In Vitro Evidence for Growth Regulation of tRNA Gene Transcription in Yeast

A ROLE FOR TRANSCRIPTION FACTOR (TF) IIIB\textsubscript{70} AND TFIIIC*

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We report in vitro studies showing that tRNA gene transcription in yeast is down-regulated during the transition from logarithmic to stationary phase growth. Transcription in a postdiauxic (early stationary) phase extract of a wild-type strain decreased 3-fold relative to a log phase extract. This growth stage-related difference in transcription was amplified to 20-fold in extracts of a strain containing a mutation (pcf1–4) in the 131-kDa subunit of TFIIIC. The reduction in transcription activity in both wild-type and mutant postdiauxic phase extracts was correlated with a decrease in the amount of TFIIIB\textsubscript{70}, the limiting factor in these extracts. However, the 3.7 \pm 0.5-fold decrease in amount of TFIIIB\textsubscript{70} in mutant extracts does not, by itself, account for the 20-fold decrease in transcription. Accordingly, transcription in the mutant postdiauxic phase extract could be reconstituted to a level equal to the mutant log phase extract by the addition of two components, TFIIIB\textsubscript{70} and TFIIIC. Addition of TFIIIB\textsubscript{70} increased transcription 10-fold, while a 2-fold effect of TFIIIC was seen at saturating levels of TFIIIB\textsubscript{70}. The data suggest that both TFIIIB\textsubscript{70} and TFIIIC play a role in coordinating the level of polymerase III transcription with cell growth rate.

The expression of genes transcribed by each of the three nuclear RNA polymerases (pol)s is down-regulated during the transition from logarithmic to stationary phase (G\textsubscript{s}) growth (1–3). The regulatory events that mediate this general decrease in transcription are complex and not well understood. In the yeast Saccharomyces cerevisiae, the transition from logarithmic growth to stationary phase is accompanied by a 50% decrease in total RNA and a decline in the number of ribosomes to less than 25% of the maximum amount (4). One of the earliest events in this process is the decrease in pol I-dependent transcription of rRNA. For cells growing in rich medium, this occurs well before the end of the log phase and is followed closely by a decrease in pol II-dependent transcription of ribosomal protein genes. The early shut-off in the synthesis of ribosomal components, specifically the large rRNAs, demonstrates that yeast cells respond to increasing cell density prior to changing their growth rate. At the present time, the nature of the signal and the manner in which it is transduced to affect transcription is unknown. However, in higher eukaryotes and in Acanthamoeba, growth regulation of pol I transcription appears to involve posttranslational modification of the polymerase or a tightly associated initiation factor (see Refs. 1 and 5, and references therein).

At the end of logarithmic growth, yeast cells undergo a diauxic shift in which the nutrients required for growth by fermentation become exhausted and the cells switch to respiratory metabolism (2). Many pol II-transcribed genes that are expressed in abundance during logarithmic growth are down-regulated to barely detectable levels following the diauxic shift. In contrast, the expression of other genes remains relatively constant or even increases in some cases. Nonetheless, by the time the cells reach stationary phase, the level of total poly(A)\textsuperscript{+} RNA has decreased 2-fold (6). Since not all pol II-transcribed genes are regulated coordinately during the growth cycle, decreased transcription of specific genes is brought about by controlling the activity of activator and/or repressor proteins. These, in turn, affect the assembly or function of the basal transcription factors on promoters under their control (reviewed in Ref. 7).

The RNAs transcribed by pol III are not limiting for cell growth under normal conditions but are available at all times for numerous cellular processes including protein synthesis, protein secretion, and RNA processing (3, 8). Despite the apparent surplus of pol III transcripts for cell growth, the expression of pol III genes is not unregulated. Indeed, the coordination of pol III gene transcription with cell growth rate is well documented in higher eukaryotic systems (reviewed in Ref. 3). Pol III gene transcription in both human and mouse cells increases when growth is stimulated by serum (9, 10). Conversely, down-regulation of pol III transcription is observed when mouse cells are treated with cycloheximide or grown to confluence (11). In vitro systems that mimic these in vivo treatments show effects on transcription of 3-8-fold and have allowed identification of the regulated factors. Growth-related differences in transcription result almost entirely from changes in one or both of the multisubunit transcription factors (TFs) IIIC and IIIB; the activity of pol III changes very little with cell growth rate (9, 11).

