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A new yeast translation initiation factor suppresses a mutation in the eIF-4A RNA helicase

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We have isolated a gene, *STM1*, which encodes a new translation initiation factor from *Saccharomyces cerevisiae*. The gene acts, if present on a multicopy plasmid, as a suppressor of a temperature-sensitive mutation in eIF-4A. The single copy *STM1* gene is not essential, but disruption causes a slow growth phenotype. Analysis of polysomes from a strain carrying a disrupted *stm1* allele shows a clear defect in translation initiation as shown by a strong reduction in polysomes and an increase in the monosomes. Sequence analysis revealed interesting features of the putative Stm1 protein. Comparison of the entire protein sequence with databanks showed some similarity with the human eIF-4B protein. The Stm1 protein has potential RNP1 and RNP2 motifs characteristic for RNA-binding proteins. The protein also contains six highly conserved direct repeats of 21–26 amino acids and one partial repeat.

Key words: eIF-4A/polysomes/RNA helicase/*Saccharomyces cerevisiae*/translation initiation

Introduction

Eukaryotic ribosomes initiate translation by binding at the capped 5′ end of the mRNA. This requires the presence of a cap-binding complex and some less defined additional factors. Biochemical analysis in reticulocytes showed that the cap-binding complex consists of a 24 kDa protein (cap-binding protein or eIF-4E), a large molecular weight component of 220 kDa and a 46 kDa protein (eIF-4A). One of the additional factors is eIF-4B, which can be also cross-linked to the cap. A free form of eIF-4A is also required (for review see Hershey, 1991). This protein is an RNA-dependent ATPase, which together with eIF-4B, functions *in vitro* as an RNA helicase to unwind potential secondary structures and allow scanning for the first AUG by the small ribosomal subunit (Lawson *et al.*, 1989; Jaramillo *et al.*, 1990; Rozen *et al.*, 1990).

Biochemical strategies and genetic approaches in yeast led to the cloning of several genes encoding translation initiation factors known from biochemical analysis in mammalian systems (eIF-2α, eIF-2β, eIF-4D, eIF-4E, eIF-2B and eIF-4A). Genes encoding new factors have also been isolated using genetic studies (*SSL1* and *SSL2*; Gulyas and Donahue, 1992; for review see Linder, 1992). We have reported the isolation and characterization of two genes, *TIF1* and *TIF2*, encoding eIF-4A from this organism (Linder and Slonimski, 1989). The yeast eIF-4A protein is highly similar to eIF-4A proteins from mammals, *Nicotiana plumbaginifolia*, *Caenorhabditis elegans* and *Schizosaccharomyces pombe* (Nielsen *et al.*, 1985; Nielsen and Trachsel, 1988; Owttrim *et al.*, 1991; Waterston *et al.*, 1992; A.Fischli and P.Linder, unpublished). The proteins encoded by the *Saccharomyces cerevisiae* genes are completely identical and essential for cell viability. Using an eIF-4A-dependent *in vitro* translation system, we could show that these genes encode a *bona fide* translation initiation factor (Blum *et al.*, 1989).

The eIF-4A protein has several sequence elements in common with other proteins involved in diverse processes such as ribosome assembly, splicing and development; their comparison revealed a new family of proteins, the DEAD box family (DEAD = Asp-Glu-Ala-Asp) (Linder *et al.*, 1989). We have shown that most of the residues in the conserved domains are essential for the *in vivo* function of the protein (Schmid and Linder, 1991). Biochemical analysis of the effect of some mutations in the ATPase A domain showed that this domain is in fact required for ATPase activity and that this activity is required for RNA helicase and *in vitro* translation (Blum *et al.*, 1992). A similar study with different mutations has also been carried out with the mammalian eIF-4A protein (Pause and Sonenberg, 1992). These results show that the A motif is involved in ATP binding, the B motif and the HRIGR motif in ATP hydrolysis and the SAT motif in RNA unwinding.

For RNA unwinding a second translation initiation factor, eIF-4B, is also required (Rozen *et al.*, 1990). This factor has been purified from mammalian cells and its corresponding cDNA has been isolated (Milburn *et al.*, 1988, 1990). The eIF-4B protein has a deduced MW of 69.8 kDa and reveals the presence of a probable RNA-binding motif. It is interesting to note that neither eIF-4B nor eIF-4A itself binds efficiently to globin mRNA, but binding is stimulated several fold in presence of both proteins and ATP (Abramson *et al.*, 1987). The eIF-4B protein contains several DRYG motifs in the middle of the protein and a C-terminus that is extremely rich in polar residues, with many serines. Many of these serines are potential targets of caseine kinase II and protein kinase C, which is in accordance with the observed multiple phosphorylation states of eIF-4B (Hershey, 1990). Based on its biochemical characteristics (cross-linking to the RNA in the presence of cap-binding proteins and stimulation of eIF-4A functions) a similar factor has also been isolated from wheat germ. This protein, which has previously been termed eIF-4G (Browning *et al.*, 1987) is smaller than its mammalian counterpart (Browning *et al.*, 1989). Thus eIF-4B is present in mammalian and plant systems and seems to play an important role in translation initiation. Nevertheless, its exact function in the helicase activity of the pair eIF-4A/eIF-4B remains unknown and the corresponding gene has not yet been isolated from yeast.

