The archaeal TFIIIE α homologue facilitates transcription initiation by enhancing TATA-box recognition

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Transcription from many archaeal promoters can be reconstituted *in vitro* using recombinant TATA-box binding protein (TBP) and transcription factor B (TFB)—homologues of eukaryal TBP and TFIIB—together with purified RNA polymerase (RNAP). However, all archaeal genomes sequenced to date reveal the presence of TFE, a homologue of the α -subunit of the eukaryal general transcription factor, TFIIIE. We show that, while TFE is not absolutely required for transcription in the reconstituted *in vitro* system, it nonetheless plays a stimulatory role on some promoters and under certain conditions. Mutagenesis of the TATA box or reduction of TBP concentration in transcription reactions sensitizes a promoter to TFE addition. Conversely, saturating reactions with TBP de-sensitizes promoters to TFE. These results suggest that TFE facilitates or stabilizes interactions between TBP and the TATA box.

INTRODUCTION

The archaeal basal transcription machinery is fundamentally related to the eukaryal RNA polymerase (RNAP) II machinery (Orphanides *et al.*, 1996; Reeve *et al.*, 1997; Bell and Jackson, 1998a). A range of *in vitro* transcription assays have demonstrated that a variety of archaeal promoters of varying strengths can be accurately transcribed in reactions containing archaeal TATA-box binding protein (TBP), transcription factor B (TFB) and RNAP (Hethke *et al.*, 1996, 1999; Qureshi *et al.*, 1997; Bell *et al.*, 1998; Darcy *et al.*, 1999; Bell and Jackson, 2000). However, it has become apparent that all archaeal genomes sequenced to date encode a homologue of a third eukaryal general transcription factor, TFIIIE (Bell and Jackson, 1998b; Aravind and Koonin, 1999; Kyripides and Ouzounis, 1999). The archaeal TFIIIE homo-

logue, TFE, is related to the N-terminal 20 kDa of the α -subunit of TFIIIE (Figure 1A). Extensive analyses of eukaryal TFIIIE function have been performed *in vivo* and *in vitro* (Ohkuma *et al.*, 1995; Kuldell and Buratowski, 1997). In particular, deletion analyses have revealed that the C-terminal portion of TFIIIE α is dispensable for viability in yeast but that the N-terminal portion is essential *in vivo* (Kuldell and Buratowski, 1997). Remarkably, the smallest truncated version of TFIIIE α , which nonetheless permits yeast growth, corresponds closely to full-length archaeal TFE (Figure 1A). In addition, similar deletion analyses that examined TFIIIE function in reconstituted *in vitro* transcription assays revealed an analogous requirement for the N-terminal portion of human TFIIIE α for basal and activated transcription (Ohkuma *et al.*, 1995). Thus, it appears that archaeal TFE corresponds to the minimal essential region of eukaryal TFIIIE α (see Figure 1A). Although the nature of the essential function remains undetermined, it has been demonstrated that this region of TFIIIE α can interact with TBP and RNAP II (Maxon *et al.*, 1994; Yokomori *et al.*, 1998). In archaea, the TFE open reading frame (ORF) contains an N-terminal, weakly conserved, helix–turn–helix motif within a leucine-rich region and a C-terminal zinc ribbon (Figure 1B). Intriguingly, while some archaeal TFEs possess putative zinc ribbons with four cysteine residues coordinating the metal ion, those of *Sulfolobus solfataricus* and *Archaeoglobus fulgidus* have the second cysteine substituted by aspartic acid or methionine, respectively. While these maintain the potential to coordinate a metal ion via polar side chains, the TFEs of *Pyrococcus* and *Methanobacterium thermoautotrophicum* possess proline or glycine in the analogous position (Figure 1A). Thus, it is possible that not all archaeal TFEs possess a coordinated metal ion.

