Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 2286–2290, April 1989
Genetics

An essential yeast protein, encoded by duplicated genes *TIF1* and *TIF2* and homologous to the mammalian translation initiation factor eIF-4A, can suppress a mitochondrial missense mutation

(*Saccharomyces cerevisiae*/suppression/mitochondria/essential duplicated genes)

PATRICK LINDER* AND PIOTR P. SLONIMSKI

Centre de Génétique Moléculaire du Centre National de la Recherche Scientifique, Laboratoire propre associé à l'Université Pierre et Marie Curie, 91190 Gif-sur-Yvette, France

Communicated by Giuseppe Attardi, December 5, 1988

ABSTRACT **We describe the isolation and characterization of two previously undescribed genes, *TIF1* and *TIF2*, from *Saccharomyces cerevisiae*. The protein-encoding sequences of the two genes are highly conserved, resulting in two completely identical proteins, whereas the flanking regions show no obvious homology. The two yeast proteins are highly similar to the translation initiation factor eIF-4A from mouse. Elevated gene dosage of *TIF1* or *TIF2* results in the suppression of a missense mutation in the mitochondrial *oxi2* gene, which codes for subunit III of cytochrome-*c* oxidase, although the sequence of the Tif protein indicates its cytoplasmic localization. Inactivation of either gene by gene disruption has no effect on cell viability or on mitochondrial functions. However, simultaneous inactivation of both genes is lethal to the cell.**

The majority of the mitochondrial proteins are encoded by the nuclear genome and synthesized in the cytoplasm prior to import into mitochondria. In addition to the direct biochemical approach, two mutually nonexclusive but complementary approaches have been used to study the nuclear–mitochondrial interactions in *Saccharomyces cerevisiae*. The first approach has been based on the loss of mitochondrial functions resulting from mutations in nuclear genes. This approach is exemplified by the study of numerous *pet*$^-$ mutations (1, 2). The other approach is based on the restoration of respiratory functions by suppression of mitochondrial mutations resulting from mutations in nuclear genes (nuclear accommodation of mitochondria, *NAM*, genes) (3). That the two approaches are not contradictory is indicated by the fact that the inactivation of a nuclear gene which has been isolated by the second approach (gain of function) leads to a *pet*$^-$ phenotype, loss of mitochondrial function (*NAM2*) (4). However, until now none of these approaches has led to the discovery of genes which are essential for basic processes in the cell because the selection of *pet*$^-$ mutants is based on the principle that they grow well by fermentation and do not grow by respiration, thus they cannot affect functions that are essential and common to both processes. Here we describe the "nuclear accommodation of mitochondria" approach, which has led to the characterization of two genes coding for an *essential nonmitochondrial* function.

Different types of nuclear suppressors of mitochondrial deficiencies have been isolated. Some have a very broad spectrum of suppression [*NAM1* (5); *NAM3* (6, 7)]. Others are more specific and suppress several mutations within one gene, as has been described for the mutations in the gene *NAM2*, coding for the mitochondrial leucyl-tRNA synthetase, which alleviates mutations in the "maturase" encoded by the fourth intron of cytochrome *b* (8). The most specific suppressor, called *NAM6*, acts only on three missense mutations in the *oxi2* gene (also called the COXIII gene, which codes for subunit III of cytochrome *c* oxidase) out of 315 different *mit*$^-$ mutations analyzed (ref. 9, unpublished data).

In this work we have isolated two different genes which encode independently the same protein, which is essential for cell viability. The protein is highly homologous to the mouse translation initiation factor eIF-4A (10). The fact that the cloned genes suppress a mitochondrial missense mutation only when the genes are cloned on a multicopy plasmid reveals the importance of a carefully regulated equilibrium of expression of nuclear and mitochondrial genes. We are unaware of any other report describing the use of mitochondria as a sensor for processes essential for cell viability.

MATERIALS AND METHODS

Bacterial Strains, Yeast Strains, Plasmids, Phages, and Media. The *Escherichia coli* strains we used were JA221 (*recA*$^-$, *hsdR*$^-$, *hsdM*$^+$, *leuB6*, *trpE5*, *lacY*$^-$) for standard cloning procedures and JM101 ([*lac*–proAB], *thi*$^-$, *supE*, F'*traD36*, *proAB*$^-$, *lacIQ*$^-$, *lacZM15*) for propagation of M13 phages. The yeast strains are listed in Table 1. Mitochondria carrying the *oxi2* mutation V382 were transferred into W303-1B/50 by cytoduction, taking advantage of the *kar1* mutation in CD8. Media were as described (3).

