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TFE, an Archaeal Transcription Factor in *Methanobacterium thermoautotrophicum* Related to Eucaryal Transcription Factor TFIIEα

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In the archaeon *Methanobacterium thermoautotrophicum*, MTH1669 encodes a protein with a sequence related to the N-terminal sequences of the α-subunits of eucaryal general transcription factor TFIIE. The recombinant MTH1669 gene product has been purified and shown to stimulate transcription in vitro from *M. thermoautotrophicum* promoters that were almost inactive or much less active in reaction mixtures that contained only *M. thermoautotrophicum* RNA polymerase, TATA-binding protein and transcription factor B. As all complete archaeal genome sequences contain an MTH1669 homolog, the protein encoded by this gene is apparently the first characterized example of a transcription activator, here designated TFE, that may be universally present in the *Archaea*.

Transcription initiation in *Archaea* appears to be a simplified version of the eucaryal RNA polymerase II (RNAPII) system, (16, 21, 29, 36). Archaeal RNAPs contain ~12 different subunits, each of which is homologous to a subunit in eucaryal RNAPII, and archaeal promoters contain a TATA-box element (7, 15, 16, 27, 36). In vitro transcription systems have been established from several different *Archaea* that contain template DNA, RNAP, and just two general transcription factors, archaeal homologs of eucaryal TATA-binding protein and transcription factor TFIIB, designated TBP and TFB, respectively (5, 7, 9, 10, 26). Consistent with this, all fully sequenced archael genomes encode an RNAP, TBP, and TFB proteins but no clear homologs of the eucaryal general transcription factors TFIIA, TFIIF, and TFIIH or of the TBP-associated factors (2, 15, 36). They do, however, all encode a protein with a sequence related to the N-terminal sequences of the α-subunits of eucaryal TFIIEs (Fig. 1), and these include the residues that form a zinc finger motif that is essential for TFIIE function (18). Eucaryal TFIIEs contain a second, unrelated β-subunit (12), but archaeal genomes do not appear to encode a homolog of this subunit nor proteins related to the C-terminal region of the α-subunits of eucaryal TFIIEs (2, 15, 36). The C-terminal region of the α-subunit of human TFIIE is not essential for basal or activated transcription in vitro (23) and can be deleted from the α-subunit of yeast TFIIE without loss of viability (14, 32). It is required for interactions with TFIIH that result in phosphorylation of the C-terminal domain of eucaryal RNAPII (17, 18, 23, 24), but *Archaea* apparently do not have a TFIIH homolog, and archaeal RNAPs do not have a phosphorylated C-terminal domain (2, 15, 16, 36).

Recently, to further investigate the regulation of methane gene transcription documented in vivo in *Methanobacterium thermoautotrophicum* (19, 28), we established an in vitro transcription system from this archaeon. Consistent with other archaeal in vitro transcription systems, this contained native *M. thermoautotrophicum* RNAP and recombinant versions of *M. thermoautotrophicum* TBP and TFB (7). Transcription initiated accurately and abundantly from the promoter of the *M. thermoautotrophicum* archaeal histone-encoding *hmtB* gene (37), but little or no transcription was observed with DNA templates that carried methane gene promoters (7). These promoters are very active in vivo (19, 28), and therefore we concluded that the in vitro system must lack an additional transcription factor(s). An obvious candidate was the protein related to the α-subunit of TFIIE, encoded by MTH1669 in *M. thermoautotrophicum* (2, 15, 35), and here we report the results of experiments that confirm that this protein, hereafter designated TFE, stimulates transcription in vitro from some but not all methane gene promoters.

MTH1669 cloning, mutagenesis, expression, and purification of recombinant TFE and TFE (C155A). MTH1669 was PCR amplified from *M. thermoautotrophicum* ΔH genomic DNA using a primer with the sequence 5′-CGAAGGTACCG TTGATTGATGAACAGGTGTTAC to add a *Kpn*I site 1 bp 5′ to the TTG translation initiation codon and a primer with the sequence 5′-CACTAAGCTTCTAGGAGTTTATTGTTG TGG to add a *Hind*III site 45 bp 3′ to the TAG termination codon (35). The PCR product (583 bp) was digested with *Kpn*I and *Hin*dIII and ligated with *Kpn*I- plus *Hin*dIII-digested pTrcHisC (Invitrogen Corp., San Diego, Calif.), generating pTrc1669-2, which encodes TFE with an N-terminal His_6 extension. Substituting alanine for cysteine at position 154 in the α-subunit of human TFIIE resulted in a C154A variant that no longer bound zinc and lost the ability to activate basal-level transcription in vitro (18). Megaprimer site-directed mutagenesis (34) was used to obtain the structurally homologous TFE(C155A) variant (Fig. 1), using the primers listed above for the PCR amplification and 5′-TTGAACCACATTCAGG GGCTGTGAAGTTTTC as the mutagenic primer. The underlined bases mismatch the wild-type codon 155 sequence. The mutated PCR product was similarly digested with *Hin*dIII and *Kpn*I and ligated with pTrcHisC to generate pTFEC155A,