To identify factors that interact with eIF-4A, we started to isolate suppressors of a temperature-sensitive *tif1* mutant.

Here we describe the isolation of a gene dosage-dependent suppressor which shows some similarity to human eIF-4B.

Results

Suppression of a temperature-sensitive eIF-4A mutation

Previously we have shown that eIF-4A from *S.cerevisiae* is essential for growth. In strain SS13-3A/pSSC120 both genomic *TIF1* and *TIF2* genes were deleted and the only *TIF1* gene (present on a *LEU2* centromere plasmid) had the valine at position 79 substituted by an alanine. This residue is very close to the ATPase A domain (residues 66–73). The strain was able to grow at low temperatures but not at 37°C. During growth the plasmid is maintained stably, since it is the only source for the essential eIF-4A protein. In order to isolate gene dosage-dependent suppressors, we transformed this strain with two libraries which were made in a multicopy vector (*URA3*; library kindly provided by R.Henriquez and M.N.Hall). After an initial selection for transformants at the permissive temperature, the plates were replica plated and incubation was carried out at 37°C (Figure 1). A total of 34 clones were further analysed. Among these clones three types were found. Transformants of type 1 were spontaneous revertants, intragenic or genomic suppressors of the *tif1* mutation. Such transformants could lose the plasmid from the library, but not the resident plasmid carrying the mutated *tif1* gene (Leu2$^+$, Ura3$^-$). Transformants of type 2 encoded either wild type *TIF1*, *TIF2* or bypass suppressors and segregation of the resident plasmid carrying the mutant *tif1* gene could be observed (Leu2$^-$, Ura3$^+$). Transformants of type 3 carried gene dosage-dependent suppressors (Leu2$^+$, Ura3$^+$). In this class of transformants both plasmids are essential for growth at the nonpermissive temperature and therefore cannot segregate (Figure 1). Eight candidates of type 3 were found in total. Restriction analysis of these candidates showed that they represent two different clones, called *STM1* and *STM2* (*STM* = *s*uppressor of *t*ranslation *m*utations). Southern analysis with the two clones revealed no sequence homology to each other (data not shown).

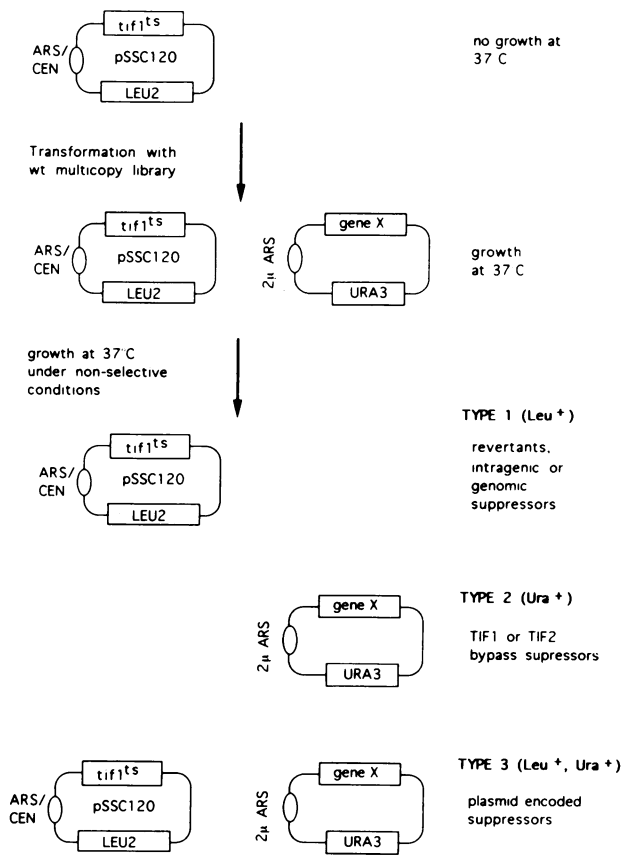

Fig. 1. Strategy of isolation of multicopy suppressors. The strain carrying a temperature-sensitive *tif1* mutation on a *LEU2* plasmid was transformed with a multicopy library made on a *URA3* plasmid. After transformation the Ura$^+$ clones were replica plated onto selective plates and incubated at 37°C. Clones that showed growth were further grown at the nonpermissive temperature in nonselective medium. The cultures were then plated on complete medium and subsequently segregation of the plasmids was followed by replica plating on SD medium lacking either leucine, uracil or both. Clones of type 1 carry only the *LEU2* plasmid, indicating that growth was not due to a plasmid-encoded suppression. These clones represent either revertants, intragenic or genomic suppressors. Clones of type 2 harboured only the *URA3* plasmid. These are most likely plasmids carrying wild type *TIF* genes or bypass suppressors. Clones of type 3 carry both plasmids, indicating that the *URA3* plasmid encodes a gene which is essential for cell viability.

