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Structure of the Yeast TAP1 Protein: Dependence of Transcription Activation on the DNA Context of the Target Gene

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Sequence data are presented for the *Saccharomyces cerevisiae TAP1* gene and for a mutant allele, *tap1-1*, that activates transcription of the promoter-defective yeast *SUP4* tRNA^{Tyr} allele *SUP4A53T61*. The degree of in vivo activation of this allele by *tap1-1* is strongly affected by the nature of the flanking DNA sequences at 5′-flanking DNA sequences as far away as 413 bp from the tRNA gene and by 3′-flanking sequences as well. We considered the possibility that this dependency is related to the nature of the chromatin assembled on these different flanking sequences. *TAP1* encodes a protein 1,006 amino acids long. The *tap1-1* mutation consists of a thymine-to-cytosine DNA change that changes amino acid 683 from tyrosine to histidine. Recently, Amberg et al. reported the cloning and sequencing of *RAT1*, a yeast gene identical to *TAP1*, by complementation of a mutant defect in poly(A) RNA export from the nucleus to the cytoplasm (D. C. Amberg, A. L. Goldstein, and C. N. Cole, Genes Dev. 6:1173–1189, 1992). The *RAT1/TAP1* gene product has extensive sequence similarity to a yeast DNA strand transfer protein that is also a riboexonuclease (variously known as *KEM1*, *XRN1*, *SEP1*, *DST2*, or *RAR5*; reviewed by Kearsey and Kipling [Trends Cell Biol. 1:110–112, 1991]). The *tap1-1* amino acid substitution affects a region of the protein in which *KEM1* and *TAP1* are highly similar in sequence.

Transcription initiation by eukaryotic RNA polymerase III (PolIII) on various types of class III genes is dependent upon a number of protein initiation factors and upon short DNA sequences of several types that specifically bind some of the factors (13). For tRNA genes, the binding of factor TFIIIC to box A and box B of the internal promoter is well documented (13, 56) and contributes to formation of the transcription initiation complex (3, 27). Similarly, for 5S rRNA genes, binding to internal sequences occurs, with TFIIIA binding first to the C box, followed by TFIIIC binding to box A and to a sequence 3′ to box C (46). Very often observed, but less intensively studied, are effects of the 5′-flanking sequence context of tRNA and 5S rRNA genes upon their level of in vitro and in vivo expression (14, 24, 26, 33, 38, 53). Several reports have described tRNA genes that are activated by removal or replacement of their natural 5′-flanking sequences (24), while in other cases, replacement of the native 5′ context leads to decreased transcription activity (14, 26, 33, 38, 53). The latter results are not easily explainable in terms of an obligatory upstream activation element that binds a single required general transcription factor; the deleted 5′-flanking sequences whose absence inactivates different tRNA genes are highly dissimilar. Whether these sequences need to be recognized by specific DNA-binding proteins for the adjacent genes to be transcribed is not clear; activation may depend upon another attribute of the DNA, such as bending, inherent flexibility, or for in vivo expression, nucleosome positioning.

We describe here the properties of a yeast transcription activator protein (the *TAP1* gene product) for which the *tap1-1* mutant allele can activate transcription of a promoter-defective yeast tRNA^{Tyr} gene. The accompanying report (15) describes our genetic characterization of the yeast *tap1-1* mutation, the rationale for its isolation, and the molecular cloning of *TAP1* by complementation of the *tap1-1* temperature-sensitive growth defect. The *tap1-1* mutation can compensate for a defect in PolIII transcription caused by the presence of a defective B block in the *SUP4A53T61* tRNA^{Tyr} gene. In the present report, we describe the DNA structure of *TAP1*, identify the single amino acid substitution in the *tap1-1* mutant, show by allelic replacement that this change activates *SUP4A53T61*, and describe tests for antigenic relatedness between the *TAP1*-encoded protein and transcription factors TFIIIC and TFIIIB. The latter study indicates that the *TAP1*-encoded protein is unrelated to these PolIII transcription initiation factors.

A clue to the mode of action of *tap1-1* activation of transcription of *SUP4A53T61* is provided by the observed dependence of this activation upon the flanking DNA context within which the *SUP4A53T61* target gene is placed. All of the contexts studied have 101 bp of 5′-flanking DNA and 50 bp of 3′-flanking DNA from the *SUP4* locus immediately adjoining the tRNA gene. The DNA that is distal to these *SUP4*-derived sequences has profound effects upon *tap1-1*-activated *SUP4A53T61* expression. As a possible explanation for these effects, we discuss recent experiments that demonstrate mutual antagonism between the formation of PolIII stable transcription complexes and the assembly of class III genes into chromatin (12, 43).

MATERIALS AND METHODS

Strains, plasmids, growth conditions, and media. *Escherichia coli* RR1 (10) was used to propagate plasmid DNA and

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was grown on Luria broth (42). In protein expression experiments, *E. coli* cells were grown on M9 (39) supplemented with 0.5% Casamino Acids and tryptophan (10 μg/ml) when necessary. Ampicillin (50 μg/ml) was added to the medium when necessary. *E. coli* JM103 (41) was used to propagate bacteriophage and was grown on M9. *E. coli* C600F$^+$ was used to propagate bacteriophage for single-stranded DNA preparation and was grown on Luria broth. *E. coli* cultures were grown at 37 or 30°C for protein expression.

Saccharomyces cerevisiae GDS1-25A (*MATα trp1-1 ura3-1 ade2-1$_o$ lys2-1$_o$ met4-1$_o$ can1-100$_o$ leu1-12 TAP1$^+$*) and a temperature-sensitive derivative of GDS1-25A, 8-14 (*tap1-1*), were used for suppression and RNA studies. The *tap1-1* mutant strain was used for complementation studies. Media were as described by Sherman et al. (54). Cultures were grown at 30°C. For studies at the nonpermissive temperature, cultures were grown at 38°C.

Plasmid YCp50 (47) was the vector for complementation tests. Plasmid pGDS1 contains the 8-kb *Eco*RI-*Eco*RI fragment bearing the *TAP1* gene (15). Bacteriophages M13mp18 and M13mp19 (44) were the cloning vectors used for sequence analysis. *trpE* fusion vectors pATH2 and pATH3 (55) were used for protein expression. Plasmid pHSS6 (52) was used as an intermediate cloning vehicle in fragment exchange cloning.

Construction of plasmids containing *SUP4A53T61*. The *SUP4A53T61* gene was cloned as a 260-bp *Bam*HI fragment into either the *Bam*HI site of yeast shuttle vector YCp50 (pTT33), the *Bgl*II site of yeast shuttle vector pTC3 (pTT42 or pTT43, depending on orientation), or an artificially created *Bam*HI site within a 1.8-kb *Eco*RI-*Eco*RI fragment containing the coding sequences of the yeast *MFα1* gene (pTT40, pTT41, pTT48, and pTT49; see Fig. 5). In pTT40 and pTT41, *SUP4A53T61* is inserted with its direction of transcription opposite to that of *MFα1*, whereas in pTT48 and pTT49, the genes have the same transcriptional orientation. Plasmids pTT40 and pTT41 (and, similarly, pTT48 and pTT49) were created by flipping an *Eco*RI piece containing both the tRNA^{Tyr} and flanking *MFα1* DNAs so that the orientations of each pair differs with respect to flanking YCp50 vector sequences. Plasmid pTT41ΔS resulted from a *Sal*I deletion and religation of pTT41. Plasmids pTT46 and pTT47 resulted from flipping of the *Eco*RI-*Eco*RI fragments in pTT42 and pTT43, respectively. Partial *Bam*HI digestion of pTT33, followed by fill-in synthesis, resulted in loss of either the 3′ (pTT53) or the 5′ (pTT54) *Bam*HI site. These plasmids and pTT41ΔS allowed the construction of pTT55, a plasmid with a 5′ *MFα1* flank and a 3′ *tet*^{r} flank (*Bam*HI-*Sal*I fragment from pTT41ΔS into *Bam*HI-*Sal*I-cut pTT53). Similarly, plasmid pTT56, with a 5′ *tet*^{r} flank and a 3′ *MFα1* flank, was constructed by cloning a *Bam*HI-*Sal*I fragment of pTT54 into *Bam*HI-*Sal*I-cut pTT41ΔS. Plasmid YCp1c-*SUP4* has the 260-bp *Bam*HI fragment of the *SUP4$_o$* gene (3, 21) cloned into the *Bam*HI site of YCp50.