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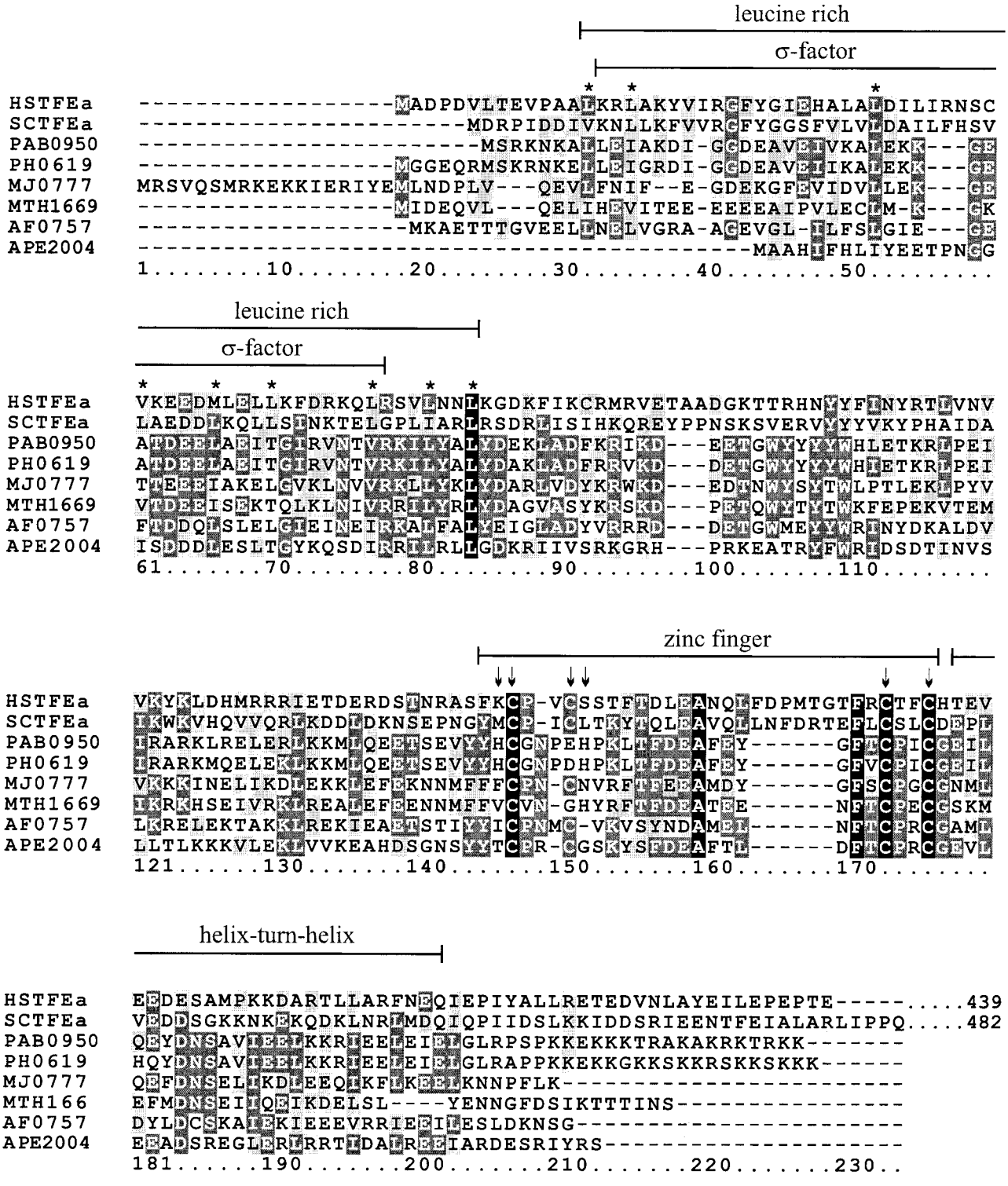

