MOLECULAR AND CELLULAR BIOLOGY, Jan. 1994, p. 822–830
0270-7306/94/$04.00+0

Vol. 14, No. 1

A Mutation in the Second Largest Subunit of TFIIIC Increases a Rate-Limiting Step in Transcription by RNA Polymerase III

GERALD RAMEAU, KAREN PUGLIA, ALEX CROWE, INDRA SETHY, AND IAN WILLIS*

Department of Biochemistry, Albert Einstein College of Medicine, Jack and Pearl Resnick Campus, Bronx, New York 10461

Received 19 July 1993/Returned for modification 25 August 1993/Accepted 22 October 1993

In previous studies, we have shown that the *PCF1-1* mutation of *Saccharomyces cerevisiae* suppresses the negative effect of a tRNA gene A block promoter mutation in vivo and increases the transcription of a variety of RNA polymerase III genes in vitro. Here, we report that *PCF1* encodes the second largest subunit of transcription factor IIIC (TFIIIC) and that the *PCF1-1* mutation causes an amino acid substitution in a novel protein structural motif, a tetratricopeptide repeat, in this subunit. In agreement with the nature of the mutation, in vitro transcription studies with crude extracts indicate that *PCF1-1* facilitates the rate-limiting step in transcription, namely, the recruitment of TFIIIB to the template. Additionally, biochemical fractionation of wild-type and mutant cell extracts shows that *PCF1-1* increases the amount of the 70-kDa TFIIIB subunit detectable by Western (immunoblot) analysis in purified TFIIIB fractions and the transcription activity of a TFIIIB″ fraction containing the 90-kDa subunit of this factor. We suggest that the effect of *PCF1-1* on TFIIIB activity in vitro is a consequence of its increased rate of recruitment in vivo.

Two multi-subunit transcription factors, IIIB and IIIC (TFIIIB and TFIIIC), are required for the synthesis of 5S RNA, tRNAs, and many other small RNAs by RNA polymerase (Pol) III (for reviews, see references 11, 12 and 37). These factors have been the subject of ongoing investigations to elucidate their structures and functions since the first in vitro transcription systems were established over a decade ago. During this period, much has been learned about the biochemical properties of the factors and the role that each plays in the assembly of a Pol III transcription complex. Recently, these data have begun to be understood in structural terms.

TFIIIC is responsible for binding to the A and B block promoter regions of tRNA-type genes and to 5S gene-TFIIIA complexes, in which it directs the assembly of TFIIIB upstream of the transcription start site. Once TFIIIB is bound to the gene, there is no further requirement for TFIIIC (or TFIIIA) in initiating transcription. These factors can be stripped from the DNA, leaving a TFIIIB-gene complex that is competent for multiple rounds of transcription (19). Accordingly, TFIIIB constitutes a general initiation factor, whereas TFIIIC and TFIIIA function as assembly factors. Recently, a new Pol III initiation factor, TFIIIE, was identified in yeast cells (9). Although the nature and function of this factor are unknown, it has been suggested to play a role at some late stage in initiation. Yeast TFIIIC is believed to comprise six polypeptide subunits with molecular sizes of 145 (138), 135 (131), 95 (100), 90, 60, and 55 kDa (the numbers in parentheses represent independent size estimates of the same subunit [2, 4, 10, 28]). Of these, all but the 60-kDa subunit have been specifically photocross-linked to probes located within and immediately flanking the *sup4* $tRNA^{Tyr}$ gene (2–4). Since the photoprobe in these studies was precisely positioned, a picture of the relative locations of these subunits along the DNA has emerged. The 131-kDa polypeptide is the only TFIIIC subunit that can be photocross-linked to the region footprinted by TFIIIB. This subunit is a likely candidate to mediate the recruitment of TFIIIB. Similarly, the 95- and 55-kDa subunits may interact with the A block, and the 138-kDa subunit may interact with the B block. To help resolve the roles of individual TFIIIC subunits in transcription complex assembly, a major effort to clone these genes has been under way. So far, this has been achieved for the 95-, 131-, and 138-kDa polypeptides (23, 26, 29, 34).

Studies with yeast and human cells indicate that TFIIIB is a multisubunit factor (20, 24, 35, 36). Detailed characterization of the yeast factor suggests that it comprises three components: the TATA-binding protein (TBP), a 70-kDa TFIIB-like polypeptide ($TFIIIB_{70}$), and a 90-kDa polypeptide ($TFIIIB_{90}$). These three polypeptides are stably associated under most conditions and copurify as a complex with TFIIIB activity over numerous columns. However, by using strong cation exchangers, specifically MonoS, TFIIIB activity has been separated into two fractions, designated TFIIIB′ and TFIIIB″ (18). By photocross-linking, these two fractions were found to contain the $TFIIIB_{70}$ and $TFIIIB_{90}$ polypeptides, respectively, which had been identified previously in less pure TFIIIB fractions with this technique (3). Following the demonstration of a universal role for TBP in eukaryotic transcription (7, 32), TBP was identified as a component of TFIIIB and traced by Western blotting (immunoblotting) to the TFIIIB′ fraction (20). This result together with the cloning of the gene for $TFIIIB_{70}$ (5, 6, 25) has permitted the demonstration that these proteins are the only TFIIIB components in the TFIIIB′ fraction. The two proteins expressed in bacteria can replace the TFIIIB′ fraction in a reconstituted transcription system (20). The TFIIIB″ fraction has not been purified to homogeneity. However, $TFIIIB_{90}$ may be the only TFIIIB subunit in this fraction, since TFIIIB″ transcription activity can be provided by sodium dodecyl sulfate (SDS) gel-eluted and renatured proteins in the 90-kDa size range (20).

Our laboratory is pursuing a genetic approach to identify components of the Pol III transcription machinery (39). A

* Corresponding author. Mailing address: Department of Biochemistry, Albert Einstein College of Medicine, Jack and Pearl Resnick Campus, 1300 Morris Park Avenue, Bronx, NY 10461.

tandem pair of nonsense suppressor tRNA genes (*sup9-e A19-supS1*) was engineered in such a manner that expression of the downstream gene (*supS1*) was dependent on transcription directed by the defective internal promoter of the upstream gene (*sup9-e A19*). This construct allowed the isolation of extragenic suppressors of the A19 promoter mutation by their ability to express *supS1*. Using this approach, we have identified two genes, *PCF1* and *PCF4*, whose products are involved in Pol III transcription (25, 39). Dominant mutations in both genes increase tRNA gene expression in vivo and increase the transcription of a large number of Pol III genes in vitro. We recently reported the cloning of the *PCF4* gene and the identification of its product as $TFIIIB_{70}$ (25). The *PCF1* gene product and the *PCF1-1* mutation are the focus of this study. Studies carried out to date indicate that the *PCF1-1* mutation influences two steps in the transcription process. Both the rate of formation of preinitiation complexes and the number of such complexes which are transcriptionally competent are increased in mutant compared with wild-type cell extracts (38, 39). Additionally, fractionation of cell extracts has shown that *PCF1-1* augments the activity of a crude TFIIIB fraction and that the activities of the TFIIIC and Pol III fractions are unaffected (38). Since these studies did not establish whether the *PCF1* gene product was present in the TFIIIB fraction, the factor was proposed to be either a component or a regulator of TFIIIB. We report here that *PCF1* encodes the second largest subunit of TFIIIC and that the *PCF1-1* mutation causes an amino acid substitution in this subunit. Furthermore, we present direct evidence that *PCF1-1* increases the amount of active TFIIIB in cell extracts.

(The presence of TPR in PCF1 was originally described at the Asilomar meeting on Pol III transcription in May 1992.)

