JOURNAL OF BACTERIOLOGY, Apr. 2005, p. 2540–2550 Vol. 187, No. 7
0021-9193/05/$08.00+0 doi:10.1128/JB.187.7.2540–2550.2005

Transcriptional Analysis of the Principal Cell Division Gene, *ftsZ*, of *Mycobacterium tuberculosis*

Sougata Roy and Parthasarathi Ajitkumar*

Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore, India

Received 23 September 2004/Accepted 23 December 2004

Multiple promoters drive the expression of the principal cell division gene, *ftsZ*, in bacterial systems. Primer extension analysis of total RNA from *Mycobacterium tuberculosis* and a *Mycobacterium smegmatis* transformant containing 1.117 kb of the upstream region of *M. tuberculosis ftsZ* and promoter fusion studies identified six *ftsZ* transcripts and their promoters in the *ftsQ* open reading frame and *ftsQ-ftsZ* intergenic region. The presence of multiple promoters reflects the requirement to maintain a high basal level of, or to differentially regulate, FtsZ expression during different growth conditions of the pathogen in vivo.

Bacterial cell division is a tightly regulated process that involves spatial and temporal control of the replication and segregation of the chromosome (karyokinesis) and cytoplasmic division of the cell (cytokinesis) (12, 27, 37). The principal cell division protein FtsZ initiates septation through polymerization to form a ring-like structure at the leading edge of the invaginating septum to guide cytokinesis (1, 5, 27, 37). Although the function of FtsZ seems to be similar in all bacterial cells, transcriptional regulation of the gene differs remarkably, apparently in compliance with the physiological demands and growth conditions of different bacterial genera and species. In *Escherichia coli*, six promoters distributed in the upstream *ftsQ*, *ftsA*, and *ddlB* coding sequences drive one-third of the total *ftsQAZ* transcripts. The remaining two-thirds of *ftsZ* transcription is driven by promoters that are placed beyond 6 kb upstream of *ddlB* gene (10, 18, 40). Some of these promoters are upregulated by protein factors, such as SdiA, which regulates the *ftsQ2p* promoter (46), and the response regulator RcsB, which regulates the *ftsA1p* promoter (8, 22). Like for *E. coli*, the presence of multiple promoters for *ftsZ* has been reported for *Bartonella bacilliformis* and *Bartonella henselae* (17), *Shewanella violacea* (31), *Neisseria gonorrhoeae* (20), and *Thermoplasma acidophilum* (48). However, in the differentiating bacterium *Caulobacter crescentus*, monocistronic *ftsZ* is driven by a single promoter under the control of the global cell cycle regulator CtrA (32). In *Bacillus subtilis*, where the organization of the genes in the *dcw* cluster is similar to that in *E. coli*, among the three promoters that drive *ftsA-ftsZ* cotranscription, two are active during vegetative growth (SigA dependent) and one is active during sporulation (SigH dependent) (24). Moreover, the response regulator YycF of the YycG/YycF two-component system binds directly to the nonessential P1 promoter upstream of *ftsAZ* and activates transcription of the gene (21, 30). In *Streptomyces*, among the three *ftsZ* promoters present in the *ftsQ-ftsZ* intergenic region, one promoter is constitutively active, the second one is active during vegetative growth, and the third one is active during sporulation (11, 19, 33, 41). A minor sporulation-specific *ftsZ* transcript was detected from the *ftsQ* open reading frame (ORF) in *Streptomyces griseus* (11, 33) and in *Streptomyces coelicolor* (19, 39). In *Corynebacterium glutamicum* ATCC 13869 (*Brevibacterium lactofermentum*), there is a less abundant short transcript originating from the *ftsQ-ftsZ* intergenic region and a more abundant transcript starting inside *ftsQ* (29, 42).

Mycobacterium tuberculosis, which is a member of the lower *Actinomycetes* group and is similar to *Corynebacterium* and *Streptomyces*, is known to shut down its proliferation inside activated macrophages (34, 35). Similarly, proliferation of the pathogen is arrested at a uniform stage of the cell cycle when the cell enters the state of dormancy and is resumed when the cell comes out of dormancy (36, 47). Thus, the regulation of cell division is a key process that is obligatory for the pathogen for the successful establishment of infection as well as for survival and proliferation inside host cells. Although the mechanism of the control of septation in this pathogen is not known, a critical intracellular concentration of FtsZ protein, the level of which decreases in stationary phase, was found to be required for productive septum formation (13). This finding suggests a regulatory mechanism that controls the critical level of FtsZ protein inside the cells. We have initiated studies on the transcriptional regulation of the *ftsZ* gene of *M. tuberculosis*, and recently we have reported the identification of three specific regions upstream of the *ftsZ* ORF, which elicited *ftsZ*-specific transcription (43). These regions were the *ftsQ-ftsZ* intergenic region (172 bp) and the 5′ 467-bp and 3′ 217-bp regions of the *ftsQ* ORF, with maximal activity coming from within the *ftsQ* ORF. In continuation of these observations, here we report the identification of multiple transcripts and their start sites at the nucleotide level, and the corresponding putative promoters, by primer extension analysis from these regions on the RNA from mid-log-phase *M. tuberculosis* cells and on *Mycobacterium smegmatis* transformant carrying the *M. tuberculosis ftsZ* upstream region.

For this study, *M. tuberculosis* H37Ra cells, the isogenic attenuated mutant strain of pathogenic *M. tuberculosis* H37Rv, and an *M. smegmatis* transformant carrying the pMN406-Q1K1 construct containing the *ftsQ* ORF–*ftsQ-ftsZ* intergenic region were grown to mid-log phase (an optical density of approximately 0.8 at 600 nm) in Middlebrook 7H9 (Difco) liquid

* Corresponding author. Mailing address: Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore-560012, India. Phone: 91-80-22932344. Fax: 91-80-23602697. E-mail: ajit@mcbl.iisc.ernet.in.

TABLE 1. Primers used in primer extension

Primer	Sequence	Annealing temperature (°C)
ZPE	5′ CGATCATTCGGTTGACGGCG TTGAC 3′	55
K1	5′ CGGGATCCCGTTCGGCTTCC TCCCTGGTGGGG 3′	61
Igf	5′ CGCGGATCCATGGCTACGAC GGTTGCTAACCGTGCAGG 3′	60
K2r	5′ GCTAACCGTGCAGGCGCGCC GATAC 3′	60–58–55
P2r	5′ CGGGATCCCGCCGCCTTGGT CGTCGGATCG 3′	58
P1P2r	5′ GGGTACTGCCGCTGCACCCG CGCAC 3′	62
Revgfp2PE	5′ CGGTGAACAGCTCCTCGCCC TTCGAC 3′	58

medium supplemented with albumin-dextrose-catalase (ADC) enrichment and hygromycin selection (50 μg/ml) wherever applicable. The isolation of RNA, removal of genomic DNA contamination from the samples, and quantitation of total RNA from *M. tuberculosis* cells and *M. smegmatis* transformants were all carried out as previously described (43). For primer extension analysis, 5′ end-labeled primers (Table 1) of 1.5×10^6 cpm were hybridized to 2 μg of total RNA for 10 min at 65°C followed by another 10 min of hybridization at a suitable annealing temperature (Table 1). Primer was extended by using Moloney murine leukemia virus RNase H^- reverse transcriptase enzyme (MBI Fermentas) at either 42 or 45°C for 1 h in the presence of either 1 or 5 mM deoxynucleoside triphosphates. All of the reactions were repeated with two different dilutions of total RNA preparations from at least three independent mid-log-phase cultures. Control primer extension reactions were performed on *E. coli* tRNA (1 μg) (Sigma) to rule out extended products due to nonspecific annealing. Similarly, in order to rule out primer-extended product being a falloff product due to GC-rich sequences, primer extension reactions were performed on single-stranded sense strand DNA (200 ng) of the corresponding regions that was amplified from the PCR product of the 1.202-kbp region (Q1-ZPE segment) from the ATG of *ftsQ* to the annealing site of the ZPE primer inside the *ftsZ* ORF by using only the forward Q1 primer (57) as described previously (16). The primer extension products were analyzed in 6 or 8% denaturing polyacrylamide gel with 7 M urea, along with a corresponding sequencing reaction, using a Thermosequenase cycle sequencing kit (USB) on the PCR product template Q1-ZPE that was amplified from genomic DNA of *M. tuberculosis* H37Ra.