Recently, an analysis of mutant yeast strains selected for their ability to increase pol III transcription identified a number of isolates in which this phenotype is recessive (12). A genetic characterization of one of these strains mapped the mutation to the gene encoding the 131 kDa subunit of TFIIIC (PCF1/TFC4). Subsequently, a mutation in this subunit (pcf1–3) was shown to increase transcription of a variety of pol III genes in vitro. One implication of these results, namely that a recessive (presumed loss of function) mutation increases pol
III transcription, is that the wild-type TFIIIC131 protein may negatively regulate this process. We therefore examined (i) whether pol III transcription in yeast is negatively regulated during the transition from logarithmic to stationary phase growth and (ii) whether a recessive mutation in TFIIIC131 perturbs this regulation. The results provide in vitro evidence that pol III transcription is down-regulated at the end of the logarithmic growth phase and show that the magnitude of this regulation is amplified in extracts of a mutant strain. The growth-related differences in transcription activity are attributed to effects on TFII1B70 and TFII1C.

EXPERIMENTAL PROCEDURES

Yeast Strains—The effect of the growth stage on transcription was examined using isogenic strains deleted for the genomic PCF1 gene and containing the wild-type or pcf1–4 mutant gene on the centromeric plasmid pRS313. The construction of these strains has been described by Sathy and Willis (12). S17pcf1–4 is Mata ura3–52-URA3(sup9-e A19-sup51) leu2–3,112 his3–11,15 trp1–1 met8–1 pcf1 and S17pCF1 is Mata ura3–52-URA3(sup9-e A19-sup51) leu2–3,112 his3–11,15 trp1–1 met8–1 LEU2 pRS313 PCF1. Strain YS810 (13) was used to prepare nuclear extracts (14) for the Western analysis in Fig. 6A.

Extract Preparation and Factor Purification—Wild-type and mutant cell extracts were prepared from cultures grown in YPD medium at 30 °C and 180 rpm. Late logarithmic phase and postlog phase cells were harvested at densities of 5 × 10⁷ cells/ml and 2 × 10⁶ cells/ml, respectively. Whole cell extracts were prepared in parallel from 9–13 g (wet weight) of cells by glass bead disruption as described by Willis et al. (15). Aliquots were stored at 70 °C following chromatography of the samples on Sephacryl S200. Protein concentrations were determined by UV spectrophotometry (16).

All transcription factors were wild-type unless otherwise noted. Recombinant yeast TBP was a gift from Dr. Michael Brenowitz. TFII1B70 was synthesized in a rabbit reticulocyte lysate as described below. TFII1C was prepared by urea extraction of chromatin pellets and was synthesized in a rabbit reticulocyte lysate as described below. TFII1B70 was expressed at approximately 10 mg/liter. The specificity of the TFII1B70 antibody was demonstrated by the lack of reactivity of the prebleed in Western analysis of yeast whole cell extracts, even at 100 times higher concentrations than the working dilution of the antibody (1:5000). The antibody, but not the prebleed, recognized a 70-kDa band in yeast nuclear and whole cell extracts, in rabbit reticulocyte lysate extracts, in vitro synthesized TFII1B70, and in E. coli extracts induced for the expression of full-length recombinant TFII1B70. These reactivities could be blocked with excess antigen at 2.5 μg/ml and with full-length recombinant TFII1B70 at 2.5 μg/ml, but not with polyhistidine at 1 μg/ml. A peptide antibody to TFII1C131 was used at a dilution of 1:500 (20). Western analysis was performed as described by Harlow and Lane (23). Nitrocellulose membranes were blocked in 5% nonfat dry milk in Tris-buffered saline, pH 7.6, 0.2% bovine serum albumin, 0.1% Tween 20 (TBS-T) for a minimum of 1 h. Antibodies were incubated with the blots for 1 h in TBS-T. Antibody-antigen complexes were detected with a horseradish peroxidase-conjugated goat anti-rabbit antibody (1:5000) using enhanced chemiluminescence (ECL) as described by the manufacturer (Amersham Corp.). Autoradiograms were quantified using a laser densitometer (Molecular Dynamics) and ImageQuant software. Detection of recombinant TFII1B70 by these methods showed a linear response over a 60-fold concentration range. Relative levels of TFII1B70 in extracts were determined from Western blots performed simultaneously over a range of protein concentrations. The resulting ECL signals were within the linear range of the standards. Differences between extracts were derived from ratios of the slopes determined by linear regression.