MATERIALS AND METHODS

Yeast strains and genetic methods. Strains IW1B6, IW2A4, and IWD1 have been described by Willis et al., (39). Strain T2D [*MAT***a** *ura3-52::URA3*(*sup9-e A19-supS1*) *leu2-3,112 arg4-17 trp1-1 met8-1 PCF1-1*] was obtained after three outcrosses of mutant 59-4 (39) to strain IWD1. The diploid strain A4B6 (*MAT***a**/*MAT*α *ura3-52/ura3-52 trp1-1/trp1-1 met8-1/met8-1 leu2-3,112/leu2-3,112 HIS3/his3-11,15 ARG4/arg4-17*) was constructed by mating strains IW1B6 and IW2A4. Strain GR1D1 (*MAT***a**/*MAT*α *ura3-52/ura3-52 trp1-1/trp1-1 met8-1/met8-1 leu2-3,112/leu2-3,112 PCF1/pcf1*Δ*::LEU2 HIS3/his3-11,15 ARG4/arg4-17*) was obtained by targeted deletion of the *PCF1* gene in strain A4B6. This was achieved by transformation with a *Hin*dIII-*Sac*I digest of plasmid pGR1. The deletion was confirmed by Southern analysis. Strain AC1 (*MAT*α *ura3-52 pcf1*Δ*::LEU2 his3-11,15 leu2-3,112 trp1-1 met8-1* pAC1W) was constructed following sporulation and random spore analysis (31) of a transformant of strain GR1D1 that contained plasmid pAC1W. Standard methods for growth and manipulation of yeasts were employed (33). Yeast transformations were performed by the dimethyl sulfoxide-lithium acetate method (15).

Molecular cloning of *PCF1-1* and *PCF1*$^+$ and plasmid constructions. A genomic library was prepared from a *PCF1-1* strain (59-4 [39]) by cloning size-fractionated (10 kb) fragments from a partial *Sau*3A1 digest into the *Bam*HI site of plasmid pRS315. A total of approximately 42,000 transformants (or six genome equivalents) were obtained in *Escherichia coli* DH5α. After amplification, the gene bank was transformed into the wild-type yeast strain, IWD1, which contains a chromosomally integrated *sup9-e A19-supS1* gene. Approximately 5,000 transformants (Leu$^+$ colonies) were analyzed by replica plating and selection for *supS1* amber nonsense suppressor activity (Trp$^+$ Met$^+$). Transformants harboring putative *PCF1-1* clones were cured of the plasmid by growth in yeast extract-peptone-dextrose and were grown separately on media containing 5-fluoroorotic acid to evict the *sup9-e A19-supS1* gene. Colonies were tested for coloss of the respective selectable markers and amber suppressor activity. Plasmid DNA was prepared from transformants that exhibited these phenotypes and used to obtain Amp^r transformants in *E. coli* DH5α. Plasmid pGR14 contains the *PCF1-1* gene on a 10-kb insert. Plasmid pGR48L was obtained by subcloning a 4.8-kb *Pst*I fragment from pGR14 into pRS315 (note that one of the two *Pst*I sites that defines this fragment is derived from the polylinker region of pGR14). The wild-type *PCF1* gene was cloned by colony hybridization in *E. coli* from a partial genomic library (strain IW1B6) of size-selected *Pst*I fragments in pRS315. Plasmid pIS1W contains the *PCF1*$^+$ gene on a 5.8-kb genomic *Pst*I fragment. Plasmid pAC1W was obtained by recloning the wild-type *PCF1* gene from pIS1W as a 5-kb *Xho*I-*Spe*I fragment into pRS313. Plasmid pGR1 contains a disrupted *PCF1* gene (*pcf1*Δ*::LEU2*) and was constructed in two steps as follows. A 1.1-kb *Hin*dIII-*Pvu*II fragment from pGR48L containing the 5′ end of the gene and upstream sequences was cloned into plasmid pJJ282 (17), which had been cleaved with *Hin*dIII-*Sal*I and blunt-ended at the *Sal*I site. Subsequently, this plasmid was cleaved with *Bam*HI-*Sac*I and blunt ended at the *Bam*HI site to receive a 0.8-kb *Sca*I-*Sac*I fragment from pGR48L containing the 3′ end of the *PCF1* gene and downstream sequences. For gene replacement, the disrupted *PCF1* gene was excised as a 4-kb fragment by digestion with *Hin*dIII and *Sac*I. Plasmid pGR2 contains a *malE-PCF1* fusion protein. A 2.7-kb *Eag*I-*Pst*I fragment (made blunt ended by Klenow) encoding a 107-kDa carboxyl-terminal fragment of PCF1 was inserted downstream of the *malE* gene in the pMAL-c2 vector (New England Biolabs), which had been linearized with *Xmn*I-*Pst*I.

Sequence analysis and data base searches. Sets of overlapping unidirectional deletions were generated in plasmid pGR48L by using the Erase-a-Base system (Promega). Deletion clones were tested for *PCF1-1* function by transformation of strain IWD1 and were then sequenced by using the Sequenase version 2 system (U.S. Biochemical). The DNA sequence was analyzed and data base searches were carried out by using programs of the University of Wisconsin Genetics Computer Group (8) and the Blast program server (1).

Transcription factor purification and assays. Extracts were prepared from 40 to 50 g of wild-type (IW1B6) and *PCF1-1* mutant (59-4) cells by using a large bead beater chamber (Biospec Products) as described by Willis et al. (38, 39). Initial fractionation of the extracts on BioRex 70 and DEAE-Sephadex followed the conditions described by Kassavetis et al. (21) with the exception that TFIIIC and Pol III were eluted from the DEAE-Sephadex column with a single 0.1 to 0.5 M NaCl step. Further purification of TFIIIB from the flowthrough fraction of this column was performed on heparin-agarose (Sigma) as described by Klekamp and Weil (22). The 0.275 to 0.6 M NaCl fraction containing TFIIIB was dialyzed and loaded on a MonoS column equilibrated with 40 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid)-KOH (pH 7.9)–100 mM NaCl–7 mM $MgCl_2$–2 mM dithiothreitol–10% glycerol–1 μg of leupeptin per ml–0.5

mM phenylmethylsulfonyl fluoride to separate the TFIIIB′ and TFIIIB″ fractions (18). This was achieved with two-step elutions at 0.35 and 0.7 M NaCl. Fractions were concentrated, dialyzed, and stored as described previously (38).

Two partially purified TFIIIC fractions were used for immunological experiments. TFIIIC was obtained from *PCF1-1* cells by chromatography on BioRex 70 and DEAE-Sephadex as described by Kassavetis et al. (21) and further purified by gel filtration on a Sephacryl S300 column equilibrated with 20 mM HEPES-KOH (pH 7.9)–0.1 mM EDTA–100 mM NaCl–2 mM dithiothreitol–10% glycerol. Heparin-agarose-purified TFIIIC was obtained from wild-type cells as described by Kassavetis et al. (19). TFIIIC activity was monitored by a mobility shift assay using a ^{32}P-labeled 375-bp *Hin*dIII fragment containing the *sup3-eST* gene (16). Binding reactions in 20 μl contained 10 mM HEPES-KOH (pH 7.9), 10 mM $MgCl_2$, 0.1 mM EDTA, 10% glycerol, 100 mM NaCl, 1 mM dithiothreitol 1 μg of poly(dI-dC) · poly(dI-dC) (Pharmacia), the DNA probe (2,000 to 7,000 cpm/ng), and TFIIIC fractions and were incubated for 5 min at 25°C before electrophoresis (27). Supershifts of TFIIIC-DNA complexes were performed by adding the PCF1 peptide antibody or the maltose-binding–PCF1 fusion protein (MBP-PCF1) antibody to preformed TFIIIC-DNA complexes and incubating for an additional 25 min at 25°C.

Multiple-round transcription assays were performed as described previously (38) by using a $tRNA_3^{Leu}$ gene template. Reactions were initiated by addition of a cocktail containing the template, nucleotides, and salts to tubes containing various transcription factor fractions. Transcription lasted 1 h at 25°C.

