The *PCF1-1* mutation increases the activity of the transcription factor (TF) IIIB fraction from *Saccharomyces cerevisiae*

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ABSTRACT

***PCF1-1* is a dominant suppressor of a tRNA gene A block promoter mutation (A19) in *Saccharomyces cerevisiae*. Transcriptional activation by *PCF1-1* was examined *in vitro* using whole-cell extracts and purified factors derived from mutant and wild-type strains. These experiments show that *PCF1* is a general activator of RNA polymerase III (pol III) gene transcription. The transcription of all pol III genes analyzed to date, including type I and numerous type II genes, is increased 3–7 fold in mutant cell extracts. Single round transcription assays indicate that the *PCF1-1* mutation increases the number of functional preinitiation complexes and suggest that this is achieved by increasing the intrinsic activity of the encoded product rather than its amount. Point mutations throughout the A block of the *sup3-e* gene and numerous B block mutations fail to abolish transcriptional activation suggesting that interactions between TFIIC and the internal promoter are unaffected by *PCF1-1*. Moreover, TFIIC purified from the mutant strain is incapable of conferring *PCF1-1* transcriptional activity to a reaction in which the remaining components are wild-type. In contrast, the activity of the TFIIB fraction is increased in *PCF1-1* extracts and can reconstitute mutant levels of transcription when added to wild-type TFIIC and polymerase. We conclude that *PCF1* is a component or regulator of TFIIB.**

INTRODUCTION

Biochemical studies have established that chromatographic fractions containing transcription factors known as IIB and IIC together with RNA polymerase III (pol III) are sufficient to reconstitute specific transcription of tRNA and other type II pol III genes *in vitro*. TFIIB and TFIIC have been purified extensively from numerous sources (reviewed in refs. 1–3). These studies indicate that TFIIC is a large complex comprising several polypeptide

subunits. Estimates of the molecular weight of yeast TFIIC (also known as τ) range from 500 kDa (the aggregate molecular mass of the subunits, 4) to 670 kDa (estimated from electron micrographs of TFIIC-tRNA gene complexes, 5). Of the various systems under investigation, the yeast factor has been characterized most extensively. These efforts notwithstanding, the total number and nature of TFIIC polypeptides is uncertain. Three polypeptides (145, 135 and 95 kDa) copurify in all preparations (4, 6, 7). Antibodies raised against each of these proteins inhibit TFIIC transcription activity. Based on immunological and UV crosslinking experiments these components are unique gene products which interact at or between the A and B blocks that comprise the internal promoter of type II pol III genes (4, 6–8). Additionally, the 135 kDa protein is accessible to photoreactive nucleotides located in 5' flanking sequences (9). Two other polypeptides (62–65 and 55 kDa) have been observed in highly purified TFIIC preparations although the amounts of these species seems to be variable. They also appear to be distinct from each other as well as from the 3 larger polypeptides. The 55 kDa protein has been crosslinked to the *SUP4* tRNA^{Tyr} gene near the A block (4) while the 62–65 kDa protein was found to comigrate with TFIIC-DNA complexes in native acrylamide gels (6). TFIIB has been reported to comprise a single polypeptide with a molecular weight of about 60 kDa (10, 11). Recently however, the TFIIB fraction was resolved into 2 components (B' and B'') both of which are necessary to reconstitute transcription in the presence of highly purified TFIIC and pol III (12). The two fractions contain polypeptides of 70 and 90 kDa respectively, that can be photocrosslinked to the *SUP4* tRNA^{Tyr} gene in a region known to be protected from DNase I digestion by the binding of TFIIB (9).

The activity of TFIIB and TFIIC is known to be regulated by a variety of cellular conditions: Alterations in cell growth rate produced by entry into stationary phase, treatment of cells with cycloheximide (13) or stimulation of cells with serum (14) have been shown to increase or decrease the activity of both factors. Similarly, changes in TFIIB and/or TFIIC activity have been described following cell differentiation (15, 16) during development (17–19) and after infection of mammalian cells by

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several viruses including SV40 (20, 21), adenovirus (14, 22) and poliovirus (23). Possible mechanisms for affecting these regulatory events have been advanced only for TFIIC and include phosphorylation and proteolysis (14, 21, 23). Presently, very little is known about the molecules immediately responsible for regulating TFIIB or TFIIC activity. However, two antagonistic activities (designated A2 and I) have been fractionated from *Xenopus* oocyte extracts that may be involved in this process (24). These activities appear to have different chromatographic properties to the known factors and pol III. Consistent with the proposed role of phosphorylation in the regulation of pol III gene transcription, ATP hydrolysis was required for transcriptional activation by the A2 fraction.