Identification of the origin of *ftsZ* transcripts from the *ftsQ-ftsZ* intergenic region. Primer extension analysis of RNA from *M. tuberculosis* cells with ZPE and K1 primers, which anneal to the *M. tuberculosis ftsZ* gene and the *ftsQ-ftsZ* intergenic region, respectively (Table 1), identified two transcripts, t1 and t2, originating from the intergenic region. However, extension using the Revgfp2PE primer, which anneals to the *mycgfp2+* gene in the pMN406-Q1K1 construct, on RNA from *M. smegmatis* transformants harboring multicopy reporter plasmid pMN406-Q1K1 (43) containing the 1.117-kb *M. tuberculosis ftsZ* promoter region (*ftsQ* ORF and *ftsQ-ftsZ* intergenic region) identified t2 and, instead of t1, two additional transcripts, ta and tb, which were not found in *M. tuberculosis*. The control experiments using a mixture of *E. coli* tRNA and single-stranded sense strand DNA did not show any corresponding bands (Fig. 1b, lane cz), indicating that the primer extension reactions did not involve either nonspecific extension or secondary structure-related falloff of the enzyme. The control experiments for the primer extension of RNA from *M. smegmatis* transformant were carried out using RNA from *M. smegmatis* transformants containing promoterless vector control (pMN406-Δpimyc) (43). Primer extension analyses using RNA from *M. smegmatis* transformants containing the individual 172-bp *ftsQ-ftsZ* intergenic region and the 3′ 217-bp and 5′ 467-bp regions of the *ftsQ* ORF in the reporter plasmid (43) were not carried out in order not to miss any transcripts that originate near the junctions of these cloned regions.

The transcript t1, which was identified in *M. tuberculosis*, starts at the A or G 12 or 13 bp upstream of the ATG of *ftsZ* and just includes the predicted ribosome binding site (RBS) GGAGGAAG of the cognate mRNA (Fig. 1b). The potential RBS where the transcription start lies can form stem-loop structures in several conformations with sequences just downstream of ATG of the *ftsZ* gene. The presence of such stem-loop structures and a strong RBS at the 5′ end of the RNA is

TABLE 2. Primers used in the cloning of promoter sequences

Primer	Sequence	Region or promoter cloned
T1f	5′ GCTCTAGAGTTGAGGTTGAGAGTTTGCCAGCAGACACAC 3′	P1
K1	5′ CGGGATCCCGTTCGGCTTCCTCCCTGGTGGGG 3′	P1
Tbf	5′ GCTCTAGACCATACGGTTCTGACTACGAGCAACTAC 3′	Pb
Tbr	5′ CGGGATCCAAACTCTCAACCTCAACCATAGGCTTAGAG 3′	Pb
K2	5′ GCTCTAGACCGAAAAAATGCCCGCCGCGTATC 3′	P2
T2r	5′ GCGGATCCATGTCAAGTAGTTGCTCGTAGTCAGAACCGTATGGC 3′	P2
Igf	5′ GCGGATCCTAAATGGCTACGACGGTTGCTAACCGTGCAGG 3′	P2Δ, P3
T3f	5′ GCTCTAGAGGCAGAACGTACGACGTGTCCAGCCCC 3′	P3
T4	5′ CTAGACCTGAAGTTGCAAGCCAGGTGGGGCGGATCGCGGCCCCGTCG 3′	P4
T4c	5′ GATCCGACGGGGCCGCGATCCGCCCCACCTGGCTTGCAACTTCAGGT 3′	P4
T5f	5′ GCTCTAGAGAGGTTCTCGACGCCGCCAG 3′	P5
T5r	5′ CGGGATCCCGATCTGCAGCAACGGCGTTGCCG 3′	P5
Q1	5′ GCGGGATCCGATATCATGACGGAACACAACGAGGACCCACAGATCGAGCGC 3′	Q1SD1
SD1	5′ GATATCGCTAGCGATGGCGATCTCGGCCGCTTTGAGCGACCGGCCTTC 3′	Q1SD1

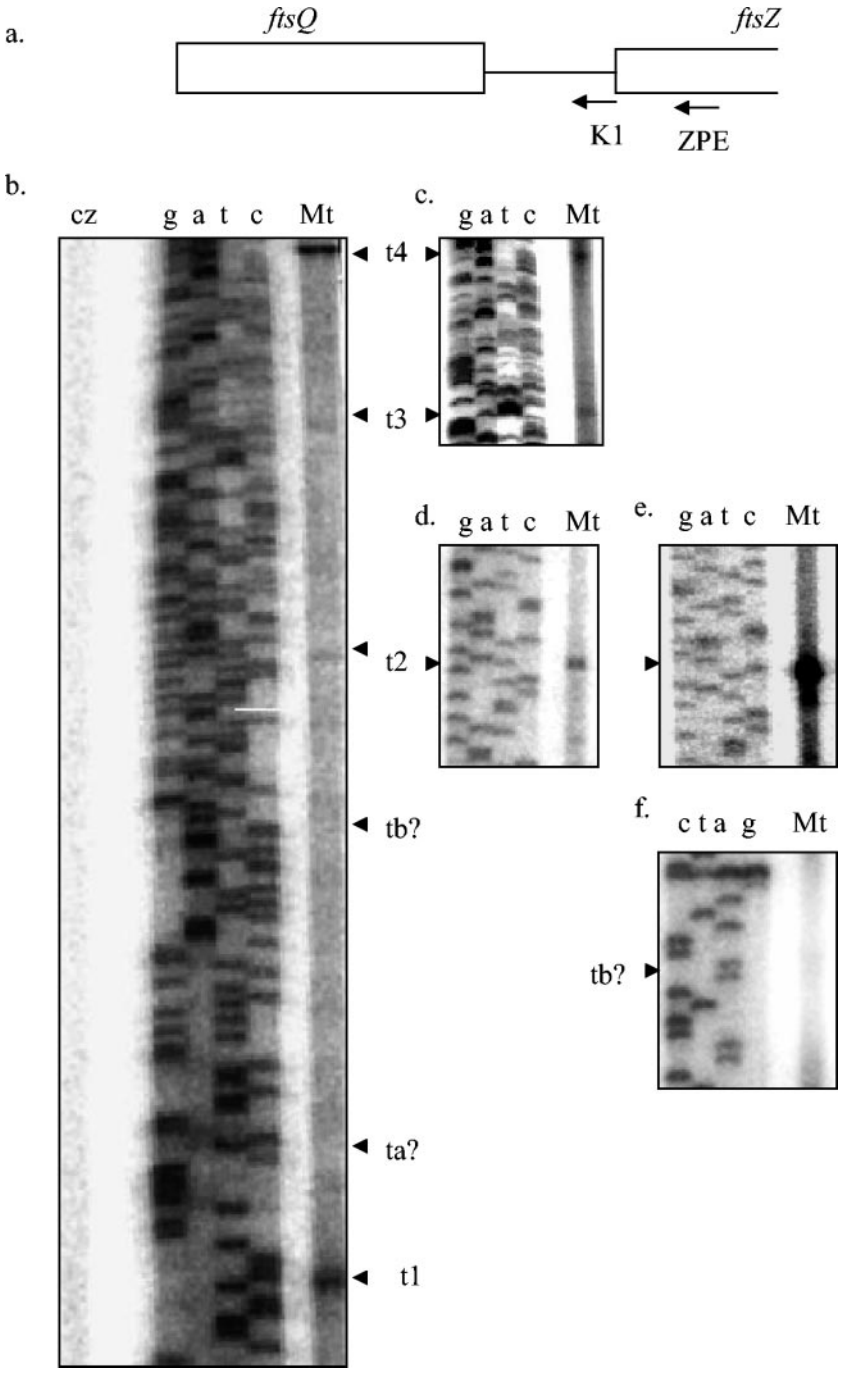

FIG. 1. Primer extension analysis of RNA from *M. tuberculosis*. (a) Schematic locations of ZPE and K1 primers. (b to d) Phosphorimaging profile of primer extension analysis using ZPE on *M. tuberculosis* RNA. Lane cz, control extension with ZPE on *E. coli* tRNA (1 μg) mixed with single-stranded sense strand DNA (200 ng) of the 1.117-kb promoter region. (e and f) Phosphorimaging profile of primer extension using K1 on *M. tuberculosis* (Mt) RNA.

known to stabilize the cognate mRNA molecule (3, 4, 26). The putative promoter P1, which drives the expression of the t1 transcript, has CGAGCCCC as the −10 sequence and TT GCCA as the −35 sequence (Table 3) (see Fig. 5). Although the −35 sequence shows a σ^A-like promoter consensus, the −10 sequence does not. Therefore, P1 could be classified under type C promoters (23). If the −10 region (CGAGCC) is considered with a −35-like (GAGAGT) sequence, with a 24-nucleotide gap between them, it shows σ^C consensus (38, 45) (see Fig. 5). However, in mycobacteria the gap between −10 and −35 sequences of various promoters reported so far are found to vary between 12 and 22 nucleotides only and not 24 nucleotides (23).