Preparation and use of antibodies. Two PCF1-specific antibodies were prepared. A synthetic 16-amino-acid peptide (NDKGKSYGRQRKERVC; Laboratory of Macromolecular Analysis, Albert Einstein College of Medicine) derived from the deduced amino acid sequence of PCF1 was coupled through a nonencoded C-terminal cysteine residue to maleimide-activated keyhole limpet hemocyanin (Pierce). The immunogen was purified by gel filtration and used to immunize two female New Zealand White rabbits. A second antigen was prepared by using plasmid pGR2 to express a truncated MBP-PCF1 in *E. coli*. After the initial injections (~1 mg of keyhole limpet hemocyanin-peptide conjugate for rabbits 458 and 459 and 1 mg of MBP-PCF1 fusion protein for rabbit 461), boosts (~500 μg) were performed at 2-week intervals. The animals were bled 10 days after each injection. Sera obtained after the fourth boost of rabbit 459 and the third boost of rabbit 461 were used for the experiments in this study. Antisera were used to screen various yeast transcription factor fractions for PCF1 immunoreactivity by Western blotting. Protein fractions were resolved on polyacrylamide-SDS gels and electroblotted for 1 h at 100 V onto nitrocellulose membranes. The blots were preincubated overnight in 25 mM Tris-HCl (pH 7.5)–0.9% NaCl–5% Carnation nonfat milk–0.1% Tween 20 (BLOTTO-Tween) and then incubated with the primary antisera at room temperature for 2 h. For the peptide antibody, specific immunoreactivity was demonstrated by preincubation of the antisera with bovine serum albumin (BSA) alone or BSA-peptide conjugate (200 μg) for 2 h on ice in BLOTTO-Tween. The specificity of the fusion protein antibody was demonstrated similarly by preincubation with Sephacryl-purified TFIIIC (144 μg) which had been denatured by boiling in 5 M urea. Primary immune complexes were visualized by using a horseradish peroxidase-conjugated goat anti-rabbit secondary antibody with either 3′,3′-diaminobenzidine tetrahydrochloride (Sigma) color development or chemiluminescence detection (Amersham). Immunoprecipitations were carried out by the method of Harlow and Lane (14) with protein A-coupled Sepharose (Pharmacia). Polyclonal antibodies to TFIIIC subunits were kindly provided by M. Parsons and P. A. Weil, and TBP antibodies were a gift from W. J. Feaver. Rabbit antibodies to $TFIIIB_{70}$ were generated with a *malE-PCF4* fusion protein. The details of their preparation will be described elsewhere. Polyclonal antibodies to the *E. coli* MBP were purchased from New England Biolabs.

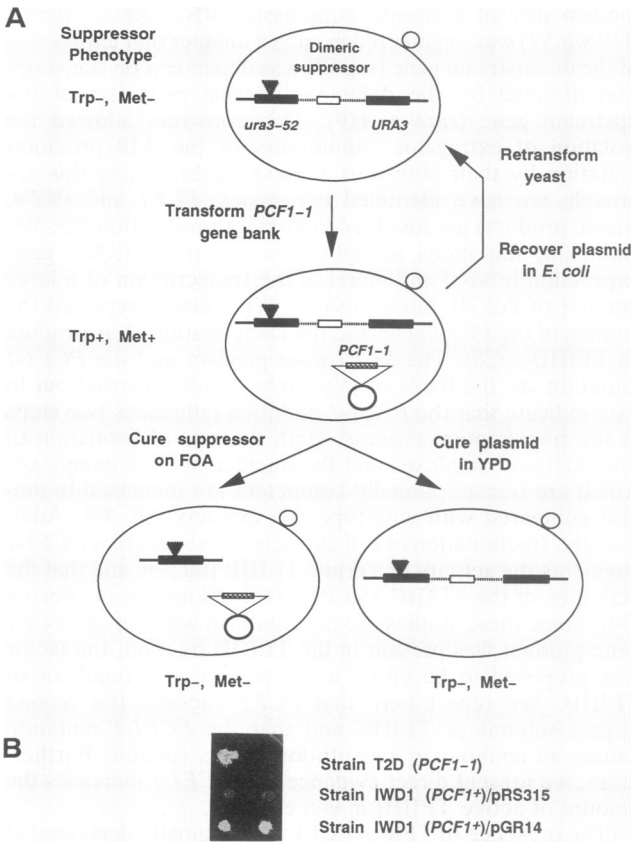

FIG. 1. Molecular cloning of *PCF1-1*. (A) Scheme for cloning the *PCF1-1* gene. The wild-type strain (IWD1) used for bank transformation contained the dimeric *sup9-e A19-supS1* gene (open box) flanked by wild-type and mutant *URA3* alleles (solid boxes), a marker for plasmid selection, and the amber nonsense mutations *trp1-1* and *met8-1*. The Trp Met phenotype at different stages during the analysis is shown. Transformants containing the *PCF1-1* gene (hatched box) were identified by independently curing cells of the plasmid and the suppressor and testing for the simultaneous loss of amber suppressor activity (Trp^- Met^-; see Materials and Methods). (B) Suppressor activity of plasmid pGR14. Plasmid DNA extracted from isolates exhibiting the phenotypes in panel A was retransformed into strain IWD1 (*PCF1*$^+$) and tested for amber suppressor activity. Control vector (pRS315) and plasmid pGR14, which contains the *PCF1-1* gene, are compared with a *PCF1-1* strain.

RESULTS

Cloning and molecular characterization of *PCF1-1*. The *PCF1-1* gene was cloned by exploiting its dominant phenotype, namely, its ability to express the *supS1* amber nonsense suppressor from the dimeric *sup9-e A19-supS1* gene in a wild-type cell (Fig. 1A). A genomic library of mutant strain

DNA was constructed in the centromeric plasmid vector pRS315 and used to transform the wild-type yeast strain IWD1. This strain contains a single copy of the *sup9-e A19-supS1* gene integrated at the *URA3* locus. IWD1 transformants that expressed the plasmid marker and had amber suppressor activity were identified. These transformants were analyzed by curing the plasmid containing the putative *PCF1-1* gene and by eviction (pop-out recombination) of the chromosomally localized *sup9-e A19-supS1* gene. Strains which showed a loss of nonsense suppressor activity in both cases indicated a dependence of the suppressor phenotype on the plasmid and the *sup9-e A19-supS1* gene. Plasmid DNA was extracted from four of these isolates and recovered by transformation in *E. coli*. Subsequent restriction mapping showed that two different but overlapping clones (pGR14 and pGR17) had been isolated. Both clones were capable of restoring *supS1* nonsense suppressor activity to wild-type (IWD1) yeast cells upon retransformation (Fig. 1B) (30). The independent isolation of these clones, their mutual dependence on the *sup9-e A19-supS1* gene for amber suppressor activity, and the dominant phenotype argue that the cloned gene was indeed *PCF1-1*.

To confirm genetically that the *PCF1-1* gene had been cloned, linkage was established between the cloned DNA and the *PCF1-1* mutation in strain T2D. This strain was crossed to strain AC1, which contains a chromosomal deletion of the cloned gene (see below) and a rescuing plasmid, pAC1W, bearing the wild-type *PCF1* gene. Diploid cells were selected and subjected to random spore analysis. Haploid progeny containing the disrupted chromosomal *PCF1* allele (Leu^+), the rescuing plasmid (His^+), and the *sup9-e A19-supS1* gene (Ura^+) were tested for *supS1* suppressor activity. Close linkage of the cloned gene to the *PCF1-1* mutation site was indicated by the fact that no suppressor-active cells were observed among 48 isolates analyzed.

The *PCF1* gene was physically mapped to the right arm of chromosome VII, 80 to 90 kb from the centromere, by hybridization to an ordered set of yeast fragments in phage lambda (provided by L. Riles and M. Olsen, Washington University). The gene was unambiguously assigned to this locus, since it fortuitously hybridized to two overlapping phage lambda clones from the minimal set.

The importance of the *PCF1* gene for cell viability was tested by constructing a null allele in which the region coding for amino acids 15 to 992 was replaced with the *LEU2* gene. The disrupted gene was integrated at the *PCF1* locus of the diploid strain A4B6 to give strain GR1D1. Sporulation of this strain was followed by random spore and tetrad analysis. Over 100 viable spores were examined. All had a Leu^- phenotype, indicating that the null allele was incompatible with cell survival. Additionally, viability segregated 2:2 in the analysis of six tetrads (30), confirming that *PCF1* is an essential gene.

