A pre-tRNA carrying intron features typical of Archaea is spliced in yeast

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ABSTRACT

Archaeal pre-tRNAs are characterized by the presence of the bulge–helix–bulge (BHB) structure in the intron stem-and-loop region. A chimeric pre-tRNA was constructed bearing an intron of the archaeal type and the mature domain of the *Saccharomyces cerevisiae* suppressor *SUP4* $tRNA^{Tyr}$. This pre-$tRNA^{ArchEuka}$ is correctly cleaved in several cell-free extracts and by purified splicing endonucleases. It is also cleaved and ligated in *S. cerevisiae* cells, providing efficient suppression of nonsense mutations in various genes.

Keywords: BHB; *M. jannaschii; RAT1; tap1-1;* tRNA splicing

INTRODUCTION

Accuracy in tRNA splicing is essential for the formation of functional tRNAs, and hence for cell viability. In both Eukarya and Archaea correct splicing depends on the specific recognition of tRNA precursors (pre-tRNAs) by tRNA splicing endonucleases, but the mechanisms for determining the cleavage sites appear to differ (Belfort and Weiner 1997; Trotta and Abelson 1999). The eukaryal tRNA splicing endonuclease interacts with the mature tRNA domain, measures the distance from the body of the mature tRNA, and cleaves near a base pair that is formed between nucleotides in the anticodon loop and the intron (the A-I pair) (Mattoccia et al. 1988; Reyes and Abelson 1988). This cleavage generates the 3′ splice site. An independent cleavage event, also at a fixed distance from the mature domain, produces the 5′ splice site (Baldi et al. 1992; Di Nicola Negri et al. 1997; Trotta et al. 1997). In contrast, the archaeal enzyme recognizes a structural element of pre-tRNAs comprised of two bulges of 3 nt each separated by 4 bp, the so-called bulge–helix–bulge (BHB) motif (Daniels et al. 1985; Diener and Moore 1998). This structure functions independently of the part of the molecule that constitutes the mature tRNA.

We recently developed a system in mouse cells and in mice that makes possible the cleavage and ligation of mRNAs having a BHB structure, provided that the archaeal endonuclease from *Methanococcus jannaschii* is present (Deidda et al. 2003; G. Deidda, N. Rossi, S. Putti, and G.P. Tocchini-Valentini, unpubl.). The BHB-dependent cleavage and ligation work both when the BHB is found in *cis* and when the BHB is formed, in *trans*, between appropriately designed RNA molecules and the targeted mRNAs. This method is not limited to mRNA, but, in principle, could be applied to destroy, modify, or restore the formation of regulatory noncoding RNA sequences.

We wish to develop a similar system in *Saccharomyces cerevisiae*, in view of the fact that RNA interference has not been observed in yeast (Aravind et al. 2000; Wood et al. 2002) and that an elegant genetics is available for that organism. As a first step, we ask whether *S. cerevisiae* cells are able to splice RNA molecules containing the intrinsically archaeal BHB motif. We use precursors of a suppressor tRNA containing the BHB structure, and we monitor the generation of a functional tRNA by means of its ability to suppress nonsense mutations in appropriate genes in *S. cerevisiae* cells. We show that yeast cells are able to correctly process, cleave, and ligate tRNA precursors containing the archaeal BHB motif. In addition, we show here that even noncanonical, long pre-tRNA molecules, containing the BHB structure, are cleaved and ligated in yeast, producing functional suppressor tRNA.

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RESULTS AND DISCUSSION

SUP4 pre-tRNA$^{\text{ArchEuka Tyr}}$ is cleaved in vitro

In order to verify the capability of the yeast tRNA splicing endonuclease to recognize and correctly cleave the archaeal bulge–helix–bulge structure in vivo (as already demonstrated in vitro), we used a chimeric suppressor tRNA. We chose the yeast *SUP4* tRNA$^{\text{Tyr}}$, which suppresses nonsense mutations of the ochre type (UAA) by inserting tyrosine (Goodman et al. 1977). *SUP4* tRNA$^{\text{Tyr}}$ was modified to bear eukaryotic tRNA structural features as well as the archaeal BHB motif. This hybrid precursor was designated *SUP4* pre-tRNA$^{\text{ArchEukaTyr}}$ (see Fig. 1).