Oligonucleotides. Synthetic oligonucleotides were obtained from the Howard Hughes DNA Synthesis Facility at the University of Washington.

Enzymes and chemicals. Restriction enzymes and T4 DNA ligase were purchased from New England Biolabs (Beverly, Mass.) and Bethesda Research Laboratories (Gaithersburg, Md.). Sequenase was purchased from United States Biochemical Corp. (Cleveland, Ohio). Avian myeloblastosis virus reverse transcriptase was obtained from Promega (Madison, Wis.). Glusulase was purchased from E. I. du Pont de Nemours & Co. (Wilmington, Del.). All enzymes were used in accordance with the manufacturers' instructions. Radionucleotides were purchased from NEN Research Products (Boston, Mass.). Indoleacrylic acid was purchased from Sigma Chemical Co. (St. Louis, Mo.).

Nucleic acid preparations. Large-scale preparation of plasmid or phage DNA from *E. coli* was done by polyethylene glycol precipitation (34). Miniprep DNA isolation from *E. coli* was performed by the method of Birnboim and Doly (8). Isolation of single-stranded M13 DNA was done by the method of Heidecker et al. (23). Chromosomal DNA from *S. cerevisiae* was prepared as described by Braus et al. (11), and plasmid DNA was recovered as described by Ausubel et al. (2). Total RNA was obtained from *S. cerevisiae* as described previously (20).

DNA sequence analysis. Restriction fragments to be sequenced were cloned into M13mp18 or M13mp19. The nucleotide sequence was determined by dideoxy-chain termination (50) with [^{32}P]dCTP, Sequenase, and M13 forward primer (New England Biolabs). In regions where there were no convenient restriction sites, 15-bp synthetic oligonucleotides were used as primers. The complete DNA sequence was determined on both strands.

Primer extension analysis. Primer extension analysis was performed as described by Ausubel et al. (2), by using a *TAP*-specific oligonucleotide (5′-TCCAATACTGGGGAT-3′). The cDNA was analyzed on a 6% polyacrylamide gel (1:20 bisacrylamide-acrylamide ratio).

Molecular and genetic techniques. Vector DNA digested with a single restriction enzyme was treated with bacterial alkaline phosphatase (1 U/μl; Boehringer Mannheim Biochemicals, Indianapolis, Ind.) to minimize vector religation (39). DNAs for fragment exchange mapping and sequence analysis were obtained from plasmids pGDS1 and pTM11. DNA for *trpE* fusions was obtained from pGDS1. Restriction fragments less than 2 kb long were purified from 5% polyacrylamide gels (1:30 bisacrylamide-acrylamide ratio) by extraction with 0.1× SSC (1× SSC is 0.15 M NaCl plus 15 mM sodium citrate) at 37°C. Larger restriction fragments were purified from 0.7% agarose gels with GeneClean (Bio 101, La Jolla, Calif.). Ligations were carried out overnight at 15°C. Preparation of competent *E. coli* cells and transformations were performed by the $CaCl_2$ method (2). Transformants of *E. coli* RR1 were selected on Luria broth plates supplemented with ampicillin, and clones were verified by restriction analysis of miniprep DNA. For complementation tests, the *tap1-1* mutant *S. cerevisiae* strain was transformed by the spheroplast method (6). Transformants were selected on plates lacking uracil and then tested for growth at 30 and 38°C. Suppression tests of the *ade2-1*, *lys2-1*, and *met4-1* ochre alleles in the *TAP1$^+$* and *tap1-1* strains were performed by replica plating cell patches onto plates lacking adenine, lysine, or methionine and scoring phenotypes after incubation at 30°C for 5 days. Redness was measured on YEPD (1% yeast extract, 2% peptone, 2% dextrose) agar.

TrpE fusion protein expression and purification. Restriction fragments from plasmid pGDS1 (see Fig. 3) were attached to the C terminus of *trpE* sequences in *trpE* fusion vectors pATH2 and pATH3. Induction of the formation of these fusion proteins was achieved as described by Spindler et al. (55). Lysates were prepared by suspending harvested cells in cracking buffer (10 mM Na_2HPO_4 [pH 7.2], 1% 2-mercaptoethanol, 1% sodium dodecyl sulfate [SDS], 6 M urea). The proteins were separated with SDS–10% polyacrylamide gels (0.8:30 bisacrylamide-acrylamide ratio) as described by Laemmli (36). The gels were stained for 2 h in nonfixing protein stain (75 mM Tris-HCl [pH 7.4], 20% methanol, 0.25% Coomassie blue) and then destained with

75 mM Tris-HCl (pH 7.4)–20% methanol. The TrpE::TAP1 fusion protein bands were cut from the gel and eluted by electrophoresis.

Antibody production and purification. Polyclonal antibodies to the TrpE::TAP1 fusion proteins were raised in two female New Zealand Black rabbits. Purified proteins (750 μg) were pooled and mixed with an equal volume of Freund's complete adjuvant (Sigma Chemical Co.) and injected subcutaneously. This was followed 21 days later with a booster of protein (750 μg) mixed with an equal volume of Freund's incomplete adjuvant (Sigma Chemical Co.). Serum was collected at 2-week intervals, beginning at day 41 and continuing until antibody titers dropped as determined by Western blot (immunoblot) analysis. Antibodies were affinity purified against the fusion proteins as follows. Gel-purified TAP1 fusion protein (500 μg) was spotted onto a nitrocellulose filter and allowed to air dry at room temperature for 1 h. The filter was blocked in TBS (20 mM Tris-HCl [pH 7.5], 250 mM NaCl) plus 1% dry skim milk for 15 min. The filter was placed in a heat-sealable bag and incubated with 700 μl of TBS plus 1% skim milk and 300 μl of rabbit serum for 4 h at room temperature. The filter was washed once in TBS, twice in TBS plus 0.1% Nonidet P-40, and once in TBS for 15 min each time to remove unbound antibodies. Bound antibodies were eluted with 2 ml of 200 mM glycine (pH 2.4) with shaking for 2 min at room temperature. Aliquots (750 ml) of the eluate were mixed with 250 μl of 1 M Tris-HCl (pH 8.5) for neutralization. The elution step was repeated. Antibodies were concentrated by using Centricon 30 microconcentrators (Amicon, Beverly, Mass.) and stored at −20°C until use.

Immunoblotting. Equivalent amounts of protein were mixed with an equal volume of solubilization solution (10 mM Tris-HCl [pH 6.8], 6% SDS, 0.002% bromophenol blue), boiled for 3 min, and electrophoresed through an SDS–10% polyacrylamide gel (0.8:30 bisacrylamide-acrylamide ratio) by the method of Laemmli (36). Following electrophoresis, the proteins were transferred electrophoretically with the Transblot System (Bio-Rad), at 80 V and 4°C, to nitrocellulose filters (0.45-μm pore size; Schleicher & Schuell). They were probed with antibodies specific for the TAP1 protein by using TBS plus 1% skim milk as the blocking agent. The presence of the TAP1 protein was detected by luminescence of a secondary (goat anti-rabbit) antibody coupled to peroxidase as described by Schneppenheim and Rautenberg (51).