Sequence analysis of *PCF1-1*. Plasmid pGR48L containing the *PCF1-1* gene on a 4.8-kb fragment was isolated by subcloning of the 10-kb insert from plasmid pGR14. Subsequently, the minimal fragment required to maintain *PCF1-1* function was delimited to a 3.3-kb region by exonuclease III deletion analysis. The DNA sequence of this region revealed a large open reading frame encoding an acidic protein (pI = 5.0) of 1,025 amino acids with a deduced molecular mass of 120 kDa. *PCF1* is identical to the recently described gene *TFC4*, which was cloned by reverse genetics on the basis of protein microsequence data derived from an abundant 131-kDa polypeptide found in highly purified preparations of TFIIIC (26).

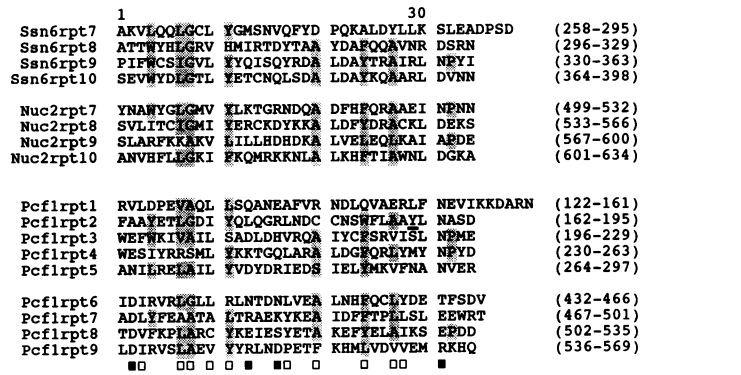

FIG. 2. Alignment of TPR sequences in PCF1-1, SSN6, and nuc2. Repeats 7 to 10 of SSN6 and nuc2 are compared with nine putative TPRs of PCF1. Repeat 5 in PCF1-1 showed similarity to sequences beyond the end of the 10th repeat in SSN6 (not shown). While this region of SSN6 has not been identified as a TPR, the corresponding region in PCF1-1 conforms to the degenerate TPR consensus. The position of the repeats in each protein is indicated in parentheses. Positions that show the best conservation among TPR family members are shaded. Positions most often occupied by charged or polar residues and those predominantly occupied by hydrophobic residues are indicated by solid and open boxes, respectively. The site of the His→Tyr substitution that identifies the dominant mutant is underlined.

Two regions of the protein encoded by *PCF1/TFC4* (amino acids 122 to 297 and 432 to 569) show significant similarity to the tetratricopeptide repeat (TPR) sequences found in the SSN6 protein of *Saccharomyces cerevisiae* and the nuc2 protein of *Schizosaccharomyces pombe*, respectively (Fig. 2). *SSN6* and *nuc2* are among the prototypes of the TPR gene family. Family members encode a functionally diverse group of proteins which are structurally related by the presence of multiple copies of a degenerate 34-amino-acid motif. Of the 10 TPR sequences predicted for SSN6 and nuc2, repeats 7 to 10 of both proteins showed the highest similarity to PCF1. Additionally, in the case of SSN6, the similarity to PCF1 extended for 40 amino acids beyond the end of the 10th repeat. The PCF1-SSN6 and PCF1-nuc2 comparisons indicated 19 and 21% identities and 61 and 63% similarities, respectively. The low identity and high similarity of these sequences are indicative of the degeneracy of the TPR motif (13). Despite the degeneracy, however, it can be seen from the alignment in Fig. 2 that the character of amino acids at corresponding positions in the proposed TPR sequences of PCF1, SSN6, and nuc2 is conserved.

In order to identify the site of the *PCF1-1* mutation, the wild-type *PCF1* gene was cloned from strain IW1B6. Subsequently, the dominant mutation was mapped to a 1.16-kb *Bln*I-*Nde*I fragment by cloning restriction fragments of the mutant gene into the wild-type gene and testing for a gain of *supS1* amber suppressor activity following transformation of yeast strain IWD1 (30). Sequence analysis of this region of $PCF1^+$ revealed a single nucleotide difference (T→C) from the dominant mutant gene in the first nucleotide of codon 190. Thus, the *PCF1-1* mutation causes a His→Tyr substitution at amino acid 190, within the second TPR sequence (Fig. 2). A comparison of the nucleotide sequence of *PCF1-1* with that of *TFC4* confirmed this finding. With the exception of the transition cited above, the two sequences were identical (30).

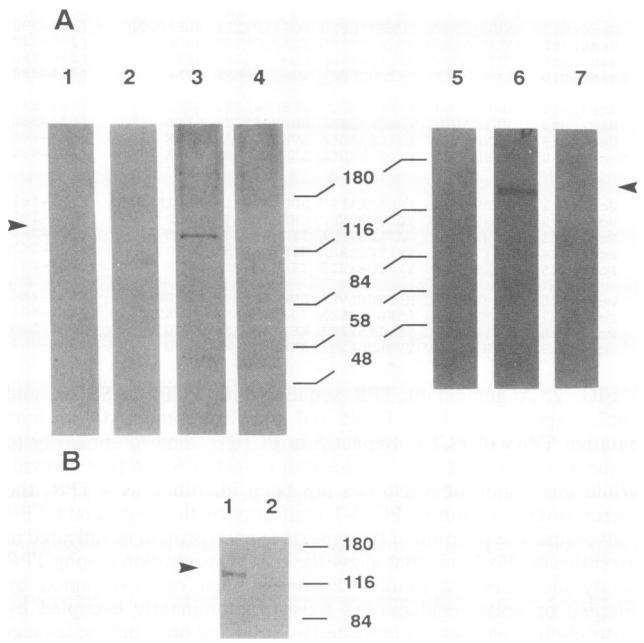

FIG. 3. PCF1-specific antisera recognize a 130-kDa polypeptide in the TFIIIC fraction. (A) Characterization of PCF1-specific antisera. Immunoblots of heparin-agarose TFIIIC (1.7 μg) purified from wild-type cells were probed with preimmune serum (lanes 1 and 2) or anti-PCF1pep serum (lanes 3 and 4) after preincubation of the sera with BSA (lanes 1 and 3) or PCF1 peptide-BSA conjugate (lanes 2 and 4). Immunoblots of Sephacryl-purified TFIIIC (2.4 μg) derived from *PCF1-1* cells were probed with preimmune serum (lanes 5) or with anti-MBPPCF1 serum that had been preincubated with buffer (lane 6) or denatured TFIIIC (lane 7). Specific bands are indicated (arrowheads). (B) The PCF1 antigen is not detectable in the TFIIIB fraction derived from *PCF1-1* cells. Sephacryl-purified TFIIIC (2.4 μg or 1 fmol; lane 1) and heparin-agarose purified TFIIIB (29 μg or 10 fmol; lane 2) derived from the mutant strain and quantitated by single-round transcription assays were analyzed by Western blotting using the anti-PCF1pep serum. The position of the PCF1 antigen is indicated (arrowhead).