In order to verify whether the same transcript is produced in

TABLE 3. Putative promoter sequences of *M. tuberculosis ftsZ*

Putative promoter	−35	−10	+1	Distance of +1 from ATG of *ftsZ*	Type
P1	GAGAGTTTGCCA	CGAGCCCC	G/A	13 or 12	C
P2	TTAGCA	TACGGT	A	100	A
P3	GTACGA	TGCCGA	A	176	C
P4	GTTGCAA	GATCGC	G	331	C
P5	TTCTCGA	GGCCGGCA	G	655	C
P6	TCGCGA	TCGGGA	GG	773 or 772	C

the *M. smegmatis* transformant, extension was carried out on RNA from the transformant by using primer Revgfp2PE, the 3′ end of which anneals against G of the ATG codon of *mycgfp2+* in the promoter probe vector (43) (Fig. 2a and Table 1). Instead of the t1 transcript, the primer extension reaction identified the transcript ta, which starts at the G that is 24 bp upstream of the ATG of *ftsZ* (Fig. 2b; see Fig. 5). Similar to P1, the corresponding Pa promoter for the ta transcript possessed TTGAGA as the −35 sequence of σ^A consensus, but there was no clear −10 region similar to the −10 consensus of σ^A (see Fig. 5). Therefore, Pa could also be considered a type C promoter (23). It may be noted that both P1 and Pa promoters overlap with each other, suggesting that the transcription initiation complex can engage only one of them at any time point if both are functional in the same organism. Such promoters, having conserved the −35 region but without −10 consensus, are known to be targets of regulation by extracellular function sigma factors (15). The *M. smegmatis* genome lacks a σ^C homologue but possesses sequences for eight additional extracellular function sigma factors which are absent in *M. tuberculosis* (38). Therefore, it is likely that the overlapping P1 and Pa promoters might be targets of regulation by different sigma factors in *M. tuberculosis* and *M. smegmatis*. Another possibility for the presence of t1 and ta transcripts is that the products originated from differential processing of a single pre-mRNA due to altered secondary structure in these two mycobacterial species, owing to the GC-rich *mycgfp2+* sequence, instead of the *ftsZ* ORF, downstream of the promoter region in the transformant. One distant possibility is sequence polymorphism, similar to a previous report (6), between the *ftsZ* promoter region of the attenuated H37Ra strain, from which RNA was prepared for primer extension, and of the virulent H37Rv strain, the DNA of which was used to generate the insert in the pMN406-Q1K1 construct (43). We ruled out this possibility, as the sequence of the Pfu polymerase (MBI Fermentas)-amplified product from the *ftsQ* ORF–*ftsQ-ftsZ* intergenic–*ftsZ* ORF region (Q1-ZPE) from H37Ra genomic DNA with the ZPE, K2r, P2r, and P1P2r primers (Table 1) was identical to the reported sequence of the *ftsQ-ftsZ* locus of *M. tuberculosis* H37Rv (9).

Extension with the same primer, Revgfp2PE, of RNA from *M. smegmatis* transformants detected yet another transcript, tb (Fig. 2b; see Fig. 5). The product starts at the A or G at 62 or 63 bp, respectively, upstream of the ATG of the *ftsZ* gene. The corresponding promoter region, Pb (−35 TTCTGA and −10 TCTAAG with a 21- to 22-bp spacer region) shows a high degree of consensus to σ^A promoters (see Fig. 5). In addition to the presence of a clear σ^A promoter consensus, there could also be a probable σ^E or σ^H consensus with −35-like GCAAC and −10-like GGTTG, but with a 24-bp spacer, just immediately upstream of the +1 site (see Fig. 5). However, the tb transcript was absent from extension using the ZPE primer on the RNA from *M. tuberculosis* mid-log-phase cells (Fig. 1b). The absence of tb in mid-log-phase *M. tuberculosis* cells could be due to the possibility that the Pb promoter might be under regulated activity, as there is a strong hairpin loop present downstream of the −10 sequence of Pb involving the start site of the tb transcript. Such secondary structure downstream of the −10 sequence might be suggestive of a repression mechanism, as repressors are shown to maximally bind to palindrome sequences that are present downstream of −30 of promoter regions and up to +20 of the ORF (25). It is also possible that the activity of Pb is below detection limits during mid-log phase in *M. tuberculosis*. The presence of a large amount of transcribed RNA from the multicopy plasmid pMN406-Q1K1 in *M. smegmatis* transformant might have enabled its detection in the transformant. Another possibility is that antibiotic selection of *M. smegmatis* transformant might have induced stress response-specific sigma factors, such as σ^E or σ^H, for which consensus exists upstream of tb, which in turn might have activated the Pb promoter. Extension reactions using the primer K1, which binds upstream of ZPE and Revgfp2PE, the 3′ end of which anneals to 24 bp upstream of the ATG of *ftsZ* (Fig. 1a and 2a and Table 1) or *mycgfp2+* in pMNQ1K1 (Fig. 2a), confirmed the observation that tb is present in *M. smegmatis* transformant only and not in *M. tuberculosis* cells (Fig. 1f and 2d).

Primer extension with the ZPE, K1, and Revgfp2PE primers identified a fourth transcript, t2, from the RNA of both *M. tuberculosis* cells and the *M. smegmatis* transformant (Fig. 1b, d, and e and 2b, d, e, and f). The +1 site starts at the A 100 bp upstream of the ATG of the *ftsZ* gene (Table 3). The corresponding promoter, P2, shows a canonical σ^A-type consensus having −35 TTAGCA and −10 TACGGT with a spacer region of 13 bp. Alternately, there could be σ^E- or σ^H-type consensus having −35-like GGAAC and −10-like GGTTC with a 14-bp spacer (see Fig. 5). Incidentally, the promoter region contains palindromic sequences upstream of as well as between the −35 and −10 regions (see Fig. 5). Such promoters are subject to regulation and could be probable transcription factor binding sites, as in the case of the *tipA-p* promoter of *Streptomyces* (44). It may be noted that the −10 region of P2 and the −35 region of Pb overlap (see Fig. 5), indicating the possibility of differential engagement of the two promoters by RNA polymerase. Another possibility is of promoter occlusion (2) of Pb by the higher level of activity of P2 in *M. tuberculosis*.

Transcripts ta and tb are not specific to *ftsZ*. In order to verify whether ta and tb represent differentially expressed true