RESULTS

In Vivo Evidence for Growth Regulation of RNA Polymerase III Transcription in Yeast—The pcf1–3 mutation is an arginine to lysine substitution at amino acid 728 in TFII1B70. This recessive mutation suppresses the effects of an A block promoter mutation in vivo and increases pol III transcription in extracts of log phase cells by 2- to 3-fold (12). One interpretation of these findings is that the arginine residue at amino acid 728 plays a negative role in transcription and that other residues at this position (e.g. lysine) are less effective in exercising this function. Subsequently, a site-directed mutation to histidine at amino acid 728 (pcf1–4) was found to have a stronger effect than the original pcf1–3 allele, a strain containing the stronger mutation was chosen together with an isogenic wild-type strain to examine the effect of growth phase on pol III-specific gene transcription in vitro. Wild-type and pcf1–4 cells were grown to densities of 5 × 10⁷ and 2 × 10⁶ cells/ml. These cell densities correspond to the late log phase and postlog phase, respectively. The latter precedes stationary phase and is characterized by several generations of respiratory growth at a greatly reduced rate (compared to log phase; Ref. 2). Both strains grew to a density of 5 × 10⁷ cells/ml with a doubling time of 90 min. Thereafter, cell growth rates decreased with the next two generations of growth requiring an additional 9 h.
Negative Regulation of tRNA Gene Transcription

Log and postdiauxic phase whole cell extracts were prepared in parallel by glass bead disruption and assayed for transcription activity. The pcf1–4 log phase extract increased pol III-specific transcription activity 9-fold over the wild-type log phase extract (Fig. 1A and Ref. 12). This effect of pcf1–4 on transcription is consistent with the strong in vivo phenotype of this mutant and is appropriate (in magnitude) relative to the transcriptional activities and in vivo phenotypes of the pcf1–3 and Pcf1–1 alleles (12). Transcription in wild-type extracts prepared from postdiauxic phase cells was reduced by 70% compared to the log phase control. A quantitatively similar low level of transcription was also observed in the mutant postdiauxic phase extracts. Consequently, transcription activity in the wild-type extracts decreased 3-fold between the log and postdiauxic phase, while transcription activity in the pcf1–4 extracts decreased by 20-fold. To examine whether the differential activity in pcf1–4 extracts resulted from a difference in the number of transcriptionally competent complexes, a modified single round initiation assay was used to determine the number of these complexes (see "Experimental Procedures"). Briefly, preinitiation complexes assembled in the absence of NTPs were allowed to transcribe for 30–90 s following NTP addition. Using this assay and a BRα fraction, the 30-, 60-, and 90-s time points were found to yield 1.8, 1.3, and 1.0 transcripts/active complex/30 s, respectively (Fig. 1B, compare lanes 3–5 with lane 2). A comparison of transcription in pcf1–4 log and postdiauxic phase extracts under these conditions yielded a 25 ± 1-fold difference in the number of active complexes (Fig. 1B, lanes 6–11). This is similar to the difference seen in multiple round assays. Thus, a difference in the number of active complexes can account for the transcriptional differential between the pcf1–4 log and postdiauxic phase extracts.