The *SUP4* pre-tRNA$^{\text{ArchEuka Tyr}}$ consists of two regions derived from yeast *SUP4* pre-tRNA$^{\text{Tyr}}$ (from the beginning to nucleotide 39 and from nucleotide 54 to the end) joined by a 17-nt insert that produces a BHB motif typical of archaeal pre-tRNAs. This hybrid pre-tRNA is analogous, in its structure, to other chimeras we used in in vitro experiments, such as the pre-tRNA$^{\text{ArchEuka Phe}}$. As we have shown previously, pre-tRNA$^{\text{ArchEuka Phe}}$ is cleaved by either the eukaryal or the archaeal endonuclease (Fabbri et al. 1998).

If the splicing endonuclease cleaves the *SUP4* pre-tRNA$^{\text{ArchEuka Tyr}}$ and the tRNA ligase ligates the two resulting halves, the product will be indistinguishable from mature *SUP4* tRNA$^{\text{Tyr}}$ and will be able to suppress ochre nonsense mutations in appropriate *S. cerevisiae* strains. Before testing the ability of the *SUP4* chimera to suppress nonsense mutations in yeast cells, we examined whether in vitro the *SUP4* pre-tRNA$^{\text{ArchEuka Tyr}}$ chimera behaves like pre-tRNA$^{\text{ArchEuka Phe}}$ and is cleaved by either the eukaryal or the archaeal endonuclease.

Splicing reactions with natural and chimeric pre-tRNAs were assayed in cell extracts (from yeast or *Xenopus laevis*) or using purified splicing endonucleases (from *X. laevis* or *M. jannaschii*). Figure 2 shows that *SUP4* pre-tRNA$^{\text{Tyr}}$ is cleaved correctly by the eukaryal enzymes (lanes 2–4,6) but not by the archaeal enzyme (lane 5), whereas all enzymes precisely cleave the *SUP4* pre-tRNA$^{\text{ArchEuka Tyr}}$ (lanes 8–12). Notice that *SUP4* pre-tRNA$^{\text{Tyr}}$ is 93 nt long, while *SUP4* pre-tRNA$^{\text{ArchEuka Tyr}}$ is 96 nt long, because of the presence of the BHB motif. Mature tRNA is present only in lanes 3 and 9, because of the higher ATP concentration (necessary for ligase activity) found in manually prepared nuclei with respect to nuclei prepared en masse. The intron in lane 9 has an additional phosphate group and therefore migrates faster than the intron in lanes 8 and 10–12. This phosphate is added by a kinase, in the presence of ATP, at the 5′-end of the tRNA intron. The intron, in fact, is left with a free OH group after the cleavage by the tRNA-splicing endonuclease, while at the 3′-end of the tRNA 5′-half a 2′,3′-cyclic phosphate remains (Gandini Attardi et al. 1985). In lane 3 the intron is only visible after long exposures and may be degraded in the crude extract. Conceivably, the ArchEuka intron (lane 9) is more stable than its normal counterpart (lane 3) (it is longer by 3 nt and differs in its sequence). The difference in migration of tRNA half-molecules seen when comparing pre-tRNA incubated in extracts (lanes 2,3,6,8,9,12) versus that incubated with pure enzymes (lanes 4,10,11) is due to the heterogeneity at the 3′-end of the precursors, caused by stuttering of the T7 RNA polymerase used to synthesize them. The added nucleotides are then removed by enzymes present in the crude extract, but not in the purified preparation.

The result shown in Figure 2 confirms that *SUP4* pre-tRNA$^{\text{Tyr}}$, a typical eukaryotic precursor, is not a suitable substrate for the archaeal enzyme, while the chimeric tRNA precursor, featuring an archaeal BHB motif, is recognized and cleaved by both eukaryal and archaeal enzymes, like pre-tRNA$^{\text{ArchEuka Phe}}$.

