A Mutation in the Largest Subunit of Yeast TFIIC Affects tRNA and 5 S RNA Synthesis

IDENTIFICATION OF TWO CLASSES OF SUPPRESSORS

(Received for publication, May 9, 1994, and in revised form, June 1, 1994)

Olivier Lefebvre, Jochen Rüth, and Andrée Sentenac

From the Service de Biochimie et de Génétique Moléculaire, CEA-Centre d’Études de Saclay
F-91191 Gif-sur-Yvette Cedex, France

We report the characterization of a mutation affecting τ150, the largest subunit of yeast transcription factor III (TFIIC). A previously described thermosensitive mutation (tuo150), tightly linked to the centromere of chromosome I (Harris, S. D., and Pringle, J. R. (1991) Genetics 127, 279-285) is shown to lie in the TFC3 gene which encodes τ150. The τ150 subunit carrying this mutation bears a single substitution of Gln for Gly at position 349 (G349E). In extracts from mutant cells, both the level of TFIIC and its affinity for tDNA were found to be reduced. The tDNA binding activity of mutant TFIIC protein was very sensitive to mild heat treatments, and its function, such as ABClOa;... 

Transcription of small genes by RNA polymerase III (or C) involves a multistep assembly of transcription factors into a preinitiation complex which is able to direct accurate initiation by RNA polymerase III (for a review, see Gabrielsen and Sentenac (1991), Geiduschek and Kassavetis (1992), Willis (1993), and White (1994)). The factor TFIIC plays a primary role in the maturation of pre-tRNA and pre-rRNA, respectively.

Based on their apparent migration rate on SDS-polyacrylamide gel electrophoresis, these polypeptides are referred to as τ150, τ131, τ90, τ57, τ50, and τ49 (Swanson et al., 1991; Conesa et al., 1993). Two subunits, τ50 and τ150, can be cross-linked to DNA by UV irradiation and are thought to play a central role in A and B block recognition, respectively. These two subunits belong to two distinct DNA binding domains of the yeast factor, τ49 (Gabrielsen et al., 1989; Conesa et al., 1993). The τ49 subunit has been mapped over the A block by photo-cross-linking experiments, while τ150 mapped to the vicinity of block B (Bartholomew et al., 1990). In the factor-DNA complex, the τ150 subunit was positioned upstream of transcription start site in a region occupied by TFIIB. This subunit, therefore, appears to be the best candidate for assembling TFIIB (Bartholomew et al., 1991). The genes encoding the three largest subunits of TFIIC have been cloned (Swanson et al., 1991; Parsons and Weil, 1992; Lefebvre et al., 1992; Marck et al., 1993). The functions of the smallest components of TFIIC remain unknown. TFIIC from animal cells is also a large complex that co-purifies with five polypeptides (Yoshinaga et al., 1989; Schneider et al., 1989; Kovelman and Roeder, 1992; Keller et al., 1992). The largest polypeptide (200 kDa) of human TFIIC, recently cloned (L'etoile et al., 1994), was found in UV cross-linking experiments to interact directly with the DNA (Yoshinaga et al., 1989; Kovelman and Roeder, 1992) and may therefore functionally correspond to the τ150 subunit of the yeast factor.

We demonstrated that TFC3, the gene encoding the τ150 subunit, corresponds to an essential transcribed region lying next to the centromere of chromosome I (Lefebvre et al., 1992). We show here that a previously described mutation that maps in this region (Harris and Pringle, 1991) lies in TFC3, changes one amino acid in τ150, and has severe effects on tRNA and 5 S RNA synthesis. This mutation was used to isolate gene-dosage-dependent suppressors that compensate for a deficiency in transcription of class III genes.

MATERIALS AND METHODS

Media, Strains, and Plasmids—Cells were grown in standard rich (YPD) or synthetic (SD-Ura, minimal medium containing all amino acids).
PCR reaction mixtures were loaded on a low melting point agarose gel, fragments with an average size of 1 kilobase. PCR experiments were performed in a Perkin-Elmer DNA thermal cycler using 30 cycles (1 min at 92 °C, 1 min at 55 °C, 1 min at 72 °C) for each cycle. PCR reaction mixtures were loaded on a low melting point agarose gel, and, after electrophoretic migration, the DNA bands of the expected size were excised. Gel slices were digested with the β-garase I enzyme (Biolabs), and DNA was purified by phenol-chloroform treatment and ethanol precipitation. DNA fragments (0.25 pmol) were directly sequenced using an improved procedure adapted to double-stranded PCR products using the Sequencing kit from Pharmacia (Casanova et al., 1990).