For construction of the gene bank we used the *E. coli*–yeast shuttle vector YEp13 (12) and for subcloning experiments, pEMBLYe31 (13). The low copy number plasmid pFL39 (*ARS–CEN–TRP1*) was kindly given to us by by F. Lacroute (Gif-sur-Yvette). The *URA3* gene (*Hin*dIII fragment) was given to us by B. Guiard (Gif-sur-Yvette), and the *HIS3* gene (*Bam*HI fragment in CMp169) was from C. Mann (Centre d'Etudes Nucleaires, Saclay, France). The plasmids constructed in this study were YEpPL2 (YEp13-*TIF1*), YEpPL3 (YEp13-*TIF2*), and YCpPL1 (pFL39-*TIF1*).

Construction of the Gene Bank. Genomic DNA was isolated from the strain CD11/1830/50 and partially digested by *Bam*HI prior to ligation into *Bam*HI-digested and phosphatase-treated YEp13 vector. Plasmid DNA from roughly 10^4 colonies was used to transform the strain W303-1B/V382. Transformants were selected for leucine prototrophy and colonies were replicated on glycerol-containing medium to screen for respiration-proficient cells. Glycerol-positive colonies were analyzed for cosegregation of the *LEU2* marker and the glycerol-positive phenotype.

The *TIF2* gene has been isolated by screening the library with a probe containing the *TIF1* gene cloned on the plasmid pGB2 (14). This plasmid has no homology to the YEp13 vector and thus only clones which have sequences homolo-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

*Present address: Microbiology Department, Biozentrum, 4056 Basel, Switzerland.

Table 1. Yeast strains used in this study

Name	Nuclear genotype	Mitochondrial genotype	Source
CD11/B1719/50	*MATa leu1 NAM6-1 kar1*	*rho*^{0}	Ref. 9
CW04	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100*	*rho*$^+$ *mit*$^+$	Ref. 11
W303-1B/50	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100*	*rho*^{0}	R. Rothstein
W303-1A/D	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100*	*rho*$^+$ *mit*$^+$	R. Rothstein
W303-1B/V382	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100*	*rho*$^+$ *oxi2*$^-$ (V382)	This work, cytoduction of V382 mitochondria from CD8
CD8	*MATa leu1 kar1*	*rho*$^+$ *oxi2*$^-$ (V382)	Ref. 9
PL37	*MATα tif2::URA3 ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100*	*rho*$^+$ *mit*$^+$	This work
PL38	*MATa tif1::HIS3 ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100*	*rho*$^+$ *mit*$^+$	This work
PLD1	*MATα/MATa TIF1/tif1::HIS3 tif2::URA3/TIF2 ade2/ade2 his3/his3 leu2/leu2 can1-100/can1-100*	*rho*$^+$ *mit*$^+$	This work, PL37 × PL38
PL49	*MATa tif1::HIS3 tif2::URA3 ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100* [YEpPL2]	*rho*$^+$ *mit*$^+$	This work, spore issue from PLD1

gous to *TIF1* should be found. Restriction analysis and hybridization of *TIF*-adjacent sequences have been used to distinguish between clones carrying *TIF1* and clones carrying *TIF2*.

General Methods. Transformation of yeast was performed according to the lithium procedure (15). Restriction analysis and Southern transfers were carried out according to standard procedures (ref. 16, pp. 104 and 382). Restriction enzymes were purchased from Appligene (Strasbourg, France) and Boehringer Mannheim.

RESULTS

Isolation of the Genes *TIF1* and *TIF2*. Using a gene bank, constructed on a multicopy vector, we have isolated a plasmid which conferred a glycerol-positive phenotype to the strain W303-1B/V382, which normally cannot grow on glycerol medium due to its mutation in the *oxi2* gene. The glycerol-positive phenotype cosegregated with the plasmid-encoded *LEU2* marker, indicating clearly that we have in fact cloned a gene which has suppressor activity. The suppressor activity is weak but clearly visible after 5–8 days of incubation at 28°C (Fig. 1). We designate the resulting plasmid YEpPL2, and the gene carried by the insert we designate *TIF1* (see also below). Restriction analysis of the insert in plasmid YEpPL2 is shown in Fig. 2. A subclone conferring suppressor activity is designated sup^{active}.