Elucidating the molecular structure of the pol III transcription factors, understanding the functions of these molecules and how their activity is regulated are among the central problems being addressed in numerous laboratories including our own. In contrast to the classical biochemical approaches that have been pursued to date, our approach to study this process has, in its initial stages, involved the application of yeast genetics. Using the phenotype of nonsense suppression, a selection was developed to isolate mutant strains in which the deleterious transcriptional effects of an A block promoter mutation were reversed (25). The selection strategy employed a pair of *Schizosaccharomyces pombe* nonsense suppressor tRNA genes arranged in tandem such that expression of the downstream gene (*supS1*) was dependent on transcription directed by the internal promoter of the upstream gene (*sup9-e*). The *sup9-e* gene however, carried a mutation at position 19 in its A block that reduced *supS1* activity (measured by suppression of the auxotrophic markers, *trp1-1* and *met8-1*) by 3 orders of magnitude. Following mutagenesis of a *Saccharomyces cerevisiae* strain containing the *sup9-e A19-supS1* construct, eight dominant mutant strains of independent origin were isolated by virtue of their ability to grow on media lacking tryptophan and methionine. A nuclear gene designated *PCF1* (Polymerase C Factor 1) was identified in one of the mutant strains. Whole-cell extracts derived from a *PCF1-1* strain were shown to transcribe the *sup9-e* tRNA gene and a 5S RNA gene at increased levels relative to wild type cell extracts.

We have begun a biochemical analysis of transcription in *PCF1-1* cell extracts. The main goals of this work are to determine the nature of the *PCF1* gene product and how the mutant protein increases pol III gene transcription. In this report we show that activated transcription in *PCF1-1* extracts is mediated by TFIIB.

MATERIALS AND METHODS

FPLC-purified nucleoside triphosphates (NTPs) were purchased from Pharmacia. Radiolabeled nucleotides were from Amersham. YCp50-based plasmids containing the *S. pombe sup3-e* tRNA^{Ser}-tRNA^{Met} dimeric gene (YCp*sup3-e*), various *sup3-e* point mutant genes and the homologous dimeric *sup9-e* gene (YCp*sup9-e*) are described elsewhere (26). *S. cerevisiae* genes used in this work include the dimeric tRNA^{Arg}-tRNA^{Asp} gene pair of plasmid pYM205 (27) and the tRNA^{Leu} gene of plasmid YEpl3.

Extract preparation and factor purification

Whole cell extracts were prepared by glass bead disruption of 12–15 g (wet weight) of cells from logarithmically grown cultures of wild-type and *PCF1-1* strains. Cells were broken in

a small (25 ml) bead beater chamber (Biospec Products) as described by Klekamp and Weil (28) with modifications (29 and below). Broken cells were extracted with 0.5 M (NH₄)₂SO₄ prior to the 100,000×g spin. After the final 70% (NH₄)₂SO₄ precipitation, the resuspended protein (5–7 ml) was fractionated on Sephacryl S200 (25). A fraction equal to one and a half sample volumes containing all of the excluded protein and some partially included material was collected and stored at –70°C in 150 µl aliquots. Protein concentrations in crude extracts were determined spectrophotometrically (30) and ranged from 4–6 mg/ml. The concentrations of purified protein fractions were determined using the Bradford method (Biorad).