DNA Binding Assays—TFIIIC-DNA interaction was monitored by gel retardation assay, essentially as described (Baker et al., 1986). TFIIIC was incubated with a 206-bp 32P-labeled BamHI-EcoRI DNA fragment carrying the yeast tRNA^\text{ Arg} gene (5-10 fmol; ~3,000–10,000 cpm). 1.7 µg of competitor DNA (Bluescript KS) in 15 µl of binding buffer containing 20 mM Tris-HCl, pH 8.0, 1 mM EDTA (pH 8.0), 150 mM KCl, 2.5 mg/ml bovine serum albumin, 10% glycerol. Binding reaction was initiated by addition of the TFIIIC fraction (1.5 µg of protein). After a 10-min incubation at 25 °C, DNA-TFIIIC complexes were analyzed by gel electrophoresis (Camier et al., 1985). Both wild type and mutant TFIIIC were partially purified by heparin-Ulrogl chromatography (Conesa et al., 1990). The relative amounts of complexed and free DNA probe were determined using a Phosphorimager (Molecular Dynamics).

The apparent dissociation constant (K_D) of wild type and mutant TFIIIC-DNA complexes were determined, using the above binding conditions, as described (Baker et al., 1986; Vignais et al., 1990). K_D was derived from Equation 1, [C] = [C]_{max} [P]/[F], where [P], [F], and [C] stand, respectively, for the concentration of active TFIIIC protein, and of the free and complexed tRNA^\text{Arg} gene. Equation 1 describes a hyperbola with [P] as the asymptotic value. K_D was determined using the Scatchard representation [C] = [P] - [C]_{max} [P]. The slope of the plot gives the apparent dissociation constant K_D, and the y intercept yields TFIIIC concentration [P] in the assay.

In Vivo Transcription Assay—TFIIIC-dependent 5 S RNA synthesis was assayed after preincubation of wild type or mutant TFIIIC at different temperatures. Reaction mixtures (40 µl) contained 20 mM Hepes, pH 7.5, 100 mM KCl, 12 mM MgCl2, 5 mM dithiothreitol, 0.1 mM EDTA, 5% glycerol, 10 µCi of [n-32P]UTP (400 mCi/mmol), 0.6 mM each of ATP, CTP, and GTP, 0.03 mM UTP, 5 units of RNAsin (Promega), 0.9 µg of TFIIIC (heparin-Ulrogl fraction), 55 ng of polymerase III, 1.4 µg of partially purified TFIIIC, the template, and the gel electrophoresis. In each case, the corresponding plasmids were ex- 

