THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 268, No. 32, Issue of November 15, pp. 24047-24052, 1993
Printed in U.S.A.

Purification and Characterization of a General Transcription Factor, aTFB, from the Archaeon *Methanococcus thermolithotrophicus**

(Received for publication, May 20, 1993, and in revised form, August 5, 1993)

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We have recently shown that cell-free transcription of homologous templates from the archaeon *Methanococcus thermolithotrophicus* requires an archaeal transcription factor (aTFA) that separated from the RNA polymerase during phosphocellulose chromatography. We report here the identification and extensive purification of a second activity, aTFB, required for *in vitro* transcription. This activity copurified with RNA polymerase during initial chromatographic steps but was positively identified as a distinct transcription factor after Superdex 200 sizing chromatography. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed that the intensity of a M_r = 28,000 polypeptide in silver-stained gels is correlated with transcription factor activity. The same polypeptide, when eluted from a denaturing polyacrylamide gel and subsequently renatured, showed the functional properties of the transcription factor. In conjunction with gel filtration and sedimentation studies, which indicated a molecular mass of 54,000 Da for the native protein, these results suggested that aTFB is a dimer with polypeptide chains of identical molecular mass. Functional studies with highly purified aTFB demonstrated that it is a general factor required for transcription of genes encoding tRNA and proteins.

Archaeal cells appear to contain only one RNA polymerase. This complex multisubunit enzyme is structurally related to eukaryotic RNA polymerases II and III (Zillig, 1991). Recent studies have shown that cell-free extracts derived from the methanogens *Methanococcus vannielii* and *Methanococcus thermolithotrophicus* and from *Sulfolobus shibatae* direct specific transcription from promoters for stable RNA- and protein-encoding genes (Frey *et al.*, 1990; Gohl *et al.*, 1992; Thomm *et al.*, 1992; Hüdepohl *et al.*, 1990). These cell-free systems have been used to identify the cis-acting DNA sequence elements necessary for initiation of *in vitro* transcription (Hausner *et al.*, 1991; Reiter *et al.*, 1990). The archaeal promoter defined by these studies is composed of an AT-rich (TATA) element 22–27 base pairs upstream of a pyrimidine/purine initiator motif. The TATA box seems to determine the site of transcription initiation. Thus, the archaeal TATA box is very similar in structure and function to that of eukaryotic class II promoters (Corden *et al.*, 1980; Xu *et al.*, 1992).

Although the basic cis-acting DNA sequences required for cell-free transcription have been identified, a detailed understanding of the mechanism underlying accurate recognition and transcription of archaeal genes also requires the identification and purification of the trans-acting components necessary for *in vitro* transcription. Direct evidence for the existence of a transcription factor has been provided by reconstitution of a cell-free system of *M. thermolithotrophicus* from fractions purified by chromatography on phosphocellulose (Frey *et al.*, 1990). In addition a factor is required for specific initiation of transcription by the RNA polymerase fraction of *S. shibatae* at two rRNA promoters of this archaeon (Hüdepohl *et al.*, 1990).

The RNA polymerase of *M. thermolithotrophicus* was contained in the 0.35 M KCl step eluate, the transcription factor, here referred to as aTFA, in the 1 M KCl step eluate from PC[1] columns. When the PC fraction containing the RNA polymerase was replaced by purified RNA polymerase and assayed for its ability to transcribe these templates in combination with aTFA, we found that these components were insufficient to effect cell-free transcription (Frey *et al.*, 1990). This finding suggests that additional factors may exist in cells of *M. thermolithotrophicus* that are separated from the RNA polymerase during chromatographic purification.

In the present study we report the identification and extensive purification of a transcription factor from *M. thermolithotrophicus*. A cell-free system reconstituted with this highly purified transcription factor, RNA polymerase, and aTFA, is able to faithfully transcribe *in vitro* a variety of archaeal genes with high activity.

EXPERIMENTAL PROCEDURES

Plasmids—The plasmid pIC-31/2, which contains the $tRNA^{Val}$ gene of *M. vannielii*, was used as template in a standard *in vitro* transcription reaction (Hausner *et al.*, 1991). Other templates used were the *nifH1* gene of *M. thermolithotrophicus*, which encodes the putative dinitrogenase reductase, and the plasmid pKS304Δ16, containing the *hmfB* gene of *Methanothermus fervidus* (Gohl *et al.*, 1992; Thomm *et al.*, 1992). DNA was purified by repeated centrifugation in CsCl density gradients as described previously (Thomm and Wich, 1988).

Enzymes, Chemicals, and Column Materials—Restriction enzymes were obtained from Boehringer Mannheim or New England Biolabs. [α-^{32}P]UTP (800 Ci/mmol) was purchased from DuPont NEN. DEAE-cellulose (DE 52) was from Whatman. Heparin-Sepharose, ssDNA-cellulose, Mono Q (HR 5/5), Superdex 75 (HR 10/30), and Superdex 200 (HR 16/60) were obtained from Pharmacia. The hydroxylapatite HPLC column (Bio-Gel HPHT, 100 × 7.8 mm) was from Bio-Rad. All other chemicals were from Merck (Darmstadt, Federal Republic of Germany (FRG)) or Serva (Heidelberg, FRG).