RESULTS

Nucleotide sequence and mRNA initiation site of *TAP1*. Preliminary deletion analysis by subcloning and complementation of the *tap1-1* temperature sensitivity (ts^-) lesion showed that the complementing gene is present on a 5.5-kb *Eco*RI-*Xba*I fragment (see Fig. 2). Within the nucleotide sequence of this fragment (Fig. 1) is a single long open reading frame predicted to encode a protein of 1,006 amino acids with a calculated molecular weight of 115,940. Northern (RNA) hybridization experiments indicated the presence of a large yeast mRNA mapping to the region of the *Eco*RI-*Xba*I fragment. To place the *TAP1* mRNA 5′ end precisely on the DNA sequence, primer extension analysis was performed. A ^{32}P-end-labeled oligodeoxynucleotide primer (TCCAATACTGGGGAT) was hybridized to total RNA isolated from *S. cerevisiae* GDS1-25A and then elongated with reverse transcriptase. The primer sequence (shown as the complementary sequence in Fig. 1) occurs at positions +54 to +68 relative to the ATG codon. Therefore, the length of 138 nucleotides measured for the elongation product, by using dideoxy sequencing reaction size standards (results not shown), places the 5′ end of *TAP1* mRNA 70 bp upstream of the ATG codon. The sequence at that position (CATAA) includes two potential transcription start sites (20). Upstream of this position, short AT-rich blocks occur only at positions −40, −108, and −158, none of which closely matches the canonical TATAAA element.

Chemical nature and phenotypic effect of the *tap1-1* mutation. DNA containing the entire coding region from the *tap1-1* mutant allele was obtained from yeast strain 8-14 (15) by the technique of gapped plasmid repair (45). DNA of plasmid pGDS1, containing the *TAP1* allele, was digested with *Xba*I nuclease to remove the *TAP1* coding region, leaving the flanking chromosomal sequences. When this linear DNA was introduced into cells of strain 8-14, nonreciprocal recombination between the chromosomal *tap1-1* locus and the homologous ends restored the original plasmid structure by filling the gap with *tap1-1* sequences. By restriction and ligation in vitro, we constructed various hybrid *tap1-1/TAP1* genes (Fig. 2), exchanging localized regions between the recovered *tap1-1*-containing plasmid and pGDS1. Each such hybrid plasmid was tested for the ability to complement the ts^- growth phenotype of *tap1-1* mutant strain 8-14. The results of this analysis (Fig. 2) map the mutant site to a position within a 1.3-kb *Eco*RV-*Sst*I fragment of the gene. When this segment of the wild-type gene was replaced with *tap1-1* DNA, complementation of the ts^- mutation was lost; conversely, when this segment of *TAP1* DNA was introduced into the gene recovered from the *tap1-1* mutant strain, the resulting DNA was able to complement the ts^- growth defect.

To identify the molecular nature of the mutation, the 1.3-kb *Eco*RV-*Sst*I segment of the *tap1-1* allele was sequenced. It proved to have the same sequence as the *TAP1* allele, except for a single thymine-to-cytosine transition that causes the mutant protein to have a histidine at amino acid 683, where the wild type has tyrosine.

To determine whether the mutation responsible for the ts^- phenotype also affects suppression, allelic replacement experiments were done by yeast transformation. The 1.3-kb *Eco*RV-*Sst*I fragment from the wild-type gene was cotransformed into *tap1-1* mutant strain 8-14 along with plasmid pTT40, a *URA3*-containing plasmid which has the *SUP4A53T61* gene (see Fig. 5) and allows the *tap1-1* mutant to grow on plates lacking lysine. Transformants were selected on plates lacking uracil. They were screened for growth at 30 and 38°C. Five ts^+ transformants were obtained. They, along with five ts^- transformants, were screened for suppression. The ts^+ transformants were unable to grow on plates lacking lysine, while the ts^- transformants could do so. This demonstrated that the nucleotide change responsible for the ts^- phenotype also determined the suppression phenotype.

Heterologous *TAP1* gene expression and antibody formation. To make possible antibody studies of the relationship between the *TAP1* protein and transcription factors previously described for yeast Pol III (32, 49), we carried out heterologous expression of the cloned sequence in *E. coli*. Fusions between the *E. coli trpE* gene and each region of *TAP1* were constructed (Fig. 3). Clones pTT3, pTT4, pTT6, and pTT9 produced large amounts of fusion proteins in *E. coli* when expression of the *trpE* gene was induced with indoleacrylic acid. Clone pTT12, however, did not produce enough protein to be detected by SDS-polyacrylamide gel electrophoresis. Since attempts to overexpress the entire

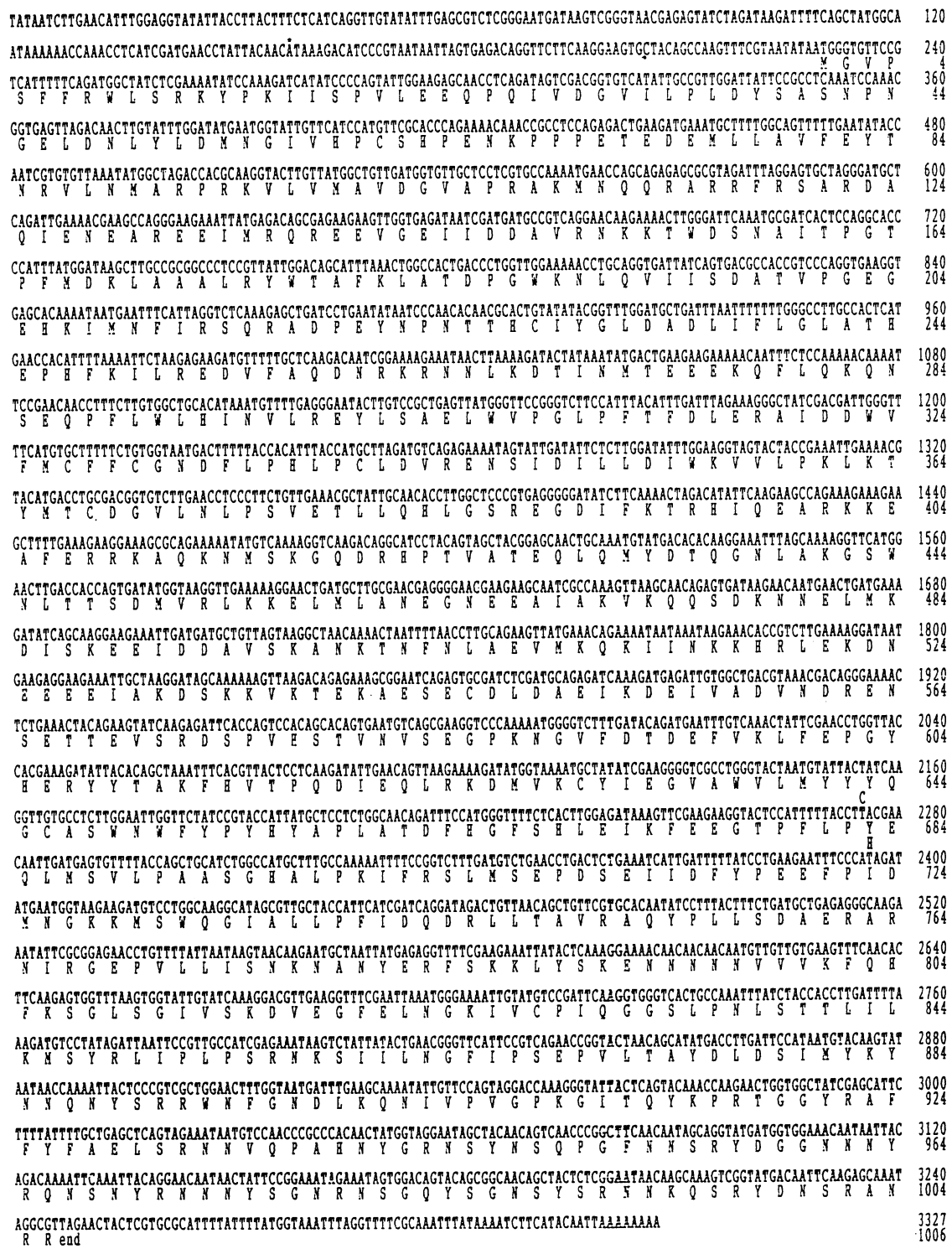

FIG. 1. Nucleotide and amino acid sequences of the *TAP1* gene. The nucleotide sequence of the coding strand of the putative *TAP1* gene is shown along with the translation of the open reading frame. Restriction sites are indicated above the DNA sequence. Positions of the nucleotides and amino acids are designated at the right of the sequences. The asterisk denotes the 5′ end of the mRNA. The single nucleotide change found in the *tap1-1* mutation is located at position 2275, and the corresponding amino acid alteration is located at position 683.

