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

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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 Mr = 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.

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 PC1 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<sup>Val</sup> gene of *M. vannielii*, was used as template in a standard *in vitro* transcription reaction (Hausner et al., 1991). Other templates used were the *nifH* gene of *M. thermolithotrophicus*, which encodes the putative dinitrogenase reductase, and the plasmid pKS304A16, 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<sup>32</sup>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 x 7.8 mm) was from Bio-Rad. All other chemicals were from Meck (Darmstadt, Federal Republic of Germany (FRG)) or Serva (Heidelberg, FRG).

**Nonspecific Transcription by RNA Polymerase**—Reaction mixtures (100 µl) contained 20 mM Tris-Cl (pH 8.0), 10 mM MgCl<sub>2</sub>, 50 mM KCl, 10 mM dNTPs, 200 µg/ml heparin, 50 µg/ml salmon sperm DNA, and 50 ng/ml isolated RNA polymerase. The reaction was started by the addition of 1 µl of transcription factor and incubated at 42°C for 12 minutes. Each reaction was stopped by the addition of 30 µl of 10% sodium dodecyl sulfate and 100 µl of 6 M urea. Samples were electrophoresed on 15% polyacrylamide gels, and the labeled RNA were detected by autoradiography.

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‡ The abbreviations used are: PC, phosphocellulose; aTFA, 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-32P]UTP (150 cpm/mol), 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 fractionation at 55 °C for 20 min, the reaction was stopped by precipitation with 1.1-ml stop solution (5.5% trichloroacetic acid, 165 mM NaCl) and filtered over glass microfiber filters (Whatman GFC). 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 reactions were monitored at 55 °C for 30 min in a buffer containing 20 mM Tris-HCl (pH 8.0), 10 mM MgCl₂, 100 mM KCl, 0.1 mM EDTA, 0.33 mM ATP, 0.33 mM CTP, 0.33 mM GTP, 2 μM [α-32P]UTP (40,000 cpm/μl), and 2 μg of closed circular plasmid pC191Z. 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 mM KCl step eluate from a phosphocelulose column (prepared as described previously; Frey et al. (1990)), that contained a second activity required for transcription. The reaction was incubated at 55 °C for 30 min and then terminated by addition of 200 μl of 6 M guanidinium hydrochloride, incubated at room temperature for 30 min and then centrifuged at 50,000 rpm, 20 °C, 18 h in a Beckman SW 60 rotor. The supernatant was loaded onto a 4.1-ml glycerol gradient (10-50%, linear) in TMK buffer containing 0.5 mM 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) served as markers. The void volume of aTFB was determined using 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 TMBK buffer containing 0.5 mM 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) served as markers. The void volume of aTFB was determined using SDS-polyacrylamide gel electrophoresis followed by silver staining.

**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 ( Hoeffer Scientific Instruments, 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 50% 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 mM Tris-HCl (pH 7.9), 0.1 mM EDTA, 0.1 mM dithiothreitol, 0.1 mg/ml bovine serum albumin, 0.15 mM 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).

**Radioassay Assay**—Protein concentration was determined 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. thermolithothrophicus*, RNA polymerase was purified according to the procedure outlined in Fig. 1. Nonspecific RNA polymerase activity was detected by an assay using polya(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 aTFB purified by PC chromatography. Labeled RNA products transcribed from a tRNAVal template were analyzed by polyacrylamide urea gel electrophoresis and identified by autoradiography.

The DEAE-cellulose fraction of the RNA polymerase (Fig. 2A, lane 1) and some fractions from the heparin-Sepharose (fractions 20–26 in Fig. 2A) were still able to synthesize pre-tRNA. However, some RNA polymerase fractions (28 in Fig. 2A) did not direct specific transcription. As depicted in the lower part of Fig. 2A, 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...
were concentrated on a Mono Q column (see "Experimental
weight) of M. thermolithotrophicus. The presence of aTFB and of the
transcriptional components from RNA
M. thermolithotrophicus,
(Fig. 2B, upper part, lane 2). Three Poly­
merase and transcription factor aTFB were purified from 20 g (wet
cell-free transcription was also observed when the Mono Q
column (Fig. 25,
4). Tb test whether this required component could be separated
from the RNA Polymerase and thus identified, fractions 20-26
fraction of RNA Polymerase was purified by Sedimentation
Separation of RNA Polymerase and an activity required for
correct transcription of this tRNA template and did not
respond to a molecular weight of 50,000-70,000 and did not
show detectable RNA polymerase activity (Fig. 2B, 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 indi­
cated in Fig. 1. The fractions with aTFB activity (15-18 in Fig.
3A) eluted from the ssDNA-cellulose column were analyzed by
SDS-polyacrylamide gel electrophoresis (Fig. 3B). Three poly­