Partial purification of TFIIB, and TFIIC was achieved by serial chromatography on BioRex 70, and DEAE-Sephadex A25 (28, 31). Whole cell extracts were fractionated on a BioRex 70 column and eluted in a step-wise manner with increasing concentrations of KCl. After dialysis all the fractions were tested for transcription activity. The 500 mM KCl step, also known as the BRα fraction (31), was found to contain all of the factors required to support tRNA gene transcription. This fraction was further purified on a DEAE-Sephadex A25 column (28) which separates the three major fractions required to reconstitute pol III transcription: The flow-through constitutes the IIB fraction, the 250 mM NaCl step comprises the IIC fraction and the 500 mM NaCl eluate contains most of the polymerase III activity. All fractions were concentrated using Centricon-10 (Amicon) and dialyzed against 20 mM Hepes-KOH pH 7.9, 20% glycerol, 100 mM NaCl, 2 mM DTT, 0.2 mM EDTA, 0.1 mM PMSF. Extracts were stored at –70°C in small aliquots. To ensure equivalence of fractions prepared from the two strains, cell growth and extract preparation was performed in parallel. For chromatography, the same columns were used for both extracts and the bed volumes were adjusted according to the amount of protein being loaded.

Transcription assays

Standard multiple round transcription assays contained excess template DNA (0.4–0.8 µg), equal amounts of extract protein (up to 100 µg) and were performed as described previously (25). A number of variations of the standard assay were routinely performed on all extracts to optimize transcription and to ensure linear kinetics. These included extract (0–100 µg), template (0–400 ng, with constant total DNA concentration) and carrier DNA (0–1.0 µg) titration experiments. The latter were performed at limiting template DNA concentrations. All reactions were extracted with an equal volume of phenol-chloroform and were subjected to successive ethanol precipitations from 0.3 M sodium acetate and 2 M ammonium acetate, respectively. After electrophoresis on denaturing 8% polyacrylamide gels and autoradiography, primary *sup9-e* transcripts or all tRNA^{Leu} gene products were excised and quantitated by Cerenkov counting. Alternatively, transcription levels were measured by 2-dimensional video densitometry.

Assays in which transcription was limited to a single round were carried out using heparin to prevent reinitiation (31). Template DNA was preincubated with extract for 30 min at 25°C under standard conditions but in the absence of one or more NTPs (see text). This condition allows complete assembly of preinitiation complexes in either wild-type or mutant extracts. Missing nucleotides were then added to the same final concentrations as for standard reactions together with heparin (300 µg/ml, unless otherwise specified) in a volume of 5 µl. The

incubation was continued for a further 10 min at 15°C. Sample processing and analysis was carried out as for the standard assay. Single round experiments with limiting amounts of template (50 ng) were performed similarly but contained 650 ng of pGem DNA (Promega) as a non-specific carrier.

Transcription reconstitution assays were performed under standard multiple round conditions (see above) with the following exceptions: NaCl (70 mM final) was used owing to the lower salt optimum for reactions containing purified components and incubations were at 25°C for 1 hour. Since at this temperature, processing of the primary transcripts is quite efficient, a monocistronic *tRNA_{3^{Leu}}* gene was used in place of the dimeric *sup9-e* gene to simplify the quantitation. We note that the transcription of both genes is increased to the same extent by *PCF1-1* extracts.

RESULTS

PCF1-1 increases the number of transcriptionally active preinitiation complexes

The effects of the *PCF1-1* mutation are discernable at two stages during the transcription process: During and after the formation of transcription preinitiation complexes. The difference at each step is approximately 2–3 fold (favoring transcription in mutant extracts) and together, this accounts for the overall difference (4–6 fold) between the extracts (25). We wished to determine whether the difference observed after complex formation was due to increased re-initiation on a constant number of transcription complexes or whether there were simply more transcriptionally competent complexes formed with the mutant extract. Single round transcription experiments were performed to answer this question. In these assays, transcription complexes are allowed to form and initiate RNA synthesis but transcription is halted after the synthesis of a nascent RNA by the omission of one or more NTPs. Subsequent addition of the missing triphosphate(s) and heparin allows completion of nascent transcripts and prevents re-initiation by polymerase (31). We first tested our extracts by performing a heparin titration experiment under conditions that were reported to limit transcription to a single initiation event per preinitiation complex (Fig. 1A, lanes 1–5). Compared to a short (10 min) multiple round reaction (lane 1), transcription under single round conditions yielded approximately 30% fewer transcripts. The level of transcription remained essentially constant over the range of heparin concentrations tested (100–600 µg/ml, lanes 2–5). In a second experiment, we observed that no full length transcripts were synthesized during a 30 min incubation of extract with *sup9-e* DNA, ATP, UTP and GTP (lane 6). However, subsequent addition of CTP with heparin led to the synthesis of full length products (lane 7). These data indicate that transcription under these conditions is limited to single initiation events. In the following experiments, we employed the same concentration of heparin used by Kassavetis *et al.*, (31), namely 300 µg/ml.