The preceding experiments suggest that pol III transcription in yeast is subject to down-regulation as the cells approach stationary phase. This conclusion is in concordance with in vitro and in vivo studies on growth regulation of pol III transcription conducted in mammalian systems (3). Interestingly, despite the high level of transcription seen in the pcf1–4 log phase extract, the pcf1–4 mutation does not impair the down-regulation of transcription in extracts of postdiauxic cells. Therefore, the mutation at amino acid 728 in TFIIIC131 does not render the protein defective in postdiauxic phase regulation. Nonetheless, the ability of the pcf1–4 mutation to amplify the transcriptional difference between log and postdiauxic phase cell extracts provides a useful system to investigate the factors and the molecular mechanisms regulating pol III gene transcription during the growth cycle.

TFIIB190 Is the Primary Limiting Factor in Wild-type Log Phase and pcf1–4 Postdiauxic Cell Extracts—The reduction in pol III transcription in postdiauxic phase extracts could be due to the presence of an inhibitor in these extracts. This possibility was tested by performing an extract mixing experiment. Transcription activity was determined as in Fig. 1 by separately assaying equal amounts of log or postdiauxic phase extract or a mixture of the two. The results from three such experiments are presented in Fig. 2. There is no significant effect on transcription when equal amounts of the mutant log and postdiauxic phase extracts are mixed compared to the mutant log phase extract alone. Therefore, the decrease in transcription observed in the postdiauxic phase extracts is not due to a diffusible inhibitor. Rather, the equivalent levels of transcription in log phase and mixed extracts suggests that the postdiauxic phase extract is deficient in one or more positively acting factors.

Transcription of a tRNA gene in yeast requires the initiation factors TFIIB (which comprises TBP, TFIIB190, and TFIIB90), TFIIC (a multisubunit complex of six polypeptides), and the 16-subunit RNA polymerase III (3, 8, 17, 24). Additionally, a requirement for a distinct new factor, TFIIE, has been dem-
shown using a different purification scheme than that used by most laboratories (25). The nature of the activities that are limiting for pol III transcription in wild-type log phase and mutant postdiauxic phase cell extracts was examined by adding various factors (or fractions) individually or in combination to these extracts. The TFIIIB components used in this experiment included recombinant yeast TBP, TFIIIB70 synthesized in a rabbit reticulocyte lysate, and a purified TFIIIB90 fraction from urea-extracted chromatin pellets (see Ref. 17 and "Experimental Procedures"). Previous studies have demonstrated the ability of two of these fractions (recombinant TBP and urea-extracted TFIIIB90) to function in pol III transcription (17, 26). The activity of the third component, in vitro synthesized TFIIIB70, is demonstrated in Fig. 3A. Addition of a rabbit reticulocyte lysate programmed with RNA encoding TFIIIB70, but not a negative lysate control, to a wild-type log phase cell extract produced a significant (5-fold in Fig. 3A) increase in transcription. This result is consistent with our previous finding that TFIIIB70 is limiting for pol III transcription in vivo (27) and extends the related observation, that TFIIIB70 is limiting for pol III activity in vitro. Other experiments have demonstrated that in vitro synthesized TFIIIB70, but not lysate alone, is active in reconstituting transcription in the presence of TBP, urea-extracted TFIIIB90, purified TFIIIC and pol III (see "Experimental Procedures").

The ability of TFIIIB70 and other pol III factors/fractions to increase transcription when added to wild-type log phase and pcf1–4 postdiauxic phase extracts is examined in Fig. 3 (B and C, respectively). The results for both extracts are similar and show a significant increase in transcription for TFIIIB70 (up to 6-fold, lanes 4–6), no effect of TBP (lanes 1–3), and a slight decrease in transcription for TFIIIB90 (at higher concentrations, lanes 7–9). Multiple round reactions were employed using 75 μg of each extract. Lane 1 contained no exogenous TFIIIB70. The amount of added TFIIIB70 was determined by single round transcription (see "Experimental Procedures"). B shows the effect of adding increasing amounts of heparin-agarose-purified TFIIIB on transcription in a pcf1–4 log phase extract (circles and lanes 1–6) and a pcf1–4 postdiauxic phase extract (squares and lanes 5–9). Multiple round reactions were employed using 75 μg of each extract. Lane 1 contained no exogenous TFIIIB70. The amount of added TFIIIB70 was determined by single round transcription (see "Experimental Procedures"). C shows that the effect of adding increasing amounts of heparin-agarose-purified TFIIIB11 on transcription in a pcf1–4 log phase extract (circles and lanes 1–6) and a pcf1–4 postdiauxic phase extract (squares and lanes 7–12). The same reaction conditions were used as in panel A.

