respect to the *oxil*-V25 mutation was very low (5×10^{-8}). Among the 7,650 Ura$^+$ transformants selected, 5 clones showed a glycerol-positive, plasmid-dependent phenotype. Further analysis resulted in two recombinant plasmids, designated pAD1 and pAD2. They carried 10.4- and 14.6-kb inserts, respectively (Fig. 1). As preliminary restriction analysis had shown that both the inserts had the 10.4-kb fragment in common, the smaller of the two plasmids was used for further molecular analysis. As revealed by Southern blot hybridization, the insert was of yeast genomic origin and it was localized by the orthogonal-field alternation gel electrophoresis technique on chromosome XIV (data not shown).

To prove that the cloned suppressor gene was *NAM9-1* and not another hypothetical suppressor acting on the target *oxil*-V25 mutation, the action spectrum of the suppressor present in the recombinant plasmids was tested. The cloned

TABLE 2. Action spectrum of the *NAM9-1* suppressor on *mit* mutations[a]

Gene (protein)	No. of *mit* mutations:		Mutation suppressed	Position in gene	Suppression efficiency		
					Chromosomal *NAM9-1*		*NAM9-1*-carrying plasmid
	Tested	Suppressed			UM9R/50	CD113/TA18/51	CD113/TA18/51
oxi1 (COX2)	29	5	V10	Exon	+	++	++
			V25	Exon	++	+++	+++
			V44	Exon	+	++	++
			V248	Exon	+++	+++	+++
			V253	Exon	++	+++	+++
oxi2 (COX3)	23	4	V53	Exon	ε	ε	+
			V76	Exon	ε	+	+
			V85	Exon	+	++	+++
			V503	Exon	++	+++	+++
oxi3 (COX1)	95	1	V221	Intron ai1	++	+++	+++
cob-box (cytb)	187	6	G5026	Intron bi2	+	++	++
			M2075	Intron bi2	+	+++	+++
			M2101	Intron bi2	++	+++	+++
			M2491	Intron bi2	++	+++	+++
			M2573	Intron bi2	+	+++	+++
			M4476	Intron bi2	+	+++	+++

[a] Three hundred and thirty-four different respiration-deficient mutations localized in four different mitochondrial genes (see Materials and Methods) were tested for the efficiency of suppression (ε = marginal, + = weak, ++ = moderate, and +++ = strong suppression) in three different nuclear backgrounds; only 16 mutations were suppressed. There is a general agreement between the suppression directed by *NAM9-1* which carries plasmid CD113/TA18/51 (last column) and that by the chromosomal *NAM9-1* gene (preceding columns).

gene suppressed the same 16 mutants as the *NAM9-1* chromosomal suppressor (Table 2), thus arguing strongly for the identity of both genes. The latter conclusion was also substantiated by the genetic mapping of the *NAM9-1* suppressor mutation in the cross with the *met4* rho^0 strain (MB23-73A × 2069-10A/50) on the right arm of chromosome XIV, 28 centimorgans (cM) from the *met4* gene.

TABLE 3. Nucleotide specificity of mitochondrial mutations suppressed by *NAM9-1*[a]

Mutation	Sequence	Reference
Suppressed		
V25	T GGA CAA ACT (Gln)	16
G5026	A AAT TAT TTA (Tyr)	17
M2075	A AAT TTA TCA (Leu)	17
M2573	A AAT TAT TTA (Tyr)	17
Not suppressed		
W91	A ATT TTA ACT (Leu)	41
G55	A GGT TTA AAA (Leu)	41
M6821	A TTA TAT TAT (Tyr)	64
G171	T GGA CAG ATG (Gln)	41

[a] All mutations suppressed to create TAA stop codons. However, several TAA stop codon mutations are not suppressed. The context surrounding the TAA codon, the original sense codon, and the mutated nucleotide are shown. The remainder of the mutations listed in Table 2 were not sequenced.

Localization of the *NAM9-1* gene in the insert. The pAD1 plasmid contained the 10.4-kb insert with many convenient restriction sites. We mapped the region required for *mit* mutation suppression by deletion analysis (Fig. 1). The set of plasmids, derived from pAD1, was introduced into the yeast strains which carry the *oxi1*-V25 target mutation, and the growth of transformants was tested on glycerol medium. Deletions resulting in the loss of suppression were in the 3′ region of the inserts. *Bam*HI, *Bgl*II, and *Hin*dIII sites, present in this region, were found to be located within the suppressor gene. The shortest plasmid which retained the suppressor activity (pH13) had a 4.7-kb insert (Fig. 1).

Inactivation of the chromosomal wild-type $NAM9^+$ allele. The *NAM9* gene exists in a single copy per haploid genome as indicated by Southern analysis (data not shown). To generate a null mutation of the *NAM9* gene and to examine its effects on cell function, we used the one-step gene disruption method (55).

The 2.1-kb *Eco*RI fragment of pH13 was transferred to pUC18, and its internal 0.35-kb *Bgl*II-*Bam*HI fragment was replaced by a 1.1-kb *Bgl*II fragment derived from the pFl44 plasmid which contained the *URA3* marker (Fig. 2). The new plasmid was digested with *Eco*RI and *Hin*dIII and used for transformation of the homozygous diploid BS504 $NAM9^+$/$NAM9^+$ *ura3*/*ura3*. The Ura^+ transformants were examined for the $NAM9^+$::*URA3* disruption by Southern analysis (data not shown). The four diploid Ura^+ transformants were sporulated, and 52 tetrads derived from them were dissected. All four spores were viable with Ura, segregating 2^+:2^-. The Ura^+ ascospores contained the disrupted copy of the $NAM9^+$ allele and the Ura^- ascospores contained the intact copy, as was confirmed by Southern analysis (data not shown). In all tetrads tested, the Ura^+ phenotype cosegregated with a respiration deficiency. This shows that the