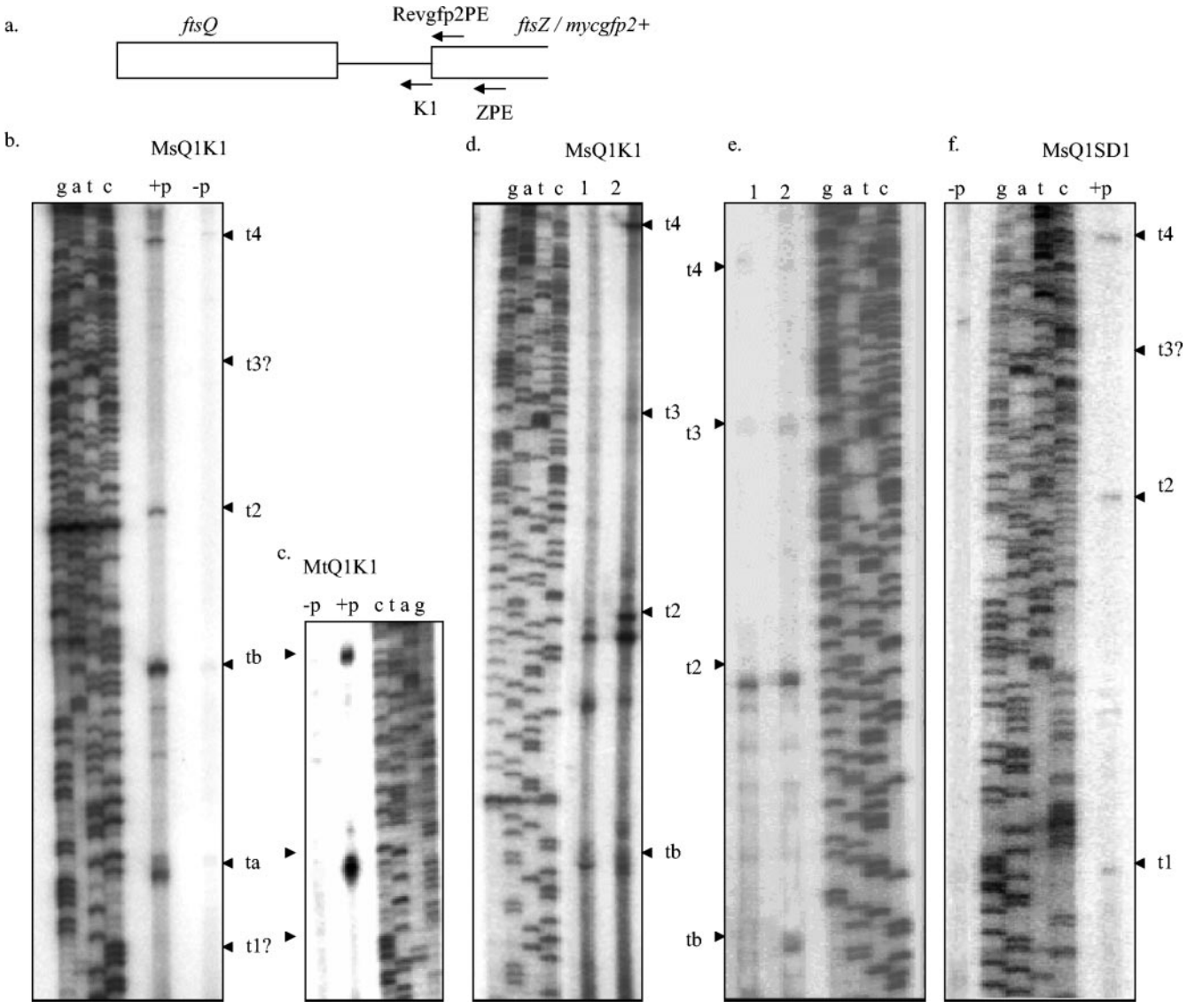

FIG. 2. Primer extension analyses of RNA from mycobacterial transformants. (a) Schematic locations of ZPE, K1, and Revgfp2PE primers. (b) Phosphorimaging profile of primer extension analysis using Revgfp2PE on *M. smegmatis* Q1K1. (c) Phosphorimaging profile of primer extension analysis with Revgfp2PE of RNA from *M. tuberculosis* Q1K1. (d) Phosphorimaging profile of primer extension analysis of RNA of *M. smegmatis* Q1K1 with K1 primer. Extension products from 500 ng (lane 1) and 2 μg (lane 2) of RNA are shown. (e) Phosphorimaging profile of primer extension analysis with K1 primer of RNA from *M. smegmatis* Q1K1 (lane 2) and *M. smegmatis* Q1SD1 (lane 1). (f) Phosphorimaging profile of primer extension analysis with ZPE primer of RNA from *M. smegmatis* Q1SD1. MsQ1K1 +p, *M. smegmatis* transformed with pMN406-Q1K1; MsQ1K1 −p, *M. smegmatis* transformed with promoterless vector control pMN406-Δpimyc; and MsQ1SD1 +p, *M. smegmatis* transformed with pMN406-Q1SD1. t1 to t4, ta, and tb are extension products representing transcripts.

transcripts, RNA was prepared from *M. tuberculosis* integrant carrying pMN406-Q1K1 derivative (unpublished data) at the L5 lambda attachment site. Primer extension analysis using the Revgfp2PE and K1 primers identified the same ta and tb transcripts, instead of t1, from the RNA of *M. tuberculosis* transformants too (Fig. 2c). The presence of these transcripts in *M. tuberculosis* transformants rules out the possibility, suggested earlier, of repression or downregulated expression from the Pa and Pb promoters in *M. tuberculosis*. However, antibiotic-induced sigma factor-mediated activation of these promoters cannot be ruled out. In order to verify this possibility, the 1,843-bp region starting from the ATG of *M. tuberculosis ftsQ* up to the 726-bp region of *M. tuberculosis ftsZ* was PCR amplified from *M. tuberculosis* genomic DNA by using Q1 and SD1 primers (Table 2) and KOD XL DNA polymerase (Novagen) and cloned into the pMN406-Δpimyc vector (43) at the SspI and NheI sites, replacing the *mycgfp2+* gene, to obtain pMN406-Q1SD1. In this construct, *M. tuberculosis ftsZ* was directly under the regulation of its own native 1.117-kb promoter region. Extension of the ZPE primer on the RNA from *M. smegmatis* transformant containing pMN406-Q1SD1 identified t1 and t2 transcripts, originating from the intergenic region, but not ta or tb transcripts (Fig. 2f). This observation was again confirmed by the extension of K1 primer on the same

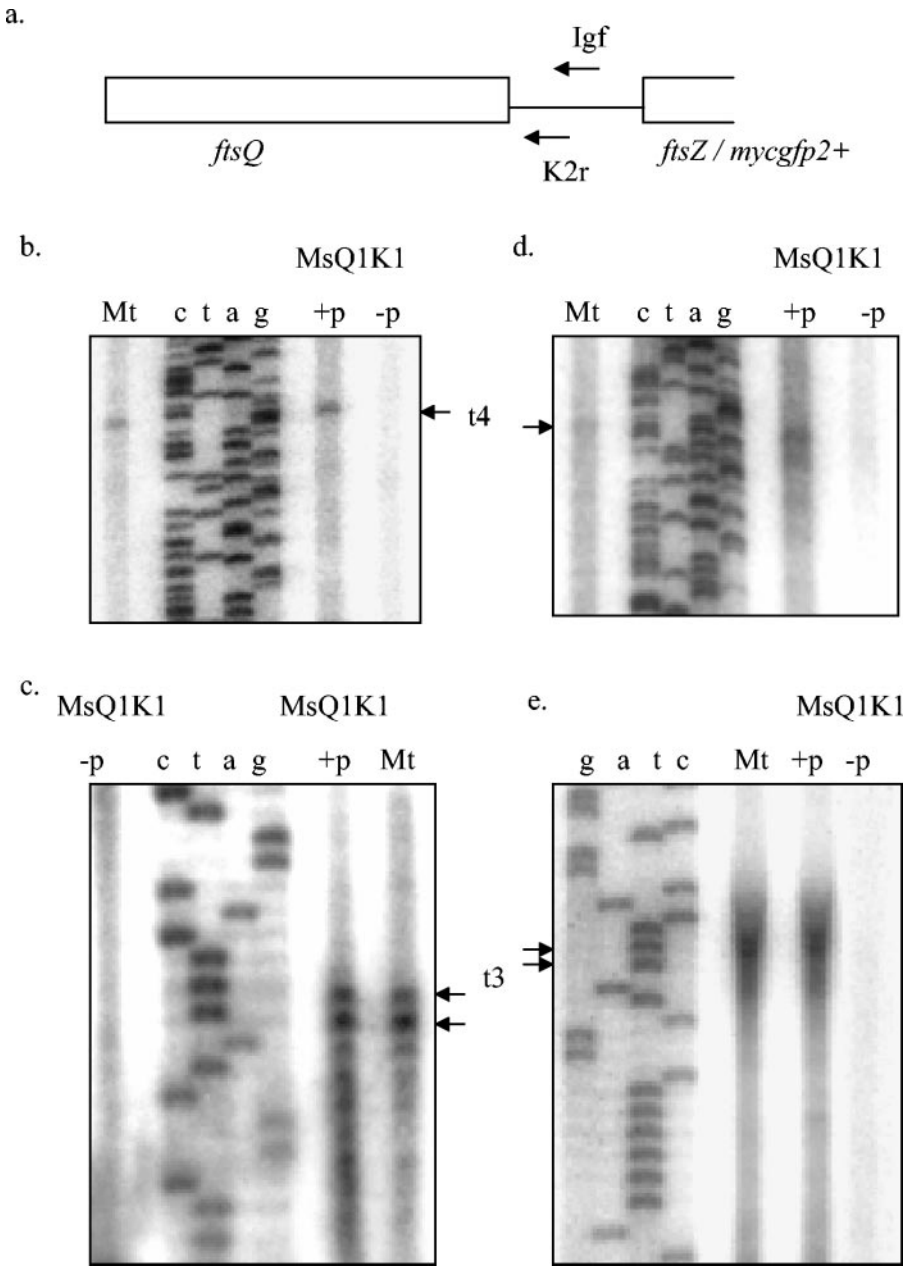

FIG. 3. Primer extension analysis in the 3′ portion of the *ftsQ* ORF. (a) Schematic locations of Igf and K2r primers. (b to e) Phosphorimaging profile of primer extension using K2r (b and c) and Igf (d and e) primers. t3 and t4 are extension products representing transcripts.