FIG. 1. Alignment of archaeal sequences with the N-terminal sequences of the α-subunits of yeast and human TFIIE. The *M. thermoautotrophicum* ΔH (MTH1669), *Pyrococcus horikoshii* (PH0619), *Pyrococcus abysii* (PAB0950), *Methanococcus jannaschii* (MJ0777), *Archaeoglobus fulgidus* (AF0757), and *Aeropyrum pernix* (APE2004) archaeal sequences are identified by their genome reading frame reference numbers (2, 15), and the yeast and human sequences are identified by SCTFEa (GenBank accession no. 607957) and HSTFEa (GenBank accession no. 5031726), respectively. Only the N-terminal 210 residues of the yeast (482 residues in total) and human (439 residues in total) proteins are shown. The alignment was generated by PILEUP (Genetic Computer Group, Inc.). Residues present in all eight sequences are identified by a black background, identical residues are identified by a dark gray background, and similar residues are identified by a light gray background. Brackets above the sequences indicate the leucine-rich region with similarity to bacterial σ factors (25), the zinc finger motif (18), and the helix-turn-helix region in human TFIIE. Conserved hydrophobic positions within the leucine-rich region are identified by asterisks (*), and arrows (↓) identify cysteine and histidine residues that could ligate the zinc atom in the proposed zinc finger domains.

which encodes His_6-TFE(C155A). MTH1669 transcripts contain 8 AGA/G and 11 AUA codons (35), and therefore pR1952, which encodes $tRNA^{AUA}$ and $tRNA^{AGG/A}$ (8), was transformed into *Escherichia coli* Top10 (Invitrogen Corp.), together with pTrc1669-2 or pTFEC155A, to increase the availability of these otherwise rare tRNAs in *E. coli*. Synthesis of His_6-TFE and His_6-TFE(C155A) was induced by addition of isopropyl-D-thiogalactoside (1 mM final concentration) to exponentially growing cultures of *E. coli*(pRI952, pTrc1669-2) and *E. coli*(pRI952, pTFEC155A). After 5 h of incubation at 37°C in SOB medium (33) that contained 200 μg of ampicillin and 30 μg of chloramphenicol per ml, the cells were lysed and the His_6-tagged proteins were purified by Ni^{2+} affinity chromatography as described previously for the purification of His_6-tagged recombinant TBP and TFB (7). Based on Coomassie blue staining after sodium dodecyl sulfate-polyacrylamide gel electrophoresis, both TFE and the TFE(C155A) variant were purified to >95% homogeneity.

In vitro transcription and templates. In vitro transcription reaction mixtures (100 μl) contained 200 ng of linear template DNA, 20 mM Tris-HCl (pH 8), 120 mM KCl, 10 mM $MgCl_2$, 2 mM dithiothreitol, 30 μM ATP, 30 μM CTP, 30 μM GTP, 2 μM UTP, 2.2 μCi of [α-^{32}P]UTP (3 kCi/mmol; ICN Pharmaceuticals Inc., Costa Mesa, Calif.), 550 ng of native *M. thermoautotrophicum* RNAP, 100 ng of recombinant TBP, and 600 ng of recombinant TFB (7). The templates used were generated by PCR amplifications directly from *M. thermoautotrophicum* ΔH genomic DNA (35) and carried either the *hmtB* promoter (37), the promoter for *rpoN* that encodes a subunit of *M. thermoautotrophicum* RNAP, or a methane gene promoter. In methanogenesis, *M. thermoautotrophicum* uses H_2 to reduce CO_2 to CH_4 (38), and templates carrying the methane gene promoters for the transcriptional units that encode the uptake hydrogenases (*frhADGB* and *mvhDGAB*), enzymes that catalyze the reductive steps between CO_2 and CH_4 (*fmdECB, fwdHFGDACB, ftr, mch, mtd, hmdI, hmdII, hmdIII, mer, mtrEDCBAFGH, mrtBDGA*, and *mcrBDCGA*), and the subunits of the heterodisulfide cofactor reductase (*hdrA* and *hdrCB*) were generated by PCR amplification (28). The primers used (sequences are available on request) resulted in DNA templates that extended 50 to 200 bp upstream from known and predicted sites of transcription initiation. Transcription reaction mixtures were incubated for 30 min at 58°C, unless otherwise noted, and the transcripts generated were purified by phenol-chloroform extraction, separated by polyacrylamide gel electrophoresis under denaturing conditions, and visualized by autoradiography as described previously (7). The amount of transcript synthesized was determined by ^{32}P-decay measurements made directly from the gel using an InstantImager 2024 (Packard Instruments, Meriden, Conn.).