<table>
<thead>
<tr>
<th>TABLE I Plasmids</th>
<th>Name</th>
<th>Parent</th>
<th>Relevant markers</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>plL</td>
<td>pL775</td>
<td>URA3, TFC3, CEN IV</td>
<td></td>
<td>Lefebvre et al., 1992</td>
</tr>
<tr>
<td>plL44L</td>
<td></td>
<td>URA3, 2 µm</td>
<td></td>
<td>Bonneaud et al., 1991</td>
</tr>
<tr>
<td>plL23</td>
<td></td>
<td>TFC3, ΔuraA, URA3, 2 µm</td>
<td>This study</td>
<td>Lefebvre et al., 1992</td>
</tr>
<tr>
<td>plL44L-C160</td>
<td>plL44L</td>
<td>TFC3</td>
<td></td>
<td>Gudenus et al., 1988</td>
</tr>
<tr>
<td>YEp352</td>
<td></td>
<td></td>
<td></td>
<td>James et al., 1991</td>
</tr>
<tr>
<td>plL44L-C53</td>
<td>YEp352</td>
<td></td>
<td></td>
<td>Mann et al., 1992</td>
</tr>
<tr>
<td>plL44L-C40</td>
<td>plL44L</td>
<td></td>
<td></td>
<td>Mann et al., 1987; Lal et al., 1993</td>
</tr>
<tr>
<td>PM316</td>
<td>plL44L</td>
<td></td>
<td></td>
<td>Stettler et al., 1992</td>
</tr>
<tr>
<td>plL44L-AC19</td>
<td>plL44L</td>
<td></td>
<td></td>
<td>Moerin et al., 1990</td>
</tr>
<tr>
<td>plL44L-RPB110c</td>
<td>plL44L</td>
<td></td>
<td></td>
<td>Dequard-Chabail et al., 1991</td>
</tr>
<tr>
<td>plRPC16c-2</td>
<td>plL44L</td>
<td></td>
<td></td>
<td>Lalo et al., 1993</td>
</tr>
<tr>
<td>plL</td>
<td>plL1</td>
<td></td>
<td></td>
<td>Treich et al., 1992</td>
</tr>
<tr>
<td>plL230</td>
<td>plL1</td>
<td></td>
<td></td>
<td>This study</td>
</tr>
<tr>
<td>plL57</td>
<td>plL233</td>
<td></td>
<td></td>
<td>This study</td>
</tr>
<tr>
<td>plL207</td>
<td>plL233</td>
<td></td>
<td></td>
<td>This study</td>
</tr>
<tr>
<td>plL233</td>
<td>plL207</td>
<td></td>
<td></td>
<td>This study</td>
</tr>
<tr>
<td>plL231</td>
<td>plL233</td>
<td></td>
<td></td>
<td>Schinzinger et al., 1989</td>
</tr>
</tbody>
</table>
harvested and chilled by mixing with an equal volume of ice-cold sterile water. RNA was extracted essentially as described by Schmitt et al. (1990). Small RNA species were separated on a 7 M urea, 6% polyacrylamide gel. Equal amounts of RNA (20 µg per lane) from wild type or mutant cells were analyzed to evaluate the relative efficiency of RNA synthesis.

RESULTS

Identification of tsu115 as a Thermosensitive Point Mutation in TFC3—We have previously characterized the TFC3 gene that encodes the 138-kDa subunit of S. cerevisiae transcription factor TFIIC (Lefebvre et al., 1992). By restriction mapping and sequence comparison, we found that a partially sequenced region, called FUN24 (for Function Unknown) was part of TFC3 (Lefebvre et al., 1992). FUN24 is an essential transcribed region immediately adjacent to the centromere, on the left arm of chromosome I (GenBank Accession number L22015). A recessive, UV-induced thermosensitive mutation, tsu115, tightly linked to the centromere of chromosome I, has been shown to map in FUN24 (Harris and Pringle, 1991). Consequently, tsu115 was a good candidate for being a mutation in TFC3.

The strain SH516 (MAT a, tsu115, ade1) which harbors the tsu115 mutation was crossed with YPH499 (MAT a, ade2-101, lys2-801, ura3-52, trp1-Δ63, his3-Δ200, leu2-Δ1) (Sikorski and Hieter, 1989). The diploid thermo resistant strain was sporulated, and a temperature-sensitive haploid segregant yOL8 (MAT a, ade1, tsu115, lys2-801, ura3-52, trp1-Δ63, his3-Δ200) was selected for this study. This strain was transformed by a multicopy plasmid pOL92 containing the TFC3 gene. All the transformants lost the thermosensitivity phenotype at 37°C which, together with the genetic linkage data, strongly suggests that the tsu115 mutation lies in TFC3.

To firmly establish this fact, the TFC3 allele of the tsu115 strain yOL8 was amplified by PCR and was entirely sequenced starting from 512 bp upstream of the ATG to 252 bp after the stop codon. Only one difference was found compared to the wild type TFC3 sequence previously reported (Lefebvre et al., 1992). The mutation substitutes an adenine for a guanine, 1136 nucleotides after the start codon. No other difference was found between the two sequences even within the promoter and the centromeric regions upstream of the ATG. To verify that this unique difference between the wild type and mutant sequences was not due to a PCR or sequence artifact, we amplified a 720-bp PCR DNA fragment, which includes this point mutation, from yOL8 genomic DNA as well as from the plasmid pOL45 which contains the wild type TFC3 gene (Lefebvre et al., 1992). Both PCR fragments were sequenced. The G → A transition at position 1136 was exclusively observed in yOL8 DNA. This G → A transition substitutes a GAA codon for a GGA codon resulting in a Gly to Glu replacement at position 349 (G349E).