RNA, wherein as expected only t2 and not tb could be detected, as K1 anneals at the P1/Pa region (Fig. 2e, lane 1). This result ruled out the possibility of firing of Pa and Pb promoters by antibiotic-induced sigma factors. The fact that ta and tb transcripts are absent in the negative control (pMN406-Δpi-myc) and that two independent primers, K1 and Revgfp2PE, have identified them in *M. smegmatis* and *M. tuberculosis* transformants only when *mycgfp2+* is present downstream of the 1.117-kb *M. tuberculosis ftsZ* promoter region (Fig. 2b and c) and not when the *ftsZ* ORF is present (Fig. 2f and 1b) proves that the two transcripts are the processed ends due to a modified conformation of the untranslated *M. tuberculosis ftsZ* leader sequence in the presence of the GC-rich *mycgfp2+* sequence downstream of the promoter region. Since the two transcripts do not pertain to *ftsZ* transcription, as evident from the primer extension result with RNA from *M. smegmatis* transformant (pMN406-Q1SD1) (Fig. 2e and f), the probable reason for their *mycgfp2+*-specific origin was not investigated.

Identification of the origin of *ftsZ* transcripts from the *ftsQ* ORF. Primer extension analysis on the RNA from *M. tuberculosis* cells and *M. smegmatis* transformants containing pMN406-Q1K1 with the primers ZPE, K1, Revgfp2PE, K2r, Igf, P2r, and P1P2r (Table 1) identified four transcripts: t3 and t4, originating in the 3′ 217-bp region of the *ftsQ* ORF, and t5

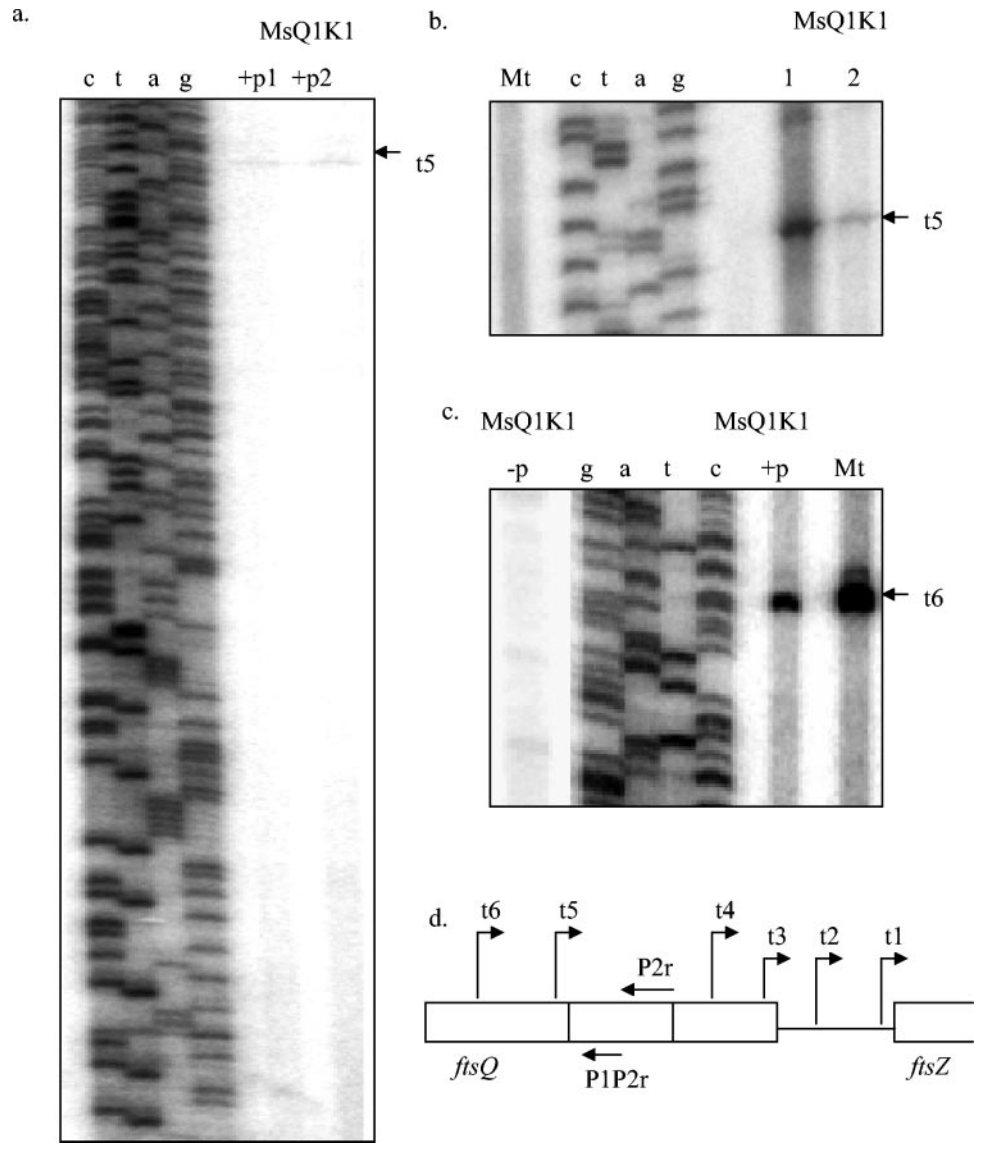

FIG. 4. Primer extension analysis in the 5′ portion of the *ftsQ* ORF. (a to c) Phosphorimaging profile of primer extension using P2r and P1P2r primers. (a) Extension with P2r of RNA from *M. smegmatis* transformed with pMN406-Q1K1. +p1 and +p2 are two different reactions from two different samples. (b and c) Extension with P1P2r primer of RNA from *M. smegmatis* Q1K1 (MsQ1K1) and *M. tuberculosis* (Mt). Primer extension products from 2 μg (lane 1) and 500 ng (lane 2) of RNA are shown. (d) Schematic locations of P2r and P1P2r primers and +1 sites of *ftsZ* transcripts (t1 to t6).

and t6, originating in the 5′ 467-bp region of the *ftsQ* ORF (Fig. 3 and 4). The extension of ZPE, K1, and Revgfp2PE primers identified transcript t3 as originating inside the *ftsQ* ORF at the A, which is 4 nucleotides upstream of the end of the stop codon of *ftsQ* and 176 bp upstream of the ATG of *ftsZ* (Fig. 1b and c and 2b, d, e, and f; Table 3). Similarly, t4 was found to originate approximately 320 bp upstream of the ATG of *ftsZ* and about 145 bp upstream of the stop codon of *ftsQ*. In order to precisely map t3 and t4 transcripts, K2r and Igf primers were used for primer-extension on total RNA from *M. tuberculosis* and *M. smegmatis* transformant containing pMN406-Q1K1 (Table 1 and Fig. 3a).