Southern hybridization experiments and genetic results (see below) suggested that there are two copies of that gene present in the genome of *S. cerevisiae*. We thus isolated the second fragment from the same gene bank to establish differences (if any) in their primary structures and functions (i.e., suppression of the *oxi2* mutation V382). Since *TIF1* was isolated as a suppressor for a mitochondrial mutation we carried out the same analysis for the *TIF1*-related sequence. As is shown in Fig. 1, this clone also has suppressor activity on the *oxi2* mutation V382. This gene thus has an activity similar or identical to that of *TIF1*. We designate this second gene *TIF2* (see below).

Gene-Dosage-Dependent Suppression. *A priori* the suppression could be due to a gene dosage effect. We therefore cloned the genomic *Bam*HI fragment encoding *TIF1* onto a low copy number *ARS-CEN* plasmid (pFL39). When this plasmid, YCpPL1 (pFL39-*TIF1*), was introduced into strain W303-1B/V382, no growth on glycerol could be observed even after prolonged incubation. The gene is situated such that in the cloned fragment there is at least 4 kb upstream of the gene in the *Bam*HI fragment, and so lack of part of the gene or distant regulatory sequences can be excluded. Thus we are convinced that the suppressor activity is solely due to the fact that the genes *TIF1* and *TIF2* are present at a high gene dosage.

One or the Other *TIF* Gene Must Be Active for Cell Viability. Since the gene was isolated as a suppressor of a mitochondrial mutation we carried out a gene disruption of *TIF1* to see whether inactivation of *TIF1* leads to a glycerol-negative (i.e., respiration-deficient) phenotype or whether the gene is essential. The gene disruption was made by insertion of a *HIS3* marker into the *Hin*dIII site (Fig. 2*B*). Upon transformation of the haploid strain CW04 with a linear *Eco*RV–*Eco*RI fragment, we isolated several clones prototrophic for histidine (His$^+$). Since the disruption was successful in a haploid strain the inactivation is not lethal. This clone (PL38) did not have a respiratory-deficient phenotype, as would be expected for the inactivation of a gene implicated in mitochondrial functions. As we have already shown, a second gene (*TIF2*) with similar or identical functions is present in *S.*

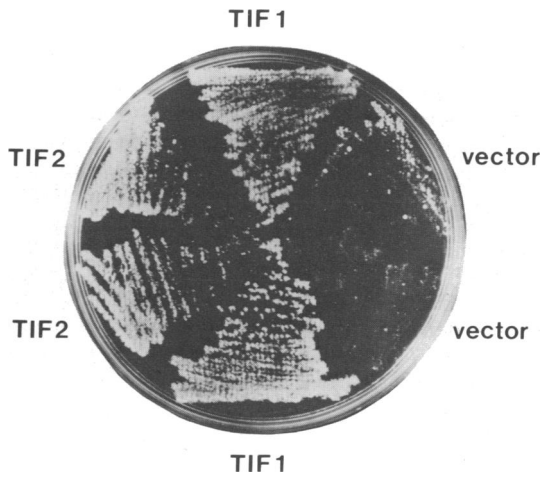

FIG. 1. Respiratory growth on glycerol of a strain carrying a missense mutation in the *oxi2* mitochondrial gene (W303-1B/V382) in presence and absence of the *TIF* gene on a multicopy plasmid. The strain W303-1B/V382 was transformed with YEp13 (vector), YEpPL2 (*TIF1*), or YEpPL3 (*TIF2*) and colonies were streaked on minimal medium selective for the plasmid and containing 2% glucose prior to replica plating on complete glycerol-containing plates. The glycerol plates were incubated 8 days at 28°C. Two examples of each type of transformant are shown.