Nonspecific Transcription by RNA Polymerase—Reaction mixtures (100 μl) contained 20 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 50 mM KCl,

* This work was supported by grants from the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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[1] The abbreviations used are: PC, phosphocellulose; aTF, archaeal transcription factor; HPEC, high performance electrophoresis chromatography; HPLC, high performance liquid chromatography; ss, single-stranded.

0.5 mM EDTA, 1 mM ATP, 0.1 mM [α-^{32}P]UTP (150 cpm/pmol), 2.5 μg of poly(dA·dT) (Boehringer Mannheim), and 5 μl of various protein fractions of the different purification steps. From the Mono Q purification 2 μl were used. After incubation at 55 °C for 20 min, the reactions were stopped by precipitation with 1.1-ml stop solution (5.5% trichloroacetic acid, 165 mM NaCl) and filtered over glass microfiber filters (Whatman GF/C). The radioactivity on the filters was measured by liquid scintillation counting.

Specific Transcription Reactions—The assay was performed in a 100-μl reaction volume according to Frey *et al.* (1990). The transcription reaction was incubated at 55 °C for 30 min and contained 20 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 100 mM KCl, 0.1 mM EDTA, 0.33 mM ATP, 0.33 mM CTP, 0.33 mM GTP, 2 μM [α-^{32}P]UTP (40,000 cpm/pmol), and 2 μg of closed circular plasmid pIC-31/2. The reactions were started by the addition of RNA polymerase, 5 μl of the Heparin-Sepharose pool (not containing aTFB) or 10 μl of the Superdex 200 fraction (see "Purification of aTFB"), and 10 μl of the 1 M KCl step eluate from a phosphocellulose column (prepared as described previously; Frey *et al.* (1990)), that contained a second activity required for cell-free transcription. In assays measuring aTFB activity (complementation assay), aliquots of various column fractions were added; otherwise, 20 μl of the Superdex 200 fraction of aTFB were used.

Purification of aTFB—The transcription factor aTFB was purified from 20 g of cells (wet weight) from *M. thermolithotrophicus* under the exclusion of oxygen in an anaerobic chamber (Coy Manufacturing Company, Ann Arbor, MI) (Thomm *et al.*, 1986). A cellular extract (S-100, 4800 mg of protein) was prepared in 40 ml of TMK buffer (50 mM Tris (pH 7.9), 10 mM $MgCl_2$, 50 mM KCl, 20% glycerol) as previously described (Frey *et al.*, 1990) and loaded onto a DEAE-cellulose column (5 × 10 cm) equilibrated with TMK buffer. After washing with three volumes, bound proteins were eluted with a 1600-ml linear gradient of KCl (50–1000 mM). Aliquots of the column fractions were analyzed for RNA polymerase activity (nonspecific transcription assay) and aTFB activity (specific transcription assay). Although aTFA activity was detected, only traces of specific transcripts were obtained without addition of external aTFA. The fractions were pooled (1613 mg of protein/125 ml), diluted with three volumes of TMK buffer, and loaded onto a Heparin-Sepharose CL-6B column (12 × 1.8 cm) equilibrated with the same buffer. Proteins were eluted with a linear gradient (200 ml/50–1000 mM KCl). RNA polymerase fractions that did not contain detectable aTFB activity were pooled and used in the complementation assay for detection of aTFB. The aTFB active fractions were pooled (23.5 mg of protein/24 ml), diluted with three volumes of TMK buffer, and applied to a Mono Q column equilibrated with TMK buffer containing 10% glycerol. Bound proteins were eluted with a linear gradient (50–1000 mM of KCl/15 ml). Fractions containing aTFB activity (12 mg of protein/2 ml) were pooled and applied to a Superdex 200 column equilibrated with a buffer containing 20 mM KPO_4 (pH 8.0), 200 mM KCl, and 10% glycerol. Aliquots of the fractions were analyzed for RNA polymerase and aTFB activities. The fractions containing aTFB were pooled (2.5 mg of protein/8 ml), diluted with three volumes of buffer (10 mM KPO_4 (pH 8.0), 10% glycerol), and then loaded onto an hydroxylapatite HPLC column equilibrated in the same buffer. Proteins were eluted with a 20-ml linear gradient (10–500 mM KPO_4). Fractions containing aTFB (1.8 mg of protein/7 ml) were diluted with three volumes of TMK buffer and applied to a single-stranded DNA-cellulose column (0.5 × 5 cm) equilibrated in the same buffer. A linear gradient (8 ml/50–1000 mM KCl) was used to elute aTFB.