Primer K2r, the 3′ end of which lies in the *ftsQ-ftsZ* intergenic region 153 bp upstream of the ATG of *ftsZ*, was annealed to RNA with a gradual reduction of temperature (Table 1 and Fig. 3a) owing to the high secondary structure in the region. The 3′ end of the primer Igf anneals 142 bp upstream of ATG (Table 1 and Fig. 3a). The extension of both of the primers detected t3 transcript (Fig. 3c and e and 5), confirming the earlier observation. The corresponding promoter, P3, lacks consensus to σ^A-, σ^C-, or σ^H-type promoters and could be grouped as a C type promoter (Table 3 and Fig. 5). Since P3 lacks consensus with any of the known sigma factor-specific promoters and t3 starts in an AU-rich region downstream of which there is a probable strong stem-loop structure, t3 could be a RNase-processed product, as found in the *ftsQAZ* transcript of *E. coli* (7). The extension of K2r and Igf mapped t4, originating at the G 331 bp upstream of the ATG of *ftsZ* and

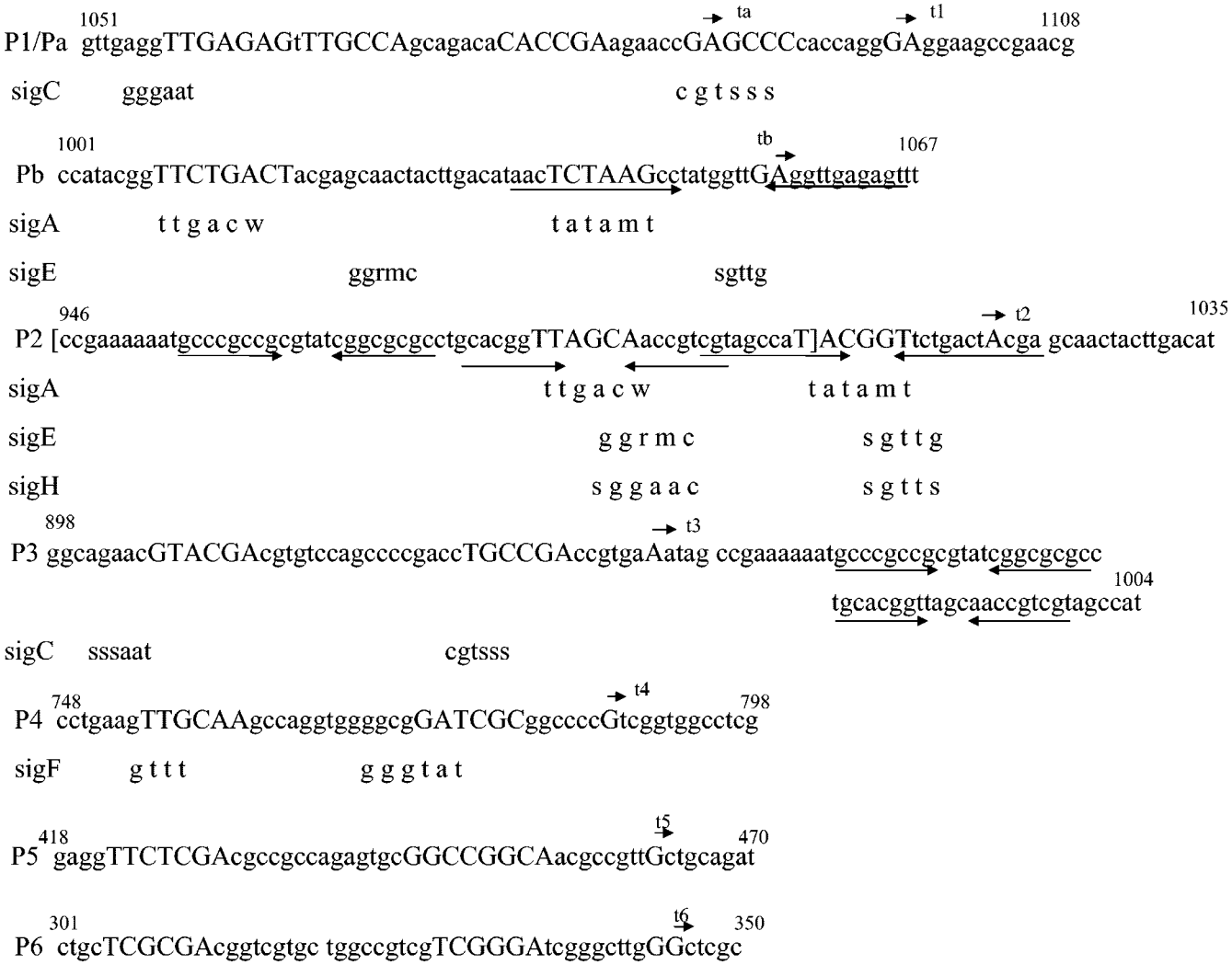

FIG. 5. Promoter sequences of *M. tuberculosis ftsZ* and comparisons with different sigma factor consensuses. The number of nucleotides are based on the *M. tuberculosis* Q1-ZPE region, with *ftsQ* ATG as 1. s denotes g or c; w denotes a or t; r denotes a or g; and m denotes a or c. The top arrow is the +1 site. Capital letters indicate −10 and −35 sequences. Convergent arrows indicate palindrome sequences. These promoter sequences were cloned by using primers shown in Table 2. The portion of the P2 sequence in square brackets is P2Δ.

158 nucleotides upstream of the end of the stop codon of *ftsQ*, confirming the earlier observation with ZPE, K1, and Revgfp2PE primers (Fig. 3b and d and Table 3). The corresponding promoter, P4, has a GC-rich −10 region (GATCGC) and a weak −35 consensus (TTGCAA) for σ^A, indicating that it could probably be a type C promoter (Table 3 and Fig. 5). The alternate possibility is a weak σ^F consensus promoter (GTTG-13 bp-GCGGAT) (Fig. 5).

In order to verify the observation of the absence of promoter activity in the 261-bp PstI fragment of the *ftsQ* ORF (43), extension was performed with the P2r primer (Table 1 and Fig. 4d), the 3′ end of which anneals at 416 bp upstream of the ATG of *ftsZ* and 244 bp upstream of the stop codon of *ftsQ*. P2r extension did not generate any product from *M. tuberculosis* RNA or from RNA of *M. smegmatis* transformant from the 261-bp region of the *ftsQ* ORF (43). Instead, it gave a faint extension product, t5, on RNA from the *M. smegmatis* transformant containing pMN406-Q1K1, but not from *M. tuberculosis*, above 650 bp upstream of the ATG of *ftsZ* that lies in the 467-bp 5′ region of the *ftsQ* ORF (Fig. 4a). In order to precisely map transcript t5, extension was carried out using the P1P2r primer (Table 1 and Fig. 4d), the 3′ end of which anneals to 597 bp upstream of the ATG of *ftsZ*. The product t5 was found to be present only in *M. smegmatis* transformant, starting at the G, 655 bp upstream of the ATG of the *ftsZ* gene and 482 nucleotides upstream of the stop codon of *ftsQ* and lies in the 467-bp 5′ portion of *ftsQ* (Fig. 4a and b). The corresponding promoter, P5, having only a weak consensus to the −35 region (TTCTCG) of σ^A, lacks consensus to any of the known sigma factor-specific promoters. Therefore, it could be a type C promoter (Table 3 and Fig. 5). The reason for the absence of t5 transcript in *M. tuberculosis* could be a low level of P5 activity or differential regulation at the mid-log phase of growth. Besides t5, one more transcript, t6, which starts at the G 772 or 773 nucleotides upstream of the ATG of *ftsZ* and 599 or 600 nucleotides upstream of the stop codon of *ftsQ* and lies

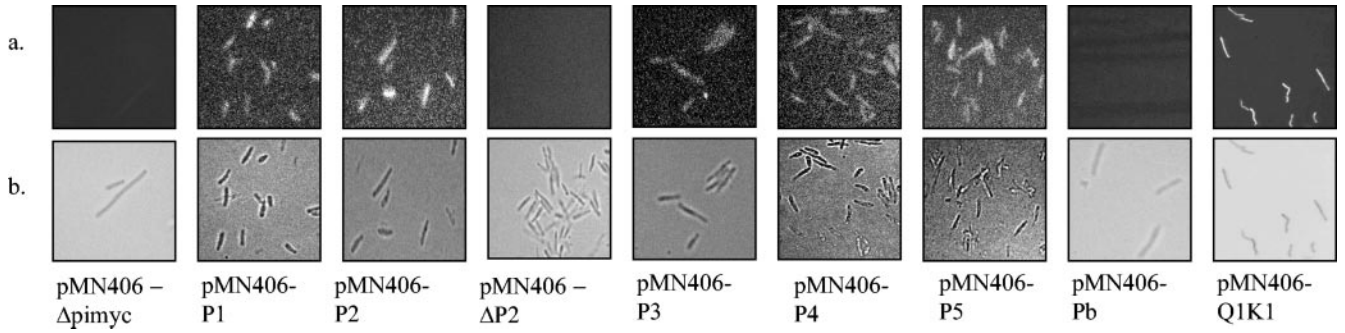

FIG. 6. Fluorescence microscopic observation of *M. smegmatis* transformants containing various promoter fusion constructs. (a) Dark-field fluorescence microscopy of the cells containing the constructs indicated in the figure. (b) Figures of phase-contrast microscopy of the corresponding fluorescent *M. smegmatis* cells. Cells are photographed and shown at ×25,000 magnification. pMN406-Q1K1 (43) was taken as the positive control, whereas pMN406-Δpimyc (43) was used as the negative control. Brightness and contrast have been modified with Adobe Photoshop 6.0.