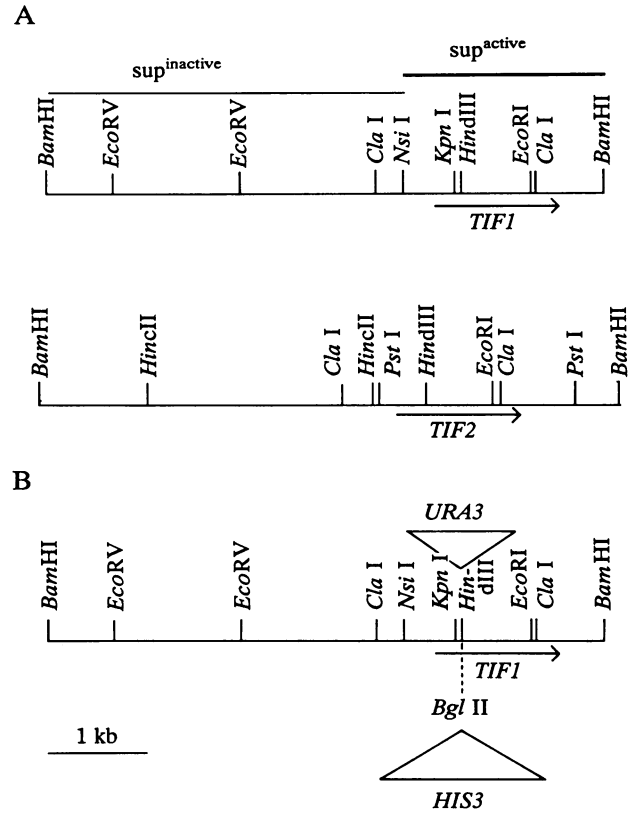

FIG. 2. Localization and inactivation of the suppressor function. (*A*) Restriction maps of the *Bam*HI fragments carrying *TIF1* and *TIF2*. Only the relevant restriction sites are shown. The activity suppressing mitochondrial missense mutations, as shown in Fig. 1, has been localized by subcloning to the *Nsi* I–*Bam*HI fragment indicated by sup^{active}. The locations of the *TIF* genes as deduced from the sequence analysis are indicated by the arrows. Note that in *TIF2* the *Kpn* I site within the gene is absent due to a base substitution. (*B*) Insertions of the *URA3* or *HIS3* genes into the *Hin*dIII sites. In the case of the *HIS3* insertion the *Hin*dIII site had first been changed in a *Bgl* II site by addition of a linker. Both insertions were made in *TIF1*, and the *Kpn* I–*Cla* I fragment (*tif1*::*URA3*) and the *Eco*RI–*Eco*RV fragment (*tif2*::*HIS3*) were used for the inactivation by replacement of the resident chromosomal genes *TIF2* and *TIF1* (see text). kb, Kilobase.

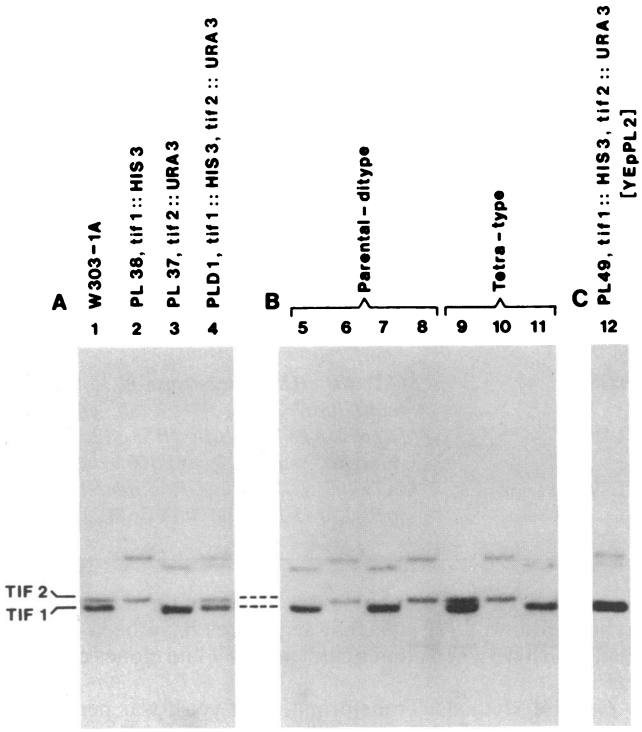

FIG. 3. Southern blot hybridization of genomic DNA digested with *Bam*HI and using *TIF1* as probe (2.1-kb *Nsi* I–*Bam*HI fragment, Fig. 2). (*A*) The parental strain W303-1A, the two strains having one of the *TIF* genes disrupted (PL38 and PL37), and the diploid strain (PLD1) having the two genes disrupted are shown from left to right. (*B*) Two examples from the tetrad analysis. In the parental ditype all four spores are viable and correspond either to one parent (spores 5 and 7 = parent 3) or the other (spores 6 and 8 = parent 2). In the tetratype one spore is missing, since it is, as predicted, a lethal (spore 11 = parent 3, spore 10 = parent 2, spore 9 = wild type 1). (*C*) Strain PL49, having both genes disrupted and carrying the plasmid YEpPL2. In *A* and *B* the probe was a pUC13 plasmid carrying the *TIF1* gene; in *C* the probe was pGB2-TIF1. Due to the high copy number of the plasmid YEpPL2, the band corresponding to the plasmid-encoded *TIF* is much stronger.

cerevisiae. This could easily explain the absence of any detectable phenotype upon inactivation of *TIF1*.