Estimation of the Native Molecular Mass of aTFB—The native molecular mass of aTFB was estimated by gel filtration chromatography on a Superdex 75 column. The Superdex 200 pool was chromatographed on a Mono Q column, and aTFB activity was recovered by step elution with TMK buffer containing 0.5 M KCl. A 100-μl aliquot of the Mono Q concentrate was loaded onto a Superdex 75 column that was equilibrated with TMK buffer containing 300 mM KCl and 10% glycerol. The elution volume (V_e) of aTFB was determined using the complementation assay, and by silver staining of SDS-polyacrylamide gels. The native molecular mass of aTFB was estimated by comparing with four different standard proteins: phosphorylase *b* (97.4 kDa; V_e = 8.1 ml), bovine serum albumin (66.2 kDa; V_e = 8.9 ml), ovalbumin (45 kDa; V_e = 9.85 ml), and carbonic anhydrase (29 kDa; V_e = 11.1 ml). The void volume (V_0) was determined using blue dextran (V_0 = 7.75 ml).

The Stokes radius of aTFB was estimated by extrapolation from a curve in which a modified partition coefficient $(-\log K_{av})^1 hs^2$ for each standard protein was plotted against the corresponding Stokes radius according to the correlation of Laurent and Killander (1964). The following proteins were used for calibration: bovine serum albumin (K_{av} = 0.07), ovalbumin (K_{av} = 0.13), chymotrypsinogen A (K_{av} = 0.25), and cytochrome *c* (K_{av} = 0.31).

Glycerol Gradient Analysis—For determination of the sedimentation coefficient ($s_{20,w}$) of aTFB, 100 μl of a Superdex 200 fraction concentrated by Mono Q chromatography was loaded onto a 4.1-ml glycerol gradient (10–30%, linear) in TMK buffer containing 0.5 M KCl. After centrifugation (50,000 rpm, 20 °C, 18 h) in a Beckman SW 60 rotor, 200-μl fractions were collected from the top of the tubes. Aliquots of 10 μl were analyzed for aTFB activity by using a specific transcription assay for aTFB activity. Carbonic anhydrase (29 kDa), ovalbumin (45 kDa), bovine serum albumin (66.2 kDa), and phosphorylase *b* (97.4 kDa) were used as calibration standards in a parallel gradient. Their sedimentation positions were determined by SDS-polyacrylamide gel electrophoresis followed by silver staining.

Sucrose Gradient Analysis—The interaction of RNA polymerase with aTFB was analyzed by sucrose gradient centrifugation. The Mono Q fraction of RNA polymerase and of aTFB were combined and 400 μl of this mixture applied to a linear gradient of 10–30% sucrose and 5–10% glycerol in TMK buffer or in TMK buffer containing 0.5 M KCl. The polypeptides were sedimented for 22 h at 41,000 rpm in a Beckman SW 41 rotor at 20 °C as described previously (Thomm and Stetter, 1985).

Renaturation of aTFB Activity—A Superdex 200 pool was concentrated by Mono Q chromatography and an aliquot of 80 μl was loaded onto a preparative tube gel (10% polyacrylamide, 0.1% SDS, 75 mM Tris phosphate (pH 7.5)). Running buffer was 75 mM Tris phosphate (pH 7.5), 0.1% SDS for the upper electrode and 75 mM Tris-HCl (pH 7.5) for the lower electrode. Electrophoresis was performed with a HPEC system (model 230 A) from Applied Biosystems according to the manufacturer's instructions (conditions: 30 min at 0.3 mA, 60 min gradient from 0.3 to 1.5 mA, 400 min at 1.5 mA, 200 min gradient from 1.5 to 2.5 mA, 200 min at 2.5 mA). After electrophoresis, aliquots (5 μl) of the collected fractions (70 μl) were analyzed by SDS-polyacrylamide gel electrophoresis. The fractions containing the polypeptides (designated I, II, and III) were pooled and renatured by a modification of the procedure of Hager and Burgess (1980). First, 1 μl of bovine serum albumin (20 mg/ml) and four volumes of cold absolute acetone (−20 °C) were added, and the sample was allowed to precipitate for 1 h in a dry ice-ethanol bath. After centrifugation using a microcentrifuge, the pellet was washed twice with 80% acetone. The dried precipitate was dissolved in 4 μl of 6 M guanidinium hydrochloride, incubated at room temperature for 30 min, and then diluted 50-fold with dilution buffer (0.05 M Tris-HCl (pH 7.9), 0.1 mM EDTA, 5 mM dithiothreitol, 0.1 mg/ml bovine serum albumin, 0.15 M KCl). After dialysis against TMK buffer (Microdialyzer System 500; Pierce Chemical Co.), aTFB activity was assayed by *in vitro* transcription.

SDS-Polyacrylamide Gel Electrophoresis—Proteins were separated in 12% SDS-polyacrylamide gels as described by Laemmli (1970) and silver-stained according to the procedure of Heukeshoven and Dernick (1988).

Protein Assay—Protein concentration was determinated with a commercial kit (Bio-Rad) according to the procedure of Bradford (1976) using bovine serum albumin as standard.