SUP4 pre-tRNA$^{\text{ArchEuka Tyr}}$ is functional in vivo

The gene encoding *SUP4* pre-tRNA$^{\text{ArchEuka Tyr}}$ was introduced into a yeast strain (PJ17-1A) bearing ochre nonsense mutations in three different loci, *met4-1$_o$*, *lys2-1$_o$*, and *ade2-1$_o$*. These mutations require increasing levels of *SUP4* tRNA$^{\text{Tyr}}$ in order to be suppressed, that is, *met4-1$_o$* is sup-

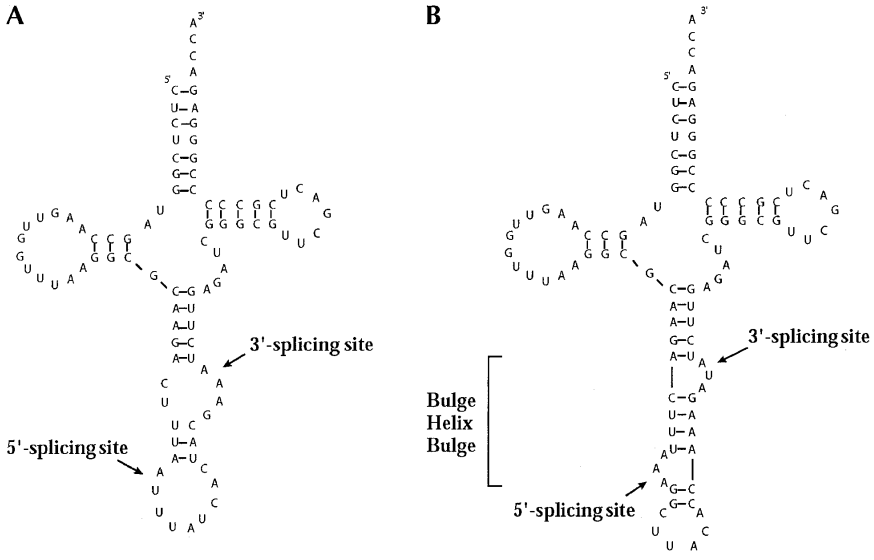

FIGURE 1. (*A*) *SUP4* pre-tRNA$^{\text{Tyr}}$. This tRNA precursor carries a mutation in the anticodon that enables it to suppress nonsense mutations of the ochre type (UAA). (*B*) *SUP4* pre-tRNA$^{\text{ArchEuka Tyr}}$. An archaeal-type intron of 17 nt forming a BHB structure has been inserted in the anticodon stem-and-loop region of *SUP4* pre-tRNA$^{\text{Tyr}}$.

pressed by low levels, *lys2-1$_o$* is suppressed by intermediate levels, and the *ade2-1$_o$* mutation requires high levels of expression of the *SUP4* gene (Hawthorne and Leupold 1974). This difference is due to the functionality of the mutated protein in which the tyrosine amino acid is inserted as a replacement for the wild-type amino acid. The use of a multiple mutated strain is important for two reasons: it permits an evaluation of the expression of the *SUP4* gene, and it also avoids the complication that revertants of the nonsense mutation would be erroneously confused with true suppression events.

The yeast strain PJ17-1A (*met4-1$_o$*, *lys2-1$_o$*, *ade2-1$_o$*), carrying the centromere-based plasmid YCp50 containing the gene for *SUP4* pre-tRNA$^{\text{ArchEuka Tyr}}$, was plated onto selective media without methionine, lysine, or adenine. As controls, we used cells with a plasmid encoding *SUP4* pre-tRNA$^{\text{Tyr}}$ and with the vector alone. The growth of cells carrying *SUP4* pre-tRNA$^{\text{ArchEuka Tyr}}$ is comparable to that of control cells with *SUP4* tRNA$^{\text{Tyr}}$ on −Met and −Lys plates; less growth is obtained on −Ade plates. Cells with the vector alone do not grow on any of the three kinds of selective plates (Fig. 3A).

Another way to determine suppression of the nonsense *ade2-1$_o$* mutation is to grow the transformed cells on rich media. On these plates, *ade2* mutant colonies are red, due to the accumulation of a pigment caused by this mutation; the congenic wild-type *ADE2*$^+$ strain is white. Depending on the level of suppression, yeast colonies carrying a suppressor tRNA will be white (good suppression level), pink (intermediate level), or red (no suppression at all). Moreover, a well-suppressed colony will be white, but red sectors will appear, especially in large colonies, due to the occasional loss of the plasmid encoding the suppressor tRNA (Di Segni et al. 1993). Colonies with *SUP4* pre-tRNA$^{\text{ArchEuka Tyr}}$, as well as with *SUP4* pre-tRNA$^{\text{Tyr}}$-encoding plasmids, are white with red sectors (Fig. 3B). This result indicates that the suppression is caused by a tRNA encoded on a plasmid and not by a chromosomal suppressor tRNA gene.