To be certain that *TIF1* inactivation was compensated by *TIF2* activity we set out to disrupt the second sequence as well. To do so we inserted into the *Hin*dIII site of *TIF1* the *URA3* gene encoded by a *Hin*dIII fragment (Fig. 2). The haploid strain W303-1A/D was transformed with a linear *Kpn* I–*Cla* I fragment carrying the *URA3* gene inserted within *TIF1*. This fragment derived from *TIF1* has a sequence practically identical to that of *TIF2* (data not shown), and replacement of *TIF2* as well as replacement of *TIF1* can be expected. Indeed, we found replacement of one or the other gene, but never both (see Fig. 3, lane 3). Growth of the strain *tif2*::*URA3* (PL37) on glycerol was perfectly normal, as we found for the strain carrying the disrupted *TIF1* gene (data not shown).

Disruption of either gene gave no mutant phenotype, indicating that the two genes are isofunctional. But what happens when both genes are disrupted in a haploid strain? To answer this question a diploid (PLD1) carrying the two disrupted genes *tif1*::*HIS3* and *tif2*::*URA3* as well as one wild-type copy of each *TIF* gene (*MATα/MATa*, *TIF1/tif1*::*HIS3*, *tif2*::*URA3/TIF2*; Fig. 3, lane 4) was constructed. Since the two genes are located on two chromosomes (data not shown), we would expect that in tetrad analysis 25% of the spores are wild-type (His$^+$, Ura$^+$) recombinants if such spores can germinate and lead to the formation of viable haploid clones. No such spore, however, could be isolated in 32 tetrads analyzed. The reciprocal recombinant spores (Ura$^-$, His$^-$) were found 16 times, indicating that, in principle, recombinant spores could be observed. Moreover, in all tetratype tetrads that issued from diploids heterozygous for both genes, only three viable spores could be found: two of the parental type and one of the wild type, and never the spore carrying the two disrupted genes (Fig. 3).

The results suggest but do not prove that either of the genes has to be active for viability of the cell. The proof is given by the fact that such spores could be isolated when a plasmid providing the *TIF1* function (YEpPL2) was introduced into the diploid prior to sporulation. In such an analysis spores carrying the two disrupted genes could be isolated (14 spores in 27 tetrads) and all the cells having both genes disrupted carried YEpPL2 (Fig. 3*C*). As expected, no segregation of the plasmid in such cells could be observed in nonselective medium, perfectly in agreement with the idea that one of the *TIF* genes must be active. To be sure that the plasmid YEpPL2 was not integrated in the genome, one such clone was transformed with the YCpPL1 plasmid (pFL39-*TIF1*), and segregation of the resident plasmid YEpPL2 could be

FIG. 4. Comparison of the protein sequence of the yeast Tif protein with the mouse eIF-4A protein (10, 18). The yeast Tif protein is shown on the upper line, and eIF-4A is shown on the lower line. Identical amino acids are indicated by vertical bars, and amino acids that are similar according to the rules of Dayhoff (17) are indicated by dots between the sequences.

observed. It is thus clear that either the *TIF1* or the *TIF2* gene has to be active to ensure the survival of the cell.

The Tif Protein Is Highly Homologous to eIF-4A from Mouse. We also established the primary sequence of the genes *TIF1* and *TIF2*. The sequence (data not shown; EMBL data bank accession numbers X12813 and X12814) reveals an open reading frame for 395 amino acids (see Fig. 4). Interestingly, the proteins encoded by the two genes are completely identical, whereas the flanking sequences share no obvious homology. Within the coding region only 5 base substitutions in 1185 nucleotides are found.