RESULTS

Identification of a Second Archaeal Transcription Factor, aTFB—To investigate the possibility that a second transcription factor may exist in cells of *M. thermolithotrophicus*, RNA polymerase was purified according to the procedure outlined in Fig. 1. Nonspecific RNA polymerase activity was detected by an assay using poly(dA·dT) as template. To monitor RNA polymerase direction of specific transcription, the active fractions from each chromatographic step were combined in cell-free transcription reactions with aTFA purified by PC chromatography. Labeled RNA products transcribed from a $tRNA^{Val}$ template were analyzed by polyacrylamide urea gel electrophoresis and identified by autoradiography.

The DEAE-cellulose fraction of the RNA polymerase (Fig. 2*A*, *lane 1*) and some fractions from the heparin-Sepharose (*fractions 20–26* in Fig. 2*A*) were still able to synthesize pre-tRNA. However, some RNA polymerase fractions (*28* in Fig. 2*A*) did not direct specific transcription. As depicted in the *lower* part of Fig. 2*A*, the unspecific RNA polymerase activity of fraction 28 was considerably higher than that of some fractions (20–24), which supported accurate transcription. Hence it appears that fractions 20–26 of the heparin-Sepharose contain a component

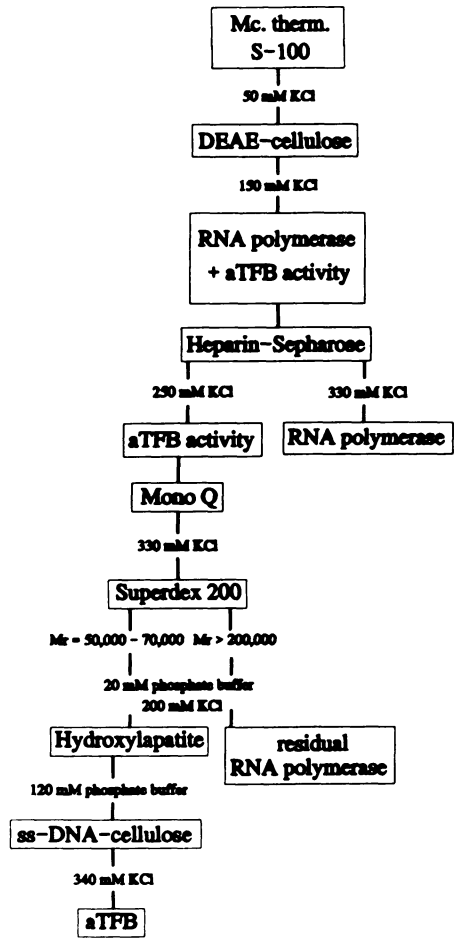


FIG. 1. **Flow chart showing the chromatographic separation of transcriptional components from *M. thermolithotrophicus*.** RNA polymerase and transcription factor aTFB were purified from 20 g (wet weight) of *M. thermolithotrophicus*. The presence of aTFB and of the enzyme in the various fractions are indicated. Column sizes, protein concentrations, and specific activities are detailed under "Experimental Procedures" and in Table I.

necessary for accurate transcription that is missing in fraction 28. To test whether this required component could be separated from the RNA polymerase and thus identified, fractions 20–26 were concentrated on a Mono Q column (see "Experimental Procedures") and applied to a Superdex 200 sizing column. The RNA polymerase eluting in fractions 28–31 from the Superdex column (Fig. 2*B*, *lower part*), was unable to synthesize distinct transcripts (Fig. 2*B*, *upper part*, *lane 2*). However, after reconstitution with fractions 36–38, the specific activity was restored (Fig. 2*B*, *upper part*). These fractions eluted at a position corresponding to a molecular weight of 50,000–70,000 and did not show detectable RNA polymerase activity (Fig. 2*B*, *lower part*). Separation of RNA polymerase and an activity required for cell-free transcription was also observed when the Mono Q fraction of RNA polymerase was purified by sedimentation through sucrose density gradients (data not shown). These data support the conclusion that a second transcription factor is required for correct transcription of this tRNA template and that the factor can be separated from RNA polymerase by sizing steps.

Purification of aTFB—The Superdex fractions containing the specific transcription factor were purified by chromatography on hydroxylapatite and single-stranded DNA-cellulose as indicated in Fig. 1. The fractions with aTFB activity (*15–18* in Fig. 3*A*) eluted from the ssDNA-cellulose column were analyzed by SDS-polyacrylamide gel electrophoresis (Fig. 3*B*). Three polypeptides were visualized by silver staining in fractions with aTFB activity. The relative intensity of one polypeptide with an apparent molecular weight of 28,000 (labeled by an *arrow* in Fig. 3*B*) paralleled the ability of these fractions to stimulate specific transcription (*cf.* Fig. 3, *A* and *B*). Hence, of the three polypeptides detectable in the DNA-cellulose fraction of aTFB, the 28-kDa protein appears to be the transcription factor.