Analysis of suppression by STM1

To show that the suppression was due to the presence of multiple copies of the *STM1* gene, an *Eco*RI fragment containing the suppressor activity was subcloned into a centromere plasmid (YCplac33; Gietz and Sugino, 1988). The presence of this plasmid in the temperature-sensitive strain did not suppress the mutant phenotype, indicating clearly that the suppression requires the presence of multiple copies of *STM1* (data not shown).

To exclude the possibility that the suppressor changes the expression of the mutant *tif1* gene or the copy number of the plasmid carrying this gene, we performed Northern analysis using the *TIF1* gene as probe. No increased expression of the *tif1* mutant gene could be observed (data not shown). This suggests that *STM1* codes for a protein which interacts directly or indirectly with the eIF-4A protein and does not change the expression of the *tif1* gene.

We have previously isolated several mutants in the *TIF1* and *TIF2* genes encoding eIF-4A (Schmid and Linder, 1991). To test the specificity of the suppression we also introduced the suppressor plasmid into strains carrying other *tif1* alleles with mutations in the different motifs conserved in the DEAD

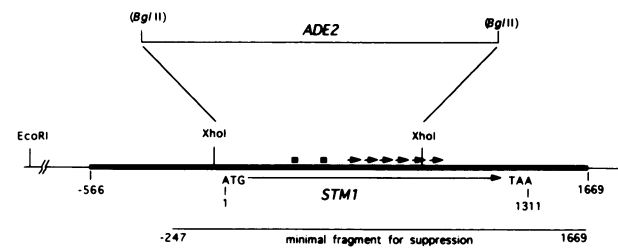

Fig. 2. The fragment encoding the *STM1* gene. The insert present in the multicopy plasmid pSEY18 is shown. The thick line represents the sequenced part of the fragment (EMBL accession number X71996). The minimal fragment required for suppression was determined by the *Exo*III deletion analysis and represents the borders which still confer suppression. The long arrow flanked by ATG and TAA indicates the position of the open reading frame. The small boxes above the thick line represent the putative RNP elements and the repeated arrows the repeated sequences present in the Stm1 protein. The insertion of the blunt ended *Bgl*II fragment carrying the *ADE2* gene in the filled-in *Xho*I site is indicated.

box family. None of these mutations in the ATPase A motif ($A_{66}V$), the ATPase B motif ($D_{170}E$) or the HRIGR motif ($R_{347}G$) could be suppressed, showing that the suppression is allele specific.

Deletions and sequence analysis of the suppressor clone

For delimitation of the suppressor activity, nested *Exo*III deletions were made from both sides of the fragment. The deletion products were used for sequencing and for testing suppressor activity. In Figure 2 the minimal region required for suppressor activity, as determined by this deletion analysis, is indicated. The sequence of the fragment which conferred suppressor activity reveals an open reading frame of 436 amino acids resulting in a potential protein of 48.5 kDa. The size of the ORF is consistent with an RNA of 1.6 kb detected in Northern analysis using the *Xho*I fragment as probe (Figure 3A). Primer extension gives two major initiation sites at −48 and −49 from the ATG (Figure 3B and C). No consensus intron sequence (TACTAAC; Teem *et al.*, 1984) is present in the entire sequence. At the 3′ end a consensus transcription termination signal as determined by Zaret and Sherman (1982) is present at coordinate 1480. A potential Abf1 binding site, found in many ribosomal protein genes (for an overview see Planta *et al.* 1993) and a pyrimidine-rich stretch are present in the promoter region.

A search of the protein sequence in the Swissprot data bank (release 24) revealed some similarity to the human eIF-4B protein (Milburn *et al.*, 1990) (Figure 4). The identical (17%) and similar (36%) amino acids are distributed all over the two proteins and no major gaps were introduced (Figure 4). The yeast protein (436 amino acids) is shorter than the human protein (611 amino acids). The Stm1 protein has, like the human eIF-4B protein, a potential RNA-binding motif consisting of the two elements RNP2 and RNP1. Structure predictions suggest that these elements lie indeed within potential β-sheets allowing an interaction with RNA (M.Görlach, E.Matunis, H.Siomi and G.Dreyfuss, personal communications).

In the middle of the protein six direct and one additional partial repeat are present (Figures 4 and 5). They are composed of two parts. The first part is highly conserved in all seven repeats. The second part is rich in charged residues and two or three serines are present in the repeats 3−6. The first serines in the elements SSR are potential phosphorylation sites for protein kinase C (determined by the program MOTIFS of the UWGCG package using the Prosite dictionary from Dr A.Bairoch, Geneva). The function of the repeats is not yet known and a search in the translated EMBL databank (release 34) did not reveal any other protein carrying this sequence.