TFE stimulates *frh* but not *hmtB* transcription in vitro. As previously documented (7), transcription from the *hmtB* promoter was abundant in reaction mixtures that contained *M. thermoautotrophicum* RNAP, TBP, and TFB, whereas very little transcription occurred in identical reaction mixtures provided with template DNAs that carried the *frh* promoter (1). However, addition of TFE increased the amounts of *frh* transcript synthesized 2- to 2.5-fold but did not further increase *hmtB* transcript synthesis (Fig. 2A). It seemed possible that TFE might substitute for TBP or TFB in *frh* transcription, but *frh* transcription, with or without TFE, was fully dependent on the presence of both TBP and TFB (B. L. Hanzelka, unpublished data).

TFE activity is dependent on an intact zinc finger motif. Substituting alanine for cysteine at position 154 (Fig. 1) generated a variant of the α-subunit of human TFIIE that no longer bound zinc and lost the ability to activate basal-level transcription in vitro (18). Consistent with conservation of a structurally homologous and essential zinc finger in TFE, the TFE(C155A) variant did not increase transcription in vitro from the *frh* promoter or from any other TFE-sensitive methane gene promoter (Fig. 2B).

TFE stimulates transcription from some but not all methane gene promoters. As observed for the *frh* promoter, very little transcription occurred in reaction mixtures that contained *M. thermoautotrophicum* RNAP, TBP, and TFB and templates that carried either the *fmd, fwd, mvh, ftr, mch, mrt, mtd, hmdI, hmdII, hmdIII, mer, mtr, mcr, hdrA*, or *hdrC* methane gene promoters (28). TFE addition resulted in 1.8- to 3.5-fold increases in transcript accumulation in reaction mixtures supplied with templates that carried the *fmd, fwd, ftr, mcr, hdrA*, and *hdrC* promoters (Fig. 2B) but had no stimulatory effect on transcription in reaction mixtures supplied with the other methane gene promoters (data not shown). In every case, the increase in transcript synthesis observed when TFE was added was not observed when the TFE(C155A) variant was added (Fig. 2B). Comparison of the different templates did not reveal any correlation between the presence or absence of a sequence element and sensitivity or insensitivity to TFE addition.

Transcription from templates carrying the *rpoN* promoter, as a second nonmethane promoter, was also investigated. As with the *hmtB* promoter, transcription was abundant in the absence of TFE, and addition of TFE did not increase transcription from the *rpoN* promoter (Fig. 2B).

Kinetics of *frh* transcript accumulation. After a lag of ~5 min, *frh* transcripts accumulated continuously for up to 30 min at 58°C in reaction mixtures that contained or lacked TFE, but the rate of transcript accumulation was ~2.3-fold higher in the presence of TFE (Fig. 3). All components of the *M. thermoautotrophicum* in vitro transcription system therefore remained active for at least 30 min at 58°C under aerobic conditions. Transcription was started by adding the DNA template, and the initial lag in transcript accumulation therefore most likely reflected the time needed to assemble the first productive transcription initiation complexes.

Conclusions. The protein encoded by MTH1669 has sequences, and apparently a zinc finger motif, in common with the α-subunits of eucaryal general transcription factor TFIIE (Fig. 1). This protein has been shown to stimulate transcription in vitro from seven different methane gene promoters and one fortuitously detected unknown promoter (Fig. 3) and has been designated TFE. As MTH1669 homologs are present in all archaeal genome sequences, which include several methanogenic and nonmethanogenic *Euryarchaeota* and nonmethanogenic *Crenarchaeota* (2, 15, 39), it seems unlikely that TFE activity is limited to methanogenesis-related gene expression. However, addition of purified His-tagged AF0757 gene product, the TFE from *Archaeoglobus fulgidus* (Fig. 1), to the *M. thermoautotrophicum*-derived in vitro transcription system did not stimulate transcription from the *frh* and *mcr* promoters (B. L. Hanzelka, unpublished data). Therefore, although all