We next compared transcription, in wild-type and mutant cell extracts, of two dimeric gene pairs encoding *S. cerevisiae* *tRNA^{Arg}-tRNA^{Asp}* and *S. pombe* *sup9-e tRNA^{Ser}-tRNA^{Met}*. These genes were incubated separately with both extracts and combinations of NTPs that were predicted to yield nascent RNAs of 5 and 7 nucleotides, respectively. Our results show that *PCF1-1* cell extracts assemble more transcriptionally competent complexes per microgram of protein than wild-type extracts (Fig. 1B). The fold increase in the number of functional

Figure 1. Single round transcription analysis in wild-type and *PCF1-1* cell extracts. **A** Conditions for single round transcription in whole cell extracts were examined in a *PCF1-1* extract using excess amounts of *sup9-e* gene as a template. Ternary complexes were formed in the presence of ATP, GTP and UTP as described in Materials and Methods. After preincubation, CTP was added with varying amounts of heparin and the incubation continued for 10 min. Lanes 1–5 contained heparin at 0, 100, 200, 300 and 600 µg/ml, respectively and yielded 1060, 320, 220, 310 and 330 cpm of transcript. Lanes 6 and 7 compare reactions in which heparin (300 µg/ml) was added but where the NTP missing during the preincubation (CTP) was not, or was, present subsequently. **B** Reactions were performed with equal amounts of extract protein, excess template DNA and NTPs as indicated above for the *sup9-e* gene. Ternary complexes on the *tRNA^{Arg}-tRNA^{Asp}* gene were formed by including ATP and CTP in the initial incubation. Nascent transcripts were elongated in the presence of heparin (300 µg/ml) and the full complement of NTPs. The use of wild-type (w) or mutant (m) extracts is indicated above each lane. This experiment yielded 690 and 1600 cpm for *sup9-e* and 500 and 800 cpm for *tRNA^{Arg}-tRNA^{Asp}* gene. **C** A limiting amount of *sup9-e* DNA (determined by template titration) was preincubated with wild-type or *PCF1-1* cell extracts and carrier DNA to form ternary complexes as above. Extension products of duplicate reactions are shown for both extracts. Data were quantified by 2-dimensional video densitometry and revealed a 2.8-fold stimulation by *PCF1-1*.

complexes (mutant versus wild-type extracts) was similar for both genes and was essentially constant from one experiment to the next. In six determinations with *sup9-e*, activation by *PCF1-1* ranged from 1.5–2.3 fold with an average of 1.8. Similar results have been obtained using the monomeric *SUP4* gene (data not shown) where preincubation in the absence of UTP allows the synthesis of a 17 nucleotide long nascent RNA (31). It is noteworthy that the magnitude of the increase in transcription by *PCF1-1* under single round assay conditions correlates with the 2-fold stimulation seen previously in multiple round experiments when preinitiation complexes are preformed (25).

In earlier work, template titration experiments were used to ascertain whether the *PCF1-1* mutation affected the amount of some wild-type component that was limiting for transcription *in vitro* or whether the intrinsic activity of the factor had been increased (25). The data indicated that the former possibility was not the case since the template concentration corresponding to half maximal transcription was the same for wild-type and mutant extracts. We have now looked at this question again by

Figure 2. Transcriptional activation of *sup3-e* A and B block mutant genes. Panel A shows the sequence of the wild-type *sup3-e* gene (italics) in the region of the A and B block and the point mutations used in this analysis. Nucleotide numbering follows standard tRNA nomenclature (26). The nucleotide sequence spans positions 6–21 and positions 48–62. Bases in upper case italics indicate positions known from mutagenesis studies to play a role in promoting transcription (3, 26). Lower case italics indicates that no transcriptional role is known at that position. The base change in each mutant is shown below the gene sequence. A dash means that no point mutation at that position was available. The mutation at position 52 is a T insertion rather than a point mutation (Ti52 in panel C). Similarly, an A insertion after nucleotide 26 (Ai26 in panel B) was also analyzed (not shown in panel A). Panels B and C compare transcription of the A and B block mutant genes, respectively, in wild-type and *PCF1-1* extracts. Transcription reactions were performed under multiple round conditions with excess amounts of template DNA and equal amounts of wild-type or *PCF1-1* extract protein.