The STM1 gene is located at the right end of chromosome XVI

The *Xho*I fragment of *STM1* was hybridized to digested yeast chromosomal DNA and to chromosomes separated by pulse field electrophoresis. The hybridization to genomic DNA digested with several enzymes revealed only one major signal if restriction enzymes were used which do not cut within the gene. A signal corresponding to a doublet of chromosomes XIII and XVI was found in the chromosomal blot. A comparison of a partial sequence from the end of the cloned fragment with sequences in the EMBL databank showed a short overlap of 105 nucleotides with the sequence of gene *KRE6*, which has been mapped by chromosome blotting to chromosome XVI (Roemer and Bussey, 1991). To localize the gene more precisely we performed a hybridization to filters containing ordered lambda and cosmid clones (obtained from L.Riles and M.Olson). Hybridization reveals three major signals corresponding to overlapping clones at the end of chromosome XVI to the right of the genes *aro7* and *SUP16*. So far, this is the right-most gene

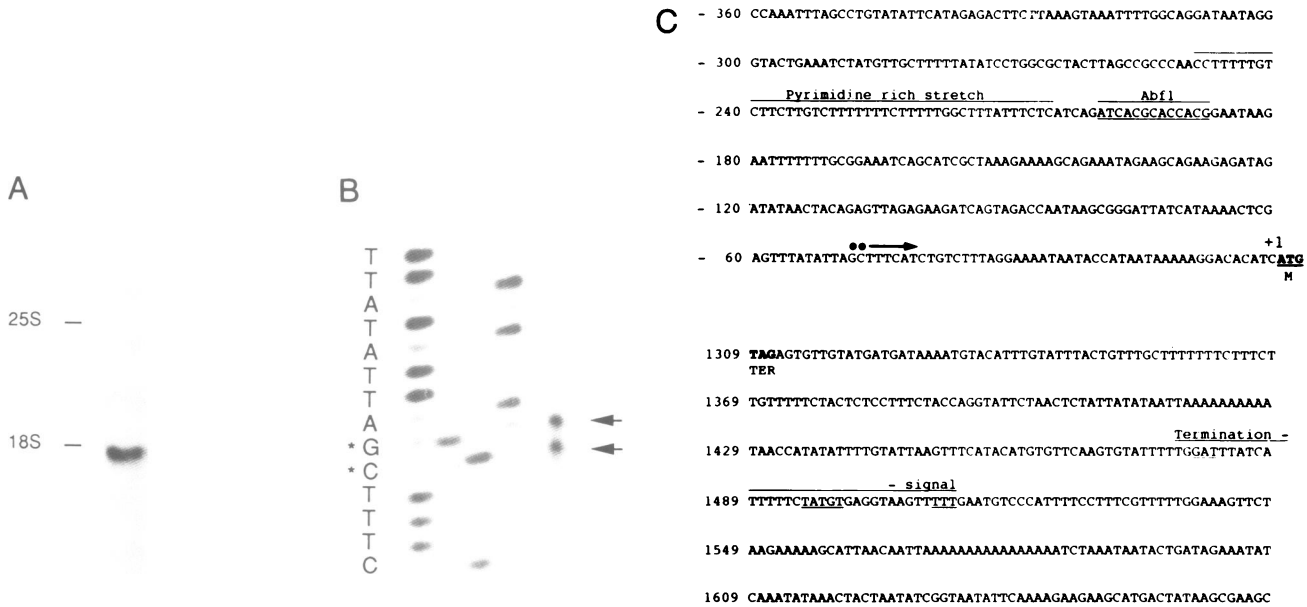

Fig. 3. Northern analysis and primer extension. (**A**) Northern hybridization with the *Xho*I fragment containing part of the promoter and most of the open reading frame as a probe. The marks indicate the positions of the 18S (1798 nt) and 25S rRNA (3392 nt). (**B**) Primer extension of the *STM1* mRNA using a primer from coordinates −33 to −17, relative to the ATG. To the left a sequence of the plasmid pSEY18-STM1 using the same oligonucleotide is shown as size standard. (**C**) Promoter and terminator region of the *STM1* gene. The main starts of the mRNA are indicated with dots and an arrow in the promoter sequence. Also indicated are a pyrimidine-rich stretch and a potential Abf1 binding site in the promoter and the tripartite termination signal in the 3′ region of the sequence.