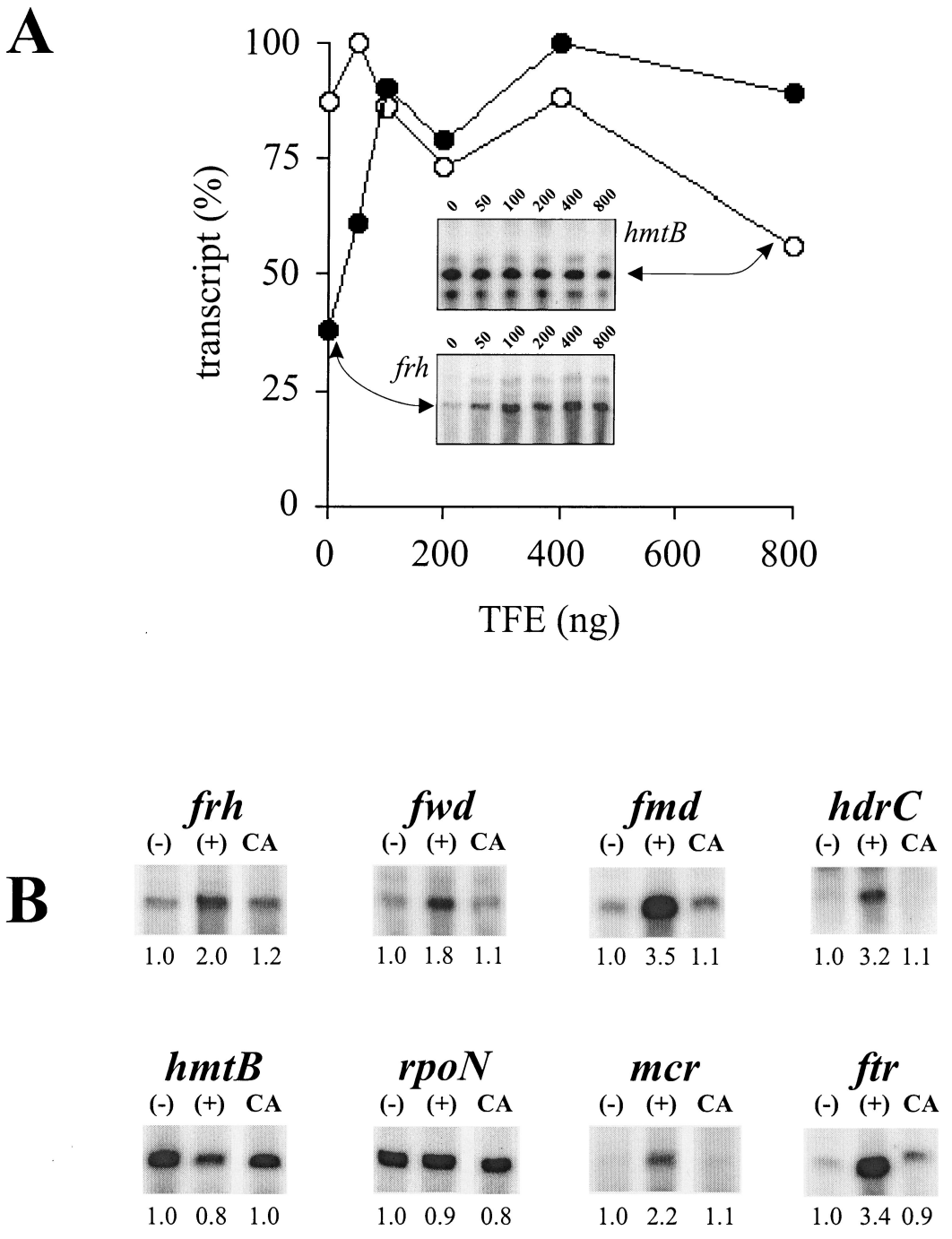

FIG. 2. Effects of TFE and TFE(C155A) addition on in vitro transcription. (A). Amounts of *frh* and *hmtB* transcripts synthesized after 30 min of incubation at 58°C in the presence of increasing amounts of TFE (listed in nanograms above the corresponding lanes in the gels shown), determined by ^{32}P-decay, plotted as percentages of the maximum amount of that transcript synthesized. (B) Run off transcripts synthesized from the promoter listed above each gel (28, 29) in reaction mixtures with no addition (−) or supplemented with 200 ng of TFE (+) or with 200 ng of TFE(C155A) (CA). The amount of each transcript synthesized was determined by ^{32}P-decay measurements, and the length of each transcript (130 to 260 nucleotides) was confirmed, based on the location of the corresponding TATA box, by adjacent coelectrophoresis of ^{32}P-labeled size standards (Boehringer-Mannheim, Indianapolis, Id.). Below the lanes are mean values, from three to seven independent experiments, of the amount of transcript synthesized in the presence of TFE (+) or TFE(C155A) (CA) relative to the amount synthesized with no addition (−).

TFEs may function similarly, TFEs from different *Archaea* may not be readily interchangeable.

In view of the apparent lack of activity with some promoters, it is not yet clear whether TFE should be considered a general transcription factor. Based on homology to TFIIE, it seems likely that TFE will play a role in promoter melting and/or promoter clearance (12, 14, 17, 18, 23, 24, 32), and the precise step at which TFE stimulates *frh* transcription is currently under investigation. Eucaryal TFIIEs also enhance the activity of promoter-specific transcription factors (14, 22, 30–32), and