We have searched in a data bank for homologies of the Tif protein with other known proteins and found a remarkable homology to the protein eIF-4A (eukaryotic translation initiation factor) from mouse (10). Out of the 395 amino acid residues from the yeast protein, 257 are identical to the residues in the mouse protein (65% identity) and 62 are similar according to the rules of Dayhoff (17), which brings the total to 81% identity + similarity. In addition to sharing this extended homology the two proteins have a similar size, only a minor gap occurring in the yeast protein. The homology is distributed over the entire protein (Fig. 4). We are thus convinced that the two genes we have cloned code for the yeast analogue of the eIF-4A protein from mouse.

DISCUSSION

We describe here the isolation and basic characterization of two previously unreported genes from *S. cerevisiae*, *TIF1* and *TIF2*. Inactivation by replacement of either gene gives no obvious phenotype, but deletion of both is lethal to the cell, indicating that the two genes are isofunctional and that neither of them represents a pseudogene.

The two genes *TIF1* and *TIF2* have an open reading frame of 395 codons. The two proteins are completely identical, whereas the flanking sequences are different. Comparison of the protein sequence with sequences in data banks revealed an extensive homology with the mouse translation initiation factor eIF-4A (10). Although we have no functional proof of the identity of these proteins, it is unlikely that the proteins encoded by the *TIF* genes do not code for the same function in *S. cerevisiae*. The homology is extremely high (65% identical and 81% identical + similar amino acids) and is distributed throughout the coding region. There exists only a minor gap of two amino acids in the yeast protein with respect to the mouse protein, and the two proteins have almost the same size. It is interesting to note that also in mouse two genes are found, which code for two eIF-4A proteins that have 98% conserved amino acids (18).

The conservation of the protein sequence from budding yeast to mouse is remarkable in the case of eIF-4A. Another highly conserved protein is the elongation factor TEF [yeast compared with *Artemia salina* (19)]. The elongation factor is conserved 80% along the entire protein and, as for *TIF1* and *TIF2*, the two genes of the elongation factor in yeast code for two completely identical proteins.

Recently the isolation and characterization of the yeast cap-binding protein (eIF-4E) from *S. cerevisiae* have been described (20). At least in mammalian cells the eIF-4E protein is found in a complex (eIF-4F) which also contains eIF-4A (21). The eIF-4F complex, which contains the cap-binding protein, a 220-kDa protein, and eIF-4A, is involved in the assembly of the mRNA and the 43S preinitiation complex (for review see ref. 22). Up to now most of the knowledge on eukaryotic translation initiation comes from *in vitro* experiments using cell extracts and purified factors from different sources. Only a few genetic data relevant to the biochemical results on the initiation process are available. The isolation of the genes coding for eIF-4A and eIF-4E from *S. cerevisiae* opens the way to a genetic analysis of the translation initiation process.

Without extensive biochemical analysis it is not possible to definitely say whether the Tif protein is really a translation initiation factor. There are, however, a number of good arguments for this, and the picture which has emerged for the *TIF* genes is reminiscent of other genes involved in translation. (*i*) There are two copies of the gene, which code for two absolutely identical proteins. Such situations have been found for many other proteins involved in translation (19, 23, 24). (*ii*) The two genes seem to be highly expressed, as judged both from the mRNA hybridization and from the highly biased codon usage (data not shown; refs. 25 and 26). (*iii*) In the 5′ region of the *TIF1* gene there are also two sequences (AACTTCCAGGCACAT and AACATCCGATGCTTG at −367 and −239 with respect to the ATG) which resemble the UASrpg,

$$\begin{array}{l} \text{TAC}\,\underset{A}{T}\,\text{G}\,\text{G}\,\text{ATTT} \\ \text{AGA}\,\underset{C}{A}\,\text{CC}\,\underset{A}{T}\,\underset{C}{A}\,\text{CCCA} \end{array},$$

a promoter element often found in ribosomal protein genes (27).

The gene *TIF1* has been isolated as a suppressor gene for a missense mutation in the mitochondrial *oxi2* gene (subunit III of cytochrome-*c* oxidase). By homology we have also isolated its twin *TIF2*, which is isofunctional to *TIF1* with respect to cell viability and suppression of the mitochondrial mutation V382. Suppression can be observed only when the genes are cloned on a high copy number plasmid, which again reveals the importance of an equilibrated expression of nuclear and mitochondrial genes for mitochondrial functions. It has already been shown that nuclear suppression of *mit*$^-$ mutations greatly depends on the copy number of the cloned gene (8, 28–30).