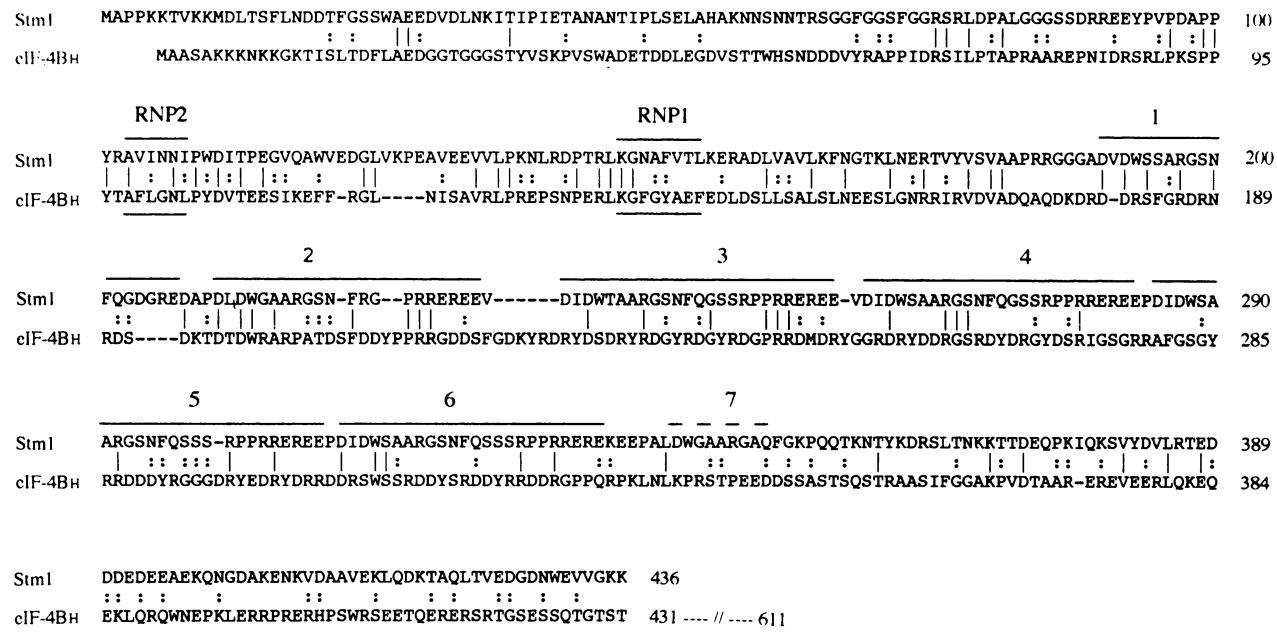

Fig. 4. Sequence comparison with the human eIF-4B protein. The two proteins Stm1 and eIF-4B (Milburn *et al.*, 1990) were aligned using the GAP program of the UWGCG package (version 7.2). Identical amino acids are indicated by a (|), similar amino acids by (:). The RNP1 and RNP2 elements and the direct repeats in Stm1 are indicated.

from chromosome XVI mapped on these filters (L.Riles and M.Olson, personal communication).

Disruption of STM1 results in a slow growth phenotype

To see whether the gene is essential for cell growth we made a disruption in the diploid strain ASZ3. For this we cloned the *ADE2* gene into the *Xho*I sites of the *STM1* gene, removing 75% of the coding sequence and part of the promoter sequences (Figure 2). After transformation in a diploid strain, the disruption was verified by PCR and Southern analysis (data not shown). The resulting strain was called RCB1. Upon sporulation all four spores were viable, although the two spores prototrophic for adenine grew very slowly. If the sporulation is made in a strain which carries the gene *STM1* on a plasmid, growth of the cells harbouring the disruption is normal. To distinguish between slow growth and delayed germination, we measured growth rate on plates and in liquid. Measurements of growth rate of strains disrupted for *STM1* revealed a drastic increase in generation time (Figure 6A and Table I). The effect is most pronounced at 18°C where the generation time increased considerably. Thus the gene is important but not essential for growth.

Stm1 is involved in translation initiation in vivo

The suppression of a mutation in translation initiation factor eIF-4A suggested the possibility that the *STM1* gene is also involved in the initiation process. A convenient method for the analysis of the translation initiation process is the measurement of polysomes in the cell. We therefore made an analysis of polysomes of wild type and *stm1* mutant strains at different temperatures. The profiles of the *stm1* mutant show a reduced polysome shoulder compared with the wild type at all temperatures used (Figure 6B). At 18°C the polysomal shoulder is practically absent, in accordance with strongly reduced growth rate. The monosome peak, however, is correspondingly higher indicating a defect in

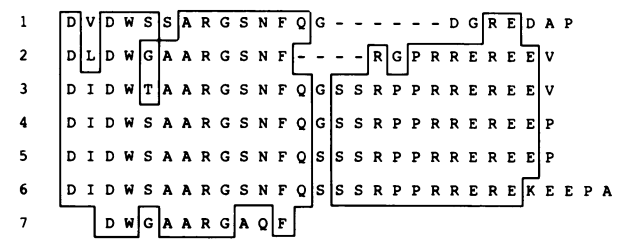

Fig. 5. Direct repeats in Stm1. The repeated sequences are aligned to give an optimal alignment. Identical amino acids are boxed. The first serines in the SRR sequences in repeats 3–6 are potential phosphorylation sites of protein kinase C.

translation initiation in the *stm1* mutant. This reduction in polysomes cannot solely be due to a reduced growth rate, since growth of the wild type at low temperature is inferior to the growth rate of the disrupted strain at 30°C.