To identify the polypeptide(s) with aTFB activity directly, the three protein bands visible in Fig. 3*B* were purified from a denaturing polyacrylamide gel using a HPEC system (see "Experimental Procedures"). The purified proteins were renatured according to the procedure of Hager and Burgess (1980) and assayed for transcription factor activity. Two of the three polypeptides present in the DNA-cellulose fraction of aTFB, designated *I* and *III* in Fig. 4, were unable to restore specific transcription. Provided that these polypeptides were renatured by this procedure, it appears that they do not have transcription factor activity. However, after addition of the renatured 28-kDa protein, designated *II* in Fig. 4, distinct RNA products were synthesized. This result demonstrates that the polypeptide of molecular weight 28,000 is a transcription factor mediating cell-free transcription of the $tRNA^{Val}$ template.

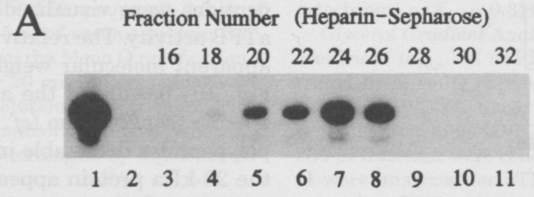
Characterization of aTFB—Gel filtration analysis on a Superdex 75 column and sedimentation analysis in a glycerol density gradient indicates a molecular weight of 54,000, a Stokes radius of 30 Å, and a sedimentation coefficient of $s_{20,w}$ = 3.9 (data not shown).

The native molecular weight of aTFB is about 2 times higher than the value derived from analysis of electrophoresis under denaturing conditions. From the discrepancy of the M_r values of denatured (28,000) and native (54,000) aTFB we conclude that native aTFB is probably a dimer of two polypeptide chains of M_r 28,000.

aTFB Is a General Transcription Factor—In this study, purified RNA polymerase supplemented with aTFA was used in combination with individual fractions of aTFB to reconstitute specific transcription from a cloned tRNA template. To investigate whether aTFB is also required for the transcription of archaeal protein-encoding genes we used various methanogen genes that had high template activities in a crude *Methanococcus* transcription system (Gohl *et al.*, 1992; Thomm *et al.*, 1992). In the transcription experiment shown in Fig. 5, linearized templates were used containing the *nifH1* promoter of *M. thermolithotrophicus* (*lanes 3* and *4*), two identical copies of the promoter of histone *hmfB* of *Methanothermus fervidus* in tandem (*lanes 5* and *6*) and, as a control, the promoter of $tRNA^{Val}$ of *M. vannielii* (*lanes 1* and *2*). When correct initiation at the corresponding promoters occurs, run-off transcripts of 75 nucleotides from *nifH1*, 154 and 81 nucleotides from *hmfB*, and 89 nucleotides from the $tRNA^{Val}$ promoter should be expected. Fig. 5 shows that none of these templates was transcribed in the absence of aTFB (*lanes 1*, *3*, and *5*, respectively). However, in the presence of the ssDNA-cellulose fraction of aTFB, distinct transcripts of correct size were synthesized with high activity. This finding demonstrated that aTFB is necessary for transcriptional activity at these promoters. From this we conclude that aTFB is a general transcription factor required for transcription from promoters for protein-encoding genes as well as tRNA genes.

DISCUSSION

We have previously reported separation of a transcription factor from the RNA polymerase of *M. thermolithotrophicus* by PC-chromatography (Frey *et al.*, 1990). In the present study, we describe the identification of a second transcription factor that coeluted with RNA polymerase during PC chromatography. Several lines of evidence indicate the existence of a second



Fraction Number (Heparin-Sepharose)	16	18	20	22	24	26	28	30	32
synthesized tRNA (fmol)	0	0.9	6.9	9.4	33.9	19.6	0.3	0	0
RNA polymerase activity (cpm x 10^3)	0	1.6	14.6	48.3	145.1	806.5	421.5	3.8	2.1