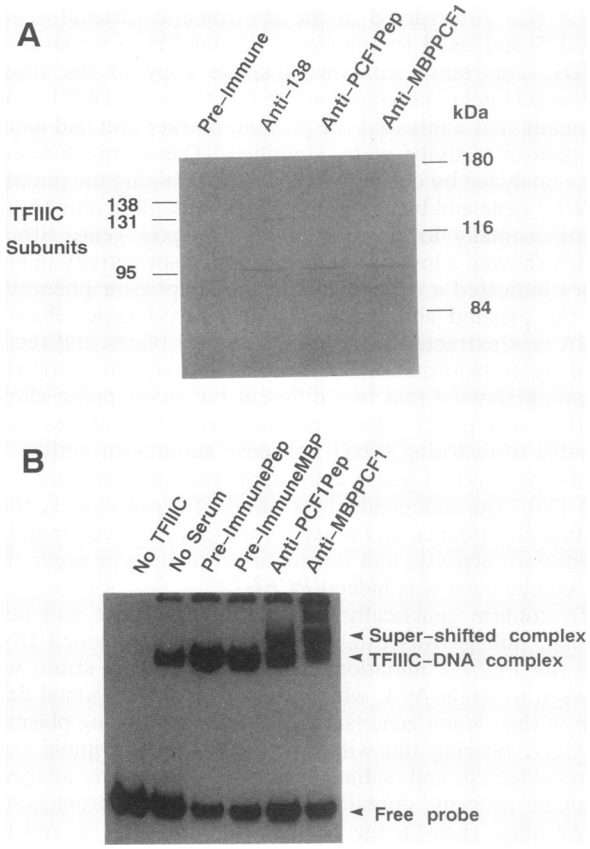

FIG. 4. *PCF1* encodes the 131-kDa subunit of TFIIIC. (A) Immunoprecipitates of heparin-agarose-purified TFIIIC (2 μg) obtained with the sera indicated above each lane were analyzed on a Western blot. The blot was probed with a mixture of sera (anti-95, anti-131, and anti-138) to detect the corresponding TFIIIC subunits (indicated on the left). (B) Native gel electrophoresis of TFIIIC-DNA complexes in the presence or absence of PCF1-specific antisera. All lanes contain TFIIIC-DNA complexes (unless otherwise indicated) and no serum, preimmune serum, or immune serum, as indicated above each lane.

***PCF1* encodes the second-largest subunit of TFIIIC.** Although the work of Marck et al. (26) strongly suggested that *TFC4* encodes the 131-kDa subunit of TFIIIC, our previous finding that *PCF1-1* affects the activity of the TFIIIB fraction (38) prompted us to seek independent confirmation of the identity of the *PCF1* gene product. To this end, antibodies were raised to a synthetic peptide derived from a hydrophilic region adjacent to the first TPR sequence (anti-PCF1pep) and to a PCF1 fusion protein expressed in bacteria (anti-MBPPCF1). Immunoblots of partially purified TFIIIC and TFIIIB fractions derived from either wild-type or mutant cells showed that both antibodies reacted only with TFIIIC fractions (Fig. 3) (30). These fractions contained a polypeptide with an estimated molecular mass of 130 kDa (Fig. 3A, lanes 3 and 6) that was not detected with the respective preimmune serum (lanes 1, 2, and 5). In addition, recognition of the 130-kDa protein by anti-PCF1pep or anti-MBPPCF1 could be specifically inhibited by prior incubation of the serum with BSA-peptide conjugate (lane 4) or denatured TFIIIC (lane 7), respectively. Immunoblots of TFIIIC fractions prepared by either heparin-agarose (lane 3) or gel filtration (lane 6) chromatography showed the same reactive band, suggesting that PCF1 was associated with the TFIIIC factor.

To determine whether *PCF1* encodes the 131-kDa subunit of TFIIIC, immunoprecipitations were performed with a TFIIIC subunit-specific antibody (anti-138; provided by M. Parsons and P. A. Weil), anti-PCF1pep, anti-MBPPCF1, or preimmune serum. Immune complexes were analyzed by Western blotting using a mixture of antisera to detect the 95-, 131-, and 138-kDa subunits of TFIIIC. Although the detection of the 138-kDa subunit was very weak, all three antigens were found in precipitates obtained with specific sera, whereas none were present in precipitates obtained with preimmune serum (Fig. 4A). We also tested whether our anti-PCF1pep and anti-MBPPCF1 antisera were capable of specifically supershifting a TFIIIC-tDNA complex. As shown in Fig. 4B, neither preimmune serum alters the mobility of a preformed TFIIIC-tDNA complex, whereas both immune sera retard the mobility of this complex. In summary, our results are in agreement with those of Marck et al. (26) and confirm that *PCF1* encodes the second largest subunit of TFIIIC.

The *PCF1-1* mutation increases the amount of transcriptionally active TFIIIB in mutant cell extracts. Previous fractionation of wild-type and *PCF1-1* mutant cell extracts on

BioRex and DEAE-Sephadex has shown that the *PCF1-1* mutation affects the biochemical activity of a crude TFIIIB fraction. Since *PCF1* clearly encodes a subunit of TFIIIC, we were interested in reproducing our initial finding and extending the purification of TFIIIB to determine whether the affected activity purifies with this factor. Fresh crude extracts were prepared from both wild-type and *PCF1-1* cells and subjected to chromatography on BioRex 70, DEAE-Sephadex, heparin-agarose, and MonoS. TFIIIB fractions obtained from the latter three columns were assayed for their abilities to reconstitute transcription in the presence of a wild-type or mutant extract-derived TFIIIC/Pol III fraction (see Materials and Methods). The results of assays of the heparin-agarose-purified TFIIIB are shown in Fig. 5. As was found for assays with less pure fractions (e.g., see reference 38), reaction mixtures containing heterologous components could reconstitute transcription at mutant levels only if the TFIIIB fraction was derived from *PCF1-1* cell extracts. Transcription activity in each set of reactions showed a linear dependence on the amount of added TFIIIB. Hence, the specific activity of the TFIIIB fraction purified from *PCF1-1* extracts was calculated to be 8.2- and 11.6-fold higher than that of the corresponding wild-type fraction in reactions in which the TFIIIC/Pol III fraction was derived from mutant and wild-type extracts, respectively. These differences in TFIIIB activity are slightly higher than the 6.6-fold difference in the specific activity of the crude extracts that were used for factor purification.

The increase in the activity of the mutant TFIIIB fraction obtained following heparin-agarose chromatography does not result from contamination by the mutant PCF1 polypeptide. This was demonstrated by Western blots of the mutant TFIIIB fraction. While both of the PCF1-specific antisera could detect PCF1 in the TFIIIC fraction, neither serum could detect PCF1 in the mutant TFIIIB fraction, even though a 10-fold molar excess of this fraction (based on single-round initiation assays) was analyzed (Fig. 3B) (30). Another possible explanation for the elevated activity of the mutant TFIIIB fraction is that this fraction may contain lower levels of some inhibitor of transcription than the corresponding wild-type fraction. This possibility was addressed by a mixing experiment. Transcription reactions were performed with a wild-type TFIIIC/Pol III fraction and approximately equal amounts of heparin-purified wild-type and/or mutant extract-derived TFIIIB. The amount of transcription observed for the mixture was slightly greater than the sum of the amounts in reactions containing one TFIIIB fraction or the other (Fig. 5C). Thus, the increased activity of mutant extract-derived TFIIIB does not result from a reduced level of inhibitors in this fraction.

Further chromatography of the heparin-purified TFIIIB fractions on MonoS was performed to resolve the TFIIIB′ and TFIIIB″ subfractions. Wild-type and mutant extract-derived TFIIIB subfractions were assayed individually and in pairwise combinations with a wild-type TFIIIC/Pol III fraction (Fig. 6A). No transcription was observed in reactions containing the TFIIIC/Pol III fraction alone or with added TFIIIB″. However, a low level of transcription was obtained when TFIIIB′ fractions were added to the TFIIIC/Pol III fraction. This result is consistent with the previously documented contamination of the TFIIIC fraction from DEAE-Sephadex with TFIIIB″ (18). Pairwise combinations of the TFIIIB subfractions show that mutant levels of transcription could be reconstituted when mutant extract-derived TFIIIB″ was present (Fig. 6A). The relative specific activities of the TFIIIB″ fractions were compared in a titration experiment in which this component was limiting for transcription (Fig. 6B). The ratio of the slopes of the two lines reveals that the mutant TFIIIB″ fraction is 11.5-fold more active than the wild type. Thus, these results indicate that the *PCF1-1* mutation increases the activity of the fraction containing $TFIIIB_{90}$.