Mutations in the RNP motifs affect suppressor activity

To investigate the function of the RNA binding domain (RBD) we made a mutational analysis of the two RNP1 (KGNAFVTL) and RNP2 (AVINNI) motifs. In the RNP1 motif we mutated the codon of the alanine residue by degenerate *in vitro* site directed mutagenesis to codons coding for tyrosine, cysteine, serine, asparagine, phenylalanine and leucine. In the RNP2 motif we mutated the codon of the first asparagine residue to codons coding for glycine, valine, serine, tyrosine and proline. The resulting plasmids were transformed into the temperature-sensitive strain SS13-3A/pSSC120 and suppressor activity was tested. Only the genes with the mutations A→C and A→S in RNP1 and N→G, N→V and N→Y in RNP2 retained suppressor activity. This strongly suggests that the RBD domain is required for function of the protein. Further experiments will be required to test *in vivo* and *in vitro* RNA binding of these mutant proteins.

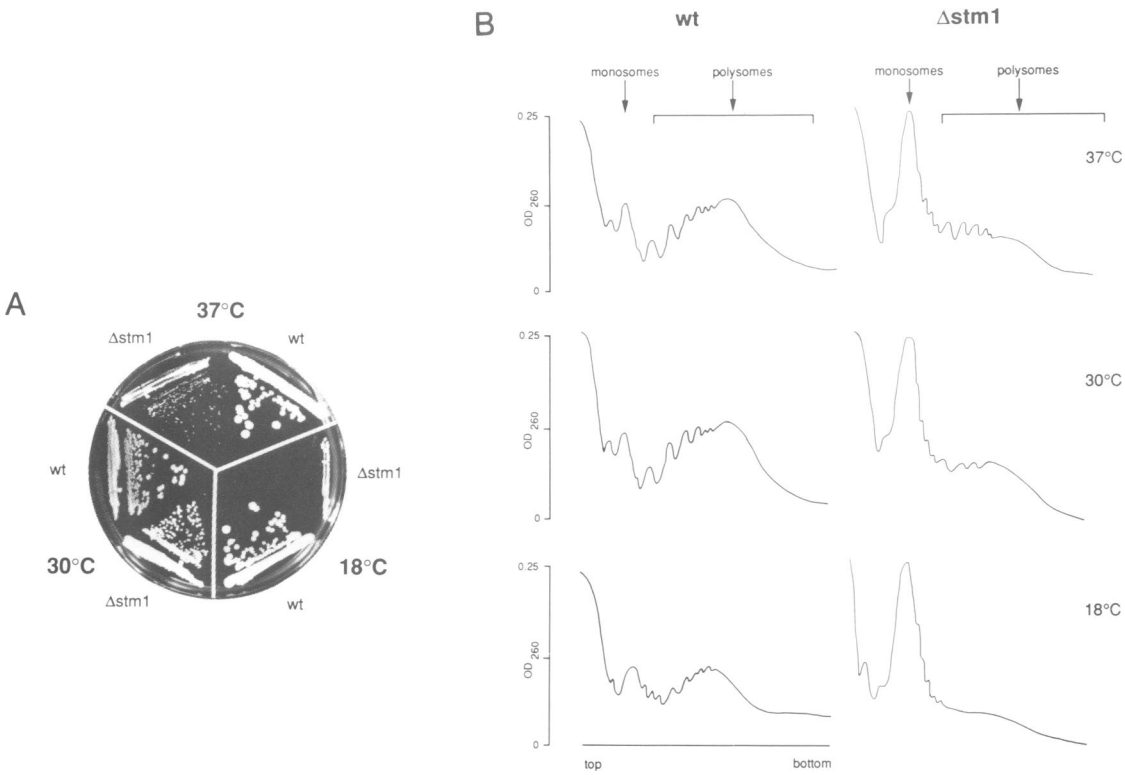

Fig. 6. The *stm1::ADE2* allele causes a reduced growth rate and a defect in translation initiation. (**A**) Representative wild type and mutant cultures were streaked for single colonies on complete medium and the plates were incubated at 18°C, 30°C and 37°C. (**B**) Polysomes of wild type and mutant strains grown at the three temperatures were separated on 17–57% sucrose gradients and the OD_{260} profile was measured.

Level of STM1 expression is similar to the TIF1 and TIF2 genes

The codon usage of yeast genes can be taken as an indication for the level of expression (Bennetzen and Hall, 1982). Highly expressed genes have an elevated codon bias (approaching 1), whereas weakly expressed genes have a low codon bias (approaching zero). The codon bias of the *STM1* gene is 0.5, which corresponds to a normal expression rate. To have an idea of the expression level, we also made a translational fusion of the *STM1* gene with the *LacZ* reporter gene. The measured β-galactosidase activities in exponential growth phase are similar to the activities measured in gene fusions of the *TIF2* gene which is in accordance with the codon bias and the abundance of the mRNA.