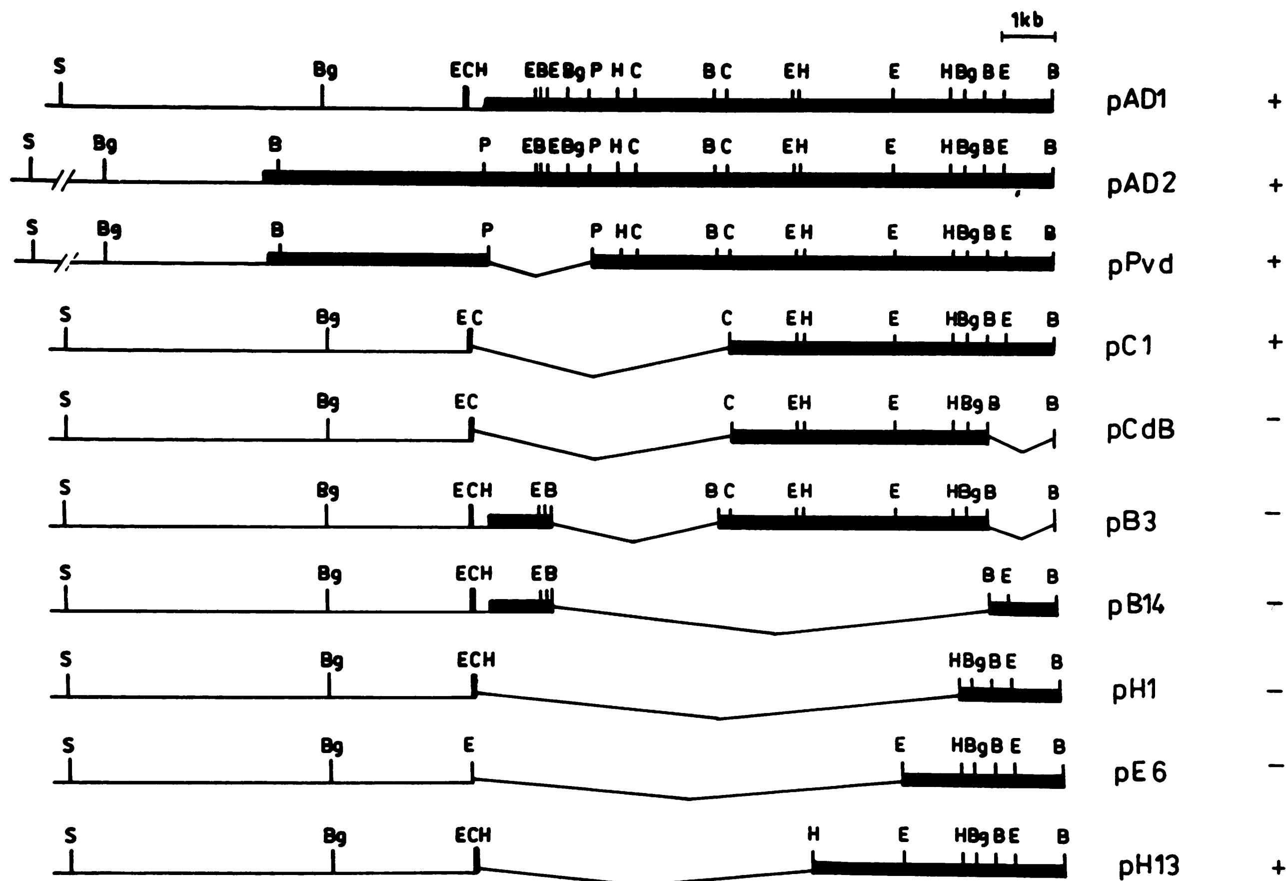

FIG. 1. Restriction maps of the pAD1 and pAD2 plasmids and their derivatives tested for suppression activity on the mitochondrial target mutation *oxi1*-V25. Thinner lines denominate YCp50 vector DNA, while the thicker ones stand for *S. cerevisiae* DNA fragments. +, active suppression present; −, active suppression absent. Abbreviations for restriction sites are as follows: B, *Bam*HI; Bg, *Bgl*II; C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; P, *Pvu*II; S, *Sal*I. In plasmid pAD2, in the fragment which extends pAD1, restriction sites for *Bam*HI, *Eco*RI, and *Cla*I are omitted.

inactivation of the $NAM9^+$ gene is not lethal to the cell but leads to a deficiency in mitochondrial function. The $NAM9^+$::*URA3* allele mapped on chromosome XIV, 28 cM from the *met4* marker. This was independent evidence that integration was in the $NAM9^+$ chromosomal locus. The fact that the *NAM9*::*URA3*/$NAM9^+$ heterozygous diploids were respiration competent indicates that the inactivated allele is recessive to its wild-type counterpart.

It was also shown that the inactivation of the $NAM9^+$ gene leads to the formation of 100% of cytoplasmic petites (either rho^- or rho^0), since all the diploids resulting from the crosses of Ura^+ Gly^- ascospore clones with the wild-type rho^0 tester strains yielded Gly^- diploids. Thus, the intact *NAM9* gene is necessary not only for the expression of the mitochondrial genome but also for its maintenance, and the disruption of the gene leads to a rapid loss of mitochondrial DNA integrity.

Sequence analysis of the *NAM9-1* suppressor gene. The sequence of the 2,400-bp subfragment of the *NAM9*-containing insert from the pH13 plasmid was determined by the dideoxy-chain termination method. The sequencing strategy is given in Fig. 3. The DNA sequence and the deduced amino acid sequence of the *NAM9-1* gene product are shown in Fig. 4. Examination of all six registers in the sequence revealed only one long open reading frame (ORF). It begins with an ATG codon and ends with a TGA opal stop codon and is able to encode a protein of 485 amino acids. The sequence surrounding the predicted initiator codon shows the presence of an A at position −3 and another A at position +4, a feature suggested to be favorable for the efficient initiation of translation (35). The predicted *NAM9-1* gene product is a basic protein (pK_i = 10.22) with a calculated molecular mass of 56,509 Da. The N-terminal stretch of 34 amino acids is devoid of acidic residues but contains positively charged and hydroxylated amino acid residues, properties that are consistent with a possible role as a leader sequence for mitochondrial targeting (29). The codon bias of 485 codons in the ORF of NAM9 is 0.09 (3). This low bias indicates that the *NAM9* gene is expressed at a low level.

The sequence preceding the start of translation of *NAM9* lacks a perfect consensus TATA box. However, at positions −48, −71, −139, and −240, four potential TATA boxes can be found. The sequences proposed to be important for the efficient termination of translation in yeast, TAG. . . TAGT. . .TTT (65), are present downstream of the coding region.

A computer-assisted search for proteins resembling NAM9, performed with FASTA and FASTP programs (42) on the MiPS Library release no. 25, discovered several

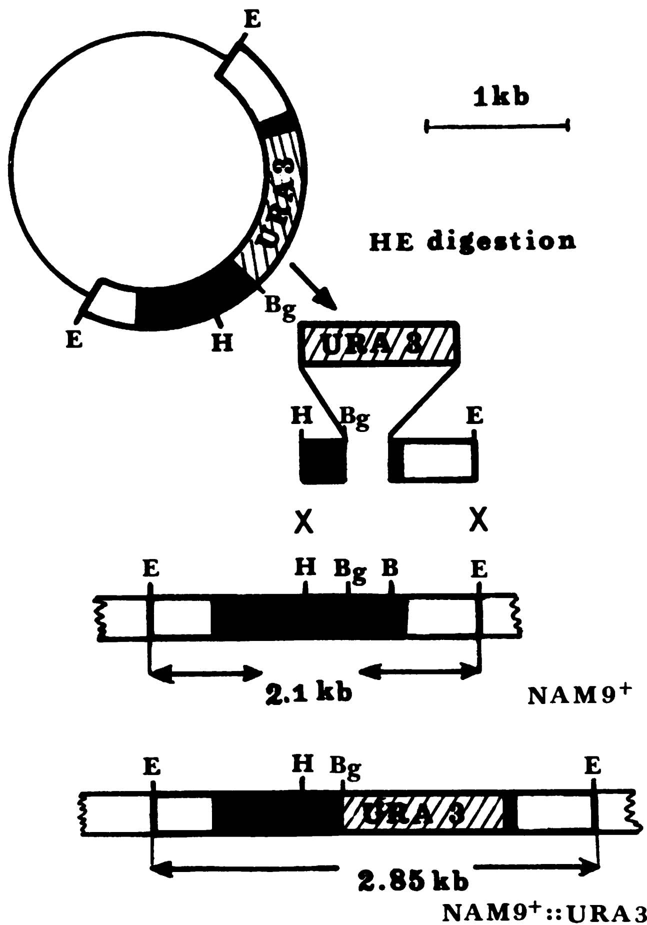

FIG. 2. Schematic representation of the disruption of the *NAM9* gene. The marked *Eco*RI fragments confirmed the presence of wild-type and disrupted copy in the Southern hybridization (data not shown). Abbreviations for restriction sites are the same as for Fig. 1. The thin line represents pUC18 vector, and the black areas represent the coding region of *NAM9*.