We conclude from this analysis that a hybrid tRNA precursor, bearing features typical of eukaryotic and archaeal pre-tRNAs, produces enough mature tRNA to be functional in suppression of nonsense mutations in yeast. Therefore,

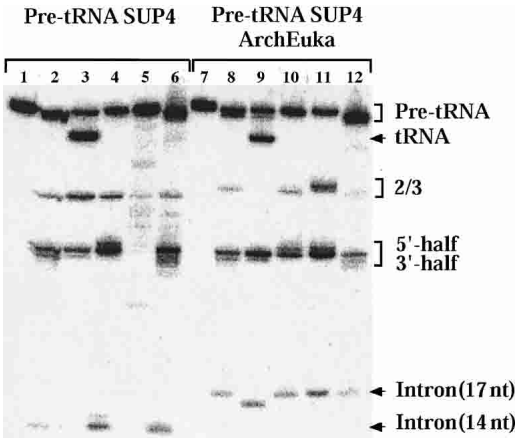

FIGURE 2. In vitro splicing of *SUP4* pre-tRNA$^{\text{Tyr}}$ and *SUP4* pre-tRNA$^{\text{ArchEuka Tyr}}$. ^{32}P-labeled tRNA precursors were incubated in cell-free extracts or with purified splicing endonucleases, electrophoresed in 8 M urea–10% polyacrylamide gels and exposed to PhosphorImager. (Lanes *1–6*) *SUP4* pre-tRNA$^{\text{Tyr}}$ (93 nt long); (lanes *7–12*) *SUP4* pre-tRNA$^{\text{ArchEuka Tyr}}$ (96 nt long). (Lanes *1,7*) Nonincubated control; (lanes *2,8*) incubation with nuclear extracts of *X. laevis* oocytes prepared en masse; (lanes *3,9*) incubation with nuclear extracts of *X. laevis* oocytes prepared manually; (lanes *4,10*) incubation with purified *X. laevis* splicing endonuclease; (lanes *5,11*) incubation with purified *M. jannaschii* endonuclease; (lanes *6,12*) incubation with *S. cerevisiae* cell-free extract. "2/3" indicates intermediates in which the 5′-half or 3′-half tRNA is still joined to the intron. The identity of the products was verified by sequencing.

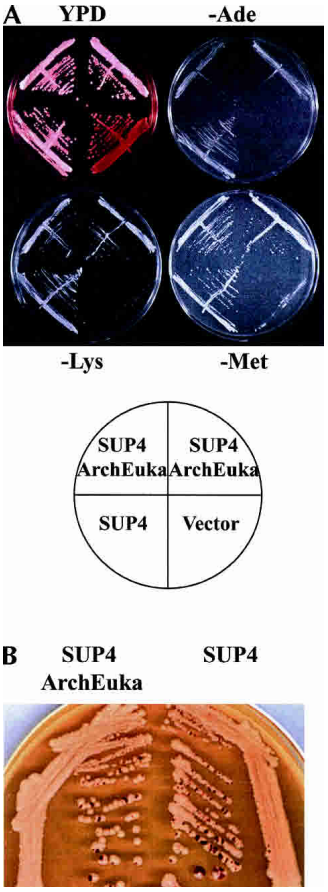

FIGURE 3. In vivo suppression in a yeast strain with a plasmid carrying the gene for *SUP4* pre-tRNA$^{\text{ArchEuka Tyr}}$. (*A*) The strain PJ17-1A, carrying the YCp50 plasmid with the *SUP4* pre-tRNA$^{\text{ArchEuka Tyr}}$ gene, was streaked onto a rich YPD plate or onto selective plates missing, respectively, methionine, lysine, or adenine, and incubated for 3 d at 30°C. Two different transformants are shown in the same plate, together with transformants containing the *SUP4* pre-tRNA$^{\text{Tyr}}$ gene or the YCp50 vector alone, as controls. The scheme of streaking in each plate is shown below. (*B*) A rich YPD plate is shown to illustrate red sectors in white colonies. A nonsense mutation in the *ade2* gene causes the accumulation of a red pigment, while suppression of the mutation restores the white color. Some colonies are white with red sectors, because of the occasional loss of the plasmid.

the hybrid ArchEuka precursor is a good substrate for the eukaryotic splicing machinery in vivo.