$TFIIIB_{90}$ has not yet been purified to homogeneity, and no immunological reagents are available for its detection. However, biochemical evidence demonstrates that $TFIIIB_{90}$, $TFIIIB_{70}$, and TBP are stoichiometrically required initiation factors (18, 20). Furthermore, $TFIIIB_{70}$ is stoichiometrically limiting for transcription in vitro and in vivo (25). Thus, to investigate whether TFIIIB″ activity in mutant cell extracts was changed qualitatively or quantitatively, we performed Western blots to examine the levels of $TFIIIB_{70}$ and TBP in wild-type and mutant TFIIIB fractions. We reasoned that an increase in the amount of some TFIIIB″ component (presumably $TFIIIB_{90}$) might be accompanied by an increase in the amount of $TFIIIB_{70}$ and perhaps TBP. Western blots containing equal amounts of protein from the heparin-agarose TFIIIB fractions were probed separately with anti-$TFIIIB_{70}$ and anti-TBP sera (Fig. 7). Although the titer of the $TFIIIB_{70}$-specific serum was quite low, a band of the size expected for $TFIIIB_{70}$ is clearly detectable in the TFIIIB fraction from mutant cells (lane 3). A discrete band of the same size is not observed, however, in the TFIIIB fraction from wild-type cells (lane 2). Other bands that are seen in both fractions also appear in the preimmune control (lane 1) and are presumably nonspecific. Since a different $TFIIIB_{70}$-specific serum (kindly provided by S. Buratowski [5]) reproduced this result (30), it appears that the amount of $TFIIIB_{70}$ in the TFIIIB fraction from mutant cells is increased. Experiments with both specific sera showed that the level of $TFIIIB_{70}$ in the wild-type TFIIIB fraction was at or below the detection limit under the conditions employed. Nonetheless, densitometric analysis of the blots was performed to estimate the relative levels of $TFIIIB_{70}$ in the wild-type and mutant fractions. The data indicate that the increase in the amount of $TFIIIB_{70}$ protein is at least comparable to the difference in transcription activity between the respective TFIIIB″ fractions. In contrast, the amount of TBP in the mutant cell TFIIIB fraction was increased only slightly (1.3-fold) over that for the wild type (Fig. 7, lanes 4 and 5). With recombinant TBP as a standard, both fractions were found to contain a significant molar excess (about 100-fold for the mutant TFIIIB fraction) of TBP polypeptide relative to TFIIIB (based on activity measurements [30]).

DISCUSSION

Using a genetic selection, our laboratory has identified two dominant mutations, *PCF1-1* and *PCF4-1*, that increase Pol III gene transcription. Studies on *PCF4-1* have shown that the gene encodes the 70-kDa subunit of TFIIIB (25; see also references 5 and 6), that the mutation site resides upstream of the protein-coding region, and that overexpression of the wild-type gene product increases transcription. Thus, this factor is stoichiometrically limiting for the expression of Pol III genes in vivo. In the present study, we have shown that the increase in transcription resulting from the *PCF1-1* mutation is caused by an amino acid substitution in the second largest subunit of TFIIIC. Furthermore, we have demonstrated that the mutation increases (i) the amount of $TFIIIB_{70}$ that can be detected by Western analysis in a purified TFIIIB fraction and (ii) the activity of a fraction containing $TFIIIB_{90}$.

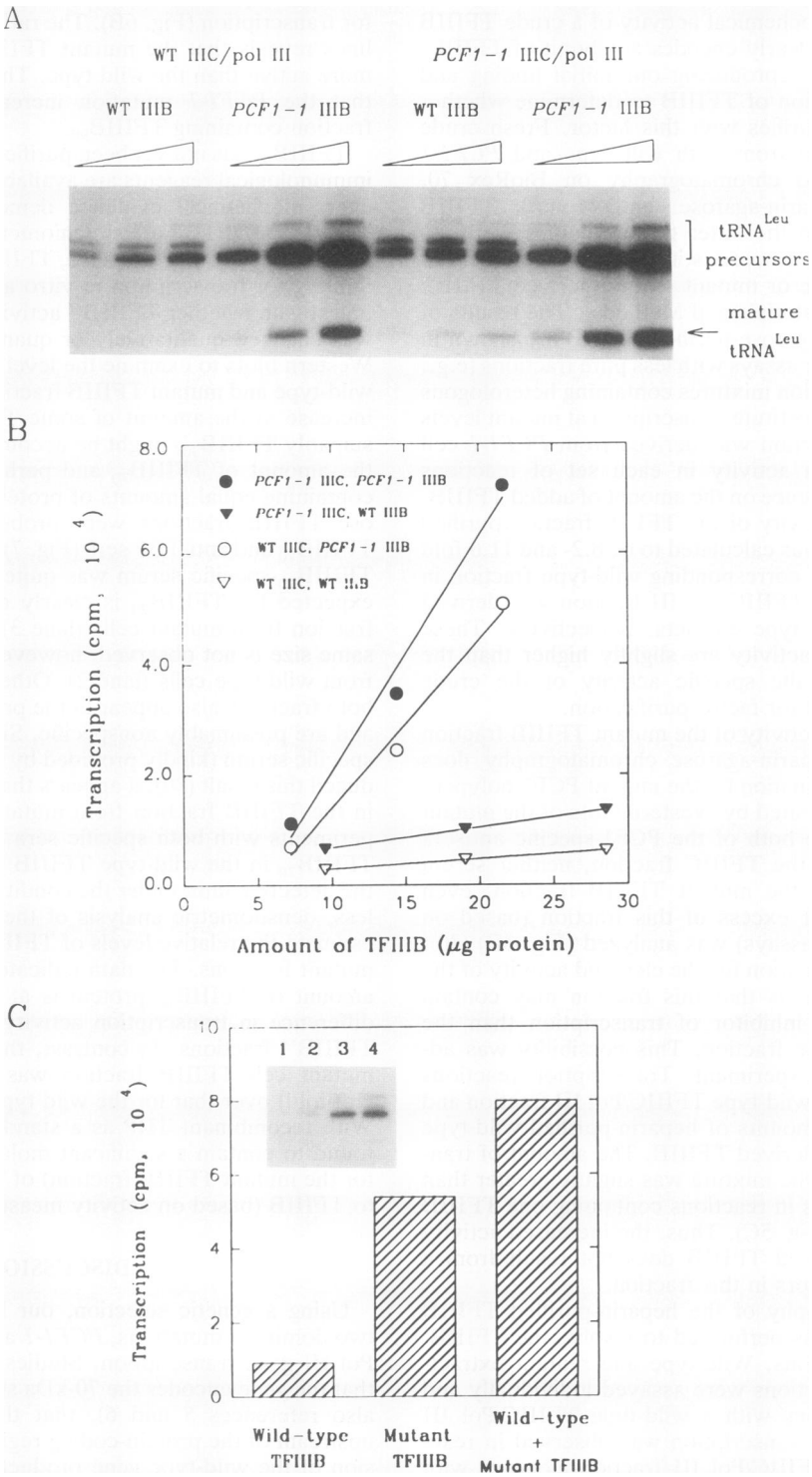

FIG. 5. Reconstitution of transcription with heparin-agarose-purified TFIIIB. (A) Autoradiogram of a multiple-round transcription experiment in which various amounts of heparin-agarose-purified TFIIIB (9.5, 19, and 28.5 μg of wild-type [WT] extract protein or 7.2, 14.4, and 21.6 μg of *PCF1-1* extract protein) were added to a constant amount of a TFIIIC/Pol III fraction (9 μg of wild-type protein or 7.8 μg of *PCF1-1* protein), nucleoside triphosphates, and an excess of a $tRNA_3^{Leu}$ gene template. Total $tRNA_3^{Leu}$ transcription was quantitated for each lane, and the data are plotted in panel B. The specific activity of TFIIIB was calculated following linear regression analysis. (C) Mixing of mutant and wild-type TFIIIB fractions. Reactions were performed as for panel A with a wild-type TFIIIC/Pol III fraction (7.2 μg) and no TFIIIB (lane 1), wild-type TFIIIB (15.2 μg; lane 2), mutant TFIIIB (11.5 μg; lane 3), or both TFIIIB fractions (15.2 and 11.5 μg, respectively; lane 4). Specific transcription (see inset) was quantified, and the results are shown in the histogram.