performing single round transcription assays under conditions where the reaction is limited by the amount of template DNA. The 2.8-fold increase in transcription that we observed (Fig. 1C) is slightly higher but still comparable to the level of activation obtained using this same assay but with excess template (Fig. 1B). We deduce that the intrinsic activity of some transcription component must be increased in the mutant cells (see Discussion).

Scanning for new transcription factor-DNA contacts in the A block

One simple way in which the transcriptional effects of the A block promoter mutation, A19, might be suppressed in *PCF1-1* strains is by the formation of a new contact between the A block and some component of the transcription apparatus. Previous work has eliminated the adenosine residue at position 19 as a potential site for this interaction, since transcription of the wild-type *sup9-e*

Figure 3. Reconstitution of transcription with TFIIIB and TFIIIC fractions purified from wild-type and *PCF1-1* cell extracts. A Pairwise mixing of TFIIIB and TFIIIC fractions. The components of each reaction are shown (+) above their respective lanes. These reactions contained wild-type pol III (2.3 μg), wild-type (65 μg) or *PCF1-1* (74 μg) extract-derived TFIIIB, and TFIIIC (8.4 μg or 7.0 μg) from wild-type and mutant extracts respectively. Transcription was assayed on a tRNA^{Leu} gene template. Total radioactivity incorporated into tRNA^{Leu}-specific transcripts was 1140, 4420, 1440 and 4570 cpm for the four lanes from left to right. (B) Titration of TFIIIB. Reactions contained constant, excess amounts of wild-type TFIIIC (2.7 μg) and pol III (1.5 μg) and varying amounts of wild-type (●) or mutant (▼) TFIIIB (0–25 μg). Solid lines represent the best fits by least squares analysis and yielded slope values of 30 and 102, respectively.

gene (G19) in *PCF1-1* extracts is stimulated to the same extent as for the *sup9-e* A19 mutant gene (25). The interaction between some factor and the A block could, however, be strengthened by forming a new contact at another position in this region. Since a mutation (especially a transversion) at the site of a new contact would be expected to abolish the activated transcription seen in *PCF1-1* extracts, we examined the effects of a series of *sup3-e* A block point mutations (26) on transcription in wild-type and *PCF1-1* extracts. The *sup3-e* mutant genes that were analyzed contained transversions at all positions (except position 19) in the A block (nucleotides 8–19) as well as mutations at positions flanking the control region that have no known role in promoting transcription (Fig. 2A). Additionally, several B block control region mutants were examined. Transcription reactions were performed in wild-type and *PCF1-1* cell extracts with equal amounts of extract protein and excess template DNA. The results are presented in Figs. 2B and C and show that the transcription of all mutant genes was activated 2.7–5.6 fold in the *PCF1-1* extract. The average value in this experiment was 4.3 ± 0.8 .

Figure 4. Activity of TFIIC fractionated from wild-type and *PCF1-1* cell extracts. Reactions were performed as in Fig. 3 but contained an excess of mutant TFIIB (63 μg), wild-type pol III (1.5 μg) and varying amounts of wild-type (●) or *PCF1-1* (▼) extract-derived TFIIC (0–1.3 μg). Solid lines represent the best fits to the data and returned slope values of 1.7 and 1.8.

The fact that no mutation was found which prevented the increase in gene transcription in the mutant cell extract suggests that the *PCF1-1* mutation does not exert its effects by forming base-specific interactions with the A block. The possibility of base-specific interactions being created at sites outside this region can also be discounted since increased transcription has been observed for a variety of pol III genes including those encoding tRNA^{Ser} (*SUQ5*), tRNA^{Leu} (*SUP53*), tRNA^{Tyr} (*SUP4*), tRNA^{Arg}, tRNA^{Asp} and 5S RNA from *S. cerevisiae* and 7SL RNA from *S. pombe* (25 and data not shown). These data indicate that *PCF1-1* is indeed a general activator of pol III gene transcription.