Properties of Mutant TFIIC—In preliminary attempts to purify the mutant TFIIC protein from the yOL8 strain, we used the standard purification procedure that includes a phosphocellulose batch adsorption of TFIIC from the cell extract followed by a heparin column chromatography step (Gabrielsen et al., 1989). The heparin column fractions were tested by gel retardation assay using the tRNA68u gene as a probe (Gabrielsen et al., 1989). No specific DNA complexes could be detected in fractions derived from mutant cells. Therefore, we used a simpler purification protocol that omitted the phosphocellulose step (Conesa et al., 1993). Under these conditions, a weakly but specifically bound complex was detected in heparin column fractions eluted with 0.35–0.45 M ammonium sulfate (Fig. 1). Thus, TFIIC purified from the mutant strain was able to bind to the tRNA68u gene. This partially purified mutant TFIIC was used for further studies, together with a wild type TFIIC preparation obtained in a similar manner.

The DNA binding properties and thermosensitivity of wild type and mutant TFIIC were compared using the same amount of protein extracts and the tRNA68u gene as a probe, in gel shift assays. As seen in Fig. 1, mutant and wild type TFIIC-DNA complex formation showed a markedly different efficiency (about 15-fold) and salt sensitivity at 25°C. This suggested that the mutant factor had a lower affinity for tDNA than the wild type protein. In the following experiment, we selected the optimal salt concentration of 150 mM KCl. The temperature sensitivity of the factor preparation was examined either in the absence of tDNA or after formation of factor-tDNA complexes. In a first series of experiments, the ts- or wt-TFIIC was preincubated for 10 min at various temperatures. The residual DNA binding activity was then measured at 25 °C. As shown in Fig. 2A, the wild type factor was relatively resistant to mild heat treatments (at 35–40°C), while ts-TFIIC lost most of its DNA binding activity upon preincubation at temperatures above 30°C. In the experiments shown in Fig. 2B, factor-DNA complexes were preformed at 25°C and then subjected to a 10-min incubation period at various temperatures, before gel electrophoresis. Both wt- and ts-TFIIC were more resistant to heat denaturation in the form of protein-DNA complexes, but, again, mutant-TFIIC-tDNA complexes were sensitive to mild heat treatments, at 35–40°C, that did not dissociate wt-
TFIIIC-tDNA complexes. The difference between the wt and ts factors was even more pronounced when complex formation was performed at different temperatures followed by a 10-min chase at 25°C in the presence of excess competitor DNA. T7 DNA to trap partially inactivated, weakly bound factor molecules (Fig. 2C).

The apparent dissociation constant ($K_{d}$) of TFIIIC-tDNA complexes was determined by titrating a constant amount of TFIIIC (wt or ts-115) with increasing concentrations of labeled tRNA$^{16S}$ gene under optimal binding conditions (25°C, 150 mM KCl) (Baker et al., 1986). The concentration of TFIIIC-DNA complex [C] and free DNA probe [F] formed in each reaction was determined using the gel shift assay (Fig. 3). When the tRNA$^{16S}$ binding data were plotted for mutant-TFIIIC and wt-TFIIIC (Fig. 3, A and B), good fits to the respective theoretical curves were obtained (see "Materials and Methods"). The linearity in the Scatchard representation (Fig. 3B) suggested the presence of a single binding component in the wt- and ts-115-TFIIIC fractions. The apparent dissociation constant ($K_{d}$) and concentration of active TFIIIC in the binding assay [F] were determined by fitting the data to the equation $F = [C] / (1 + [C] / K_{d})$ (see "Materials and Methods,") Baker et al. (1986). Vignais et al. (1990) by linear regression. The slope of the curve (Fig. 3B) yielded a value for the apparent dissociation constant ($K_{d}$) of $1 \times 10^{-10}$ M for the wt- and $5 \times 10^{-10}$ M for ts-115-TFIIIC-tRNA$^{16S}$ complexes. The concentration of active TFIIIC in the binding assay given by the y intercept of the curve was: $-0.7 \times 10^{-10}$ M for wt-TFIIIC and $-0.2 \times 10^{-10}$ M for ts-115-TFIIIC. Therefore, the 15-fold weaker binding signal obtained with ts-TFIIIC was due both to lower levels of active factor in the extract and to its lower DNA binding affinity. Note, however, that due to the weak binding signal, the DNA affinity of the mutant factor was estimated with lower accuracy than for the wt factor.