It is not clear, however, how a cytoplasmic translation initiation factor could suppress missense mutations in a

mitochondrial gene. As judged from the DNA sequence, the protein is unlikely to be targeted to the mitochondria. No strict rule has been found for mitochondrial targeting sequences (31), and so we cannot exclude that the protein is imported into mitochondria. In some cases two alternative initiation codons have been found, one for a cytoplasmically located protein and the other for a mitochondrially located protein (32). But in the case of the *TIF* genes, neither up- nor downstream could we find a likely candidate for such an alternative initiation codon. From the absence of a consensus intron sequence [TACTAAC (33)] and the absence of an appropriate reading frame 5′ to the ATG initiation codon we can also exclude the presence of an intron, as has been observed for many ribosomal protein genes (34). Furthermore, the mitochondrial translation machinery is quite different from the cytoplasmic one, and so the implication of a cytoplasmic factor in suppression of a mitochondrial mutation is difficult to explain. It is, however, possible that the Tif protein has an additional function in the cell which is not yet known and that this function is responsible for the suppression of the *oxi2* mutation. Dual functions have recently been reported for mitochondrial tRNA synthetases (8, 35). As the Tif protein is expected to be abundant in the cytoplasm, it will be difficult to prove unambiguously a mitochondrial localization even by cell fractionation and antibodies. The genetic analysis of the *TIF* genes should be able to answer this question.

At present, however, we favor a hypothesis of indirect suppression. It is known from biochemical analysis that the mammalian eIF-4A protein is involved in unwinding and selection of the 5′ region of the mRNA (36, 37). It is thus possible that under normal conditions the initiation factor eIF-4A is limiting and that not all mRNAs are translated efficiently. In our case this would imply that, in cells in which the *TIF* genes are present at a high copy number, an increased quantity of Tif protein is available and mRNAs that under normal conditions are poorly translated become more efficiently translated. Such a gene could code for a cytoplasmically translated, mitochondrially located protein. So, in cells having a high *TIF* copy number, this mitochondrial protein would be more abundant and lead to suppression of the mitochondrial missense mutation.

Note Added in Proof. (*i*) A third *Cla* I site is present 3′ to the gene *TIF1*. (*ii*) The Tif protein belongs to a large family of nucleic acid unwinding proteins characterized by the D E A D box (38).

We thank J. Banroques-Blot, A. Gargouri, A. Kruszewska, and J. Lazowska for helpful discussions and friendly collaboration; C. Mann, B. Guiard, and F. Lacroute for strains and plasmids; and J. Renowicki for help in computer analysis. P.L. acknowledges the receipt of fellowships from the Fonds National Suisse de la Recherche Scientifique and the European Molecular Biology Organization. This work was supported by grants from the Centre National de la Recherche Scientifique, Action Thématique Programée Biologie Moléculaire du Gène, the Institut National de la Santé et de la Recherche Médicale, la Ligue Nationale Française contre le Cancer (to P.P.S.), and, in the final stage, by Grant 3.568-0.87 from the Fonds National Suisse (to P.L.).