TAP1 protein with a T7 system (57) also failed, it appears that the carboxy-terminal region of this protein is toxic to *E. coli*.

Polyclonal antibodies were raised against a pool of gel-purified proteins produced by pTT3, pTT4, pTT6, and pTT9. Antibody directed against each fusion protein was affinity purified against its respective immunogen. Because each of the affinity-purified proteins contained antibody to the TrpE protein, TrpE-specific antibodies were included as negative controls in all experiments using these antibodies.

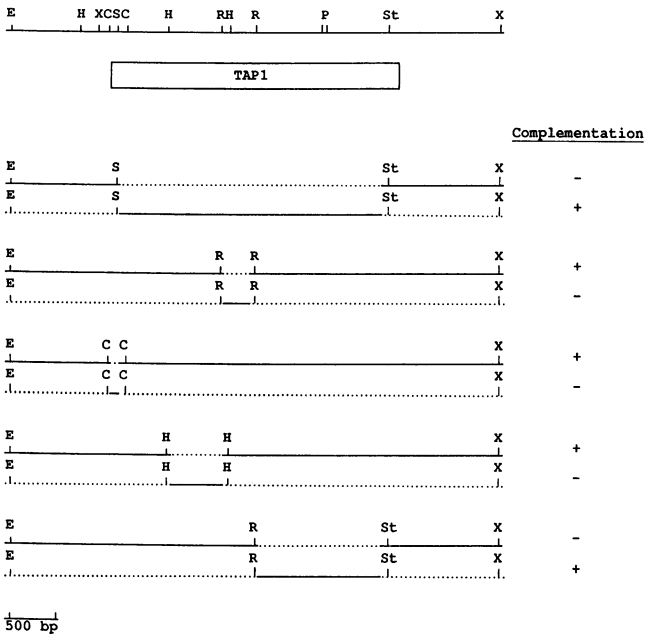

FIG. 2. Restriction fragment exchange between *TAP1* wild-type and *tap1-1* mutant strains. A restriction map of the *TAP1* gene is shown. Restriction sites are abbreviated as follows: E, *Eco*RI; H, *Hin*dIII; X, *Xba*I; C, *Cla*I; S, *Sal*I; R, *Eco*RV; P, *Pvu*II; St, *Sst*I. The open reading frame that encodes the putative *TAP1* gene is represented by the rectangle. DNA from the wild-type strain is shown as solid lines, and DNA from the mutant strain is shown as dotted lines. Complementation of the *tap1-1* mutation by the constructs is shown on the right. Complementation was determined by growth at 38°C (+); a minus sign indicates no growth at this temperature.

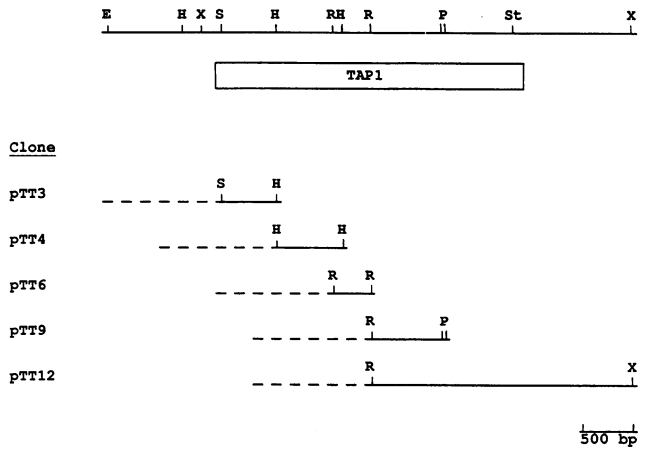

FIG. 3. *TrpE::TAP1* fusion constructs. Restriction fragments from the *TAP1* gene were fused to the *trpE* gene for expression in *E. coli*. The restriction map of the *TAP1* gene is shown. Restriction sites are abbreviated as indicated in the legend to Fig. 2. The putative *TAP1* open reading frame is represented by the rectangle. The truncated *E. coli trpE* gene is represented by dashed lines; *TAP1* DNA is represented by the solid lines.

Is the TAP1 polypeptide present in TFIIIB or TFIIIC? While the *tap1-1* mutant selection involved transcriptional activation of a yeast $tRNA^{Tyr}$ gene defective in binding transcription factor TFIIIC, in vitro tests of TFIIIC from the mutant strain did not disclose any change in its tDNA-binding behavior (15). Definitive tests at the protein level of the relationship between *TAP1* and TFIIIC, as well as TFIIIB, are made possible by the anti-TAP1 antibodies described above. To determine whether the TAP1 polypeptide was a component of either transcription factor, we carried out Western blot analysis of purified factor preparations, kindly provided by J. Huet and A. Sentenac. In each case, column fractions that covered and bracketed the peak for transcription factor activity were analyzed (Fig. 4). For TFIIIB fractionated by Cibacron Blue chromatography (Fig. 4, left lanes), a strong band equal in molecular weight to the predicted *TAP1* product was obtained in the fraction loaded in lane 1. However, this fraction contained no TFIIIB activity, its peak being in the fractions loaded in lanes 3 and 4. No cross-reaction with anti-TAP1 was seen for any fraction from the TFIIIC DEAE-Sephadex chromatographic fractionation. Thus, the TAP1 protein is definitely not a subunit of TFIIIC and also is unlikely to correspond to the major DNA-binding component present in TFIIIB preparations (5, 32).

Flanking DNA context essential for *tap1-1* transcription activation. Our immunological studies reinforce the conclusion (15) that the *tap1-1* mutation does not affect the properties of TFIIIC; indeed, *tap1-1* seems not to encode any part of this transcription factor. Nonetheless, the mutant protein somehow enables the PolIII machinery to transcribe *SUP4A53T61*, a gene that cannot efficiently bind TFIIIC, an essential component for PolIII transcription. This compensatory mutation must therefore allow the TAP1 protein to assist or supplant TFIIIC in its essential role in PolIII transcription. Because TFIIIC does not bind with high

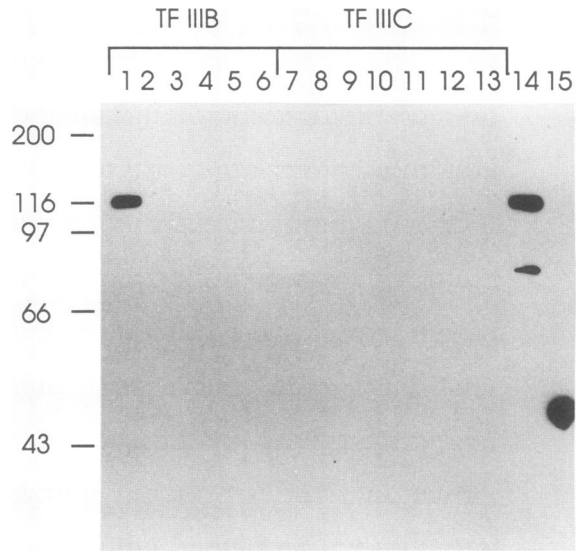

FIG. 4. Immunoblot of RNA PolIII transcription factors. Partially purified TFIIIB fractions (lanes 1 to 6, containing 3, 6, 7, 7, 5, and 3 mg of total protein, respectively) and TFIIIC (lanes 7 to 13, containing 1, 2, 2, 2, 2, 3, and 3 mg of total protein, respectively) were immunoblotted by using antibodies against the TAP1 protein. Lane 14 contained 26 mg of protein from the *tap1-1* mutant partially purified on a DEAE ion-exchange column. Lane 15 contained 10 ng of gel-purified pTT6-encoded fusion protein. Positions of the molecular size markers, indicated on the left in kilodaltons, were determined by Coomassie staining. The 116-kDa TAP1 protein was detected in one of the TFIIIB fractions (lane 1). Both the 116-kDa protein and an 80-kDa protein were detected in the *tap1-1* mutant extract (lane 14).