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10 μ g of *S. solfataricus* whole-cell extract, prior to incubation with Ni-NTA-agarose. Beads were washed extensively and bound protein eluted by boiling in SDS-PAGE loading buffer. Following electrophoresis, eluted proteins were detected by western blotting using antisera generated against TBP, TFB or the B-subunit of RNAP (Qureshi *et al.*, 1997). The interaction assays were carried out in the presence of 50 μ g/ml ethidium bromide to exclude the possibility that interactions were bridged by DNA rather than being direct (Lai and Herr, 1992). The results of these assays show that TFE can interact with TBP and RNAP, but not TFB in whole-cell extracts (Figure 2A). The assays were repeated using purified RNAP and purified recombinant TBP in place of extract (Figure 2B). These assays confirmed the results of the pull-downs from extract, indicating that TFE can interact directly with TBP and RNAP in the absence of DNA.

Archaeal TFE interacts with RNAP and TBP

TFE stimulates transcription on some promoters *in vitro*

To determine whether TFE plays a role in transcription, reconstituted transcription assays, using highly purified *Sulfolobus* RNAP and purified recombinant TBP and TFB, were performed on six different promoters, either with no added TFE or supple-

Fig. 2. TFE interacts with RNAP and TBP. **(A)** Ni-NTA pull-down assays were performed with 10 μ g of *S. solfataricus* extract, as detailed in Methods, in the presence (TFE beads) or absence (control beads) of 100 ng recombinant TFE. The proteins pulled-down in the assay were detected by western blotting with antisera raised against TBP, TFB or RNAP B-subunit (Qureshi *et al.*, 1997). Lanes contain 5% of input (In), unbound material (Un), wash (W) or material eluted from the beads (El). **(B)** Assays performed as above but with purified TBP and RNAP in place of extract.

largely unaffected by TFE. However, the yield of transcript from three other promoters (*ArgC*, *5S* and *16S*) is stimulated 2- to 3-fold by the addition of TFE. Examination of the sequence of the core elements of these three TFE-responsive promoters reveals a common deviation from the TATA-box consensus in having a G at position 8 of the template strand of the TATA box, or the dyad-related C at position 1 of the element (Figure 3B). Previous work with human TFII α has revealed that the α -subunit of TFII α can stimulate the binding of TBP to the TATA element (Yokomori *et al.*, 1998). We reasoned, therefore, that some non-consensus TATA-elements may confer sensitivity to TFE in our experiments. We note, however, that the *Sdt* promoter, which appears to be unaffected by TFE addition, also deviates from the TATA consensus. To test our hypothesis we made a series of guanine substitutions at positions 5, 6, 7 and 8 of the TATA box of the *T6* promoter, and tested the effect of TFE on transcription of these promoter derivatives (Figure 4A and B). Somewhat surprisingly, substitution of guanine for adenine at positions 6 or 8 had only a small effect on transcription compared to wild-type *T6* promoter. Furthermore, these promoters were largely insensitive to the addition of TFE. In contrast, guanine substitution at position 7 and 5 resulted in a significant reduction of transcription relative to wild type. Importantly, these promoters were now stimulated by the addition of TFE to transcription reactions. This suggests that the relative affinity of TBP for the TATA box may impart sensitivity to TFE addition to a promoter. In light of the above, we reasoned that by altering TBP concentration in transcription assays, promoters may become either TFE-sensitive (under limiting TBP concentrations) or TFE-insensitive (under saturating TBP concentrations). To test this idea, we varied TBP concentration in the transcription assays programmed with *5S* or *T6* promoters (Figure 4C and D). As demonstrated above, when the *5S* promoter is transcribed in reactions containing 20 ng of TBP, the addition of TFE stimulates transcription ~2-fold. However, as increasing TBP is added to a maximum of 180 ng the stimulatory effect is no longer detected (Figure 4C). Similarly, we varied TBP levels in transcription reactions programmed by the *T6* promoter. The promoter is TFE-independent at 20 ng of TBP; however, at lower TBP levels the reactions are now stimulated by TFE (Figure 4D). In contrast, experiments in which TFB concentration was varied and TBP levels kept constant at a saturating level showed no significant differences in the presence or absence of TFE (Figure 4E). Together with the TATA-box substitution experiments in Figure 4, these data support the hypothesis that TFE can stimulate transcription under conditions where there are sub-optimal interactions between TBP and the TATA box.