Having shown that BHB-containing pre-tRNAs are processed in *S. cerevisiae*, we then asked if noncanonical tRNA molecules, carrying the BHB structure, could also be spliced in yeast. To this end, we designed a very long precursor of *SUP4* tRNA different from the natural pre-tRNA. In this long precursor, the two regions pairing to form the BHB structure are encoded in two DNA regions that lie far away from each other. The main feature of this RNA, which we named "long pre-tRNA^{ArchEuka}," is the replacement of the intron stem-and-loop of *SUP4* pre-tRNA^{Tyr} by a very long stem that can form a BHB structure. The long stem is open at its end, while the mature 5′-end of the tRNA is joined to an artificially introduced CCA sequence at the 3′-end (see Fig. 4). In order to obtain a mature tRNA from this precursor, a cleavage by RNase P, leaving a mature 5′-end and a 3′-terminal CCA, must occur. We have already shown that a tRNA molecule where the 5′- and 3′-ends are joined together is recognized and correctly cleaved by RNase P (Carrara et al. 1995). In addition, the splicing endonuclease should cut at the correct sites of the BHB motif, followed by ligation of the two halves (Fig. 4A).

In designing such a construct we had to avoid the decreased efficiency of transcription caused by the long distance between the A-box and the B-box of the Pol III transcription promoter (Baker et al. 1987; Fabrizio et al. 1987). Accordingly, we introduced an artificial A-box at the same distance from the B-box as it is in the wild-type *SUP4* gene (Fig. 4C). As a control, we designed the same construct without the sequences forming the BHB structure (Fig. 4B).