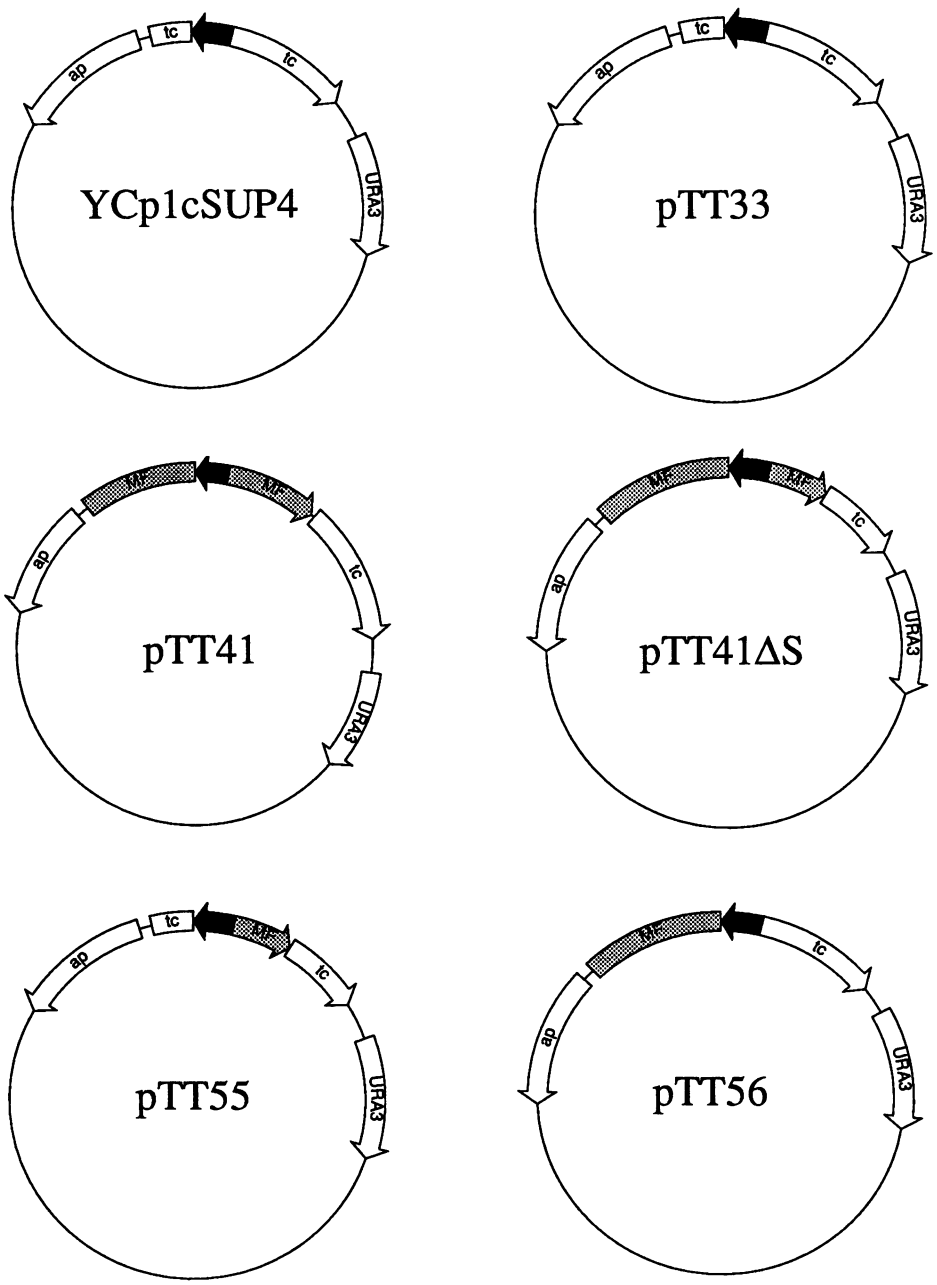

FIG. 5. Plasmid constructs used to test the effect of flanking DNA on suppression. The solid area of each plasmid represents the *SUP4A53T61* or *SUP4* gene. The *MFα1* gene is represented by the stippled area (MF). The open area and thin line represent sequences of the YCp50 vector used for cloning. The arrows point in the direction of transcription for the genes. ap, ampicillin resistance; tc, tetracycline resistance.

affinity to sequences within *SUP4A53T61*, we considered the possibility that transcription activation by *tap1-1* involves sequences outside the gene.

While the removal of 5′-flanking sequences further than 45 bp upstream of the yeast *SUP4* gene normally has little if any effect upon its transcription either in vitro or in vivo (33, 53), we found that the extent of in vivo suppression by the *SUP4A53T61* allele is strongly dependent upon the nature of the DNA sequences which flank the $tRNA^{Tyr}$ gene insert (Fig. 5 and Table 1). This was measured by placing the *SUP4A54T61* fragment in several different surrounding DNA environments in CEN plasmid vector YCp50 (Fig. 5), transforming the plasmids into the *tap1-1* strain, and measuring the effectiveness with which the *met4-1*, *lys2-1*, and *ade2-1* ochre alleles were suppressed (Table 1). In the initial studies, the *SUP4A53T61* gene was inserted as a 260-bp *Bam*HI fragment into either the *Bam*HI site of yeast shuttle vector YCp50 (Fig. 5) or a *Bam*HI site within a 1.8-kb *Eco*RI-*Eco*RI fragment of yeast *MFα1* DNA in YCp50 (*Eco*RI site) (35). The point of $tDNA^{Tyr}$ insertion in the latter sequence was an artificially created *Bam*HI site within the coding sequence for the *MFα1* precursor (Fig. 5), 170 bp upstream of the first *Hin*dIII site (35). Previously, an insertion of two tandem copies of the *SUP4A53T61* gene (head to tail) at this site in

TABLE 1. Suppression phenotypes produced by *SUP4* and *SUP4A53T61* in plasmids YCp50 and YCp50 carrying *MFα1* in mutant strain 8-14

Plasmid	Growth of transformants on media lacking:			Color on YEPD	Suppression index[a]
	Met	Lys	Ade		
pTT33	0	0	0	Dark red	0
YCp1c*SUP4*	1	4	4	White	9
pTT41 (=pTT40)	1	3	0	Dark pink	4
pTT41ΔS	1	2	0	Dark pink	3
pTT48 (=pTT49)	1	1	0	Red	2
pTT55	0	0	0	Dark red	0
pTT56	0	0	0	Dark red	0

[a] The suppression index of six Ura$^+$ transformants with each plasmid was determined in the following way: one point for growth without Met, zero to four points for strength of growth without Lys, and zero to four points for growth without Ade. In each case, all six were identical.

an *MFα1* gene within YCp50 was used as the target gene for selection of the *tap1-1* mutation (15). Our studies of the flanking DNA context were done with yeast centromere plasmids bearing one single *A53T61* gene to avoid ambiguities caused by having two *A53T61* genes with different flanking contexts within the same plasmid.