FIG. 3. TFIIIB70 is the limiting component in wild-type log phase and pcf1–4 postdiauxic phase extracts. Transcription was assayed under multiple round initiation conditions as in Fig. 1. A, stimulation of transcription by in vitro synthesized TFIIIB70. A wild-type log phase extract (65 μg of protein) was supplemented with a negative control rabbit reticulocyte lysate (1.5 and 3 μl, lanes 2 and 3) or a lysate that had been programmed with RNA encoding TFIIIB70 (1.5 and 3 μl, lanes 4 and 5). No lysate was added to the reaction in lane 1. B, a wild-type log phase extract (100 μg of protein) was supplemented with exogenous factors as follows: lanes 1–3, 20, 40, and 80 fmol of recombinant yeast TBP; lanes 4–6, 0.7, 1.4, and 2.1 fmol of in vitro synthesized TFIIIB70; lanes 7–9, 0.85, 1.7, and 3.4 fmol of TFIIIB90; lane 10, 80 fmol of TBP + 2.1 fmol of TFIIIB70; lane 11, 80 fmol of TBP + 3.4 fmol of TFIIIB90; lane 12, 2.1 fmol of TFIIIB90 + 3.4 fmol of TFIIIB70; lane 13, 80 fmol of TBP + 2.1 fmol of TFIIIB70 + 3.4 fmol of TFIIIB90; lane 14, 32 fmol of TFIIIC; lane 15, 100 ng of pol III. C, a pcf1–4 postdiauxic phase extract was supplemented with exogenous factors as in panel B.
Reconstitution of Log Phase Transcription in pcf1–4 Postdiauxic Cell Extracts—To investigate whether the factor that becomes limiting in TFIIIB70-supplemented extracts is one of the other TFIIIB components or possibly TFIIIE, which is known to copurify with partially purified TFIIIB fractions (25), increasing amounts of heparin-agarose-purified TFIIIB were added to the postdiauxic phase extract (Fig. 4B). The results are essentially identical to those observed with in vitro synthesized TFIIIB70 (Fig. 4A). Transcription in the mutant postdiauxic phase extract increased 10-fold, whereas the corresponding log phase extract increased only 1.3-fold. Thus, the increase in transcription resulting from TFIIIB addition is most likely due to the increased levels of TFIIIB70 rather than elevated levels of TBP, TFIIIB90 or other activities (i.e. TFIIIE) in this fraction. Accordingly, addition of TBP or TFIIIB90 to a TFIIIB-supplemented pcf1–4 postdiauxic phase extract gave no increase in transcription (Fig. 5, lanes 2–4). Another noteworthy feature, illustrated in Fig. 4B, is the similar slope of the TFIIIB titration curves. This indicates that the activity of the exogenous TFIIIB is not affected differentially by the two extracts.