In TFIIIC-TFIIIA-DNA complexes formed with the S RNA gene, the $\gamma_{s}$ subunit is located in the vicinity of box C and further downstream through yet undefined protein-protein interactions with TFIIA (Braun et al., 1992). Since TFIIIA contacts the box C region (Wang and Gudenus, 1989), TFIIA might conceivably contribute to the recognition of the TFIIIA-S RNA complex. Therefore, we investigated whether the ts-115 mutation affected the transcription of the S RNA gene in vitro. Samples of mutant or wild type TFIIIC factor were preincubated at different temperatures for 10 min and then tested for their ability to promote transcription of the yeast S RNA gene in the presence of TFIIA, TFIIIB, and RNA polymerase III. As shown in Fig. 4A, the mutant factor preparation lost most of its transcription activity upon heating at 35-40°C while the wild type factor was practically unaffected under the same conditions. These results suggest that the region mutated in $\gamma_{s}$ is not only involved in B block binding but also in the formation of TFIIIC-TFIIIA-S RNA complexes. The finding of three mutations of the ts-115 mutation of S RNA and tRNA 3'-terminal 5'-terminal 5' S RNA was at least 2-fold lower in mutant cells. In addition, the maturation of tRNA species appeared to be incomplete in spite of the chase period (see the trailing of radioactivity over the tRNAs). Remarkably, there was also a lag in the formation of S RNA in mutant cells (see Fig. 4B, lane 3 min) which was indicative of a problem in RNA processing. Defects in RNA processing were previously observed in RNA polymerase III mutants (Hermann-Le Denmat et al., 1994). Note that after 15 min the ratio of uracil incorporation into S RNA versus 5.8 S RNA (an RNA polymerase I transcript) was the same in wt and ts-115 mutant cells. The mechanisms involved in the co-regulation of these two RNA species were not understood (Gudenus et al., 1988; Stettler et al., 1992).

Extragenic Suppression of the ts-115 Mutation — In order to search for heterologous genes that could suppress the ts-115 mutation when expressed on high copy plasmids, strain yOL8 was transformed with an FL100 genomic library constructed in the URA3 multicopy vector pFL44L (Stettler et al., 1993). After selection on SD-Ura plates at 37°C, 85 transformants that grew at 37°C were selected. Forty-four different plasmids recovered from these transformants restored growth at 37°C when reintroduced into the strain yOL8. Restriction mapping indicated that six plasmids harbored a TFC3 insert. The remaining 38 plasmids were analyzed by restriction mapping and we verified that chasing these plasmids by 5-fluoro-orotic acid resulted in thermosensitivity at 37°C. Although the DNA insert of these plasmids was different, several of them harbored overlapping inserts with one apparently common region, as judged from restriction mapping. The restriction map of one group of suppressors (four plasmids) corresponded to PCC4/BFR1/TDA4 which encodes the 70-kDa component of TFIIIB. A partial sequence (300 bp) of a restriction fragment common to these four plasmids showed 100% identity with the sequence of

![Figure 2](https://example.com/fig2.png)
PCF4/BRF1/TDS4 (Colbert and Hahn, 1992; Buratowski and Zhou, 1992; Lopez-De-Leon et al., 1992). By restriction and PCR analysis we could also identify two plasmids harboring TFC1 (one plasmid) and TFC4 (one plasmid), the genes for the TFIIIC subunits $\tau_{\alpha}$ and $\tau_{\beta}$, respectively. Common restriction fragments of three other groups of suppressors were cloned and partially sequenced. Each sequence was then compared with the “nonredundant” nucleic acids data base of the NCBI using the BLAST network service (Altschul et al., 1990). One group (three plasmids) was shown to harbor RPR1, which encodes the RNA component of nuclear RNase P (Lee and Engelke, 1989). The identity of the latter suppressor with RPR1 was verified by transforming the yOL8 mutant strain with a multicopy plasmid harboring only the RPR1 insert.