With the simplest plasmid construct, *SUP4A53T61* in YCp50 (pTT33; Fig. 5 and Table 1), ochre suppression was not observed in either *tap1-1* or *TAP1* cells. Contrasting with this was the high level of suppression obtained with a single copy of the $SUP4_o$ gene at the *Bam*HI site of YCp50 (Table 1, plasmid YCp1c *SUP4*). Transformants bearing this latter plasmid were Ade$^+$ in either a *TAP1* or a *tap1-1* background. There was a low but significant level of ochre suppression when the orientation of the *Bam*HI *SUP4A53T61* fragment was reversed in YCp50.

When the *SUP4A53T61* gene was inserted at a site within the yeast *MFα1* gene, its expression was greatly increased (pTT41 in Table 1), but only in yeast cells of the *tap1-1* genotype. Expression of the tRNA^{Tyr} suppressor gene was highest when *SUP4A53T61* was inserted with its direction of transcription opposite to that of *MFα1* (pTT40 and pTT41, Table 1), compared with two plasmids having the same transcriptional orientation of the two genes (pTT48 and pTT49). Within each pair, the members differ in overall orientation of the composite 2-kb *Eco*RI-*Eco*RI fragment bearing both tRNA^{Tyr} and *MFα1* genes, with respect to the flanking YCp50 vector sequences. This gross orientation has no effect upon *SUP4A53T61* gene expression (Table 1). It seems clear that the largest effects on expression involve changes in the nature of those sequences which adjoin the 260-bp *SUP4A53T61* insert; these changes occur either by substitution of the flanking sequences, replacing *tet*^{r} DNA by *MFα1* sequences, or by inversion of the 260-bp *Bam-Bam* tDNA insert, switching its 5′- and 3′-flanking sequences within a given plasmid.

To determine which of the *MFα1* sequences permits *tap1-1* to activate transcription of *SUP4A53T61*, we made plasmids that are intermediate in structure between pTT33 and pTT41. As an initial step, pTT41 was cut with *Sal*I and religated to form pTT41ΔS (Fig. 5). This step deleted a *Sal-Sal* fragment containing both *MFα1* and pBR322 sequences that flank the right-hand *Eco*RI site of pTT41. The remaining *MFα1* DNA in the 5′-flanking region of *SUP4A53T61* extends from 102 to 515 bp upstream of the tRNA^{Tyr} transcription start site. Plasmid pTT41ΔS exhibits a level of in vivo tRNA^{Tyr} expression that is only slightly less than that of pTT41 (Table 1). Because both pTT41ΔS (active) and pTT33 (inactive) contain the *SUP4A53T61 Bam-Bam* fragment between a 5′-flanking *Sal-Bam* fragment and a 3′-flanking *Bam-Eco*RI fragment, it is possible to make intermediate forms that have 5′-*MFα1-A53T61-tet*^{r}-3′ (pTT55) and 5′-*tet*^{r}-*A53T61-MFα1*-3′ (pTT56). The results of transformation with these plasmids and assay for in vivo suppression (Table 1) do not identify either the 3′- or 5′-flanking *MFα1* sequence as being solely responsible for activation. Surprisingly, both the pTT55 and pTT56 plasmids showed no detectable *SUP4A53T61* expression by growth tests. We conclude that replacement of *MFα1*-flanking DNA by either the 5′ or the 3′ pBR322-derived sequences is sufficient to prevent activation of the *SUP4A53T61* gene by the *tap1-1*-encoded mutant protein.

***TAP1-1* activates chromosomal *SUP4A53T61*.** The influence of the *TAP1* genotype upon the suppressor activity of *SUP4A53T61* was also analyzed in the context of an *MFα1* gene in the chromosome. An integrating plasmid was constructed by inserting the *Eco*RI-*Eco*RI *MFα1* fragment, containing two copies of *SUP4A53T61*, into *URA3*-carrying vector YIp5, in which the *Sal*I site in the middle of the *tet* gene had previously been destroyed. The *SUP4A53T61* tandem genes were in the opposing transcriptional orientation with respect to the *MFα1* gene. Integration of this construct into the *MFα1* locus was accomplished by cutting the DNA at the single *Sal*I site, 3′ to the *MFα1* gene, and selecting for Ura$^+$ transformants, both in the *tap1-1* mutant and in the *TAP1*$^+$ strain. Transformants from each strain were patched onto plates lacking uracil and then replica plated to plates lacking methionine or lysine. While the *tap1-1* integrants grew actively on plates lacking methionine and fairly well on plates lacking lysine, the *A53T61* integrants in *TAP1* cells did not grow at all, even on plates lacking methionine. This difference in growth indicates that, for chromosomal and plasmid-borne *SUP4A53T61*, the *tap1-1* mutant gene can activate transcription.

Sequence comparison between the proteins encoded by *TAP1* and *SEP1*. Our experiments show that the *TAP1*-encoded protein is not a part of TFIIIC, the box A and box B recognition factor that normally activates tRNA gene transcription (13; Fig. 4). Because *TAP1* is unrelated to the RNA PolIII transcription factors previously identified by in vitro assays, the *tap1-1* mutation appears to have disclosed a new aspect of in vivo transcription activation. To understand the possible mechanism for this activation, we compared the inferred amino acid sequence of the *TAP1*-encoded protein with other sequences in the protein data base. A significant match was found in only one case, that encoded by a yeast gene variously named *SEP1*, *KEM1*, *XRN1*, *DST2*, or *RAR5* (Fig. 6). The different functions demonstrated for this protein (reviewed in reference 28) include in vitro DNA strand transfer (17, 25), 5′→3′ exonuclease activity (25, 37), and in vivo effects upon karyogamy (30) and plasmid copy number (31). The calculated molecular mass of the *SEP1* gene product is 160 kDa, compared with 116 kDa for TAP1. The multiple regions of sequence similarity between the two proteins occur in the N-terminal part of SEP1; that is, SEP1 contains a C-terminal extension relative to TAP1. Most striking are the perfect matches of 18, 14, and 11 amino acids in the first three lines of Fig. 6.

A possible nucleic acid-binding site within one of the conserved blocks (overlined in Fig. 6) clearly differentiates the two proteins from one another. The TAP1 amino acid

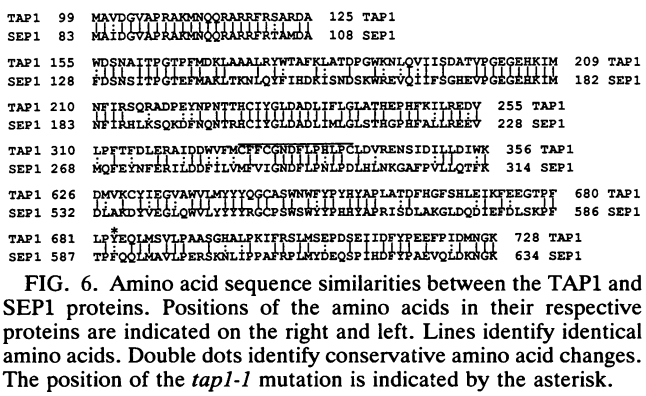

FIG. 6. Amino acid sequence similarities between the TAP1 and SEP1 proteins. Positions of the amino acids in their respective proteins are indicated on the right and left. Lines identify identical amino acids. Double dots identify conservative amino acid changes. The position of the *tap1-1* mutation is indicated by the asterisk.

sequence (**CFFCGNDFLPHLPC**) falls within the broad category of potential metal-binding motifs identified by Berg (7). While this sequence occurs in a region with 10 of 15 residues identical between TAP1 and SEP1, the key metal-binding residues (**C** and **H**) are all lacking in SEP1. Therefore, if this region has a metal-binding role in TAP1 function, we expect that SEP1 lacks this function.