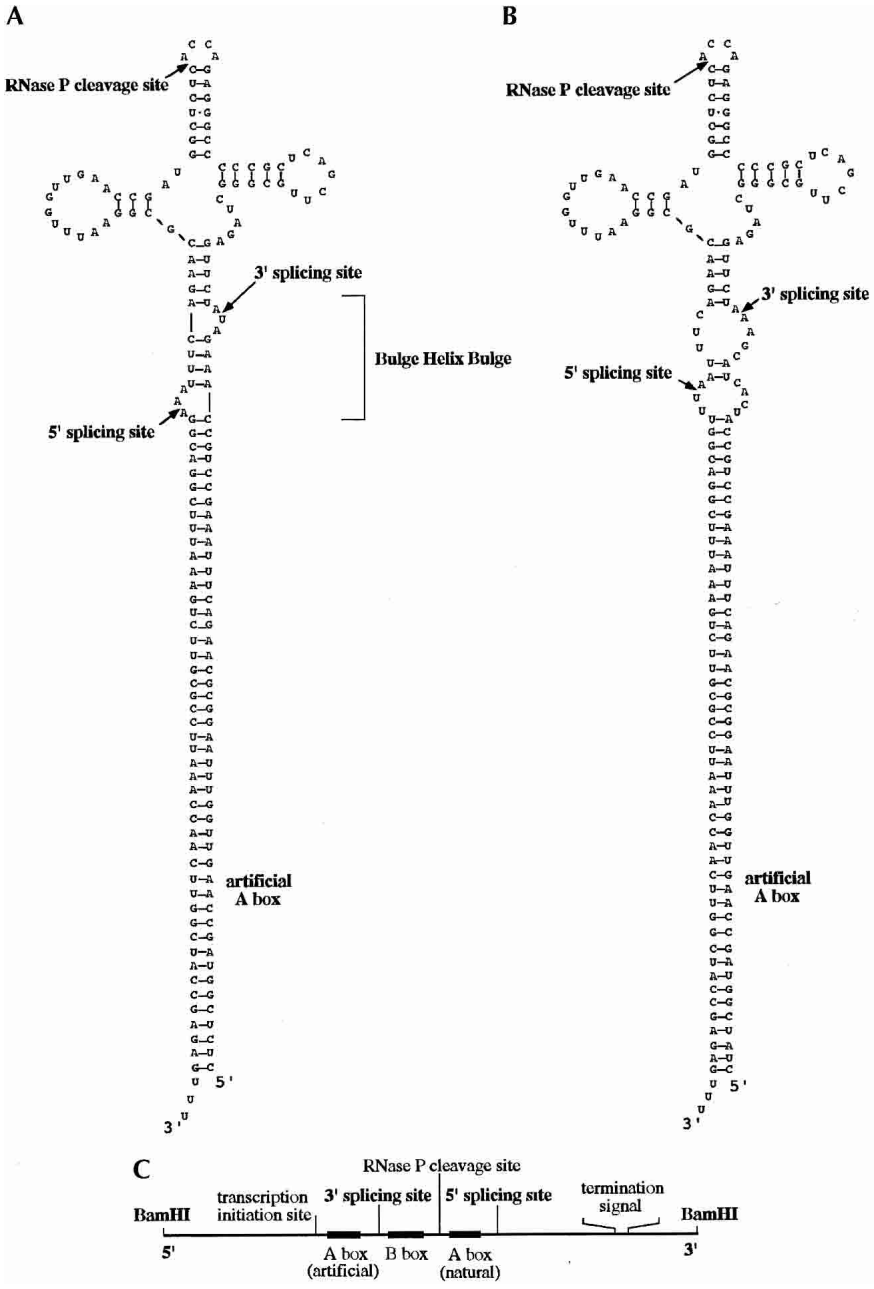

FIGURE 4. (*A*) Long *SUP4* pre-tRNA$^{ArchEuka\ Tyr}$. After processing by RNase P and splicing of the BHB-containing intron, this long precursor produces a bona fide *SUP4* tRNA^{Tyr}. (*B*) Long *SUP4* pre-tRNA^{Tyr}. This precursor is like the one in panel *A* except that the intron does not contain the BHB. (*C*) Schematic structure of the gene encoding the long tRNA precursor.

The genes encoding the long pre-tRNAs were cloned in the low-copy *URA3-CEN* plasmid YCp50 and transformed into the PJ17-1A strain (*met4-1$_o$*, *lys2-1$_o$*, *ade2-1$_o$*). As mentioned before, the ability of transformants to grow on plates without methionine, lysine, or adenine is an indication of the amount of mature *SUP4* tRNA^{Tyr} and, therefore, of correct splicing.

Ura$^+$ transformants were replica-plated on selective media, and growth was checked after 7 d. Some growth was observed on plates lacking methionine, whereas no growth was seen on plates without lysine or adenine. Cells containing the plasmid encoding the control long pre-tRNA, without the BHB structure, grew more than those carrying the plasmid encoding the long pre-tRNA with the BHB, for which growth was hardly visible (Fig. 5A).

The lack of suppression, even for the control construct, could derive, in principle, from the inefficiency in any one of the reactions necessary to get and process the precursor.