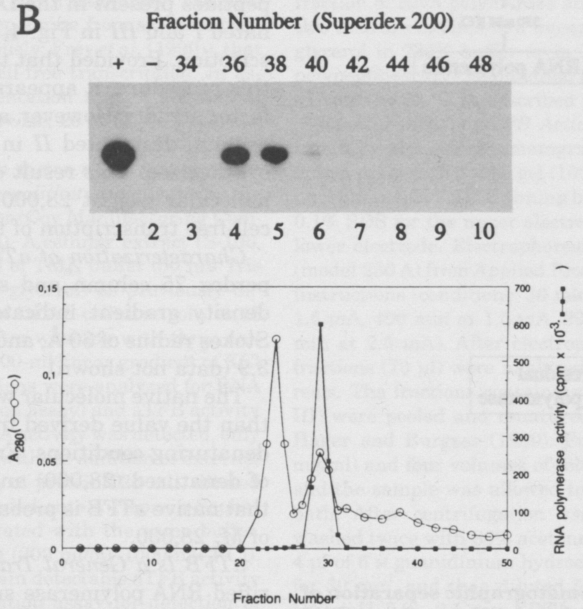


FIG. 2. **Identification of a second archaeal transcription factor.** Fractions eluted from heparin-Sepharose (*A*) and Superdex 200 (*B*) were assayed for RNA polymerase activity and for specific transcription as described under "Experimental Procedures." Assay results for selected protein fractions are shown. The autoradiograms shown in the *upper panel* of *A* and *B*, respectively, show the gel analyses of RNAs. In *panel A*, *lane 1* shows transcript synthesized by the DEAE-fraction of RNA polymerase in the presence of aTFA, and *lane 2* is a control lane not containing RNA polymerase. *Lanes 3–11*, transcription reactions contained 10 µl of phosphocellulose 1 M step eluate (aTFA), 10 µl of the pooled Superdex fraction of the RNA polymerase (fractions 28–31; see *panel B*, *lower part*) and 5 µl of the heparin-Sepharose fractions indicated above these lanes. *Lower panel*, analysis of nonspecific and specific (pre-tRNA) RNA polymerase products synthesized by 5 µl of the corresponding RNA polymerase fractions with, respectively, poly(dA·dT) and the $tRNA^{Val}$ gene as templates. The synthesized pre-tRNA (30 min at 55 °C) was quantified by liquid scintillation counting (Cerenkov radiation) of excised radioactive transcripts. A counting efficiency of 40% was used, and incorporation of 33 UMP is required for each pre-$tRNA^{Val}$ product. *B*, *upper panel*, specific transcription reactions contained the Superdex fraction (28–31) of RNA polymerase and aTFA as indicated in A. *Lanes 1* and *2* show RNA products synthesized in the presence (+) and absence (–) of pooled Mono Q fractions. In *lanes 3–10*, 20 µl of the Superdex 200 fractions indicated on top of the lanes were added. *Lower panel*, elution (A_{280}) profile of the Superdex 200 column. Shown is the UV monitor recording at 280 nm and nonspecific activity of RNA polymerase. Each fraction was 2 ml.

archaeal transcription factor. First, the RNA polymerase purified as described previously (Thomm and Stetter, 1985) was unable to synthesize distinct RNA products *in vitro* when combined with the PC fraction of aTFA (Frey *et al.*, 1990). Second, when the RNA polymerase from various steps of the purification scheme (Fig. 1) was tested for its ability to mediate specific transcription in the presence of aTFA, it was evident that some RNA polymerase fractions retained their specificity, whereas some (*e.g.* a fraction with 27-fold higher nonspecific RNA polymerase activity) had lost this potential (compare RNA products synthesized by fraction 20 and 28 and their RNA polymerase activities; Fig. 2*A*). Additionally, after Superdex 200 chromatography and centrifugation through sucrose density gradients, the RNA polymerase was incapable of specific transcription (Fig. 2*B*). This enzyme fraction regained the potential for specific RNA synthesis when low molecular weight fractions (50,000–70,000) that lacked RNA polymerase activity were added to cell-free transcription reactions.

We suggest that purified native aTFB is a homodimer (two 28-kDa subunits) on the basis of three lines of evidence. First, a polypeptide of M_r 28,000 was found in transcription factor fractions whose intensity after silver staining in denaturing gels was correlated to transcription factor activity (Fig. 3). Second, this polypeptide, isolated from the SDS gel and renatured, was able to mediate transcription by RNA polymerase in the presence of the PC fraction of aTFA (Fig. 4). These findings unequivocally demonstrate that aTFB activity is associated with this M_r 28,000 polypeptide. Third, native molecular weight of aTFB determined by gel filtration columns or glycerol gradient sedimentation in the presence of 0.3–0.5 M KCl (data not shown) is approximately 2-fold higher than that of the aTFB active polypeptide eluted from denaturing polyacrylamide gels.

Substantial purification of aTFB protein (to about 40% purity) was achieved via our purification scheme (Fig. 1). The apparent overall recovery of activity after the last purification step was low (on the order of 2%; Table I). However, after the Superdex 200 step, the recovery was still 23%, and the specific activity of aTFB increased 522-fold. At this stage of purification, the RNA polymerase was completely separated from aTFB