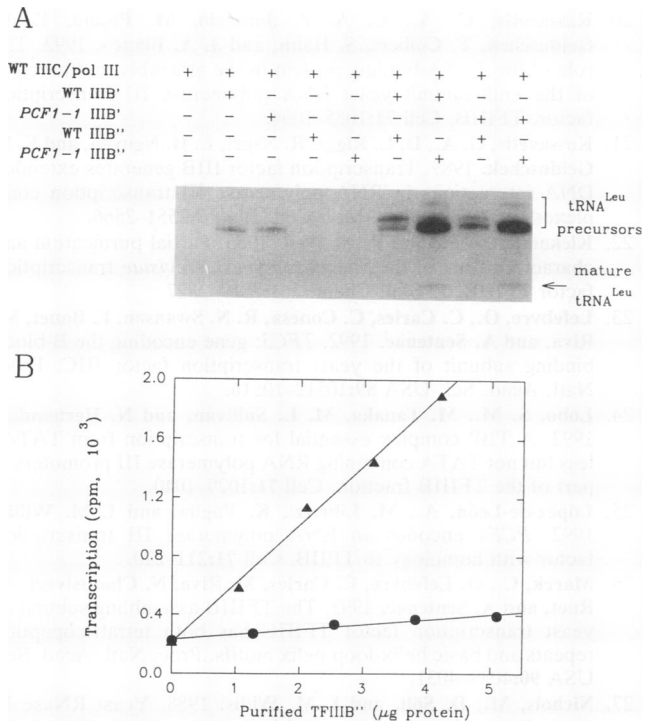

FIG. 6. The *PCF1-1* mutation increases the activity of the TFIIIB'' fraction. (A) Assays were performed as for Fig. 5 by using wild-type (WT) TFIIIC/Pol III (7.2 μg) and wild-type or mutant TFIIIB' (20 μg) and TFIIIB'' (4 μg) in the combinations indicated. (B) The difference in specific activity between wild-type TFIIIB'' and *PCF1-1*-derived TFIIIB'' was measured in assays containing wild-type TFIIIC/Pol III (5.4 μg), wild-type TFIIIB' (20 μg), and wild-type (circles) or mutant (triangles) TFIIIB'' (0 to 5.1 μg).

Considerable evidence supporting the conclusion that *PCF1-1* facilitates a rate-limiting step in transcription complex assembly now exists. A kinetic effect is indicated by our previous finding that *PCF1-1* reduces the lag phase in transcription (39). This is an indirect measure of the rate of preinitiation complex assembly. Further evidence is suggested by experiments in which complex assembly is allowed to reach equilibrium before addition of the full complement of nucleoside triphosphates. Under these conditions, the difference in transcription between mutant and wild-type

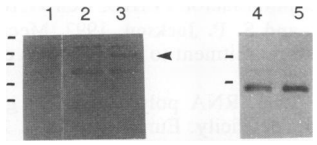

FIG. 7. *PCF1-1* increases the amount of $TFIIIB_{70}$ in purified TFIIIB fractions. Heparin-agarose-purified TFIIIB (8 μg of protein) from wild-type (lanes 1, 2, and 4) or mutant (lanes 3 and 5) cells was electrophoresed on a 12.5% polyacrylamide–SDS gel. After transfer to nitrocellulose, the blots were probed with preimmune serum (lane 1), anti-$TFIIIB_{70}$ serum (lanes 2 and 3), or anti-TBP serum (lanes 4 and 5). Immune complexes were detected by chemiluminescence. The position of $TFIIIB_{70}$ is indicated (arrowhead). The positions of prestained molecular weight markers (116, 84, 58, 48, and 36 kDa in the left-hand panel and 36 and 26 kDa in the right-hand panel) are indicated. Note that the dye on the prestained markers increases their molecular masses by about 5 kDa.

cell extracts is reduced from about six- to threefold (38, 39). A rate effect of *PCF1-1* is also consistent with the nature of the mutation: the His→Tyr substitution at residue 190 implies that the activity of the factor rather than its amount has been changed. Indeed, multiple copies of $PCF1^+$ do not confer a *PCF1-1* phenotype (30). This was anticipated, since a different factor ($TFIIIB_{70}$) is stoichiometrically limiting in vivo (25). Biochemical studies of yeast and other systems have shown that TFIIIB recruitment is the rate-limiting step in the assembly of an active transcription complex (12, 40). We suggest, therefore, that *PCF1-1* affects the rate of recruitment of TFIIIB. This role for the *PCF1* gene product is in agreement with the prediction from photocross-linking experiments that the 131-kDa subunit of TFIIIC interacts with some part of TFIIIB (3). The biochemical basis of the *PCF1-1* rate effect is currently under investigation.

In addition to increasing the rate of preinitiation complex assembly, single-round initiation assays have demonstrated that the *PCF1-1* mutation also increases the number of transcriptionally active complexes in crude cell extracts (38). Our present findings that TFIIIB fractions purified from mutant cells contain more $TFIIIB_{70}$ and have elevated TFIIIB'' activity suggest that these observations are related. Indeed, considering that TFIIIB is stoichiometrically limiting in whole-cell extracts (21, 25), our data suggest that the amount of active TFIIIB complex is increased in mutant cell extracts. How this increase is brought about by the *PCF1-1* mutation is not yet clear. However, several feasible models can be proposed. For example, the *PCF1-1* gene product, in addition to affecting a rate-limiting step in Pol III transcription, may function as an activator in the Pol II-dependent transcription of $TFIIIB_{70}$ and $TFIIIB_{90}$. In this scenario, the dominant mutation would stimulate transcription by both polymerases. Several factors exhibiting this dual function have been described (37). Alternatively, the *PCF1-1* mutation could simply destabilize the interaction of TFIIIB with the region upstream of Pol III genes, thereby allowing more of this factor to be extracted from the mutant cells. This possibility is difficult to reconcile, however, with the fact that transcriptionally active TFIIIB-DNA complexes assembled in vitro are remarkably stable under high salt concentration conditions such as those used in preparing whole-cell extracts (19, 20). A more likely hypothesis, in our opinion, is that the elevated TFIIIB levels in vitro are a direct consequence of the rate effect of *PCF1-1* in vivo. Specifically, we speculate that the steady-state level of TFIIIB-DNA complexes in *PCF1-1* cells is increased relative to the wild type and suggest that this permits more TFIIIB to be extracted. Unlike the first two models, this explanation does not require that the amount of some TFIIIB component (or components) be increased (although the latter is not excluded). A difference in the activity of one or more TFIIIB subunits before and after recruitment to DNA would be sufficient to account for the effect. If this is this case, Western blots of crude cell extracts from wild-type and mutant strains should show no change in the amount of specific TFIIIB subunits. The preparation of affinity-purified antibodies to $TFIIIB_{70}$ is under way to address this possibility.

ACKNOWLEDGMENTS

We thank Alexander Oksman for assistance in generating the exonuclease deletion clones and Marie Parsons, Tony Weil, and Steve Buratowski for providing antisera.

This work was supported by grants to I.W. from the National Institutes of Health (GM42728) and the Alexandrine and Alexander Sinsheimer Fund and BRSG funds to the Albert Einstein College of Medicine.