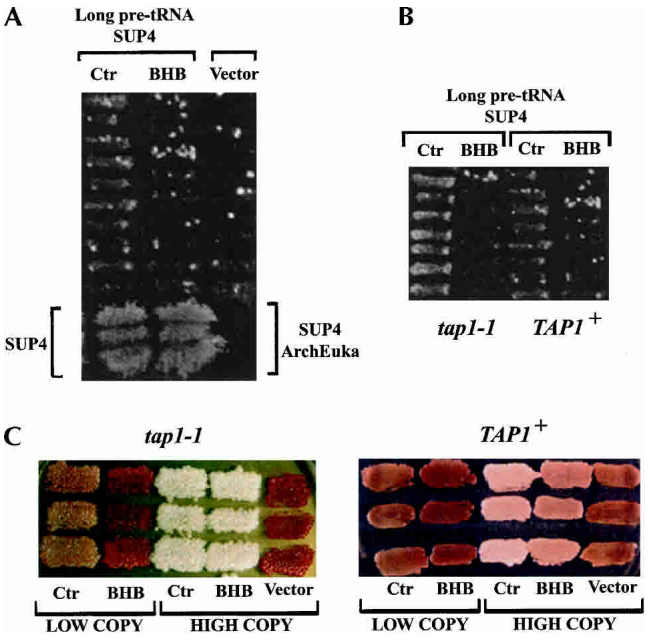

FIGURE 5. (*A*) Several transformants of the strain PJ17-1A with the low-copy *URA3–CEN* plasmid YCp50 encoding the long *SUP4* pre-tRNA$^{ArcheEuka\ Tyr}$ with the BHB or encoding, as a control (Ctr), the long *SUP4* pre-tRNA^{Tyr} without the BHB were patched on −Ura plates and, after 2 d, replica-plated on −Met plates. The photo was taken after 7 d following the replica. Transformants with the vector alone, as well as transformants with the plasmid encoding *SUP4* pre-tRNA^{Tyr} and *SUP4* pre-tRNA$^{ArchEuka\ Tyr}$ are shown. (*B*) Effect of the *tap1-1* mutation. YCp50 plasmids containing the long *SUP4* pre-tRNAs (BHB or Ctr) were transformed in the *tap1-1* mutant GDS4-16D and compared to transformants in the wild-type *TAP1*$^+$ (*RAT1*$^+$) strain PJ17-1A. Growth on −Met plates is shown. (*C*) Comparison of the suppression level provided by long pre-tRNA (BHB or Ctr) encoded by the high-copy plasmid pYX212 or by the low-copy plasmid YCp50, in the *tap1-1* mutant or in the wild-type *TAP1*$^+$ strain. Growth on rich YPD medium is shown.

In order to increase the amount of mature *SUP4* tRNA, a yeast mutant strain previously selected, *tap1-1* (Aldrich et al. 1993; Di Segni et al. 1993), was used. This mutant is characterized by the ability to increase the expression of a tRNA gene with a defective transcription promoter. Plasmids encoding the long pre-tRNAs were transformed into a *tap1-1* mutant strain (GDS4-16D) and, indeed, we obtained increased suppression with the control plasmid, but still very low suppression for the construct bearing the BHB motif (Fig. 5B). The genes encoding the long pre-tRNAs were then cloned into the high-copy 2μ-plasmid pYX212. Using this vector, very good levels of suppression were observed, with both the control plasmid and the construct coding for the BHB structure (Fig. 5C), especially in the *tap1-1* mutant. Table 1 summarizes the growth levels on different culture media for all strain/plasmid combinations. The presence of *SUP4* tRNA^{Tyr} was verified by an acidic Northern blot analysis (data not shown). The long pre-tRNA, lacking the BHB structure, gives rise to a higher level of suppression as compared to the BHB-containing long pre-tRNA. This finding is consistent with previous work, where we showed that hybrid ArchEuka pre-tRNAs are less efficiently spliced when they are incubated in cell extracts or are injected into *X. laevis* nuclei (Fruscoloni et al. 2001).

We conclude that long pre-tRNA^{ArchEuka} is correctly spliced in yeast cells.

In previous in vitro experiments we could show that the BHB constitutes an autonomous structural element when challenged with the yeast or the *X. laevis* splicing endonuclease. We were able to discriminate between the eukaryal and the archaeal mode of processing, typical of the enzyme, by increasing the distance of the intron from the mature domain of the pre-tRNA, thereby giving to the eukaryal enzyme the possibility to operate in the mature-domain independent mode. We cannot utilize this method in vivo, since we have to take into account the constraint derived from the need to produce a functional specific tRNA. There is always the requirement to locate the BHB in a fixed position with respect to the mature domain of the tRNA. The experiments reported here, therefore, do not

TABLE 1. Suppression phenotypes of long pre-tRNAs encoded by high- and low-copy plasmids in different strains

	Growth medium									
	− Ura		− Met		− Lys		− Ade		YPD	
	Strain/plasmid									
	tap1-1	*TAP1*$^+$	*tap1-1*	*TAP1*$^+$	*tap1-1*	*TAP1*$^+$	*tap1-1*	*TAP1*$^+$	*tap1-1*	*TAP1*$^+$
pYX212-Ctr	+++	+++	+++	++	+++	++	+++	+	W	P
pYX212-BHB	+++	+++	+++	++	+++	+	+++	+/−	W	P/R
pYX212 alone	+++	+++	−	−	−	−	−	−	R	R
YCp50-Ctr	+++	+++	+	+/−−	+/−	−	−	−	P	R/P
YCp50-BHB	+++	+++	−	−	−	−	−	−	R	R

High-copy *URA3*-2μ plasmid pYX212 or low-copy *URA3-CEN* plasmid YCp50, containing the gene encoding the long pre-tRNA with the BHB structure or without it (Ctr), were transformed in the *tap1-1* mutant GDS4-16 or in the wild-type *TAP1*$^+$ (*RAT1*$^+$) strain PJ17-1A. Transformants were patched onto selective plates lacking uracile (– Ura), methionine (– Met), lysine (– Lys), or adenine (– Ade), or onto rich plates (YPD). Growth was scored after 3 d. (W) white; (P) pink; (R) red. The number of + indicates the relative growth level.

prove that the yeast enzyme can work in vivo in the archaeal way, but they do show that an archaeal structure (the BHB motif) can be correctly spliced in *S. cerevisiae* cells.