in the 5′ 467-bp portion of *ftsQ*, could be detected by P1P2r primer extension of RNA from *M. tuberculosis* and *M. smegmatis* transformant containing pMN406-Q1K1 (Fig. 4c). The corresponding P6 promoter did not show consensus to any of the known sigma factor-specific promoters (Table 3 and Fig. 5).

Activity of the mapped putative promoter sequences in vivo. Although our previous study (43) showed promoter activity from the individual 5′ 467-bp (initiating t5 and t6 transcripts) and 3′ 217-bp (initiating t3 and t4 transcripts) regions of *M. tuberculosis ftsQ* as well as the *ftsQ-ftsZ* intergenic region (initiating t1 and t2 transcripts), the presence of multiple origins of transcripts from each cloned region and also the presence of potential stem-loop structures in some of the promoter regions raise the possibility that some of the 5′ ends identified could be due to RNA processing. Due to the lack of RNase mutant strains for mycobacteria, the transcripts with secondary structure near their origin could not be tested to determine whether they were RNase-processed products. Since the majority of the mycobacterial promoters are not active in *E. coli* (23), *E. coli* RNase mutant strains also could not be used for this purpose. Instead, the activity of the putative promoters was determined in the reporter fusion study using the promoter probe vector pMN406-Δpimyc (43). Since P6 is the most distal promoter detected, there is no promoter sequence beyond P6 in the *M. smegmatis* transformant containing pMN406-Q1K1. Therefore, t6 transcript cannot be a processed product. Hence, except P6, other putative promoter sequences, namely, P1, P2, P3, P4, and P5, encompassing exclusively the −10, −35, and +1 start sites of the respective transcripts (Fig. 5), were PCR amplified with primers (Table 2) and cloned in pMN406-Δpimyc vector, and the sequences were confirmed by using Revgfp2PE primer (Table 1) and analyzed for their activity in *M. smegmatis* transformants. The respective sequences of the P1, P2, P3, P4, and P5 promoters that were cloned are indicated in Fig. 5. Since P2 has secondary structure regions overlapping with the −10 and −35 sequences, in order to confirm the promoter activity of P2, the −10 sequence was deleted by cloning a 59-bp PCR-amplified truncated P2 (P2Δ) (Fig. 5). As an internal negative control, a 67-bp Pb sequence was PCR amplified and cloned. Except truncated P2Δ and the negative control Pb, all of the specific promoter sequences, namely, P1 to P5, drove the expression of *mycgfp2+* (Fig. 6). These observations confirmed that the regions, predicted based on 5′ end mapping of *ftsZ* transcripts, are true promoters and the transcripts t1 to t5 (and t6) are true transcripts and not RNase-processed products. These observations indicate that the palindrome sequences in these promoter regions are not RNase processing sites but might be involved in promoter regulation. Careful mutational analyses of the palindrome structures are needed to fully understand the transcriptional regulation of the *M. tuberculosis ftsZ* gene.

In summary, we have identified and mapped six transcripts (t1 to t6) and their putative promoters (P1 to P6) for the *ftsZ* gene of *M. tuberculosis*. Among them, P1 and P2 are in the *ftsQ-ftsZ* intergenic region, while P3 and P4 are in the 3′ 217-bp region and P5 and P6 are in the 5′ 467-bp region of the *ftsQ* ORF. The identification of these transcripts, their respective putative promoters, and their distribution are in concurrence with our previous study (43), which demonstrated promoter activity in the *ftsQ-ftsZ* intergenic region and 3′ 217-bp and 5′ 467-bp regions of the *ftsQ* ORF. The absence of any transcript from the 261-bp P1P2 region of *ftsQ* also correlates with the absence of promoter activity noted for this region in our previous study (43). The quantitation of the relative intensities of the extension products of t1, t2, t3, and t4 transcripts (Fig. 1b and 2f) showed that both in *M. tuberculosis* cells and in *M. smegmatis* transformants, the promoter strength of the 3′ 217-bp region of the *ftsQ* ORF is 1.3 to 1.8 times higher than that of the 172-bp *M. tuberculosis ftsQ-ftsZ* intergenic region. These values are comparable to the values (1.4 to 1.8) obtained in the semiquantitative reverse transcription-PCR analyses of the *M. tuberculosis ftsZ* transcripts originating from the respective regions in *M. tuberculosis* (43) and in *M. smegmatis* transformant containing pMN406-Q1K1 (Fig. 6 of reference 43). The promoters in the *ftsQ* ORF are GC rich and lack consensus to promoters that are recognized by the plethora of characterized sigma factors present in *M. tuberculosis*. Their high GC content also shows concurrence with their activity in *M. smegmatis* but lack of activity in *E. coli* (43). The presence of multiple promoters for *ftsZ* might facilitate differential regulation of gene expression in response to various environmental signals corresponding to different stages of the life cycle of the pathogen. Alternately, considering the slow rate of transcription in mycobacteria (28), multiple promoters might enable the maintenance of the high intracellular concentration of FtsZ

required for cell division in *M. tuberculosis* and *M. smegmatis* (13, 14).

This work was supported by a research grant from the Council of Scientific & Industrial Research, Government of India.

We express sincere thanks to H. S. Rajeswari for technical assistance, R. Srinivasan for discussions, and P. D. V. Krishna for the technical support in phosphorimaging. We acknowledge the DBT-Supported Phosphorimaging Facility, Division of Biological Sciences, Indian Institute of Science (IISc), and the infrastructure support from the ICMR-Funded Centre for Advanced Study in Molecular Medical Microbiology at the Department of Microbiology and Cell Biology, IISc, from the FIST Programme, Department of Science and Technology, Government of India, and from the DSA Programme, University Grants Commission, Government of India. S.R. acknowledges a Senior Research Fellowship from the Council of Scientific & Industrial Research, Government of India.