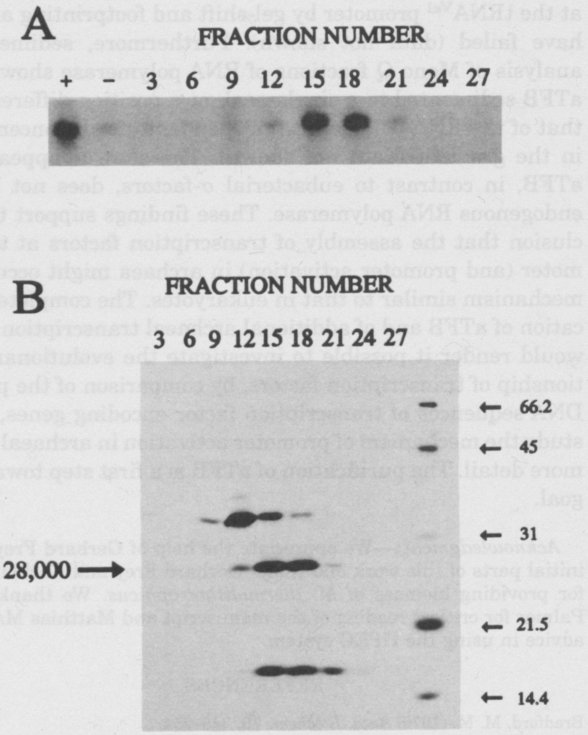


FIG. 3. **Analysis of polypeptides in a highly purified aTFB fraction.** Fractions eluted from ssDNA-cellulose (see Fig. 1) were assayed for aTFB activity (*A*) and analyzed by SDS-polyacrylamide gel electrophoresis (*B*). *Panel A*, transcription reactions contained 4-μl DNA-cellulose fractions as indicated above the lanes and RNA polymerase and aTFA as indicated in Fig. 2*A*. *Lanes 1* and *2* show RNA products synthesized in the presence (+) and absence (–) of the pooled hydroxylapatite fraction of aTFB. *Panel B*, 8 μl each of DNA-cellulose fractions were analyzed by SDS-polyacrylamide gel electrophoresis. Acrylamide concentration was 3% in the stacking gel and 12% in the separating gel. Markers at *right* indicate the molecular weight × 10^{-3} of protein standards. The *arrow* at *left* indicates the polypeptide whose intensity of silver staining correlated with aTFB activity analyzed in *panel A*.

TABLE I
Purification of transcription factor aTFB

	Protein	Units[a]	Specific activity
	mg		*units/mg protein*
S-100	4266	ND[b]	ND[b]
DEAE-cellulose	1613	5569	3.4
Heparin-Sepharose	23.5	4320	183.8
Mono Q	12	2358	196.5
Superdex 200	2.5	1306	522.4
Hydroxylapatite	1.84	800	434.8
ssDNA-cellulose	0.11	120	1091

[a] One unit of activity is defined as 1 pmol of UMP incorporated into specific pre-$tRNA^{Val}$ transcript per 30 min at 55 °C.

[b] ND, not detected. We were not able to quantitate the activity of aTFB activity in the S-100. When aliquots of this extract were assayed in cell-free transcription reactions for aTFB activity, no specific transcripts were detected. The activities of ribonucleases and unknown inhibitors most likely prevented detection.

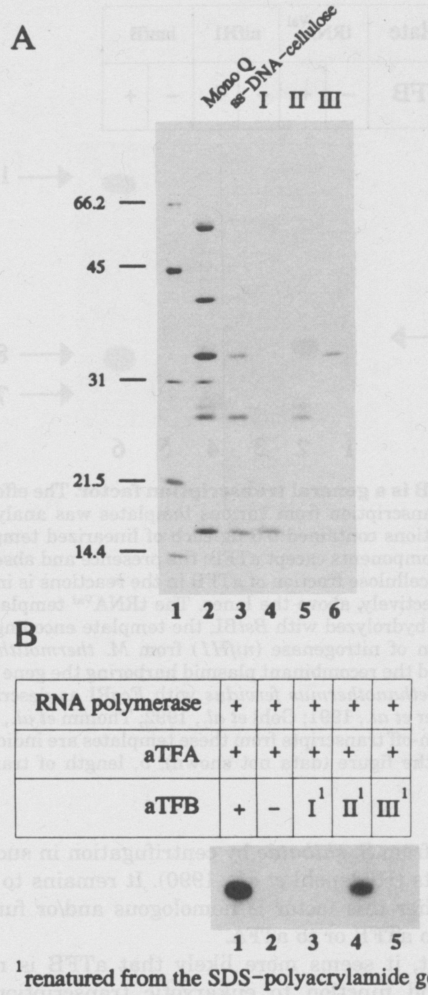


FIG. 4. **aTFB is a 28-kDa polypeptide.** 80 μl of a concentrated Superdex fraction of aTFB were purified by preparative SDS-polyacrylamide gel electrophoresis using an HPEC system (see "Experimental Procedures"). *Panel A*, SDS-polyacrylamide gel electrophoresis of marker proteins (*lane 1*), Mono Q (*lane 2*), and ssDNA-cellulose fraction of aTFB (*lane 3*) and of three low molecular weight fractions eluted from the preparative polyacrylamide gel (*lanes 4–6*), designated *I*, *II*, and *III* at the top of the lanes. *Panel B*, analysis of aTFB activity (as indicated in Fig. 2*A*) in renatured polypeptides eluted from the polyacrylamide gel. The presence (+) and absence (–) of the various transcriptional components in transcription reactions is indicated at the top of the autoradiogram. The ssDNA-cellulose fraction of aTFB (4 μl) was used for the experiment analyzed in the lane at *left*. Note that aTFB can be replaced by the preparative electrophoresis fraction (*II*) that contains the 28-kDa polypeptide.