In the most C-terminal sequence block conserved between these two proteins, including residues 626 to 728 of TAP1 and 532 to 634 of SEP1, there is a sequence identity of 50% with no gaps. This region is particularly rich in aromatic amino acids in both proteins. Of their combined total of 38 (W + Y + F), 32 occur at corresponding positions in the two proteins. One of these positions, Tyr-683 of TAP1 (opposite Phe-589 of SEP1), is the site of the *tap1-1* mutation, which substitutes a histidine for the tyrosine.

DISCUSSION

Our data show that *TAP1* does not encode any of the known PolIII general transcription factors, TFIIIB, TFIIIC, or TFIID (58); for TFIID, this conclusion is based upon a comparison of the gene sequences. The possibility remains open, however, that the TAP1 protein is a previously unrecognized general PolIII transcription factor. Because of the extensive common or closely related amino acid sequence regions between the TAP1 protein and the previously characterized yeast SEP1 protein, it seems likely that each of these proteins interacts with DNA in a similar manner. In both its activities as a DNA strand transfer protein and as a double-stranded exonuclease, the SEP1 protein appears to recognize particular types of DNA structures rather than act upon specific DNA sequences. Therefore, we are inclined toward the view that *tap1-1* affects transcription by facilitating an alteration in the DNA structure in or near the *SUP4A53T61* gene. On the basis of the nature of the transcription defect of the *SUP4A53T61* allele and the interplay we have observed between distant sequences flanking the tRNA gene and the suppression phenotype conferred by the *tap1-1* mutation, we propose that the function of *TAP1* involves changes in chromatin structure. In the paragraphs that follow, we review the findings and logical steps that led us to this suggestion.

The *SUP4A53T61* transcription defect. Unlike $SUP4_o$ and other tRNA genes with high affinity internal promoters, *SUP4A53T61* is unable to bind TFIIIC tightly (15) or competitively block transcription of another tRNA gene (yeast $tRNA_3^{Leu}$) that binds TFIIIC with high affinity. Nonetheless, in an S100 system that includes TFIIIB, TFIIIC, and PolIII, the *SUP4A53T61* gene by itself is efficiently transcribed.

Even when transcription has been initiated on the *SUP4A53T61* template, TFIIIC molecules continue to be available for transcription of a subsequently added $tRNA_3^{Leu}$ gene. In a preemption experiment (4) in which either $SUP4_o$ or *SUP4A53T61* was first added to a transcription extract, followed 2 min later by a reference $tRNA_3^{Leu}$ gene, $SUP4_o$ DNA totally prevented subsequent $tRNA_3^{Leu}$ transcription while *SUP4A53T61* was ineffective at blocking $tRNA_3^{Leu}$ transcription (data not shown). While the extent of TFIIIC-*A53T61* complex formation is sufficient to allow normal transcription initiation, the failure of *A53T61* to preempt implies that its complex with TFIIIC is readily dissociable. When the *SUP4A53T61* and $tRNA_3^{Leu}$ genes are simultaneously added to the transcription extract, dissociation of the complex between TFIIIC and *SUP4A53T61* allows the $tRNA_3^{Leu}$ gene to form stable transcription complexes, depressing the *SUP4A53T61* transcript yield (15). If the level of TFIIIC were limiting in vivo as it is in these in vitro reactions, the failure of *SUP4A53T61* to be transcribed might readily be explained as competition for TFIIIC by the 350 other tRNA genes present in the yeast nucleus. In comparison, the number of TFIIIC molecules per haploid nucleus is estimated to be 1,000 (52a), far exceeding the number of tRNA and 5S rRNA genes combined. Therefore, competition for TFIIIC per se is unlikely to be a major factor in determining the transcriptional output of the *SUP4A53T61* gene in yeast nuclei. However, the instability of the TFIIIC complex on the *SUP4A53T61* gene leads to another important consequence: an increased probability of occupancy of the gene by a nucleosome monomer.

The ability of stable transcription complexes between 5S rDNA, TFIIIA, and TFIIIC to exclude nucleosome formation has been well documented, as has the prevention of transcription complex formation on 5S rRNA genes by preassembled nucleosomes (9, 18, 22, 46). For the *SUP4A53T61* gene in vivo, we suggest that the instability of its complex with TFIIIC makes it uniquely susceptible to transcriptional inactivation by nucleosome formation. In this regard, there is a striking parallel between the template behavior of the yeast *SUP4A53T61* $tRNA^{Tyr}$ gene and the *Xenopus laevis* oocyte 5S rRNA gene (59). Both genes can act as templates for nearly normal levels of in vitro transcription, but both are incapable of stable transcription complex formation and both suffer a very strong degree of in vivo transcriptional repression relative to their functional counterparts, the $SUP4_o$ allele and the *X. laevis* somatic 5S rRNA gene (15, 59). Interestingly, the sequence differences between oocyte and somatic 5S rRNA genes that are believed to differentially affect complex stability and in vivo expression occur in a region of the 5S rRNA gene sequence involved in the binding of TFIIIC (46), as is the case with the *A53* and *T61* mutations of the *SUP4* gene (15).

Effects of sequences flanking the *SUP4A53T61* gene. If, as we hypothesize, the *SUP4A53T61* gene in a wild-type yeast cell is subject to inactivation by incorporation into a nucleosome, then the *tap1-1* mutant protein is able to prevent this inactivation. Moreover, its ability to do this is strongly dependent upon distant flanking DNA sequences. By testing for *tap1-1*-dependent ochre suppression by the *SUP4A53T61* allele in various contexts, we found that sequences more than 100 bp upstream of the transcription start and more than 50 bp downstream of the transcript terminus strongly affect its expression. All of the plasmid constructs tested (Fig. 5 and Table 1) contain exactly the same sequence within the

region of *SUP4* that can be footprinted by or cross-linked to either TFIIIB or TFIIIC (5, 27). For this reason, we consider it unlikely that a direct interaction occurs between these proteins and the sequences of *MFα1* that permit active transcription of *SUP4A53T61*. Instead, it appears that these distant flanking sequences exert an influence upon the *A53T61* template—specifically, upon its transcription activation by the *tap1-1* mutation. Two obvious possibilities for such a transmissible effect are (i) that some aspect of DNA structure (or protein binding) in the flanking sequence affects DNA topology within the TFIIIB and/or TFIIIC binding sites and thus favors transcription complex formation or (ii) that sequences in the flanking DNA, by affecting the positioning and/or avidity of the nucleosomes bound to them, influence the stability of the nucleosome that covers the *SUP4A53T61* gene and its transcription start signal. By this indirect means, the distant flanking sequence determines the potential template activity of *SUP4A53T61*.

Although these two types of effect may not be mutually exclusive, we choose to emphasize the second of these possibilities because of the precedents that exist for it in the PolIII system. Burnol et al. (12) have recently found that the yeast U6 small nuclear RNA gene is transcriptionally inactivated by nucleosome assembly in vitro when the U6 gene lacks the specific binding sequence for TFIIIC; when this sequence is present, the U6 gene binds TFIIIC and is not inactivated when chromatin is assembled on it (12).

These results imply that a normal function of TFIIIC binding to a B block may be to preserve an "open" state of the class III gene by protecting against nucleosome formation over its transcription control sequences. For the yeast U6 small nuclear RNA gene, this protective function of TFIIIC can be distinguished from its role in transcription complex assembly (27), since transcription of U6 in vitro does not require TFIIIC (40).

Recent in vivo studies of the chromatin structure surrounding a yeast *SUP4* $tRNA^{Tyr}$ gene clearly demonstrate an antagonism between PolIII transcription (or TFIIIC binding) and nucleosome formation. When a full-strength *SUP4* allele was placed next to either of two DNA sequences that strongly position nucleosomes in *S. cerevisiae*, the tRNA gene remained uncovered by nucleosomes and was actively transcribed (43). However, with *SUP4* alleles that were transcriptionally inactivated by mutation in either internal control element, positioned nucleosomes were found on the gene (43). This shows that the internal sequence elements that normally bind TFIIIC (5, 13, 56) and thereby promote PolIII transcription are required to protect the *SUP4* gene from being covered by nucleosomes. It seems probable from our data that a *SUP4A53T61* gene within a *TAP1* yeast strain is similarly incorporated into nucleosomes, with consequent inactivation, because of its failure to bind TFIIIC.