The mutant activity in *PCF1-1* extracts copurifies with TFIIB

Using a transcription factor exclusion assay, we have shown previously that the *in vitro* effects of *PCF1-1* are mediated by a factor or factors that are stably sequestered by tRNA genes (25). To determine whether TFIIB, TFIIC or perhaps both factors are responsible for the increased transcription in mutant cell extracts, wild-type and *PCF1-1* extracts were subjected to fractionation according to established protocols (15, 16, see Materials and Methods). In short, mutant and wild-type whole cell extracts were chromatographed on BioRex70 and DEAE-Sephadex to separate TFIIB, TFIIC and pol III. Since minor amounts of a TFIIB component (TFIIB^{''}) are known to contaminate the TFIIC fraction at this stage, TFIIC was further purified by rechromatography on DEAE-Sephadex (12). As a qualitative measure of the equivalence of fractions derived from the different extracts, protein recoveries were compared at each step. The final TFIIC fraction comprised 1.7% and 1.5% of the total protein in wild-type and *PCF1-1* crude extracts, respectively. Similarly, the final TFIIB fraction contained 5.2% and 6.2% of the material that was loaded onto BioRex70. Transcription assays performed with these fractions and pol III indicated that all three components were required to reconstitute high levels of transcription (data not shown).

Transcription assays were carried out using all four combinations of mutant and wild-type extract-derived TFIIB and TFIIC (Fig. 3A). For reactions containing homologous TFIIB and TFIIC components, a 4-fold increase in transcription was observed when these fractions were derived from *PCF1-1* as opposed to wild-type extracts. This result compares favorably

with the 5-fold increase in specific transcription that was measured using the crude extracts which served as the source of these fractions. Mixtures of wild-type and mutant, TFIIB and TFIIC fractions, clearly show that the mutant TFIIB fraction is able to confer mutant levels of transcription (Fig. 3A); a 3.9-fold increase in transcription was measured with respect to the homologous wild-type reaction. A more precise determination of the relative specific activities of the TFIIB fractions was obtained upon titration of wild-type and mutant TFIIB in reactions containing wild-type TFIIC and pol III (Fig. 3B). A comparison of the slopes of the resulting regression lines revealed a 3.5-fold increase in the specific activity of the mutant IIB fraction.

Although the data in Fig. 3 demonstrate that *PCF1-1* affects the activity of the TFIIB fraction, these experiments do not exclude the possibility of an additional effect on TFIIC. To ascertain whether or not the *PCF1-1* mutation affects only the TFIIB fraction we carried out a titration of TFIIC under conditions where this factor was limiting for transcription. As can be seen in Fig. 4, linear regressions of the TFIIC titration curves gave essentially the same slope indicating that there is no effect of *PCF1-1* on the activity of this fraction. Together, the results of Figs. 3 and 4 lead us to conclude that *PCF1* is a component or a regulator of TFIIB.

DISCUSSION

PCF1-1 is a general activator of pol III gene transcription. All of the pol III genes analyzed to date are transcribed at higher levels in extracts derived from the mutant strain than in wild-type extracts. The list of yeast genes examined so far includes a 5S RNA gene, a variety of monomeric and dimeric tRNA genes from both *S. cerevisiae* and *S. pombe* and a *S. pombe* 7SL RNA gene. The reproducibility of these findings and of other observations involving experiments with crude extracts has been borne out over 5 independent extract preparations. Overall, specific transcription activity assayed using our standard *sup9-e* template has increased from 4 to 7 fold in the mutant extracts.