Fig. 3. TFE stimulates transcription from some promoters. **(A)** *In vitro* transcriptions were carried out with the indicated promoters in reactions containing 20 ng TBP, 25 ng TFB and 200 ng RNAP for 10 min at 65°C. RNA products were detected by primer extension. **(B)** Sequence of TATA box and BRE of the promoters used in (A).

TFE stimulates early stages in transcription initiation

We next sought to determine whether TFE stimulates early stages in promoter recognition by performing DNase I footprinting of TBP–TFB–DNA complex formation on the *T6* promoter at a range of TBP concentrations, in the presence or absence of TFE. As seen in Figure 5, TFE modestly enhances protection of the TATA-box region of the promoter; specifically, protection is observed at a 2-fold lower TBP concentration in the presence of TFE. This level of stimulation is in agreement with the level of stimulation of transcription seen on the *T6* promoter under limiting TBP conditions (Figure 4C).

mented with 50 ng of TFE. As these assays contained RNAP purified from cells, western blotting was performed using anti-TFE antisera to confirm that the RNAP preparation was essentially free of endogenous TFE (see Supplementary data available at *EMBO reports* Online). As seen in Figure 3A, under the reaction conditions employed, three promoters (*T6*, *Lrs14* and *Sdt*) are

Fig. 4. TFE stimulates transcription under sub-optimal TBP-TATA-box interactions. (A) The sequence of wild-type and mutant *T6* promoters is shown. The TATA box and BRE are boxed and the positions of sequence substitutions shown in bold and underlined. (B) The products of *in vitro* transcription of the promoters shown in (A) were detected by primer extension analysis and electrophoresed on a 8% denaturing polyacrylamide gel. (C) *In vitro* transcription reactions were performed on the 5S promoter, either with no TFE or supplemented with 50 ng of TFE as indicated. The reactions contained 25 ng TFB, 200 ng of RNAP and varying amounts of TBP as indicated. (D) *In vitro* transcription reactions were performed on the *T6* promoter, either with no TFE or supplemented with 50 ng of TFE as indicated. Reaction conditions were as (C). (E) *In vitro* transcription assays performed on the *T6* promoter. Reactions contained 20 ng TBP, 200 ng RNAP and either 40, 20, 10, 5 or 2.5 ng TFB.

DISCUSSION

We find that TFE maximally exerts a 2- to 3-fold stimulatory effect on transcription, dependent on the promoter tested and

Fig. 5. TFE stimulates TBP-TFB-DNA complex formation. DNase I footprinting analysis was performed on the *T6* promoter in the presence or absence of 25 ng TFE and 20 ng TFBc (TFB core domain) as indicated. TBP was present at 20, 15, 10 or 5 ng per reaction (lanes 4–7 and 8–11).

reaction conditions. There appears to be a general correlation between detection of TFE-mediated stimulation and conditions where TBP-TATA-box interactions are sub-optimal. In agreement with this, we find that TFE interacts with TBP and that TFE appears to facilitate or stabilize recognition of the basal promoter elements by the basal machinery. How might this stimulation be mediated? One possibility is that TFE binds a DNA element in addition to TBP itself, and thereby facilitates cooperative binding of TBP and itself to DNA. However, extensive cross-linking analyses have been performed with the eukaryal basal transcription machinery, and there is no evidence for direct interactions between TFIIE α and promoter DNA in the vicinity of the TATA box (Kim *et al.*, 2000). Consistent with this, we observe that there is no detectable change in the TBP/TFB footprint observed in the presence or absence of TFE (Figure 5). A second possibility is that TFE induces a conformational change in TBP which facilitates DNA binding. This too seems unlikely, as the crystal structures of TBP in solution and bound to DNA have been determined and there is very little structural difference between the two forms of the protein (DeDecker *et al.*, 1996; Kosa *et al.*, 1997). A related possibility is suggested by the observation that TBP from eukarya and archaea exists as a dimer in solution. It has been proposed that dimerization of TBP prevents TBP-DNA interactions. It is possible that the function of TFE may prevent dimerization of TBP and so favour TATA-box recognition, indeed such behaviour has recently been described for eukaryal TFIIA (Coleman *et al.*, 1999).