The data in Fig. 4B suggest that either TFIIIC or pol III becomes limiting in the postdiauxic phase extract at saturating levels of TFIIIB70. An additional add-back experiment was performed to resolve these possibilities. Saturating levels of TFIIIB (i.e. TFIIIB70) were determined from Fig. 4B and were confirmed by comparing transcription at two different concentrations of this factor (Fig. 5, A, lanes 1 and 2, and B). Addition of a pol III fraction to the TFIIIB-supplemented extract showed no stimulation of transcription. However, two different concentrations of TFIIIC increased transcription 2.4- and 2-fold, respectively, over the TFIIIB-supplemented control. The addition of TFIIIC together with pol III also showed a 2-fold effect. Importantly, the transcription observed in these reactions is comparable to that seen in the log phase extract of the mutant strain (Fig. 5, lane 9). Thus, the transcription activity of the mutant postdiauxic phase extract can be increased to the level obtained in the corresponding log phase extract by adding both TFIIIB70 and TFIIIC. A similar high level of transcription can also be achieved by adding both factors to the wild-type postdiauxic phase extract (Fig. 5C). Addition of saturating amounts of heparin-agarose-purified TFIIIB (1.0 fmol except for lane 3, which contained 1.2 fmol) and various other fractions as follows: lane 3, TFIIIB90 (2.5 fmol); lane 4, TBP (80 fmol); lane 5, pol III (175 ng); lane 6, TFIIIC (6.4 fmol); lane 7, TFIIIC (19.2 fmol); lane 8, TFIIIC (10 fmol) and pol III (90 ng). Transcription in the pcf1–4 log phase extract is shown in lane 9. B, the bar graph shows the quantitation of the data in panel A. Bar designations correspond to the lanes in panel A. The presence (+) or absence (–) of supplementary components in each reaction is indicated above the graph. The 20-fold differential between pcf1–4 log and postdiauxic phase extracts is shown as a dotted line. C, the wild-type postdiauxic phase extract was supplemented with TFIIIB70 as follows: lane 2, 0.6 fmol; lane 3, 1.2 fmol; lane 4, 1.8 fmol; lane 5, 2.4 fmol; lanes 6 and 9–11, 3.0 fmol. In addition, lanes 10 and 11 contained 0.45 and 0.9 μg of heparin-agarose TFIIIC. Lanes 1 and 8 contained no exogenous factors. D, the wild-type log phase extract (lane 2) was supplemented with increasing amounts (0.4–1.2 fmol) of heparin-agarose-purified TFIIIB (lanes 2–5).
of TFIIIB\textsubscript{70} yields a 12–14-fold increase in transcription over the unsupplemented wild-type extract. Transcription is further stimulated upon addition of TFIIIC for an overall increase of 26–28-fold. We note that the ability to increase transcription in the wild-type postdiauxic phase extract is slightly higher than for the corresponding mutant extract. This correlates with the slightly lower activity of unsupplemented wild-type extract (Fig. 1A).

In contrast to the postdiauxic phase extracts, TFIIIB alone is sufficient to increase transcription of the wild-type log phase extract to a level equivalent to (and slightly above) the unsupplemented mutant log phase extract. Amounts of heparin-agarose-purified TFIIIB that were sufficient to saturate the postdiauxic extracts (Fig. 4B and data not shown) show a linear response in the wild-type log phase extract (Fig. 5D). At 1.5 fmol of exogenous TFIIIB, transcription was 11-fold higher than the unsupplemented level (recall that the unsupplemented log phase extracts showed a 9-fold differential, Fig. 1A). This result suggests that TFIIIC is not limiting to the same extent in log phase extracts as it is in the postdiauxic phase extracts.

**TFIIIB\textsubscript{70} Levels Are Reduced in pcf1–4 Postdiauxic Cell Extracts—**The most substantial contribution to the difference in transcription activity between log and postdiauxic phase pcf1–4 cell extracts is provided by TFIIIB\textsubscript{70} (Figs. 4 and 5). Therefore, we investigated whether the amount of this factor varied between the extracts. For this and other experiments, a high titer TFIIIB\textsubscript{70}-specific antibody was required to detect the protein in whole cell extracts. To this end, antibodies were raised in rabbits to the unique carboxyl-terminal half of TFIIIB\textsubscript{70} (i.e. the region lacking the TFIIIB homology, see “Experimental Procedures”). The specificity of the immune serum is demonstrated in Fig. 6A. Western blots containing increasing amounts of two yeast nuclear extracts were probed at a dilution of 1:5000 with preimmune and immune serum. One major band corresponding in size to recombinant TFIIIB\textsubscript{70} and two smaller, minor bands were detected by the immune serum in the wild-type nuclear extract. The preimmune serum showed no reactivity at this dilution. The specificity of the two largest bands is confirmed by the fact that both bands were present at higher levels in nuclear extracts from a TFIIIB\textsubscript{70} overexpressing strain (Fig. 6, PCF4-MC). Moreover, preincubation of the immune serum with the antigen but not polyhistidine effectively competed out the signal (see “Experimental Procedures”).