and no activities interfering with analysis of transcripts (such as tRNA-processing enzymes, nucleases, and aTFA) were detectable in the aTFB preparation. Hence, we have developed a highly productive procedure to prepare active aTFB. The second (first reported) factor, aTFA, is sometimes contained in the RNA polymerase fractions after the DEAE-cellulose and heparin-Sepharose steps. However, this aTFA activity was low, and all attempts to purify it from the DEAE-cellulose eluate failed. The most efficient procedure to prepare this factor is PC chromatography from an S-100 preparation as described previously (Frey *et al.*, 1990). In routine experiments in our laboratory, we use the Superdex fraction of RNA polymerase and of aTFB, as well as the 1 M KCl step eluate from PC chromatography, as sources of the three components necessary to reconstitute a cell-free transcription system. Both the RNA polymerase and the aTFB used in cell-free transcription experiments have been purified nearly to homogeneity. However, the PC fraction of aTFA is still very crude and contains many components. This implies that the two factors reported thus far as archaeal transcription factors provide only a lower estimate of the number of factors actually necessary for transcription of purified archaeal templates. Further fractionation of the PC fraction of aTFA may yield additional highly specific factors. However, the factor reported here (aTFB) is strictly required for transcription of tRNA genes, as well as protein-encoding genes (Fig. 5) and is therefore an essential part of the archaeal transcriptional machinery. Zillig and co-workers recently reported on the detection of a transcription factor that was separated from the RNA

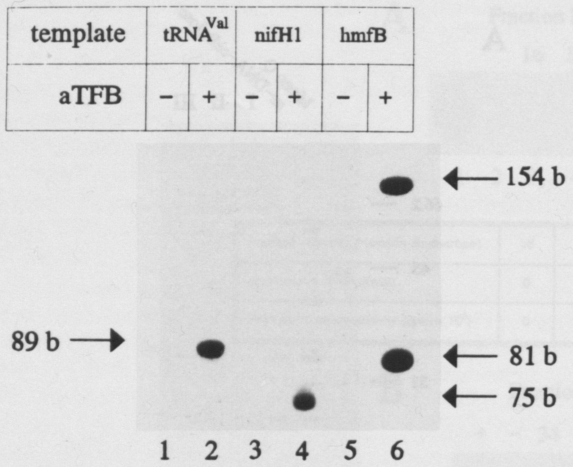


FIG. 5. **aTFB is a general transcription factor.** The effect of aTFB on specific transcription from various templates was analyzed. Transcription reactions contained 0.5 μg each of linearized templates, and all essential components except aTFB; the presence and absence of 7 μl of the ssDNA-cellulose fraction of aTFB in the reactions is indicated by + and –, respectively, above the lanes. The tRNA^{Val} template from *M. vannielii* was hydrolyzed with *Bst*BI, the template encoding the putative Fe-protein of nitrogenase (*nifH1*) from *M. thermolithotrophicus* with *Bst*NI and the recombinant plasmid harboring the gene for histone hmfB from *Methanothermus fervidus* with *Eco*RI as described previously (Hausner *et al.*, 1991; Gohl *et al.*, 1992; Thomm *et al.*, 1992). The sizes of the run-off transcripts from these templates are indicated at *left* and *right* of the figure (data not shown); *b*, length of transcripts in nucleotides.

polymerase from *S. shibatae* by centrifugation in sucrose density gradients (Hüdepohl *et al.*, 1990). It remains to be determined whether this factor is homologous and/or functionally equivalent to aTFB or to aTFA.

At present, it seems more likely that aTFB is related in structure and function to eukaryotic transcription factors. First, a TATA box is a common structural feature of both archaeal and eukaryotic class II promoters. Secondly, a gene with homology to transcription factor TFIIB was recently identified in the archaeon *Pyrococcus woesei* (Ouzounis and Sander, 1992). These similarities in transcriptional machineries suggest that a counterpart to the eukaryotic TATA box binding transcription factor TFIID (Sawadogo and Roeder, 1985; Reinberg *et al.*, 1987) may also exist in archaeal cells. On the other hand, the *Methanococcus* RNA polymerase has been shown to bind specifically to DNA fragments harboring archaeal promoters (Thomm and Wich, 1988; Brown *et al.*, 1988; Thomm *et al.*, 1989). Although aTFB binds to single-stranded (Fig. 1) and native DNA, all our attempts to detect specific binding of aTFB at the tRNA^{Val} promoter by gel-shift and footprinting analyses have failed (data not shown). Furthermore, sedimentation analysis of Mono Q fractions of RNA polymerase showed that aTFB sedimented in a single peak at a position different from that of the RNA polymerase independent of salt concentration in the gradient (data not shown). Therefore it appears that aTFB, in contrast to eubacterial σ-factors, does not bind to endogenous RNA polymerase. These findings support the conclusion that the assembly of transcription factors at the promoter (and promoter activation) in archaea might occur via a mechanism similar to that in eukaryotes. The complete purification of aTFB and of additional archaeal transcription factors would render it possible to investigate the evolutionary relationship of transcription factors, by comparison of the primary DNA sequences of transcription factor encoding genes, and to study the mechanism of promoter activation in archaeal cells in more detail. The purification of aTFB is a first step toward this goal.

Acknowledgments—We appreciate the help of Gerhard Frey during initial parts of this work and thank Gerhard Frey and Karl O. Stetter for providing biomass of *M. thermolithotrophicus*. We thank Robert Palmer for critical reading of the manuscript and Matthias Marget for advice in using the HPEC system.

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