We wish to develop a system in yeast that makes possible the BHB-dependent cleavage and ligation both when the BHB is found in *cis* and when the BHB is formed, in *trans*, between appropriately designed targeting RNAs and a targeted mRNA. We will now try to see if yeast cells are able to process a BHB that does not have in its vicinity a correctly positioned mature tRNA domain. If such an attempt fails, as in mouse cells, we will have to express in yeast cells an archaeal splicing endonuclease in order to reconstruct the complete archaeal splicing system dependent on the BHB.

MATERIALS AND METHODS

Strains, plasmids, and growth media

Escherichia coli strain DH5-α was used to propagate plasmid DNA and was grown on LB medium containing ampicillin. *S. cerevisiae* strains were PJ17-1A (*Mat**a**, trp1*, *ura3-1*, *ade2-1*$_o$, *lys2-1*$_o$, *met4-1*$_o$, *can1-100*$_o$, *gal10-1*$_u$, *his5-2*$_u$, *leu2-1*$_u$) (James and Hall 1990) and GDS4-16D, a derivative of PJ17-1A that carries the mutation *tap1-1* in the *TAP1/RAT1* gene (Amberg et al. 1992; Aldrich et al. 1993; Di Segni et al. 1993; Kenna et al. 1993). Yeast strains were grown on rich YPD medium or drop-out selective media, prepared according to Ausubel et al. (1998). *E. coli* was transformed with plasmid DNA using the $CaCl_2$ method or by electroporation; yeast was transformed using the lithium acetate procedure. Bacterial plasmid PUC19 and the yeast shuttle vectors YCp50 (a *URA3-CEN* plasmid) and pYX212 (a *URA3*–2μ plasmid; R&D System) were used.

tRNA genes

The *SUP4* tRNA^{Tyr} gene, contained in a 266-bp BamHI fragment inserted in YCp50, was used (Aldrich et al. 1993; Di Segni et al. 1993). The *SUP4* tRNA$^{ArchEuka\ Tyr}$ gene was constructed by using eight pairs of annealed oligonucleotides; the long *SUP4* tRNA^{Tyr} gene was constructed with 12 pairs of annealed oligonucleotides. The pairs of oligonucleotides were ligated together and cloned, first into PUC19, between the EcoRI and HindIII sites, and successively into the yeast plasmids. The oligonucleotides were synthesized using the 392 DNA/RNA Synthesizer (Applied Biosystem); they were phosphorylated, annealed, ligated, and digested with restriction enzymes according to standard procedures.

In vitro transcription and processing

tRNA gene sequences were amplified by PCR using two primers bearing, respectively, the T7-promoter sequence at the 5′-end and the sequence complementary to CCA at the 3′-end. Pre-tRNAs were synthesized in vitro by T7 RNA polymerase in the presence of [α-^{32}P]UTP; labeled RNA molecules were purified by phenol-chloroform extraction and ethanol precipitation, followed by electrophoresis on 8 M urea–10% polyacrylamide gels. RNA was eluted from the gel, filtered, and ethanol-precipitated (Di Nicola Negri et al. 1997).

Pre-tRNAs were processed in nuclear extracts of *X. laevis* stage 6 oocytes prepared en masse (Mattoccia et al. 1979; Gandini Attardi et al. 1990) or manually prepared (Lund and Paine 1990). *X. laevis* RNA endonuclease was purified and assayed according to Gandini Attardi et al. (1990). *M. jannaschii* RNA endonuclease was purified according to Li and Abelson (2000) and assayed at 65°C for 30 min in 25 mM Tris-HCl (pH 7.5), 50 mM NaCl, 5 mM $MgCl_2$, and 10% glycerol. Yeast cell extract was prepared and assayed according to Ho et al. (1990). RNA splicing products were purified by phenol-chloroform extraction, ethanol precipitation, and electrophoresis on 8 M urea–10% polyacrylamide gels; the gels were exposed to PhosphorImager and/or to X-ray films at −70°C.

Northern blot analysis was performed under acidic conditions according to Sarkar et al. (1999) and Varshney et al. (1991). A 21-nt-long probe was used for hybridization. The blot was exposed to PhosphorImager after a stringent wash for 1 min at 56°C.

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