proteins belonging to the S4-S13 family of the small ribosomal subunit which are found in chloroplasts of algae or higher plants, procaryotes, and cytoplasmic ribosomes from the yeast. In our computer search, we have been able to add to this family genes from *Dictyostelium discoideum* and *Trypanosoma* species, which have not been, until now, classified as S4 ribosomal proteins (58, 62). The alignment of the most conserved regions of the nine representatives of the S4 family (residues 62 to 142 of the NAM9 protein) is shown in Fig. 5. Five residues (E-R-L-R-S) are completely invariant, and 18 residues show conservative replacements. Figure 6 demonstrates that all nine proteins are members of the same class, since any one of them is significantly similar to all the remaining ones, as judged by the 36 pairwise comparisons in which all display a z value greater than 5. Among all the pairwise comparisons performed, the greatest similarities are observed within three subsets. The first one comprises, as expected, three proteins coded by chloroplast genomes from two higher plants and one alga (several other S4 proteins from tobacco, maize, and spinach chloroplasts belong to this class, but they are almost identical to the preceding ones and therefore are not shown). The second subset comprises S4 proteins from *B. subtilis* and *E. coli* (note that the former is systematically more similar to the chloroplast proteins than the latter). The third subset comprises the cytoplasmic ribosomal S13 protein from *S. cerevisiae*, the gene for which has been isolated as an omnipotent suppressor *SUP46* (63), the r-protein rp1024 from *Dictyostelium discoideum* (58), and a putative protein coded by an ORF interspersing the region encoding fructose biphosphate aldolase in *Trypanosoma brucei* (62). The three latter proteins are practically as similar to each other as are the chloroplast or procaryote S4 proteins, and there is no doubt that they are homologous. Mitochondrial ribosomal NAM9 displays a greater similarity to the chloroplast-procaryote group than to its cytoplasmic counterparts, including the yeast. This closer resemblance of NAM9 to chloroplast/procaryote proteins is consistent and significant since all the z values are between 9 and 16 instead of between 5 and 6 (Fig. 6). We have verified our calculations by using a different computer program based on the profile analysis developed by Gribskov et al. (21). The quality parameter is always 10 units greater in the comparison of NAM9 with chloroplast and/or procaryote proteins than with the cytoplasmic counterparts (data not shown). Possible implications of these relations will be analyzed in the Discussion.

Sequence comparisons described here indicate that the NAM9 protein is a mitochondrial ribosomal homolog of the S13 protein of yeast cytoplasmic ribosomes and the S4 r-proteins from *E. coli* and chloroplasts. However, all the S4 proteins discussed here are much smaller than the *NAM9* gene product, being approximately 200 amino acids long and having a molecular mass of about 23 kDa. This is also true for the SUP46 protein, which is only 196 amino acids long (63). In contrast, NAM9 protein extends for some additional 250 amino acids beyond the region of homology. The function of this long C-terminal extension is unknown, but it is probably essential for the NAM9 activity since the disruption which abolishes it is located in this region.

DISCUSSION

We describe here the genetic characterization, molecular cloning, and sequence analysis of the previously unreported *NAM9* suppressor gene, which alleviates the effects of several mutations in different mitochondrial genes in *S. cerevisiae*.

The deduced 485-amino-acid *NAM9* gene product exhibits all the general properties of an r-protein that can function in the mitochondrial matrix. Moreover, the N-terminal part of the NAM9 protein, approximately 200 amino acids long,