The relative amounts of TFIIIB\textsubscript{70} present in whole cell extracts of log and postdiauxic phase wild-type and mutant strains were determined by quantitative Western blot analysis. A representative experiment and the resulting densitometric analysis is shown in Fig. 6B and C. The data in Fig. 6C reveal a 2-fold difference in the amount of TFIIIB\textsubscript{70} between log phase extracts of the mutant and wild-type strains (circles and squares). Over five experiments, a 2.1 ± 0.3-fold difference was determined. These extracts, however, differ in transcription activity by a factor of 9 (Fig. 1A). A similar disparity is seen when comparing log and postdiauxic phase extracts of the mutant strain. Although these extracts differ in transcription by 20-fold, the amount of TFIIIB\textsubscript{70} protein shows only a 3.6-fold difference in Fig. 6C (circles and triangles) and a 3.7 ± 0.5-fold difference over four experiments. Because TFIIIB\textsubscript{70} is limiting in whole cell extracts and is stoichiometrically required for initiation (27, 28), these data suggest that changes in the amount of TFIIIB\textsubscript{70} are not sufficient to account entirely for the differences in transcription. In contrast, a good correlation is observed between the amount of TFIIIB\textsubscript{70} and the transcriptional activity of wild-type extracts from the log and postdiauxic phase. The TFIIIB\textsubscript{70} levels in these extracts differ by an average of 2.9 ± 0.4-fold over four experiments (in Fig. 6C, compare squares and inverted triangles), while transcription differs by 3-fold.

Quantitative Western analysis was also performed on the
pcf1–4 cell extracts using an antibody to the 131-kDa subunit of TFIIIC (Fig. 6D). The ratio of the slopes obtained by linear regression of the densitometry data from two experiments showed a 1.1-fold and a 1.2-fold difference in the amount of TFIIIC90 in mutant log and postdiauxic phase extracts. This result confirms the differences in the TFIIIB70 levels and the transcriptional capacities of the extracts.

**DISCUSSION**

In human and mouse systems, the level of pol III transcription is known to vary according to the cell growth rate (reviewed in Ref. 3). The present study indicates that growth regulation of pol III transcription also exists in yeast. Specifically, transcription of a tRNA gene was shown to be reduced in extracts of cells approaching stationary phase (Fig. 1). Over two generations of growth in which the cell doubling time increased from 90 min to greater than 270 min, tRNA gene transcription decreased 3-fold in extracts of a wild-type strain and 20-fold in extracts of a pcf1–4 strain. The magnitude of the effect in the wild-type extracts is similar to that observed in extracts of confluent or growth-inhibited mouse cells (11). In the mouse extracts, decreased pol III transcription was attributed largely to a reduction in the amount or activity of TFIIIB (11). Interestingly, the yeast factor most responsible for the difference in transcription between pcf1–4 log and postdiauxic phase extracts is a subunit of TFIIIB (TFIIIB70, Figs. 4 and 5). Thus, the mouse and yeast in vitro systems appear to behave similarly in response to changes in cell growth rate. This observation, together with the ability of the yeast in vitro system to reproduce in vivo phenomena (at least for TFIIIB70, discussed below), suggests that the log and postdiauxic phase extracts mimic the growth-related changes occurring in the cell.

Previous studies in our laboratory have shown that the general initiation factor TFIIIB70 is stoichiometrically limiting for pol III transcription in vivo (27). This result together with studies showing that TFIIIB activity is limiting in extracts of wild-type log phase cells (e.g. 18) suggested that the amount of TFIIIB70 might also be limiting for transcription in vitro. The experiments presented here confirm and extend this observation to include log phase extracts of a pcf1–4 mutant strain where pol III transcription is elevated 9-fold over wild-type (Fig. 3A and B, and 4A). Additionally, we have shown that TFIIIB70 is limiting in extracts of wild-type and pcf1–4 strains that are approaching stationary phase (postdiauxic phase extracts, Figs. 3C and 5D). Thus, both in vivo and in vitro, TFIIIB70 is limiting for transcription.