To uncover potential interactions between TFIIIC and other components of the class III transcription apparatus, yOL8 cells were transformed by multicopy plasmids harboring RPC160, RET1, RPC82, RPC53, RPC40, RPC34, RPC31, RPC19, RPC10, RPB10, or SPT15 that encode, respectively, the 160-, 128-, 82-, 53-, 40-, 34-, 31-, 19-, 10-, and 10-kDa subunits of the RNA polymerase III and TBP (for a review, see Thuriaux and Sentenac (1992)). These plasmids were tested for their ability to suppress the temperature-sensitive phenotype of strain yOL8. yOL8 cells were transformed at permissive temperature, and the transformants were grown at restrictive temperature. A suppression was obtained in two cases, by overexpression of the SPT15 and RPC10 genes. Fig. 5 shows the growth of yOL8 cells carrying different multicopy suppressor genes at different temperatures. One plasmid of each group of suppressors has been selected for this study. Plasmids pLR30, pLR207, and pLR233 harbor the RPC160, RET1, RPC82, RPC53, RPC40, RPC34, RPC31, RPC19, RPC10, RPB10, and SPT15 insert. Twenty plasmids remain to be characterized to determine the number of independent suppressor genes they may contain. In view of the variety of suppressors already identified, one can expect that other pol III-related genes will be found among them. Table II summarizes all the suppressors identified.

**DISCUSSION**

TFIIIC is a multisubunit, multifunctional transcription factor that plays a pivotal role in transcription of most class III genes by recruiting the initiation factor TFIIIB to the region upstream of these genes. During this process, TFIIIC engages in a variety of interactions with specific DNA sequences and transcription components. A single point mutation in the gene encoding $\tau_{\beta}$, the largest subunit of yeast TFIIIC, drastically impairs the function of this factor. Availability of this cond-
Suppressors of a Mutation in Yeast TFIIIC

Figure 5. Extragenic suppression of tsu115 mutation by multicopy suppressors. Exponentially growing yO8L cells expressing different suppressor genes were diluted in water, and 10 μl of cell suspension was spotted on four SD-Ura plates. From top to bottom on each plate, the cell suspension was diluted 10-, 10²-, 10³-, and 10⁴-fold. The plates were incubated at different temperatures (30 °C, 33 °C, 34.5 °C, or 35.5 °C) for 4 days. The plasmids used to transform the yO8L strain carrying the tsu115 mutation are indicated. The plasmid pFL44L corresponds to the vector without insert, pLR57 to pFL44L harboring a TFC9 wild type gene, and pLR311 to pFL44L harboring the NOP1 gene.

Table II
Suppressors of tsu115

<table>
<thead>
<tr>
<th>Suppressor</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I</td>
<td></td>
</tr>
<tr>
<td>TFC1</td>
<td>τ60, subunit of TFIIIC</td>
</tr>
<tr>
<td>TFC4</td>
<td>τ60, subunit of TFIIIC</td>
</tr>
<tr>
<td>PCF4/BRF1/TDS4</td>
<td>TFIIIBb</td>
</tr>
<tr>
<td>SPT15</td>
<td>TATA box binding protein</td>
</tr>
<tr>
<td>RPC110</td>
<td>ABC10a, subunit of pol I, II, and III</td>
</tr>
<tr>
<td>SRP40</td>
<td>Serine-rich protein (suppressor of a pol I and pol III mutation)</td>
</tr>
<tr>
<td>Class II</td>
<td></td>
</tr>
<tr>
<td>NOP1</td>
<td>Fibrillarin homolog (rRNA maturation)</td>
</tr>
<tr>
<td>FHL1</td>
<td>Forkhead-like protein (rRNA maturation)</td>
</tr>
<tr>
<td>RPR1</td>
<td>RNA subunit of Rnae P (rRNA maturation)</td>
</tr>
</tbody>
</table>

A functional mutant allowed the isolation of a collection of extragenic suppressors involved in class III gene expression.

τ138 is believed to participate in B block recognition (Gabrielsen et al., 1989; Bartholomew et al., 1990), and B block binding is predominant in factor-DNA interaction (Geiduschek and Kasavetis, 1992). Therefore, we hypothesized that the mutation might affect the DNA binding properties of the factor. Indeed, upon moderate heat treatments, a partially purified factor preparation rapidly lost its ability to bind to a tRNA gene in vitro. Specific tDNA binding in vitro was also defective at permissive temperatures under high salt conditions. These observations indicate that the tsu115 mutation (G349E) alters directly or indirectly a DNA binding domain in mutant TFIIIC and most probably in the τ138 subunit itself. Under optimal binding conditions, the binding affinity constant for complex formation was decreased only 5-fold. Therefore, it is more likely that the Gly → Glu mutation alters the structural stability of the B block binding domain rather than affecting an amino acid residue directly involved in protein-DNA interaction. Indeed, factor bound to DNA proved to be more thermoresistant than the free factor, as if DNA stabilized the DNA binding domain. Sequence analysis did not reveal any known DNA binding motif in the region of the mutation. τ138 has two putative high mobility group (HMG) boxes, one in an N-terminal region and another in a C-terminal region, that were thought to participate in DNA binding (Lefebvre et al., 1992), but the mutation is not located in either of these regions.