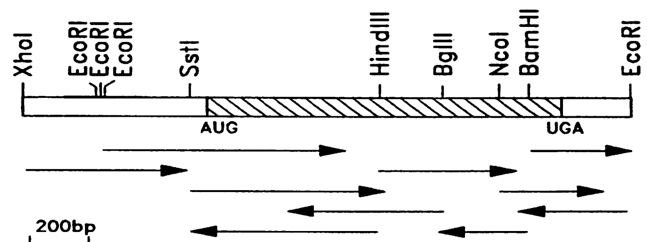

FIG. 3. Restriction map and sequencing strategy for *NAM9*. The arrows indicate the length and direction of the sequences determined by the method of Sanger (56) by using M13 clones. The *NAM9* ORF is indicated by the hatched area.

```
-769 CTCGAGGATACTCTTCGTTAGAGTTTGGACTTTTTCTTGCATTTTCTAAGATCAACTCACCGTATTCCTCCCAAGGTAATGTAAAATCTTGCTTGCGTACTCTTATTCGATTATCAATT

-650 CAAGTAAAGTTTCTTCATGAATTTCTGCATCGTCACCATTTCCATTGCCATTCCGTGAACTCTCTGTAGTGGCTTCCAAGGAATATGTATTCTCCATTTCATCAATTTTCTCTAAATTG

-531 TAAGATTGGCTCAACTTATCCCATATGTCTTTTGCAGTAAATACCTTCCCCAACTTCATGGTTTCTTTCTGTAATAGCGTCACTGGATACTTATCTGGCGAATTCATTCTTTCCACTAT

-412 GCAAAACATGTGGAAATGCTTATGAATTCCGGCCGGTTTGAATTCAGATGCCCATCTAAGAAGTCGTATTTCATCCACTATTGTCCAATGTACTACCATCACATCCACCGATTCCCTTT

-293 TCTTCACTAGCAGCTAGCAACTTCTAATAAACTCCGCCAATCCATCTTCATAGATTATATGATTGATTTCTTCGTTAAGCACGATTTTACTTTTCATGGTGGCAGCCGGATACGTTTCG

-174 CTTCGCAGTATTAGTTAAGGAACTATGCTGAAGATATACAAAAAGAGAGAGCGAACGGTCAAAAAGGTGAAGGTTTCCCCAGAGGAAGTGCAACTAATTCGAATATATATATCGAGTAGGA

-55  GCTCCTTTATACTTTTTTGGTACGGAAGACTGGTTACAATTAAAATACAGAGATA ATG CCA AGA AAG GCT AAT TTG CTA AAA TCT TTG GCA AGA GGA CGA GTA
                                                             Met Pro Arg Lys Ala Asn Leu Leu Lys Ser Leu Ala Arg Gly Arg Val   16

49   CGC ACT TCT TTC AAT AAG TAT AAT TTG TTT AAT TTG TAC AAA AAG GGT GGT GTA GAC TTG AAG TCT AAA TCT TTG TAT CAA CAG AAG TGG
     Arg Thr Ser Phe Asn Lys Tyr Asn Leu Phe Asn Leu Tyr Lys Lys Gly Gly Val Asp Leu Lys Ser Lys Ser Leu Tyr Gln Gln Lys Trp   46

189  ACG GCA AAG CAG GAA ACC CGA GCT TAT CAT GGT GAA CAT TTG ACA GAA AAG AGA TGG CAA ACC GTT TTC AAA CCC AAA TTG GAT TCA GTA
     Thr Ala Lys Gln Glu Thr Arg Ala Tyr His Gly Glu His Leu Thr Glu Lys Arg Trp Gln Thr Val Phe Lys Pro Lys Leu Asp Ser Val   76

229  GCA CAT TGG ATG CTT TAC TGC GTG GAG GTG AAA TCA AGG AAA CAC CAA TTT TTA TTA CAA ACT TTT GCA GTA TTA GAA AAA AGG CTT GAT
     Ala His Trp Met Leu Tyr Cys Val Glu Val Lys Ser Arg Lys His Gln Phe Leu Leu Gln Thr Phe Ala Val Leu Glu Lys Arg Leu Asp  106

319  TTT GCC CTT TTC AGG GCT ATG TTT GCT TCG TCA GTA AGA CAA GCT CGT CAG TTC ATC TTG CAT GGA AAT GTT CGC GTT AAT GGT GTA AAA
     Phe Ala Leu Phe Arg Ala Met Phe Ala Ser Ser Val Arg Gln Ala Arg Gln Phe Ile Leu His Gly Asn Val Arg Val Asn Gly Val Lys  136

409  ATT AAG CAT CCA AGC TAT ACT TTA AAG CCT GGA GAT ATG TTC AGT GTC AAA CCG GAC AAA GTG CTG GAA GCT CTC GGT GCC AAG AAA CCA
     Ile Lys His Pro Ser Tyr Thr Leu Lys Pro Gly Asp Met Phe Ser Val Lys Pro Asp Lys Val Leu Glu Ala Leu Gly Ala Lys Lys Pro  166

499  AGT TTC CAA GAA GCG CTA AAG ATC GAT AAG ACT CAA ATA GTT TTA TGG AAC AAG TAT GTT AAG GAA GCG AAA ACG GAG CCA AAG GAA GTT
     Ser Phe Gln Glu Ala Leu Lys Ile Asp Lys Thr Gln Ile Val Leu Trp Asn Lys Tyr Val Lys Glu Ala Lys Thr Glu Pro Lys Glu Val  196

589  TGG GAG AAG AAG TTG GAA AAT TTT GAA AAG ATG TCG GAT TCT AAC CCA AAG AAA CTT CAG TTT CAA GAA TTT TTG AGG CAA TAT AAT AAG
     Trp Glu Lys Lys Leu Glu Asn Phe Glu Lys Met Ser Asp Ser Asn Pro Lys Lys Leu Gln Phe Gln Glu Phe Leu Arg Gln Tyr Asn Lys  226

679  AAT CTG GAG TCA CAG CAA TAT AAC GCA TTA AAG GGA TGC ACA CAG GAA GGT ATC TTG AGA AAG CTT TTA AAT GTA GAG AAG GAA ATA GGT
     Asn Leu Glu Ser Gln Gln Tyr Asn Ala Leu Lys Gly Cys Thr Gln Glu Gly Ile Leu Arg Lys Leu Leu Asn Val Glu Lys Glu Ile Gly  256

769  AAG TCG AAT AAT GAG CCC TTG TCG ATA GAT GAA CTC AAA CAG GGT CTC CCT GAG ATC CAG GAC TCT CAG TTG TTA GAA AGT CTA AAT AAC
     Lys Ser Asn Asn Glu Pro Leu Ser Ile Asp Glu Leu Lys Gln Gly Leu Pro Glu Ile Gln Asp Ser Gln Leu Leu Glu Ser Leu Asn Asn  286

859  GCT TAT CAA GAG TTT TTC AAA TCC GGT GAA ATT AGA AGA GAA ATC ATT TCT AAA TGT CAG CCT GAC GAG TTG ATT TCA CTG GCT ACA GAA
     Ala Tyr Gln Glu Phe Phe Lys Ser Gly Glu Ile Arg Arg Glu Ile Ile Ser Lys Cys Gln Pro Asp Glu Leu Ile Ser Leu Ala Thr Glu  316

949  ATG ATG AAT CCT AAC GAA ACC ACA AAA AAA GAA TTA TCT GAT GGA GCC AAA TCC GCT TTA AGA TCT GGA AAA GAT CAT AGC GGA AAG CGT
     Met Met Asn Pro Asn Glu Thr Thr Lys Lys Glu Leu Ser Asp Gly Ala Lys Ser Ala Leu Arg Ser Gly Lys Asp His Ser Gly Lys Arg  346

1039 GAA ACT ATG GAC GAA AAT ATA CAG ACC ACT TTC AAA ACT AGG ATG AGT GAT ATT TCT GAT GGT TCA CTA ACG TTC GAC CCC AAA TGG GCC
     Glu Thr Met Asp Glu Asn Ile Gln Thr Thr Phe Lys Thr Arg Met Ser Asp Ile Ser Asp Gly Ser Leu Thr Phe Asp Pro Lys Trp Ala  376

1129 AAA AAT TTA AAA TAT CAT GAT CCG ATT AAA TTA TCT GAA TTG GAA GGT GAT GAA CCA AAA GCA CGT AAA TTG ATA AAC TTG CCG TGG CAG
     Lys Asn Leu Lys Tyr His Asp Pro Ile Lys Leu Ser Glu Leu Glu Gly Asp Glu Pro Lys Ala Arg Lys Leu Ile Asn Leu Pro Trp Gln  406

1209 AAA AAT TAT GTT TAT GGT AGG CAA GAT CCT AAA AAA CCC TTT TTC ACA CCA TGG AAG CCA AGA CCA TTT TTA TCG CCT TTC GCC ATT TTA
     Lys Asn Tyr Val Tyr Gly Arg Gln Asp Pro Lys Lys Pro Phe Phe Thr Pro Trp Lys Pro Arg Pro Phe Leu Ser Pro Phe Ala Ile Leu  436

1299 CCT CAT CAT TTG GAA ATA TCT TTC AAG ACA TGC CAC GCT GTA TAC CTA AGG GAT CCC GTC GCT CGA CCA GGC CAA TCC GAA GTA ATT TCA
     Pro His His Leu Glu Ile Ser Phe Lys Thr Cys His Ala Val Tyr Leu Arg Asp Pro Val Ala Arg Pro Gly Gln Ser Glu Val Ile Ser  466

1389 CCA TTT GAT GTT CCT GTT CAT GAA CGT GCA TAT ATG TAT TAC TTG AGA AAT GGT AAA TGA TCGTCGTGTATTGTTGCACGCGTCTTTTGACTGATCTTC
     Pro Phe Asp Val Pro Val His Glu Arg Ala Tyr Met Tyr Tyr Leu Arg Asn Gly Lys ---

1489 AGCCTCTCTTTACACTGTAAATATTTAATTTTTTTTGTTTTTAAGTTCTACTCGAACGATCAAGTAGTCACTTAGTCGCTCCTCGTTTTCCTGTTACTTCTTTAAGAAAAGGTTACACA
```

FIG. 4. Nucleotide sequence of the *NAM9* gene. The gene is shown with its upstream and downstream flanking sequences. The nucleotide sequence is numbered on the left. The deduced amino acid sequence of the *NAM9* ORF is indicated under the DNA sequence and numbered on the right.

displays highly significant similarity to various members of the S4 superfamily of r-proteins from different organisms (Fig. 5 and 6). These findings allow us to conclude that NAM9 codes for a mitochondrial r-protein which is an analog of the yeast cytoplasmic S13 r-protein as well as the S4 r-proteins from chloroplasts and bacteria.