Table I. Influence of the *stm1::ADE2* allele on growth rates

Strain	Generation doubling time (h)		
	18°C	30°C	37°C
Wild type	6	1.5	1.4
stm1::ADE2	19	3	2.8

Cultures were grown in liquid complete medium and growth was followed by measuring OD_{600}. The isogenic wild type and *stm1::ADE2* strains were all grown in triplicate.

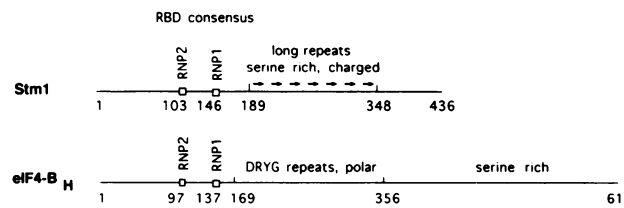

Fig. 7. Schematic representation of features of the Stm1 protein and comparison with the human eIF-4B protein. The location of the potential RNA-binding domains (RBD) with the hexa- and octapaptides RNP2 and RNP1 is indicated by open squares. The region of the direct repeats is indicated by arrows in Stm1. The corresponding region in the human eIF-4B also has repeated sequences, but they are not as conserved as in Stm1. The regions discussed are indicated by coordinates of amino acid residues.

Discussion

Here we describe the isolation of a novel gene involved in translation initiation in the yeast *S.cerevisiae*. The *STM1* gene suppresses a temperature-sensitive mutation in the translation initiation factor eIF-4A from yeast which functions *in vitro* together with human eIF-4B as RNA helicase. The Stm1 protein shows 36% similarity (17% identity) to the human eIF-4B and is therefore a candidate for the yeast eIF-4B protein. The low degree of homology is somewhat surprising since so far all yeast and mammalian translation initiation factors show a degree of identity ranging from 33% (eIF-4E, yeast–mouse) to 65% (eIF-4A, yeast–mouse). Although the alignment of the protein reveals only a low percentage identity, no major gaps have to be introduced to obtain an alignment over the entire length of the Stm1 protein. But the Stm1 protein has characteristic features present in the human eIF-4B (Figure 7). Both proteins have an RNA-binding domain (RBD) consisting of the RNP1 and RNP2 motifs. By a mutational analysis of these motifs in the *STM1* gene we could show that they are required for suppressor

function. In RNP1 the A→C and A→S substitutions did not affect the suppressor activity. A cysteine can in fact be found in some other RNP1 motifs. The substitutions of the alanine by tyrosine, asparagine, phenylalanine and leucine, however, abolish the suppressor activity. These amino acids are not found at the corresponding position in other RBD proteins. The substitutions of the asparagine in RNP2 by glycine, valine and tyrosine did not affect the activity either, whereas substitution by serine or proline abolished the suppressor activity. Glycines and tyrosines can be found in the corresponding positions in other RBD proteins (Kenan *et al.*, 1991). Although these results are in favour of an RNA binding activity of the Stm1 protein, we cannot rule out the possibility that these mutations affect the function by destroying the general structure of the protein. *In vitro* and *in vivo* RNA binding experiments are required to demonstrate a function of these domains definitively in RNA binding. The Stm1 protein and the mammalian eIF-4B have both repeated sequences in the middle domain. They are short and not highly conserved in the human protein, but long and highly conserved in the Stm1 protein. It has been speculated that the many charged and polar residues present in these repeats are involved in the interaction of the protein with mRNA (Milburn *et al.*, 1988). The overall amino acid compositions of the two proteins are very similar, which is also reflected by a high content of serine residues. Some of these residues in the human protein are targets for phosphorylation by caseine kinase II and protein kinase C.

It is intriguing that the Stm1 protein is considerably shorter than the human eIF-4B protein (calculated molecular weight of 48.5 kDa). In plants the eIF-4B protein is also shorter (59 kDa apparent molecular weight; Browning *et al.*, 1989) compared with the 80 kDa human protein (calculated molecular weight of 69 kDa). The aberrant gel mobilities of these proteins could be due to the highly polar domains present in the human and the yeast proteins. It will be interesting to compare the sequence of the Stm1 protein with the plant eIF-4B protein.