The role of the τ138 subunit is not limited to B block binding since the mutant TFIIIC proved to be equally affected in tDNA binding and in 5 S rRNA gene activation at nonpermissive temperatures in vitro. As there is no B block in 5 S rDNA, TFIIIC has to recognize a preformed TFIIIA-5 S rDNA complex. The coarse localization of the τ138 subunit in TFIIIC-TFIIIA-5 S rDNA complexes indicated that τ138 lies close to the C box that is the major binding site for TFIIIA (Braun et al., 1992). This observation suggested a role for the τ138 subunit in TFIIIA binding, a hypothesis that is strongly supported by the defect of mutant TFIIIC in 5 S rDNA transcription.

The cells harboring the tsu115 mutation ceased dividing within a few generations after a shift to nonpermissive temperatures and were severely affected in tRNA synthesis. The residual level of active TFIIIC was clearly not sufficient to provide the minimal gene activity compatible with cell growth. Therefore, we searched for suppressors in order to uncover the class III transcription components that could, when overexpressed, compensate for the TFIIIC deficiency. These suppressors could encode any component participating in a rate-limiting step in transcription complex formation. Increased gene dosage of PCF1 (τ60) or TFC4 (τ138) partly suppressed the mutant phenotype probably by increasing the efficiency of TFIIIC assembly and, indirectly, TFIIIC level. PCF4/BRF1/TDS4 that encodes TFIIIBb was cloned as a multicopy suppressor of a mutation in the A box of a tRNA gene, and this observation was interpreted in terms of kinetics of TFIIIB assembly and gene-III-IIIb complex formation (Willis et al., 1989; Lopez-De-Leon et al., 1992). Overexpression of PCF4/BRF1/TDS4 has also been shown to suppress a defect in TBP (Colbert and Hahn, 1992; Buratowski and Zhou, 1992). This result was interpreted to indicate a direct interaction between TFIIIBb and TBP. Increasing the concentration of either component may increase the rate of B' assembly (TFIIIBb-TBP complex) (Kassavetis et al., 1991, 1992) and thereby the final level of TFIIIBb factor. Alternatively (or concurrently), formation of the ternary complex TFIIIB-TFIIIA-5 S rDNA may also occur by successive step additions of the components of yeast TFIIIB: TFIIIBb-TBP, and B' (TFIIIBb), in which case, increased concentrations of TFIIIBb-TBP, or B' would increase the rate of TFIIIBb assembly onto DNA. There is some evidence in favor of a weak interaction of TFIIIBb with TFIIIC-DNA complexes (Kassavetis et al., 1991). This interaction could well be a rate-limiting step compensated for by overproduction of TFIIIBb. Our suppression results support the contention that free TFIIIBb levels are limiting in vivo (Lopez-De-Leon et al., 1992) and strengthen the tacitly accepted idea that all essential TFIIIC-dependent class III genes are transcribed via TFIIIBb. Suppression by TBP, although weaker than by TFIIIBb, suggests that free TBP concentration also influences the rate of transcription complex formation on class III genes. At this point, we want to stress that suppression effects do not prove a direct physical interaction between TFIIIB components and the τ138 subunit of TFIIIC. This interaction may well occur through a distinct subunit of TFIIIC, likely subunit τ131 (Bartholomew et al., 1991; Marck et al., 1993). As other suppressors remain to be analyzed, the other component(s) of TFIIIB may be found among them, specifically TFIIIBb, which has been identified by photo-cross-
linking experiments to be in fr action $B$ (Barthólmew et al.,
1991; Kassavetis et al., 1991) and remains to be characterized.