The resemblance of the effects of the *NAM9-1* mutation to the effects of the mutations in the *E. coli ramA* and the yeast *SUP46* genes gives further support to this conclusion. The *ramA* mutation in the *E. coli* gene coding for the S4 protein and the *SUP46* mutation in the yeast gene coding for the S13 cytosolic r-protein are both, like the *NAM9-1* mutation, gene-nonspecific, allele-specific informational suppressors, acting by decreasing the accuracy of translation (30, 44, 54, 67). In line with our conclusion that the *NAM9* suppressor gene encodes the mitochondrial ribosomal counterpart of the S4 protein is the action spectrum of the *NAM9* suppressor. Table 3 shows that all mutations which are suppressed by *NAM9-1* are ochre, resulting from a single nucleotide substitution. Interestingly, several other TAA stop codon mutations are not suppressed. The molecular basis of the selective effects of *NAM9-1* is not known, but several hypotheses can already be eliminated. (i) It is not the nature of the original codon and/or amino acid, which gave rise to the stop codon since the mutation M2075 is suppressed while W91 and G55 are not (and all create TAA from TAT); furthermore, the mutation M2573 is suppressed while M6821 is not (TAA from TAT). (ii) It is not the nature of the upstream codon, ATT in M2075 (which is suppressed) and in W91 (which is not suppressed). (iii) It is not the nature of the downstream codon, since V25 is suppressed while W91 is not and both have ACT. It is well established that informational suppression depends on the nucleotide context surrounding the suppressed stop codon (see discussion in reference 37). Inspection of Table 3 suggests such a possible context effect. All the suppressed mutations have the same

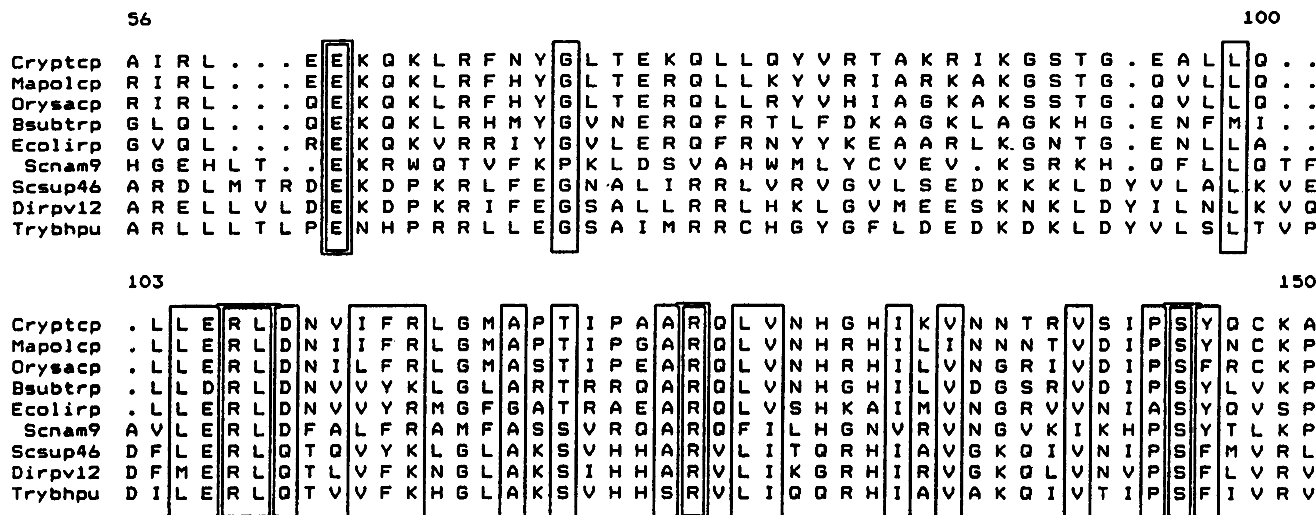

FIG. 5. Alignment of nine r-proteins from different organisms belonging to the S4 family. Sequences were aligned by using the program CLUSTAL (28) (Sharp, 1988). Abbreviations: Cryptcp, chloroplast S4 proteins from *Cryptomonas* sp. (12); Mapolcp, chloroplast S4 proteins from *Marchantia polymorpha* (50); Orysacp, chloroplast S4 proteins from *Oryza sativa* (20); Ecolirp, S4 protein from *E. coli* (1); Scnam9 *S. cerevisiae* NAM9 protein (this work; Fig. 4); Scsup46, S13 protein from the cytosolic yeast ribosome (63a); Bsubtrp, r-protein from *B. subtilis* (25); Dirpv12, *Dictyostelium* r-protein (58); Trybhpu, *Trypanosoma brucei* hypothetical protein (62). Boxed residues show similarity within the single group of Dayhoff classification. Residues boxed with double lines show identity among all nine sequences. The numbers refer to the NAM9 protein.

nucleotide 5′ and 3′ around the TAA (either T. . .T or A. . .A), while none of the nonsuppressed ones displays this property (G171, which is A. . .A, is amber, not ochre). However, this context effect should be viewed with caution until it is further substantiated, since the sample of mutations sequenced is still limited.

What we have cloned and sequenced is the active suppressor gene (*NAM9-1*) and not the wild-type gene ($NAM9^+$),

	Orysacp	Mapolcp	Bsubrp	Ecolirp	Scnam9	Scsup46	Dirpr12	Trybhpu
Cryptcp	60	106	47	21	15	13	11	16
	66	79	47	39	11	9	8	11
Orysacp		82	40	23	14	17	11	13
		82	43	25	11	10	8	10
Mapolcp			40	18	12	12	10	15
			34	36	9	9	8	9
Bsubrp				51	16	15	14	15
				58	12	9	8	12
Ecolirp					12	12	8	11
					9	8	5	8
Scnam9						5	5	6
						5	6	6
Scsup46							78	66
							56	63
Dipr12								88
								57
Trybhpu								

FIG. 6. Sequence similarity matrix for nine r-proteins from different organisms belonging to the S4 family. The figure shows 36 pairwise comparisons between the r-proteins from plant chloroplasts, bacteria, yeast mitochondria, yeast cytosol, and protists. The statistical significance of each comparison of two sequences was estimated by the RDF program of Lipman and Pearson (41), which generates the z value (similarity score, mean of random scores)/standard deviation of random scores (Ktup = 1,100 shuffled sequences). The upper values are the z values corresponding to the initial scores, and the lower values are z values corresponding to the aligned scores. A z value > 4 is believed to be biologically significant. Other abbreviations are as in the legend to Fig. 5.