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

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. 2B, lower part), was unable to synthesize distinct transcripts (Fig. 2B, upper part, lane 2). However, after reconsti­
butution with fractions 36-38, the specific activity was restored
(Fig. 2B, upper part). These fractions eluted at a position cor­
responding to a molecular weight of 50,000-70,000 and did not
show detectable RNA polymerase activity (Fig. 2B, 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 indi­
cated in Fig. 1. The fractions with aTFB activity (15-18 in Fig.
3A) eluted from the ssDNA-cellulose column were analyzed by
SDS-polyacrylamide gel electrophoresis (Fig. 3B). Three poly­
peptides 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. 3B) 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. 3B were purified from a
denaturing polyacrylamide gel using a HPEC system (see "Ex­
perimental 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, design­
nated I and III in Fig. 4, were unable to restore specific tran­
scription. 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 tRNAVal template.

Characterization of aTFB—Gel filtration analysis on a Super­
dex 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, pu­
rified RNA polymerase supplemented with aTFB was used in
combination with individual fractions of aTFB to reconstitute
specific transcription from a cloned tRNA template. To inves­
tigate whether aTFB is also required for the transcription of
archaeal protein-encoding genes we used various methanogen
genles that had high template activities in a crude Methanococ­
cus transcriptions (Göhl 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. ther­

nifH1, hmfB, nifD and nifK promoters occurs, run-off transcripts of 75
nucleotides from the tRNA
Val
promoter should be expected.

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templates were used containing the nifH1 promoter of M. ther­

nifH1, hmfB, nifD and nifK promoters occurs, run-off transcripts of 75
nucleotides from the tRNA
Val
promoter should be expected.
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(dAdT) and the tRNAVal 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-tRNAVal 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 (A280) 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. 2A). Additionally, after Superdex 200 chromatography and centrifugation through sucrose density gradients, the RNA polymerase was incapable of specific transcription (Fig. 2B). 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 Mr, 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 Mr, 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
Purification of an Archaeal Transcription Factor

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 DEAE-cellulose fraction 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 aTFA, 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 aTFA 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 transcription machinery. Zillig and co-workers recently reported on the detection of a transcription factor that was separated from the RNA

### Table I

<table>
<thead>
<tr>
<th>Protein</th>
<th>Specific activity</th>
<th>Units a mg</th>
<th>Units a mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-100</td>
<td>ND b</td>
<td>4266</td>
<td>ND b</td>
</tr>
<tr>
<td>DEAE-cellulose</td>
<td>1613</td>
<td>5589</td>
<td>3.4</td>
</tr>
<tr>
<td>Heparin-Sepharose</td>
<td>23.5</td>
<td>4320</td>
<td>183.8</td>
</tr>
<tr>
<td>Mono Q</td>
<td>12</td>
<td>2358</td>
<td>196.5</td>
</tr>
<tr>
<td>Superdex 200</td>
<td>2.5</td>
<td>1306</td>
<td>522.4</td>
</tr>
<tr>
<td>Hydroxylapatite</td>
<td>1.84</td>
<td>800</td>
<td>434.8</td>
</tr>
<tr>
<td>ssDNA-cellulose</td>
<td>0.11</td>
<td>120</td>
<td>1091</td>
</tr>
</tbody>
</table>

a One unit of activity is defined as 1 pmol of UMP incorporated into specific pre-rRNA 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. 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 tRNAVal template from M. vannieli was hydrolyzed with BstBI, the template encoding the putative Fe-protein of nitrogenase (nifH1) from M. thermolithotrophicus with BstNI and the recombinant plasmid harboring the gene for histone hmfB from Methanothermus fervidus with EcoRI as described previously (Hausner et al., 1991; Gohi 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 tRNAVal 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.

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