To explain our findings on the pattern of *tap1-1*-mediated in vivo *SUP4A53T61* expression in various contexts, we make a distinction between two different chromatin states in the flanking DNA sequences. For convenience, we will designate these Str. and Wk., to denote strongly and weakly organized chromatins, respectively. These are exemplified by studies of the yeast TRP-ARS plasmid, which has nucleosomes positioned at defined locations over the *ARS1* region but loosely positioned nucleosomes over the *TRP1* gene (48). In preliminary experiments (results not shown), we studied the effects of the *TRP1* and *ARS1* flanking contexts upon *SUP4A53T61* expression by inserting the 260-bp $tDNA^{Tyr}$ fragment into the *Bgl*II site of yeast vector pTC3 (53). With *ARS1* sequences located 5′ to the *SUP4A53T61* insert, expression was low to undetectable (0 to 1.5 in the terms of Table 1), while with 5′-flanking *TRP1* sequences, expression was high (3 to 4). These results can be related to the recent chromatin studies of Morse et al. (43), because both our studies and theirs used precisely the same *Bgl*II site for insertion of *SUP4*, although the two sets of plasmids were different in overall structure. We found that *SUP4A53T61* (a B-block mutant) was not expressed when inserted at *Bgl*II with its 5′ end adjoining the *ARSC* element. The chromatin analysis showed that both A-block and B-block mutant alleles of *SUP4* in that same orientation at the *Bgl*II site were incorporated into nucleosomes, while a transcriptionally competent *SUP4* gene was not (43). From these findings on two mutually exclusive states of the *SUP4* gene, transcribed versus incorporated into nucleosomes, we infer that the inactivity we observed, with *SUP4A53T61* in pTC3, was a consequence of nucleosome organization.

Many of our studies on flanking DNA context have placed the *SUP4A53T61* gene within a segment of pBR322 DNA previously shown to direct nucleosome formation to rigidly defined positions (16). In plasmids pTT33, pTT54, and pTT55, the $tDNA^{Tyr}$ insert is flanked by sequences lying between the *Hin*dIII and *Sal*I sites of pBR322. Chromatin was reconstituted on the major part of this sequence by Drew and McCall (16). They found that with reconstitution on either a 445-bp segment or three smaller fragments, the same preferred positions were occupied by the nucleosomes (16). In our studies, when the *SUP4A53T61* subclone adjoined this segment of pBR322 DNA it was very poorly expressed in either *TAP1* or *tap1-1* mutant yeast cells (Fig. 5 and Table 1).

Contrasting with the repressive effects of the *ARS1* and pBR322 contexts mentioned above is the active expression of *SUP4A53T61* observed when it is placed within coding sequences of the yeast *MFα1* gene. High-level *tap1-1*-dependent transcription of *SUP4A53T61* occurs in plasmids pTT40, pTT41, and pTT41ΔS (Fig. 5 and Table 1), all of which have *MFα1* sequences in both 5′- and 3′-flanking regions. Therefore, we categorize the chromatin that contains the *ARS1* and pBR322 sequences as Str. and that which contains the *TRP1* and *MFα1* sequences as Wk.

How does the mutation in *TAP1* affect gene expression? The *SUP4A53T61* gene is inactive in Str. contexts, where nucleosomes have been observed to form over mutant *SUP4* genes. In Wk. contexts, *SUP4A53T61* is active only when the yeast cell is a *tap1-1* mutant. From this, we infer that the *tap1-1* mutational alteration of the TAP1 protein brings about a change in chromatin structure over the *SUP4A53T61* gene, provided it is in a Wk. context. By conventional genetic reasoning, one might ascribe to the TAP1 protein the function of forming nucleosomes on and near Wk. DNA sequences. Because these nucleosomes either do not form or form improperly in *tap1-1* mutant cells, *SUP4A53T61* is not sequestered in nucleosomes and thus is active. However, it is known that *TAP1* (known also as *RAT1* and *HKE1* [1, 29]) is an essential gene in *S. cerevisiae*; therefore, *tap1-1* cannot be a null mutation. Conceivably, the *TAP1*-negative null phenotype of nucleic acid synthesis is that which we observed in the *tap1-1* mutant strain at 38°C. Under these conditions, shutoff of transcription of both RNA PolIII and PolI occurs (15).

As an alternative possibility, we propose that the TAP1 protein has a normal function in altering the structure of Wk. chromatin (i.e., nucleosomes that cover transcribed regions) so as to allow transcription to proceed. The effectiveness of this nucleosome removal or rearrangement by the wild-

type TAP1 protein is, however, insufficient to open the *SUP4A53T61* gene for transcription. By altering an essential part of this protein through a Tyr→His substitution, the *tap1-1* mutation may have increased the ability of the TAP1 protein to restructure Wk. chromatin, specifically, that which covers the *SUP4A53T61* gene. A strong prediction made by this view of *TAP1* function is that other mutant alleles of the gene that are simple loss-of-function alleles would not have the ability to activate *SUP4A53T61* transcription. While we have no direct evidence for *tap1-1* effects upon the chromatin structure of the *SUP4A53T61* gene, this hypothesis can be directly tested by the techniques used to demonstrate alterations in chromatin structure for other *SUP4* alleles (43).

The same yeast gene that we describe here (*TAP1*) was found to complement a mutation, *rat1-1*, that causes poly(A)$^+$ RNA to accumulate in yeast nuclei (1). In fact, *tap1-1* and *rat1-1* are alleles of the same gene. The phenotypes underlying selection of the two mutations are quite different [PolIII transcription versus poly(A) RNA trafficking]; Amberg et al. (1) have proposed that these apparently distinct functions of the *TAP1/RAT1*-encoded protein derive from a single molecular interaction—binding of the protein to RNA. In their view, the *TAP1/RAT1*-encoded protein associates with PolII and PolIII transcripts while they are being made and exerts a continuing function as the pre-tRNAs and pre-mRNAs move toward the periphery of the nucleus. While this explanation provides a link between transcription and poly(A) RNA export, we find it difficult to reconcile with our data. The *A53T61* target gene we have employed is defective in binding TFIIIC, which is required for transcription initiation, while the hypothesized transcriptional role of *RAT1* action upon transcript displacement (1) can be carried out only during elongation or subsequent to elongation. As an alternative explanation for the two facets of *TAP1/RAT1* activity, we propose that mRNA 3′-end formation normally involves a structural transition in the chromatin template that is catalyzed by the *TAP1/RAT1*-encoded protein. In a ts$^-$ *rat1-1* mutant yeast strain, this protein is inactive, the chromatin change fails to occur, and as a consequence, poly(A)$^+$ RNA is not properly released from chromatin and exported to the cytoplasm.

Very similar to the pleiotropic effects of *tap1-1/rat1-1* mutations that we attribute to chromatin is the set of phenotypes observed for the yeast *prp20* mutation. These include defects in mRNA 3′-end formation, pre-mRNA splicing, transcription initiation, and mRNA transport (19). Because *prp20* is homologous to human and *Schizosaccharomyces pombe* genes involved in chromosome condensation, Forrester et al. have proposed that these multiple lesions in nucleic acid synthesis and transport in *prp20* mutant strains are all manifestations of an underlying defect in chromatin structure (19). Although the proteins encoded by the *PRP20* and *TAP1* genes are unrelated in sequence, the correspondence between their associated phenotypes (1, 15, 19) suggests that the two proteins have parallel roles in yeast chromatin structure and chromosome-associated functions.

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