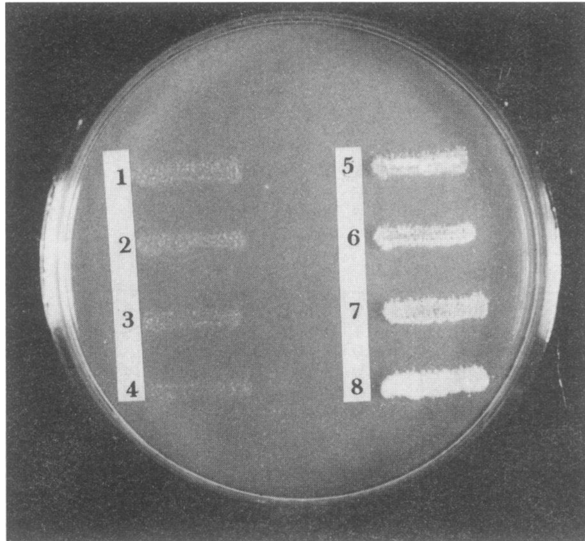

FIG. 7. The restoration of respiratory growth on glycerol medium due to the *NAM9-1* mutation. (1) CD113 (*NAM9*$^+$ *oxi1*-V25, haploid); (2) CD113 transformed with the control YCp50 vector; (3) BS501 (*NAM9*$^+$/*NAM9*$^+$ *oxi1*-V25, diploid); (4) BS504/TA3-1B (*NAM9*$^+$::*URA3*, haploid); (5) CD113 transformed with pH13 plasmid which carries *NAM9-1*; (6) MB19-2A (*NAM9-1 oxi1*-V25, haploid); (7) MB19-2A/50 × AB1-4D/V25 (*NAM9-1*/*NAM9*$^+$ *oxi1*-V25, diploid); (8) 11D, wild-type *mit*$^+$, haploid. The genotypes of strains are given in Table 1. The plate was incubated for 4 days at 28°C.

which is devoid of suppressor activity. In *E. coli*, one of the *ram* mutations which acts as an informational suppressor is due to the replacement of glutamine 58 by leucine 58 (61). The experiments leading to the establishment of the molecular basis of the *NAM9-1* mutation are in progress.

Our data clearly demonstrate that *NAM9-1* is dominant. This is in contrast to the S4 *ram* mutations in *E. coli*, in which the ambiguity mutants are recessive (19). We found that the growth of the heterologous diploid *NAM9-1*/*NAM9*$^+$ *oxi1*-V25 is even better than that of haploid *NAM9-1 oxi1*-V25 (Fig. 7). This can be explained by the presence of both types of r-proteins, one ensuring the suppression of the stop codon (*NAM9-1*) and the other ensuring the nonambiguous translation of the remaining mRNAs.

The NAM9 protein is approximately twice as large as its counterparts found in the other systems. At present, we do not know the role of the extra C-terminal part of the NAM9 protein, which lacks significant homology to any known sequence. However, the disruption experiments presented here indicate that this part of the *NAM9* gene product is indispensable for mitochondrial function and maintenance of the mitochondrial genome. The disruption of the *NAM9* gene with the *URA3* marker in its 3′ half led to the respiration-deficient phenotype and the mitochondrial petite (*rho*$^-$ or *rho*^{0}) genotype. Respiration deficiency accompanied by a rapid loss of mitochondrial genome integrity was found to be a characteristic feature of the inactivation of the genes coding for the elements of the mitochondrial translation apparatus (33, 40, 47).

Interestingly, of four yeast mitochondrial r-proteins reported so far to reveal significant homology to some *E. coli* r-proteins, three (MRP7, MRPL20, and MRPS28p) are much larger than their bacterial counterparts (10, 14, 33). Similarly, for the NAM9 protein, the sequence homology included only the N-terminal part of the protein. Multifunctionality has been postulated to be a possible explanation for why these proteins are larger than their *E. coli* counterparts. Single proteins with multiple enzymatic activities were already reported for yeasts, and it was proposed that they might have arisen by fusion of the genes for smaller proteins (32).

It was already suggested that there are two groups of mitochondrial r-proteins with different degrees of evolutionary divergence: those with conserved primary sequence domains due to functional constraints and those which evolve faster (46). On this basis, NAM9 protein in its N-terminal half can be assigned to the first group. A good conservation of the S4 protein throughout different systems is consistent with its crucial role for the ribosome assembly found in *E. coli*. In reconstitution experiments, *E. coli* r-protein S4 is among the first components to bind with the 16S rRNA in its 5′ domain, which appears to be essential for the structural integrity of the 30S subunit (66).

The evolutionary origin of mitochondria was the subject of many controversies, but at present the endosymbiotic theory of their descendence appears the most plausible (20, 22). The strongest arguments in favor of this theory came from the resemblance of the mitochondrial and procaryotic translation systems as well as from the finding that some mitochondrial enzymes resemble their bacterial counterparts more than their cytoplasmic analogs acting in the same eucaryotic organism. It is relevant in this regard to underline the fact that the NAM9 protein is significantly more similar to its bacterial and chloroplast homologs than to the eucaryotic cytoplasmic ones (Fig. 6).

In conclusion, we found here that the nuclear *NAM9* gene codes for a mitochondrial r-protein belonging to the superfamily of S4 r-proteins common to chloroplasts, bacteria, and lower eucaryotes. The *NAM9-1* suppressor acts by decreasing the fidelity of translation due to the changes in the mitochondrial ribosomal counterpart of S4, but the exact mechanism of the suppressor action, which is selective for a specific subset of ochre mutations, remains to be elucidated.

ACKNOWLEDGMENTS

We are grateful to R. Maleszka for help in the initial part of the work and to C. Herbert for helpful discussions and the critical reading of the manuscript. J. Brzywczy is gratefully acknowledged for help in conducting computer homology searches. We are grateful to S. Liebman for communicating the SUP46 sequence prior to publication.

This work was supported by the Polish Academy of Sciences under project 3.13 and by a grant from CNRS Jumelage Franco-Polonais.

REFERENCES

1. **Bedwell, D., G. Davis, M. Gosink, L. Post, M. Nomura, H. Kestler, J. M. Zengel, and L. Lindah.** 1985. Nucleotide sequence of the alpha ribosomal protein operon of *Escherichia coli*. Nucleic Acids Res. **13**:3891–3903.
2. **Ben-Asher, E., O. Groudinsky, G. Dujardin, N. Altamura, M. Kermorgant, and P. P. Slonimski.** 1989. Novel class of nuclear genes involved both in mRNA splicing and protein synthesis in *Saccharomyces cerevisiae* mitochondria. Mol. Gen. Genet. **215**:517–528.
3. **Bennetzen, J. F., and D. D. Hall.** 1982. Codon selection in yeast. J. Biol. Chem. **257**:3026–3031.
4. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1523.
5. **Boguta, M., M. Mieszczak, and W. Zagorski.** 1988. Nuclear omnipotent suppressors of premature termination codons in

mitochondrial genes affect the 37S mitoribosomal subunit. Curr. Genet. **13**:129–135.

6. **Claisse, M. L., and P. F. Pajot.** 1974. Presence of cytochrome c in cytoplasmic "petite" mutants of *Saccharomyces cerevisiae*. Eur. J. Biochem. **49**:49–59.
7. **Clevell, D. B., and D. R. Helinski.** 1969. Supercoiled circular DNA-protein complex in *Escherichia coli*: purification and induced conversion to an open circular DNA form. Proc. Natl. Acad. Sci. USA **62**:1159–1166.
8. **Conde, J., and G. R. Fink.** 1976. A mutant of *Saccharomyces cerevisiae* defective for nuclear fusion. Proc. Natl. Acad. Sci. USA **73**:3651–3655.
9. **Constanzo, M. C., and T. D. Fox.** 1990. Control of mitochondrial gene expression in *Saccharomyces cerevisiae*. Annu. Rev. Genet. **24**:91–113.
10. **Dang, H., and S. R. Ellis.** 1990. Structural and functional analyses of a yeast mitochondrial ribosomal protein homologous to the ribosomal protein S15 of *Escherichia coli*. Nucleic Acids Res. **18**:6895–6901.