REFERENCES

1. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215**:403–410.
2. **Bartholomew, B., G. A. Kassavetis, B. R. Braun, and E. P. Geiduschek.** 1990. The subunit structure of *Saccharomyces cerevisiae* transcription factor IIIC probed with a novel photocrosslinking reagent. EMBO J. **9**:2197–2205.
3. **Bartholomew, B., G. A. Kassavetis, and E. P. Geiduschek.** 1991. Two components of *Saccharomyces cerevisiae* transcription factor IIIB (TFIIIB) are stereospecifically located upstream of a tRNA gene and interact with the second-largest subunit of TFIIIC. Mol. Cell. Biol. **11**:5181–5189.
4. **Braun, B. R., B. Bartholomew, G. A. Kassavetis, and E. P. Geiduschek.** 1992. Topography of transcription factor complexes on the *Saccharomyces cerevisiae* 5S RNA gene. J. Mol. Biol. **228**:1063–1077.
5. **Buratowski, S., and H. Zhou.** 1992. A suppressor of TBP mutations encodes an RNA polymerase III transcription factor with homology to TFIIB. Cell **71**:221–230.
6. **Colbert, T., and S. Hahn.** 1992. A yeast TFIIB-related factor involved in RNA polymerase III transcription. Genes Dev. **6**:1940–1949.
7. **Cormack, B. P., and K. Struhl.** 1992. The TATA-binding protein is required for transcription by all three nuclear RNA polymerases in yeast cells. Cell **69**:685–696.
8. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12**:387–395.
9. **Dieci, G., L. Duimio, F. Coda-Zabetta, K. U. Sprague, and S. Ottonello.** 1993. A novel RNA polymerase III transcription factor fraction that is not required for template commitment. J. Biol. Chem. **268**:11199–11207.
10. **Gabrielsen, O. S., N. Marzouki, A. Ruet, A. Sentenac, and P. Fromageot.** 1989. Two polypeptide chains in yeast transcription factor tau interact with DNA. J. Biol. Chem. **264**:7505–7511.
11. **Gabrielsen, O. S., and A. Sentenac.** 1991. RNA polymerase III (C) and its transcription factors. Trends Biochem. **16**:412–416.
12. **Geiduschek, E. P., and G. P. Tocchini-Valentini.** 1988. Transcription by RNA polymerase III. Annu. Rev. Biochem. **57**: 873–914.
13. **Goebl, M., and M. Yanagida.** 1991. The TPR snap helix: a novel protein repeat motif from mitosis to transcription. Trends Biochem. Sci. **16**:173–177.
14. **Harlow, E., and D. Lane.** 1988. Antibodies: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
15. **Hill, J., K. A. I. G. Donald, and D. E. Griffiths.** 1991. DMSO-enhanced whole cell yeast transformation. Nucleic Acids Res. **19**:5791.
16. **Hottinger-Werlen, A., J. Schaack, J. Lapointe, J.-I. Mao, M. Nichols, and D. Söll.** 1985. Dimeric tRNA gene arrangement in *Schizosaccharomyces pombe* allows increased expression of the downstream gene. Nucleic Acids Res. **13**:8739–8747.
17. **Jones, J. S., and L. Prakash.** 1990. Yeast *Saccharomyces cerevisiae* selectable markers in pUC18 polylinkers. Yeast **6**:363–366.
18. **Kassavetis, G. A., B. Bartholomew, J. A. Blanco, T. E. Johnson, and E. P. Geiduschek.** 1991. Two essential components of *Saccharomyces cerevisiae* transcription factor TFIIIB: transcription and DNA-binding properties. Proc. Natl. Acad. Sci. USA **88**:7308–7312.
19. **Kassavetis, G. A., B. R. Braun, L. H. Nguyen, and E. P. Geiduschek.** 1990. *S. cerevisiae* TFIIIB is the transcription initiation factor proper of RNA polymerase III, while TFIIIA and TFIIIC are assembly factors. Cell **60**:235–245.
20. **Kassavetis, G. A., C. A. P. Joazerio, M. Pisano, E. P. Geiduschek, T. Colbert, S. Hahn, and J. A. Blanco.** 1992. The role of the TATA-binding protein in the assembly and function of the multisubunit yeast RNA polymerase III transcription factor, TFIIIB. Cell **71**:1055–1064.
21. **Kassavetis, G. A., D. L. Riggs, R. Negri, L. H. Nguyen, and E. P. Geiduschek.** 1989. Transcription factor IIIB generates extended DNA interactions in RNA polymerase III transcription complexes on tRNA genes. Mol. Cell. Biol. **9**:2551–2566.
22. **Klekamp, M. S., and P. A. Weil.** 1986. Partial purification and characterization of the *Saccharomyces cerevisiae* transcription factor TFIIIB. J. Biol. Chem. **261**:2819–2827.
23. **Lefebvre, O., C. Carles, C. Conesa, R. N. Swanson, F. Bouet, M. Riva, and A. Sentenac.** 1992. *TFC3*: gene encoding the B-block binding subunit of the yeast transcription factor IIIC. Proc. Natl. Acad. Sci. USA **89**:10512–10516.
24. **Lobo, S. M., M. Tanaka, M. L. Sullivan, and N. Hernandez.** 1992. A TBP complex essential for transcription from TATA-less but not TATA containing RNA polymerase III promoters is part of the TFIIIB fraction. Cell **71**:1029–1040.
25. **López-de-León, A., M. Librizzi, K. Puglia, and I. M. Willis.** 1992. *PCF4* encodes an RNA polymerase III transcription factor with homology to TFIIB. Cell **71**:211–220.
26. **Marck, C., O. Lefebvre, C. Carles, M. Riva, N. Chaussivert, A. Ruet, and A. Sentenac.** 1993. The TFIIIB-assembling subunit of yeast transcription factor TFIIIC has both tetratricopeptide repeats and basic helix-loop-helix motifs. Proc. Natl. Acad. Sci. USA **90**:4027–4031.
27. **Nichols, M., D. Söll, and I. M. Willis.** 1988. Yeast RNase P: catalytic activity and substrate binding are separate functions. Proc. Natl. Acad. Sci. USA **85**:1379–1383.
28. **Parsons, M. C., and P. A. Weil.** 1990. Purification and characterization of *Saccharomyces cerevisiae* transcription factor IIIC. J. Biol. Chem. **265**:5095–5103.
29. **Parsons, M. C., and P. A. Weil.** 1992. Cloning of *TFC1*, the *Saccharomyces cerevisiae* gene encoding the 95 kDa subunit of transcription factor TFIIIC. J. Biol. Chem. **267**:2894–2901.
30. **Rameau, G., K. Puglia, and I. M. Willis.** Unpublished data.
31. **Rockmill, B., E. J. Lambie, and G. S. Roeder.** 1991. Spore enrichment. Methods Enzymol. **194**:146–149.
32. **Schultz, M. C., R. H. Reeder, and S. Hahn.** 1992. Variants of the TATA-binding protein can distinguish subsets of RNA polymerase I, II and III promoters. Cell **69**:697–702.
33. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1986. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
34. **Swanson, R. N., C. Consea, O. Lefebvre, C. Carles, A. Ruet, E. Quemeneur, J. Gagnon, and A. Sentenac.** 1991. Isolation of *TFC1*, a gene encoding one of two DNA-binding subunits of yeast transcription factor tau (TFIIIC). Proc. Natl. Acad. Sci. USA **88**:4887–4891.
35. **Taggart, A. K. P., T. S. Fisher, and B. F. Pugh.** 1992. The TATA-binding protein and associated factors are components of pol III transcription factor TFIIIB. Cell **71**:1015–1028.
36. **White, J. R., and S. P. Jackson.** 1992. Mechanism of TATA-binding protein recruitment to a TATA-less class III promoter. Cell **71**:1041–1053.
37. **Willis, I. M.** 1993. RNA polymerase III: genes, factors and transcriptional specificity. Eur. J. Biochem. **212**:1–11.
38. **Willis, I. M., A. Oksman, and A. López-de-León.** 1992. The *PCF1-1* mutation increases the specific activity of the transcription factor (TF) IIIB fraction from *Saccharomyces cerevisiae*. Nucleic Acids Res. **20**:3725–3730.
39. **Willis, I. M., P. Schmidt, and D. Söll.** 1989. A selection for mutants of the RNA polymerase III transcription apparatus: *PCF1* stimulates transcription of tRNA and 5S RNA genes. EMBO J. **8**:4281–4288.
40. **Wolffe, A., and D. D. Brown.** 1988. Developmental regulation of two 5S ribosomal RNA genes. Science **241**:1626–1632.