1. Sherman, F. & Slonimski, P. P. (1964) *Biochim. Biophys. Acta* **90,** 1–15.
2. Tzagoloff, A. & Myers, A. M. (1986) *Annu. Rev. Biochem.* **55,** 249–285.
3. Dujardin, G., Pajot, P., Groudinsky, O. & Slonimski, P. P. (1980) *Mol. Gen. Genet.* **179,** 469–482.
4. Labouesse, M., Dujardin, G. & Slonimski, P. P. (1985) *Cell* **41,** 133–143.
5. Groudinski, O., Dujardin, G. & Slonimski, P. P. (1981) *Mol. Gen. Genet.* **184,** 493–503.
6. Kruszewska, A. & Slonimski, P. P. (1984) *Curr. Genet.* **9,** 1–10.
7. Kruszewska, A. & Slonimski, P. P. (1984) *Curr. Genet.* **9,** 11–19.
8. Herbert, C. J., Labouesse, M., Dujardin, G. & Slonimski, P. P. (1988) *EMBO J.* **7,** 473–483.
9. Kruszewska, A. & Szczesniak, B. (1985) *Curr. Genet.* **10,** 87–93.
10. Nielsen, P. J., McMaster, G. K. & Trachsel, H. (1985) *Nucleic Acids Res.* **13,** 6867–6880.
11. Banroques, J., Delahodde, A. & Jacq, C. (1986) *Cell* **46,** 837–844.
12. Broach, J. R., Strathern, J. N. & Hicks, J. B. (1979) *Gene* **8,** 121–133.
13. Baldari, C. & Cesareni, G. (1985) *Gene* **35,** 27–32.
14. Churchward, G., Belin, D. & Nagamine, Y. (1984) *Gene* **31,** 165–171.
15. Ito, H., Fukuda, Y., Murata, K. & Kimura, A. (1983) *J. Bacteriol.* **153,** 163–168.
16. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
17. Dayhoff, M. O., Schwartz, R. M. & Oncutt, B. C. (1978) in *Atlas of Protein Sequence and Structure*, ed. Dayhoff, M. O. (Natl. Biomed. Res. Found., Silver Spring, MD), Vol. 5, Suppl. 3, pp. 345–352.
18. Nielsen, P. J. & Trachsel, H. (1988) *EMBO J.* **7,** 2097–2105.
19. Schirmaier, F. & Philippsen, P. (1984) *EMBO J.* **3,** 3311–3315.
20. Altmann, M., Handschin, C. & Trachsel, H. (1987) *Mol. Cell. Biol.* **7,** 998–1003.
21. Edery, I., Hümbelin, M., Darveau, A., Lee, K. A. W., Milburn, S., Hershey, J. W. B., Trachsel, H. & Sonenberg, N. (1983) *J. Biol. Chem.* **258,** 11398–11403.
22. Pain, V. M. (1986) *Biochem. J.* **235,** 625–637.
23. Abovich, N. & Rosbash, M. (1984) *Mol. Cell. Biol.* **4,** 1871–1882.
24. Molenaar, C. M. T., Woudt, L. P., Jansen, A. E. M., Mager, W. H. & Planta, R. J. (1984) *Nucleic Acids Res.* **12,** 7345–7358.
25. Bennetzen, J. L. & Hall, B. D. (1982) *J. Biol. Chem.* **257,** 3026–3031.
26. Hoekema, A., Kastelein, R. A., Vasser, M. & deBoer, H. A. (1987) *Mol. Cell. Biol.* **7,** 2914–2924.
27. Lambertus, P. W., Mager, W. H., Nieuwint, R. T. M., Wassenaar, G. M., van der Kuyl, A. C., Murre, J. J., Hockman, M. F. M., Brockhoff, P. G. M. & Planta, R. J. (1987) *Nucleic Acids Res.* **15,** 6037–6048.
28. Labouesse, M., Herbert, C. J., Dujardin, G. & Slonimski, P. P. (1987) *EMBO J.* **6,** 713–721.
29. Koll, H., Schmidt, C., Wiesenberger, G. & Schmelzer, C. (1987) *Curr. Genet.* **12,** 503–509.
30. Schmidt, C., Söllner, T. & Schweyen, R. J. (1987) *Mol. Gen. Genet.* **210,** 145–152.
31. van Heijne, G. (1986) *EMBO J.* **5,** 1335–1342.
32. Natsoulis, G., Hilger, F. & Fink, G. R. (1986) *Cell* **46,** 235–243.
33. Teem, L. J., Abovich, N., Kaufer, N. F., Schwindinger, W. F., Warner, J. R., Levy, A., Woolfort, J., Leer, R. J., Raamsonk-Duin, M. M. C., Mager, W. H., Planta, R. J., Schultz, L., Friesen, J. D., Fried, H. & Rosbach, M. (1984) *Nucleic Acids Res.* **12,** 8295–8312.
34. Fink, R. G. (1987) *Cell* **49,** 5–6.
35. Akins, R. A. & Lambowitz, A. M. (1987) *Cell* **50,** 331–345.
36. Ray, B. K., Brendler, T. G., Adya, S., Daniels-McQueen, S., Miller, J. K., Hershey, J. W. B., Grifo, J. A., Merrick, W. C. & Thach, R. E. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 663–667.
37. Panniers, R., Stewart, E. B., Merrick, W. C. & Henshaw, E. C. (1985) *J. Biol. Chem.* **260,** 9648–9653.
38. Linder, P., Lasko, P. F., Ashburner, M., Leroy, P., Nishi, K., Schnier, J. & Slonimski, P. P. (1989) *Nature (London)* **337,** 121–122.