10a. **Dang, H., G. Franklin, K. Darlak, A. Spatola, and S. R. Ellis.** 1990. Discoordinate expression of the yeast mitochondrial ribosomal protein MRP1. J. Biol. Chem. **265**:7449–7459.

11. **Dmochowska, A., D. Dignard, D. Henning, D. Thomas, and H. Bussey.** 1987. Yeast KEX1 gene encodes a putative protease with a carboxypeptidase-like function involved in killer toxin and α factor precursor processing. Cell **50**:573–584.
12. **Douglas, S. E., and D. G. Durnford.** 1990. Nucleotide sequence of the genes for ribosomal protein S4 and tRNA-Arg from the chlorophyll c-containing alga *Cryptomonas phi*. Nucleic Acids Res. **18**:1903–1903.
13. **Dujardin, G., P. Pajot, O. Groudinsky, and P. P. Slonimski.** 1980. Long range control circuits within mitochondria and between nucleus and mitochondria. I. Methodology and phenomenology of suppressors. Mol. Gen. Genet. **179**:469–482.
14. **Fearon, K., and T. L. Mason.** 1988. Structure and regulation of a nuclear gene in *Saccharomyces cerevisiae* that specifies MRP7, a protein of the large subunit of the mitochondrial ribosome. Mol. Cell. Biol. **8**:3636–3646.
15. **Feinberg, A. P., and B. Vogelstein.** 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132**:6–13.
16. **Fox, T. D., and S. Staempfli.** 1982. Suppressor of yeast mitochondrial ochre mutations that maps in or near the 15S ribosomal RNA gene of mtDNA. Proc. Natl. Acad. Sci. USA **79**:1583–1587.
17. **Gargouri, A. F.** 1989. Recherches sur les introns de l'ADN mitochondial chez la levure Saccharomyces cerevisiae: mutations, suppressions et deletions genomiques d'introns. These de Doctorat. Institute of Molecular Genetics, Gif-sur-Yvette, France.
18. **Gorini, L.** 1969. The contrasting role of *strA* and *ramA* gene products on ribosomal functioning. Cold Spring Harbor Symp. Quant. Biol. **34**:101–112.
19. **Gorini, L.** 1974. Streptomycin and misreading of genetic code, p. 791–803. *In* N. Nomura, A. Tissieres, and P. Lengyel (ed.), Ribosomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. **Gray, M. W., and W. F. Doolittle.** 1982. Has the endosymbiont hypothesis been proven? Microbiol. Rev. **46**:1–42.
21. **Gribskov, M., A. D. McLachan, and D. Einsenberg.** 1987. Profile analysis: detection of distantly related proteins. Proc. Natl. Acad. Sci. USA **84**:4355–4358.
22. **Grivell, L. A.** 1983. Mitochondrial gene expression, p. 25–46. *In* R. J. Schweyen, K. Wolf, and F. Kaudewitz (ed.), Mitochondria 1983. Walter de Gruyter, Berlin.
23. **Grohman, L. H., H. Graack, and M. Kitakawa.** 1989. Molecular cloning of the nuclear gene for mitochondrial ribosomal protein YmL31 from *Saccharomyces cerevisiae*. Eur. J. Biochem. **183**:155–160.
24. **Groudinsky, O., G Dujardin, and P. P. Slonimski.** 1981. Long range control circuits within mitochondria and between nucleus and mitochondria. II. Genetic and biochemical analyses of suppressors which selectively alleviate the mitochondrial intron mutations. Mol. Gen. Genet. **18**:493–503.
25. **Groundy, F. J., and T. M. Henkin.** 1990. Cloning and analysis of the *Bacillus subtilis rpsD* gene encoding ribosomal protein S4. J. Bacteriol. **172**:6372–6379.
26. **Herbert, C., G. Dujardin, M. Labouesse, and P. P. Slonimski.** 1988. The NAM2 protein from *S. cerevisiae* and *S. douglassi* are mitochondrial leucyl-tRNA synthetases and are involved in mRNA splicing. EMBO J. **7**:473–483.
27. **Hiaratsuka, J., H. Shimada, R. Whittier, T. Ishibashi, M. Sakamoto, M. Mori, C. Kondo, Y. Honji, C.-R. Sun, B.-Y. Meng, Y.-Q. Li, A. Kanno, Y. Nishizawa, A. Hirai, K. Shinozaki, and M. Sugiura.** 1989. The complete sequence of the rice (*Oryza sativa*) chloroplast genome: intermolecular recombination between distinct tRNA genes accounts for a major plastid DNA inversion during evolution. Mol. Gen. Genet. **217**:185–194.
28. **Higgins, D. G., and P. M. Sharp.** 1988. CLUSTAL: a package for performing multiple sequence alignment on a microcomputer. Gene **73**:237–244.
29. **Hurt, E. C., B. Pesold-Hurt, K. Suda, W. Opplinger, and G. Schatz.** 1985. The first twelve amino acids (less than half of the presequence) of an imported mitochondrial protein can direct mouse cytosolic dihydrofolate reductase into the yeast mitochondrial matrix. EMBO J. **4**:2061–2068.
30. **Ishiguro, J., B. I. Ono, M. Masurekar, C. S. McLaughin, and F. Sherman.** 1981. Altered ribosomal protein S11 from the *SUP46* suppressor of yeast. J. Mol. Biol. **147**:391–397.
31. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153**:163–168.
32. **Jones, E. W., and G. R. Fink.** 1982. Regulation of amino acid and nucleotide biosynthesis in yeast, p. 181–299. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*: metabolism and gene expression. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
33. **Kitakawa, M., L. Grohmann, H. Graack, and K. Isono.** 1990. Cloning and characterization of nuclear genes for two mitochondrial ribosomal proteins in *Saccharomyces cerevisiae*. Nucleic Acids Res. **18**:1521–1529.
34. **Kotylak, Z., and P. P. Słonimski.** 1977. Mitochondrial mutants isolated by a new screening method based upon the use of the nuclear mutation op1, p. 83–90. *In* W. Bandlow, R. J. Schweyen, K. Wolf, and K. Kaudewitz (ed.), Mitochondria 1977. Walter de Gruyter, Berlin.
35. **Kozak, M.** 1981. Possible role of flanking nucleotides in recognition of the AUG initiation codons by the eucaryotic ribosomes. Nucleic Acids Res. **9**:5233–5262.
36. **Kruszewska, A., and P. P. Słonimski.** 1984. Mitochondrial and nuclear mitoribosomal suppressors that enable misreading of ochre codons in yeast mitochondria. I. Isolation, localization and allelism of suppressors. Curr. Genet. **9**:1–10.
37. **Kruszewska, A., and P. P. Slonimski.** 1984. Mitochondrial and nuclear mitoribosomal suppressors that enable misreading of ochre codons in yeast mitochondria. II. Specificity and extent of suppressor action. Curr. Genet. **9**:11–19.
38. **Kruszewska, A., and B. Szczesniak.** 1985. Functional nuclear suppressor of mitochondrial oxi2 mutations in yeast. Curr. Genet. **10**:87–93.
39. **Kruszewska, A., B. Szczesniak, and M. Claisse.** 1980. Recombinational analysis of oxi1 mutants and preliminary analysis of their translation products in *S. cerevisiae*. Curr. Genet. **2**:45–51.
40. **Labouesse, M., G. Dujardin, and P. P. Slonimski.** 1985. The yeast nuclear gene *NAM2* is essential for mitochondrial DNA integrity and can cure a mitochondrial RNA maturase deficiency. Cell **41**:133–143.
41. **Lazowska, J., C. Jacq, and P. P. Slonimski.** 1980. Sequence of introns and flanking exons in wild-type and *box3* mutants of cytochrome b reveals an interlaced splicing protein coded by an intron. Cell **22**:333–348.
42. **Lipman, D. J., and W. R. Pearson.** 1985. Rapid and sensitive protein similarity searches. Science **227**:1435–1441.
43. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