The fact that a small subunit of RNA polymerase III, shared
with RNA polymerases I and II, was also able to suppress partially
the TFIIFC deficiency when encoded on a multicopy plasmid is intriguing since it was not expected that an excess of free polymerase subunit could influence transcription complex formation. ABC10a could be a critical subunit limiting the rate of enzyme assembly, and its synthesis rate might therefore regulate the final levels of RNA polymerases. Overexpression of ABC10a in mutant cells would increase the polymerase concentration and, thereby, the efficiency of transcription complex formation. Suppression by a direct interaction between unasa-
mbedding ABC10a and the preinitiation complex appears less likely. We expect that among the suppressors not yet identified, there should be other effectors that increase the levels or the activity of pol III, TFIIFIII, or TFIIFC. This could be achieved by favoring the assembly of these multisubunit proteins, their nuclear translocation, or post-translational modifications (for instance, protein kinases may positively regulate TFIIFC and pol III since both proteins are phosphorylated in vivo; Conesa et al., 1993; Bell et al., 1977; Van Zyl et al., 1992). SRP40 probably belongs to this class of suppressors since this gene was originally isolated as a multicopy suppressor of a ts mutation in mini. This gene is transcribed in vivo by RNA polymerase III and contains a 1RNA-like promoter in its 5' leader region with an A and B box combination that binds TFIIIC (Table 11). It is remarkable that all identified suppressors since this gene was origi-
nally isolated as a multicopy suppressor of a ts mutation in mini. This gene is transcribed
in vivo by RNA polymerase III and contains a 1RNA-like promoter in its 5' leader region with an A and B box combination that binds TFIIIC (Table 11). It is remarkable that all identified suppressors since this gene was origi-
nally isolated as a multicopy suppressor of a ts mutation in mini. This gene is transcribed in vivo by RNA polymerase III and contains a 1RNA-like promoter in its 5' leader region with an A and B box combination that binds TFIIIC (Table 11). It is remarkable that all identified suppressors since this gene was origi-
nally isolated as a multicopy suppressor of a ts mutation in mini. This gene is transcribed in vivo by RNA polymerase III and contains a 1RNA-like promoter in its 5' leader region with an A and B box combination that binds TFIIIC (Table 11). It is remarkable that all identified suppressors since this gene was origi-
nally isolated as a multicopy suppressor of a ts mutation in mini. This gene is transcribed in vivo by RNA polymerase III and contains a 1RNA-like promoter in its 5' leader region with an A and B box combination that binds TFIIIC (Table 11). It is remarkable that all identified suppressors since this gene was origi-
nally isolated as a multicopy suppressor of a ts mutation in mini. This gene is transcribed in vivo by RNA polymerase III and contains a 1RNA-like promoter in its 5' leader region with an A and B box combination that binds TFIIIC (Table 11). It is remarkable that all identified suppressors since this gene was origi-
nally isolated as a multicopy suppressor of a ts mutation in mini. This gene is transcribed in vivo by RNA polymerase III and contains a 1RNA-like promoter in its 5' leader region with an A and B box combination that binds TFIIIC (Table 11). It is remarkable that all identified suppressors since this gene was origi-
nally isolated as a multicopy suppressor of a ts mutation in mini. This gene is transcribed in vivo by RNA polymerase III and contains a 1RNA-like promoter in its 5' leader region with an A and B box combination that binds TFIIIC (Table 11). It is remarkable that all identified suppressors since this gene was origi-
nally isolated as a multicopy suppressor of a ts mutation in mini. This gene is transcribed in vivo by RNA polymerase III and contains a 1RNA-like promoter in its 5' leader region with an A and B box combination that binds TFIIIC (Table 11). It is remarkable that all identified suppressors since this gene was origi-
nally isolated as a multicopy suppressor of a ts mutation in mini. This gene is transcribed in vivo by RNA polymerase III and contains a 1RNA-like promoter in its 5' leader region with an A and B box combination that binds TFIIIC (Table 11). It is remarkable that all identified suppressors since this gene was origi-
nally isolated as a multicopy suppressor of a ts mutation in mini. This gene is transcribed in vivo by RNA polymerase III and contains a 1RNA-like promoter in its 5' leader region with an A and B box combination that binds TFIIIC (Table 11). It is remarkable that all identified suppressors since this gene was origi-
nally isolated as a multicopy suppressor of a ts mutation in mini. This gene is transcribed in vivo by RNA polymerase III and contains a 1RNA-like promoter in its 5' leader region with a
Suppressors of a Mutation in Yeast TFIIIC