TFIIIB70, as a limiting initiation factor, is a likely target for global regulation of pol III transcription (27). Consistent with this expectation, we have shown (i) that the amount of TFIIIB70 decreases in both wild-type and pcf1–4 postdiauxic phase extracts relative to the corresponding log phase extracts (Fig. 6) and (ii) that addition of TFIIIB70 to the pcf1–4 postdiauxic phase extract increases transcription (10-fold) to a level equal to half that of the log phase extract (Figs. 4 and 5). These results indicate a role for TFIIIB70 in growth regulation of pol III transcription and suggest that regulation may be achieved by affecting the amount of this factor. Other data, however, suggest that growth regulation of pol III transcription may be more complex. For example, whereas the calculated 2.9 ± 0.4-fold decrease in the level of TFIIIB70 could potentially account for the 3-fold change in transcription in wild-type extracts, a 3.7 ± 0.5-fold change in the amount of this factor is unlikely to account for the entire 20-fold decrease in transcription observed for the pcf1–4 extracts. Consistent with this view, saturating levels of exogenous TFIIIB70 do not reconstitute pcf1–4 log phase-type transcription in postdiauxic phase extracts of the wild-type or the mutant strain (Figs. 4 and 5). The participation of a factor other than TFIIIB70 in growth regulation is indicated by the ability of a TFIIIC fraction (but not other fractions) to restore mutant log phase-type transcription to TFIIIB70-supplemented postdiauxic phase extracts (Fig. 5, B, lanes 6–9, and C, lanes 8–10). Notably, TFIIIC is not required to achieve this result in a wild-type log phase extract (Fig. 5D). Thus, TFIIIC appears to be subject to growth regulation although the effect is only modest (2-fold). Finally, while it is not yet known whether the activity or the amount of TFIIIC is subject to changes during the growth cycle, the 131-kDa subunit of this factor is present in the same amount in both log and postdiauxic phase extracts (Fig. 6D).

As noted above, the log to postdiauxic ratios of TFIIIB70 are similar in extracts of both wild-type and mutant strains (2.9 ± 0.4 and 3.7 ± 0.5, respectively) even though the ratios of their transcriptional activities differ significantly. Nonetheless, the reduced level of TFIIIB70 in the postdiauxic extracts and the limiting nature of this factor suggest that changes in the amount of TFIIIB70 may account, in part, for the observed differences in transcription. However, an additional (or alternative) explanation not excluded by our data is that TFIIIB70 may also be limiting during the growth cycle. Regulation of TFIIIB activity has been demonstrated during the cell cycle in Xenopus where phosphorylation of a TFIIIB component was shown to inhibit pol III transcription during mitosis (29). Mitotic repression of TFIIIB has also been reported in human cells although the mechanism has not been determined (30). Further support for the regulation of TFIIIB activity during the growth cycle in yeasts is provided by in vivo fingerprinting studies of tRNA genes in logarithmically growing and saturated cultures (31). Both growth stages exhibit identical patterns of protection in the region between –40 and –10 relative to the SUP53 tRNA gene transcription start site. This protected region corresponds precisely to that protected in TFIIIB-DNA complexes assembled in vitro (28, 32). Interestingly, cell growth-related changes in the in vivo footprint on the SUP53 gene map immediately downstream of the TFIIIB complex to a region (–10 to +15), where a number of pol III subunits and the 131-kDa subunit of TFIIIC have been photo-cross-linked in vitro (33, 34). These data indicate reduced occupancy of the DNA by pol III and/or TFIIIC in the stationary phase and suggest that pol III transcription may be regulated after TFIIIB has bound to the DNA. Accordingly, modification of TFIIIB activity (on the DNA) could potentially control the rate of initiation by pol III in non-dividing cells. In this regard, one obvious future direction of our work is to determine whether the specific activity of TFIIIB70 is different in log and postdiauxic phase extracts.

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