44. **Masurekar, M., E. Palmer, B. I. Ono, J. M. Wilhelm, and F. Sherman.** 1981. Misreading of the ribosomal suppressor *SUP46* due to an altered 40S subunit in yeast. J. Mol. Biol. **147**:381–390.
45. **Matsuhita, Y., M. Kikawa, and K. Isono.** 1989. Cloning and analysis of the nuclear genes for two mitochondrial ribosomal proteins in yeast. Mol. Gen. Genet. **219**:119–124.
45a. **McMullin, T. W., P. Haffter, and T. D. Fox.** 1990. A novel small-subunit ribosomal protein of yeast mitochondria that interacts functionally with an mRNA-specific translational activator. Mol. Cell. Biol. **10**:4590–4595.
46. **Myers, A. M., M. D. Crivellone, and A. Tzagoloff.** 1987. Assembly of the mitochondrial membrane system, *MRP1* and *MRP2*, two yeast genes coding for mitochondrial ribosomal proteins. J. Biol. Chem. **262**:3388–3397.
47. **Myers, A. M., L. K. Pape, and A. Tzagaloff.** 1985. Mitochondrial protein synthesis is required for maintenance of intact mitochondrial genomes in *Saccharomyces cerevisiae*. EMBO J. **4**:2087–2092.
48. **Nasmyth, K. A., and S. I. Reed.** 1979. Isolation of genes by complementation in yeast: molecular cloning of a cell-cycle gene. Proc. Natl. Acad. Sci. USA **77**:2119–2132.
49. **Norrander, J., T. Kempe, and J. Messing.** 1983. Construction of improved M13 vectors using oligodeoxynucleotide-directed mutagenesis. Gene **26**:101–106.
50. **Ohyama, K., H. Fukuzawa, K. Takayuki, H. Shirai, T. Sano, S. Sano, K. Umesono, Y. Shiki, M. Takeuchi, Z. Chang, A. Schinichi, H. Inokuchi, and H. Ozeki.** 1986. Chloroplast gene organization deduced from complete sequence of liverwort *Marchantia polymorpha* chloroplast DNA. Nature (London) **322**:572–574.
51. **Partaledis, J. A., and J. L. Mason.** 1988. Structure and regulation of a nuclear gene in *Saccharomyces cerevisiae* that specifies MRP13, a protein of the small subunit of the mitochondrial ribosome. Mol. Cell. Biol. **8**:3647–3660.
52. **Planta, R. J., M. H. Mager, R. J. Leer, L. P. Woudt, H. A. Raue, and T. T. El-Baradi.** 1986. Structure and expression of ribosomal protein genes in yeast, p. 699–718. *In* B. Hardesty and G. Kramer (ed.), Structure, function, and genetics of ribosomes. Springer-Verlag, New York.
53. **Rose, M. D., P. Novick, J. H. Thomas, D. Botstein, and G. R. Fink.** 1987. A *Saccharomyces cerevisiae* genomic plasmid bank based on a centromere containing shuttle vector. Gene **60**:237–243.
54. **Rosset, R., and L. Gorini.** 1969. A ribosomal ambiguity mutation in yeast. J. Mol. Biol. **39**:95–112.
55. **Rothstein, R. J.** 1983. One-step gene disruption in yeast. Methods Enzymol. **101**:202–211.
56. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
57. **Smolinska, U.** 1987. Mitochondrial mutagenesis in yeast: mutagenic specificity of EMS and the effects of *RAD9* and *REV3* gene products. Mutat. Res. **179**:167–174.
58. **Steel, L. F., A. Smyth, and A. Jacobson.** 1987. Nucleotide sequence and characterization of the transcript of a *Dictyostelium* ribosomal protein gene. Nucleic Acids Res. **15**:10285–10289.
59. **Tzagoloff, A., and C. L. Dieckmann.** 1990. *PET* genes of *Saccharomyces cerevisiae*. Microbiol. Rev. **54**:211–225.
59a. **Tzagoloff, A., and A. M. Myers.** 1986. Genetics of mitochondrial biogenesis. Annu. Rev. Biochem. **55**:249–285.
60. **Ulrich, A., J. Shine, J. Chirgwin, R. Picket, E. Tischner, W. Rutter, and H. Goodman.** 1977. Rat insulin genes: construction of plasmids containing the coding sequences. Science **196**:1313–1319.
61. **van Acken, U.** 1975. Protein chemical studies on ribosomal proteins S4 and S12 from *ram* (ribosomal ambiguity) mutants of *Escherichia coli*. Mol. Gen. Genet. **140**:61–68.
62. **Vijayasarathy, S., I. Ernest, J. E. Itzhaki, D. Sherman, M. R. Mowatt, P. A. M. Michels, and C. E. Clayton.** 1990. The genes encoding fructose biphosphate aldolase in *Trypanosoma brucei* are interspersed with unrelated genes. Nucleic Acids Res. **18**:2967–2975.
63. **Vincent, A., and S. W. Liebman.** 1990. The yeast omnipotent suppressor *SUP46* encodes yeast ribosomal protein S13, which is homologous to the *E. coli* S4 *ram* protein, abstr. 08-19A. *In* W. H. Mager and R. J. Planta (ed.), 15th International Conference on Yeast Genetics and Molecular Biology. Wiley Press, New York.
63a. **Vincent, A., and S. W. Liebman.** Personal communication.
64. **Weiss-Brummer, B., H. Sakai, and F. Kaudewitz.** 1984. Leakiness of termination codons in mitochondrial mutants of the yeast *Saccharomyces cerevisiae*. Mol. Gen. Genet. **198**:62–68.
65. **Zaret, K. S., and F. Sherman.** 1982. DNA sequence required for efficient transcription termination in yeast. Cell **28**:563–573.
66. **Zimmermann, R. A.** 1980. Interactions among protein and rRNA components of the ribosome, p. 135–169. *In* G. Chamblis, G. R. Craven, J. Davis, K. Davis, L. Kahan, and M. Nomura (ed.), Ribosomes: structure, function and genetics. University Park Press, Baltimore.
67. **Zimmermann, R. A., R. T. Garvin, and L. Gorini.** 1971. Alteration of a 30S ribosomal protein accompanying the *ram* mutation in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **68**:2663–2667.
68. **Zoładek, T., M. Boguta, and A. Putrament.** 1985. Nuclear suppressors of mitochondrial mutation *oxi1*-V25 in *Saccharomyces cerevisiae*. I. Phenotypes of some suppressors. Curr. Genet. **9**:427–433.