The effect that we observe with TFE is relatively weak: while withholding TBP or TFB from transcription assays essentially abolishes transcription on most promoters (Qureshi *et al.*, 1997), the omission of TFE exerts a small quantitative effect *in vitro*. It is conceivable that a range of archaeal promoters exist that are more TFE dependent; based on our findings, we would propose

that such promoters would contain TATA boxes that correspond poorly to the consensus sequence. In addition it is possible that *in vivo*, under physiological TBP concentrations, TFE plays a more significant effect. Additionally, in archaeal cells, DNA is compacted by interaction with a range of small basic proteins, including direct homologues of eukaryal histones in some species (Reeve *et al.*, 1997). Some of these proteins have been observed to repress transcription *in vitro* (Soares *et al.*, 1998). It is possible that some of this repression results from competition for access to the TATA box between TBP and these non-specific DNA-binding proteins. If so, TFE could stimulate transcription *in vivo* by facilitating interaction between TBP and the TATA box. Experiments to reconstitute archaeal chromatin *in vitro* are underway to address this and other related issues.

METHODS

Cloning and purification of *S. solfataricus* P2 TFE. The ORF of TFE was identified by BLAST search of the publicly available *S. solfataricus* genome sequence (www.niji.imb.nrc.ca/sulfolobus) using the sequence of *A. fulgidus* TFE as query (Klenk *et al.*, 1997). The ORF was amplified by PCR using primers TFE5 (5'-GGGGATCC-CATATGGTTAACGCAGAGGATCGTTT-3') and TFE3 (5'-GCCC-TGACTCGAGATGATTTTATTAGCTCCAAG-3'). The PCR product was digested with *Nde*I and *Xho*I, and ligated to *Nde*I-*Xho*I digested pET30a (Novagen). The resultant expression plasmid pET-ssTFE was transformed into BLR RIL and expression of His₆-tagged TFE was induced during logarithmic growth of cells by the addition of isopropyl- β -D-thiogalactopyranoside (IPTG) to 1 mM for 3 h. Cells were pelleted and resuspended in N300 (50 mM Tris pH 8.0, 300 mM NaCl, 10 mM β -mercaptoethanol), lysed by sonication and clarified by centrifugation. The supernatant was heated to 70°C for 30 min and re-centrifuged. The resultant supernatant had imidazole added to 20 mM and was then applied to a column containing Ni-NTA-agarose matrix (Qiagen). The matrix was washed with 10 column volumes of N300 + 45 mM imidazole, followed by elution by N300 + 500 mM imidazole. One millilitre fractions were collected and the presence of TFE determined by SDS-PAGE and Coomassie blue staining. Positive fractions were pooled and dialysed extensively against N300 + 10% glycerol + 10 μ M ZnSO₄. Polyclonal antisera were generated in a rabbit against TFE (three consecutive injections of 100 μ g TFE at three-weekly intervals). Serum was collected and tested for the presence of anti-TFE antibodies using standard methodologies (Lane and Harlow, 1988).

Purification of TBP, TFB and RNAP. These proteins were purified as described previously (Qureshi *et al.*, 1997; Bell and Jackson, 2000).

Transcription assays, EMSA and DNase I footprinting. These were performed as described previously (Bell *et al.*, 1999) using TBP, TFB, TFBc and RNAP purified as described (Bell and Jackson, 2000).

Protein-protein interaction studies. These were performed in a volume of 500 μ l of BB [90 mM KCl, 20 mM Tris pH 8.0, 10 % glycerol, 10 mM β -mercaptoethanol, 50 μ g/ml ethidium bromide, 0.1% Triton X-100, 20 mM imidazole, 10 μ g/ml bovine serum albumin (BSA)]. The reactions contained 100 ng purified TFE and either 10 μ g *S. solfataricus* whole-cell extract, 1 μ g of purified TBP or RNAP, as indicated in Figure 2. Reactions were shaken at room temperature together with 20 μ l of a 50% slurry of Ni-NTA-

agarose in BB. Beads were collected by centrifugation and washed 5 times with 1 ml of BB prior to resuspension in 40 μ l 1 $\times$ SDS-PAGE loading buffer and boiling. Eluted protein was subjected to SDS-PAGE and detected by western blotting as described previously.

Supplementary data. Supplementary data are available at *EMBO reports* Online.

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