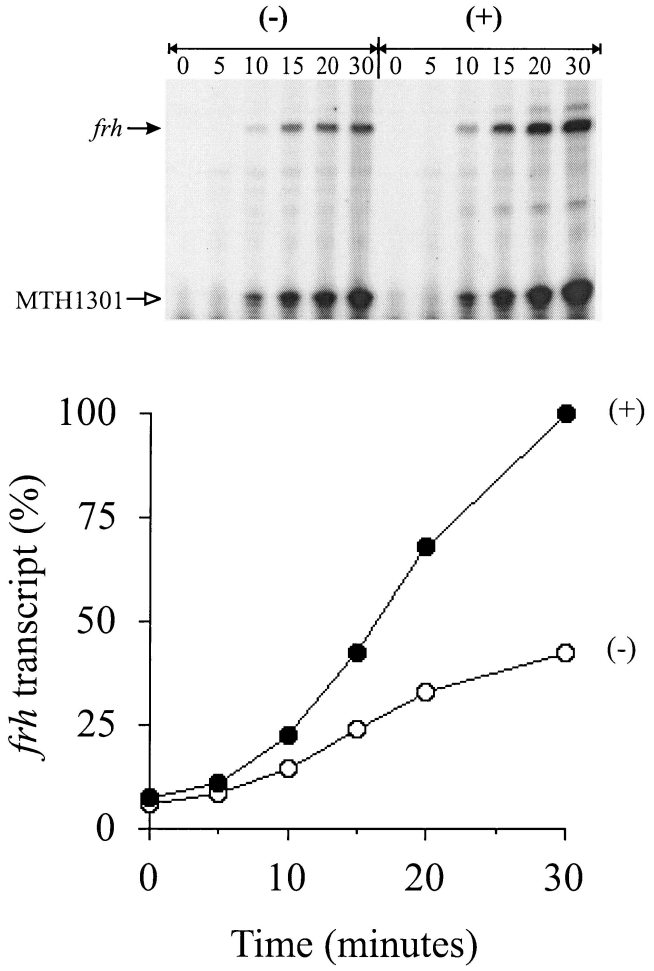

FIG. 3. Kinetics of in vitro transcription in the presence and absence of TFE. The amounts of *frh* transcript present after increasing times of in vitro transcription with (+) or without (−) 200 ng of TFE were determined, and the mean values from two independent experiments are shown in the graph as percentages of the amount synthesized after 30 min in the presence of TFE. The arrows to the left of the gel identify the *frh* transcript and a transcript that is transcribed from the opposite strand from the promoter of the adjacent MTH1301 gene (35). MTH1301 encodes a protein of unknown function, but as illustrated, transcription from the MTH1301 promoter was stimulated ~2.5-fold by TFE addition.

transcription from some archaeal promoters may therefore also involve a collaboration between TFE and a promoter-specific activator. In this regard, although there is evidence for archaeal transcription activators (11, 13), only negative regulation of transcription by repressor binding has so far been directly demonstrated (3, 4, 6, 20, 36). However, with the addition of TFE, archaeal in vitro transcription systems may now be available that will facilitate the identification and functional characterization of promoter-specific activators of archaeal transcription.

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