If Stm1 really functions as eIF-4B in yeast, one would expect that the deletion of the gene would be lethal to the cell. We have shown that this is not the case. A possible explanation might be that two related genes encoding the same or similar factors are present in the genome. In fact, duplication of translation factor genes [*TIF1/TIF2* (Linder and Slonimski, 1989), *TIF51A/TIF51B* (Schnier *et al.*, 1991), *TIF463A/TIF463B* (Goyer *et al.*, 1993), *TEF1/TEF2* (Cottrelle *et al.*, 1985)] is not rare. In some cases the genes are perfectly conserved and isofunctional under laboratory conditions (for example *TIF1* and *TIF2*), whereas in other cases the proteins differ and the phenotypes of inactivation of one of the genes are not identical (for example *TIF51A* and *TIF51B*). In Southern hybridization experiments with an *STM1* probe, however, no second strong signal could be observed, suggesting that this gene is a single copy gene, although weak bands are visible under moderately stringent conditions. Therefore a second gene with a relatively high divergence in nucleotide sequence might be present and would be required for the residual growth in the *stm1* mutant strain, if the function of this gene is essential.

If the Stm1 protein is involved in translation initiation, as suggested from the suppression of a mutation in the *TIF1* gene and from its homology to the human eIF-4B, a decrease in polysomes should be observed. In fact analysis of polysome profiles clearly showed a reduction in polysomes and increase in monosomes from a strain disrupted for *STM1* at all temperatures tested, with the most drastic effect at low temperatures. This *in vivo* result is supported by *in vitro* experiments reported by Altmann *et al.* (1993). These authors isolated the same gene by screening an expression library with polyclonal antibodies made against cap-binding proteins from yeast. They could show that a disruption of the *STM1* gene affects translation *in vitro*. Moreover, disruption of one of the genes encoding the large subunit of the cap-binding complex in this background is lethal (P.Müller, personal communication).

In conclusion, we present evidence for the isolation of a new gene from yeast involved in translation initiation. The gene might represent the fungal form of the human eIF-4B gene. The gene was isolated as a multicopy suppressor of a eIF-4A mutation close to the ATPase A motif. At present it is not clear if the defect is indirectly due to a change in the conformation of the protein or if this mutation affects directly the ATPase activity. Assuming that the interaction of the Stm1 protein with eIF-4A^{ts} is affected, one could imagine that overproduction of the Stm1 protein pushes the equilibrium towards the normal state. This could be from a dissociated to an associated form. Or, assuming that the interaction between Stm1 and the mutant eIF-4A is too strong, the overproduction of Stm1 is required to have some free protein which is not trapped by the temperature-sensitive eIF-4A protein. Further analysis of the *STM1* gene and the isolation of its gene product for *in vitro* assays together with eIF-4A will certainly help to elucidate better the functions of eIF-4A and the Stm1/eIF-4B proteins in proposed unwinding of secondary structures in the 5′ untranslated regions of mRNAs.

Materials and methods

Strains, media and genetic methods

All yeast strains used in this study were, unless otherwise described, derivatives of the wild type strain CW04 (*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3*) (Banroques *et al.*, 1986). Standard genetic procedures and media for yeast were used (Sherman *et al.*, 1986). Yeast transformations were carried out by the LiCl method (Ito *et al.*, 1983). The genomic one-step transplacements were performed as described by Rothstein (1983). Strain RCB1-1C is a meiotic product from the diploid strain RCB1 carrying a disrupted *STM1* gene. For this disruption the plasmid pSEY18-STM1 was digested with *Xho*I, the ends were filled with Klenow and a blunt-ended *Bgl*II fragment carrying the *ADE2* gene was inserted. The resulting plasmid was digested with *Eco*RI liberating the disrupted *stm1::ADE2* gene and transformed into ASZ3, a diploid derivative of CW04.

ExoIII deletions and sequence analysis

The pSEY18-STM1 plasmid originally obtained from the library was digested with *Sal*I and *Sph*I which leaves one end susceptible for *Exo*III digestion. For digestion of the other side, a *Eco*RI fragment from the original pSEY18-STM1 plasmid was recloned in pFL44 (Bonneaud *et al.*, 1991) and digested with *Sal*I and *Sph*I. *Exo*III and S1 digestions were performed as in (Stotz and Linder, 1990). The resulting plasmids were used for sequencing and analysis of suppressor activity.

In vitro mutagenesis of RNP motifs

The two motifs were mutated by *in vitro* site directed mutagenesis using the degenerate oligonucleotides 5′ CCAACAAGATTAAAGGGTAAT (A/T/C)(A/G/T)TTTCGTTACTTTG 3′ (RNP1) and 5′ AGGGCTGTC-ATA (T/C/G)(G/C/T)CAACATTCCA 3′ (RNP2). The mutagenesis was performed using the Clontech Transformer Mutagenesis Kit and the mutants were sequenced before transformation in yeast.

Polysome analysis

The preparation of polysomes was according to Baim *et al.* (1985) using 17–57% gradients loaded with 20 OD_{260} units. The gradients were run for 4 h at 23 000 r.p.m. in an SB110 swing-out bucket rotor.

Submission of the sequence to the databank

The sequence of the *STM1* gene was submitted to the EMBL databank (accession number X71996).

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Note added in proof

This paper is dedicated to the memory of Pierre Prentki, a very good friend and scientist.