In this work we have examined the relative contributions of TFIIB and TFIIC towards the activated transcription in *PCF1-1* extracts. Fractionation of both factors from mutant and wild-type extracts followed by transcription reconstitution experiments showed that the mutant activity in *PCF1-1* extracts copurifies with the TFIIB fraction (Fig. 3). The difference in the specific activity of mutant and wild-type crude extracts could be accounted for in large measure by the difference in the specific activity of TFIIB. In contrast, no detectable change in the specific activity of the TFIIC fraction was observed. These observations are supported by fractionation studies performed with a second, independently prepared set of wild-type and *PCF1-1* extracts (data not shown). Since the mutant activity in *PCF1-1* extracts is stably sequestered upon preincubation with template (25) and fractionates with TFIIB but not TFIIC, we conclude that *PCF1* either encodes a component of TFIIB or a positive regulator of this factor. This result is somewhat unexpected since *PCF1-1* was selected as a suppressor of a mutation in a *cis*-acting element (the A block) known to be a binding site for TFIIC (see refs. 1–3). TFIIB, on the other hand, is known to footprint 5S and tRNA genes from position –3 to –43 relative to the transcription start site (32). Thus, we deduce that a defect in one step of transcription may be overcome by improvements to another step in this process.

From the results of single round transcription experiments performed at limiting and excess template concentrations (Fig. 1C), we infer that the *PCF1-1* mutation increases the intrinsic activity of the factor it encodes and not simply the amount of this factor. This conclusion follows from the fact that (i) when template is limiting, all of the factors required for transcription must, by definition, be present in excess amounts and (ii) transcription under template-limiting conditions was stimulated to a similar extent as when excess amounts of template were used (2.8-fold versus 2-fold, respectively, Figs. 1B and 1C). We note that if the amount of a limiting wild-type protein had been increased by the *PCF1-1* mutation, the level of activation (mutant versus wild-type transcription) would have been higher under conditions of template excess. Additional data consistent with the idea that *PCF1-1* affects gene product activity is provided by an *in vivo* experiment where a high copy number plasmid was used to assess the effect of elevated *PCF1-1* gene dosage on *sup9-e A19-supS1* activity. No change in suppressor strength was observed suggesting that the amount of PCF1 is not limiting *in vivo* (Rameau and Willis, unpublished observations). This contrasts with a similar experiment involving a different mutant, *PCF4-1*. In this case, suppression by *sup9-e A19-supS1* is conferred by increasing the wild-type gene dosage (López-De-León, Librizzi, Puglia and Willis, submitted for publication).

The effects of the *PCF1-1* mutation do not result from the formation of base-specific contacts with the internal promoter or other coding sequences in the DNA template. This conclusion was reached by analyzing the transcription of numerous *sup3-e* point mutants and other pol III genes and is consistent with the involvement of TFIIB in the function of *PCF1*. TFIIB is known to bind upstream of the transcription start site in a region where no sequence conservation is observed (31). Another correlation between *PCF1* and TFIIB worth noting involves the shorter lag phase that precedes linear transcription in *PCF1-1* versus wild-type cell extracts (25). The lag phase in pol III transcription has been attributed to the slow binding of TFIIB to TFIIA-TFIIC-DNA complexes in the case of 5S genes or to TFIIC-DNA complexes on tRNA genes (3, 18).

Whether *PCF1* encodes a component of TFIIB or a regulator of TFIIB activity, it is necessary to explain how two activities of the factor, namely the rate of complex assembly and the functionality of the assembled complexes can be affected in the dominant mutant strain. If *PCF1* is a catalytic regulator, these effects can be readily explained by posttranslational modifications to multiple sites or domains of TFIIB polypeptides. On the other hand, if *PCF1* is a component of TFIIB, two simple explanations may account for the bifunctional effect of the mutation: There could be more than one mutation in the *PCF1* gene. A comparison between the wild-type and the recently cloned mutant gene sequences will be required to resolve this issue. Alternatively, a single mutation could be responsible for both effects since TFIIB is known to bend DNA upon binding (33) and presumably undergoes a conformational change in the process. Clearly, some change in the factor appears to be necessary to explain the lack of detectable binding of TFIIB to DNA in the absence of TFIIC and the remarkable stability of the TFIIB-DNA complexes that form in the presence of TFIIC (32). Although the mechanism remains obscure, the *PCF1-1* mutation may initially facilitate interactions between TFIIB and either the DNA or TFIIC in order to increase the rate of formation of preinitiation complexes. Subsequently, as a result of a proposed conformational change to the factor, the *PCF1-1* mutation could increase initiation by

providing a new site of interaction for the polymerase or by affecting an equilibrium between active and inactive TFIIB-DNA conformers.

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