REFERENCES

1. **Addinall, S. G., and B. Holland.** 2002. The tubulin ancestor, FtsZ, draughtsman, designer and driving force for bacterial cytokinesis. J. Mol. Biol. **318:** 219–236.
2. **Adhya, S., and M. Gottesman.** 1982. Promoter occlusion: transcription through a promoter may inhibit its activity. Cell **29:**939–944.
3. **Agaisse, H., and D. Lereclus.** 1996. STAB-SD: a Shine-Dalgarno sequence in the 5′ untranslated region is a determinant of mRNA stability. Mol. Microbiol. **20:**633–643.
4. **Belasco, J. G., G. Nilsson, A. von Gabain, and S. N. Cohen.** 1986. The stability of *E. coli* gene transcripts is dependent on determinants localized to specific mRNA segments. Cell **46:**245–251.
5. **Bi, E. F., and J. Lutkenhaus.** 1991. FtsZ ring structure associated with division in *Escherichia coli*. Nature **354:**161–164.
6. **Brosch, R., W. J. Philipp, E. Stavropoulos, M. J. Colston, S. T. Cole, and S. V. Gordon.** 1999. Genomic analysis reveals variation between *Mycobacterium tuberculosis* H37Rv and the attenuated *M. tuberculosis* H37Ra strain. Infect. Immun. **67:**5768–5774.
7. **Cam, K., G. Rome, H. M. Krisch, and J. P. Bouché.** 1996. RNase E processing of essential cell division genes mRNA in *Escherichia coli*. Nucleic Acids Res. **24:**3065–3070.
8. **Carballes, F., C. Bertrand, J. P. Bouche, and K. Cam.** 1999. Regulation of *Escherichia coli* cell division genes *ftsA* and *ftsZ* by the two-component system *rcsC-rcsB*. Mol. Microbiol. **34:**442–450.
9. **Cole, S. T., R. Brosch, J. Parkhill, T. Garnier, C. Churcher, D. Harris, S. V. Gordon, K. Eiglmeier, S. Gas, et al.** 1998. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. Nature **393:** 537–544.
10. **Dewar, S. J., and R. Dorazi.** 2000. Control of division gene expression in *Escherichia coli*. FEMS Microbiol. Lett. **187:**1–7.
11. **Dharmatilake, A. J., and K. E. Kendrick.** 1994. Expression of the division-controlling gene *ftsZ* during growth and sporulation of the filamentous bacterium *Streptomyces griseus*. Gene **147:**21–28.
12. **Donachie, W. D.** 2001. Co-ordinate regulation of the *Escherichia coli* cell cycle or the cloud of unknowing. Mol. Microbiol. **40:**779–785.
13. **Dziadek, J., M. V. Madiraju, S. A. Rutherford, M. A. Atkinson, and M. Rajagopalan.** 2002. Physiological consequences associated with overproduction of *Mycobacterium tuberculosis* FtsZ in mycobacterial hosts. Microbiology **148:**961–971.
14. **Dziadek, J., S. A. Rutherford, M. V. Madiraju, M. A. Atkinson, and M. Rajagopalan.** 2003. Conditional expression of *Mycobacterium smegmatis ftsZ*, an essential cell division gene. Microbiology **149:**1593–1603.
15. **Fernandes, N. D., Q. L. Wu, D. Kong, X. Puyang, S. Garg, and R. N. Husson.** 1999. A mycobacterial extracytoplasmic sigma factor involved in survival following heat shock and oxidative stress. J. Bacteriol. **181:**4266–4274.
16. **Finckh, U., P. A. Lingenfelter, and D. Myerson.** 1991. Producing single stranded DNA probes with the Taq DNA polymerase: a high yield protocol. BioTechniques **10:**35–39. (Erratum, **12:**382, 1992.)
17. **Fiskus, W., I. Padmalayam, T. Kelly, C. Guibao, and B. R. Baumstark.** 2003. Identification and characterization of the DdlB, FtsQ and FtsA genes upstream of FtsZ in *Bartonella bacilliformis* and *Bartonella henselae*. DNA Cell Biol. **22:**743–752.
18. **Flardh, K., P. Palacios, and M. Vicente.** 1998. Cell division genes *ftsQAZ* in *Escherichia coli* require distant *cis*-acting signals upstream of *ddlB* for full expression. Mol. Microbiol. **30:**305–315.
19. **Flärdh, K., E. Leibovitz, M. J. Buttner, and K. F. Chater.** 2000. Generation of a non-sporulating strain of *Streptomyces coelicolor* A3(2) by the manipulation of a developmentally controlled *ftsZ* promoter. Mol. Microbiol. **38:** 737–749.
20. **Francis, F., S. Ramirez-Arcos, H. Salimnia, C. Victor, and J. R. Dillon.** 2000. Organization and transcription of the division cell wall (*dcw*) cluster in *Neisseria gonorrhoeae*. Gene **251:**141–151.
21. **Fukuchi, K., Y. Kasahara, K. Asai, K. Kobayashi, S. Moriya, and N. Ogasawara.** 2000. The essential two-component regulatory system encoded by *yycF* and *yycG* modulates expression of the *ftsAZ* operon in *Bacillus subtilis*. Microbiology **146:**1573–1583.
22. **Gervais, F. G., P. Phoenix, and G. R. Drapeau.** 1992. The *rcsB* gene, a positive regulator of colanic acid biosynthesis in *Escherichia coli*, is also an activator of *ftsZ* expression. J. Bacteriol. **174:**3964–3971.
23. **Gomez, M., and I. Smith.** 2000. Determinants of mycobacterial gene expression, p. 111–129. *In* G. F. Hatfull and W. R. Jacobs, Jr. (ed.), Molecular genetics of mycobacteria. ASM Press, Washington, D.C.
24. **Gonzy-Treboul, G., C. Karmazyn-Campelli, and P. Stragier.** 1992. Developmental regulation of transcription of the *Bacillus subtilis ftsAZ* operon. J. Mol. Biol. **224:**967–979.
25. **Gralla, J. D., and J. C. Collado-Vides.** 1996. Organization and function of transcription regulatory elements, p. 1232–1245. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed., vol. 1. ASM Press, Washington, D.C.
26. **Hansen, M. J., L. H. Chen, M. L. Fejzo, and J. G. Belasco.** 1994. The *ompA* 5′ untranslated region impedes a major pathway for mRNA degradation in *Escherichia coli*. Mol. Microbiol. **12:**707–716.
27. **Harry, E. J.** 2001. Bacterial cell division: regulating Z-ring formation. Mol. Microbiol. **40:**795–803.
28. **Harshey, R. M., and T. Ramakrishnan.** 1976. Purification and properties of DNA-dependent RNA polymerase from *Mycobacterium tuberculosis* H37Rv. Biochim. Biophys. Acta **432:**49–59.
29. **Honrubia, M. P., F. J. Fernandez, and J. A. Gil.** 1998. Identification, characterization, and chromosomal organization of the *ftsZ* gene from *Brevibacterium lactofermentum*. Mol. Gen. Genet. **259:**97–104.
30. **Howell, A., S. Dubrac, K. K. Andersen, D. Noone, J. Fert, T. Msadek, and K. Devine.** 2003. Genes controlled by the essential YycG/YycF two-component system of *Bacillus subtilis* revealed through a novel hybrid regulator approach. Mol. Microbiol. **49:**1639–1655.
31. **Ishii, A., K. Nakasone, T. Sato, M. Wachi, M. Sugai, K. Nagai, and C. Kato.** 2002. Isolation and characterization of the *dcw* cluster from the piezophilic deep-sea bacterium *Shewanella violacea*. J. Biochem. (Tokyo) **132:**183–188.
32. **Kelly, A. J., M. J. Sackett, N. Din, E. Quardokus, and Y. V. Brun.** 1998. Cell cycle-dependent transcriptional and proteolytic regulation of FtsZ in *Caulobacter*. Genes Dev. **12:**880–893.
33. **Kwak, J., A. J. Dharmatilake, H. Jiang, and K. E. Kendrick.** 2001. Identification and differential regulation of *ftsZ* transcription during septation of *Streptomyces griseus*. J. Bacteriol. **183:**5092–5101.
34. **Lagrange, P. H.** 1984. Cell-mediated immunity and delayed-type hypersensitivity, p. 681–720. *In* G. P. Kubica and L. G.Wayne (ed.), The mycobacteria: a sourcebook, part B. Marcel Dekker, Inc., New York, N.Y.
35. **Lefford, M. J.** 1984. Diseases in mice and rats, p. 947–977. *In* G. P. Kubica and L. G. Wayne (ed.), The mycobacteria: a sourcebook, part B. Marcel Dekker, Inc., New York, N.Y.
36. **Lim, A., M. Eleuterio, B. Hutter, B. Murugasu-Oei, and T. Dick.** 1999. Oxygen depletion-induced dormancy in *Mycobacterium bovis* BCG. J. Bacteriol. **181:**2252–2256.
37. **Lutkenhaus, J., and S. G. Addinall.** 1997. Bacterial cell division and the Z ring. Annu. Rev. Biochem. **66:**93–116.
38. **Manganelli, R., R. Provvedi, S. Rodrigue, J. Beaucher, L. Gaudreau, and I. Smith.** 2004. Sigma factors and global gene regulation in *Mycobacterium tuberculosis*. J. Bacteriol. **186:**895–902.
39. **McCormick, J. R., and R. Losick.** 1996. Cell division gene *ftsQ* is required for efficient sporulation but not growth and viability in *Streptomyces coelicolor* A3(2). J. Bacteriol. **178:**5295–5301.
40. **Mengin-Lecreulx, D., J. Ayala, A. Bouhss, J. van Heijenoort, C. Parquet, and H. Hara.** 1998. Contribution of the P*mra* promoter to expression of genes in the *Escherichia coli mra* cluster of cell envelope biosynthesis and cell division genes. J. Bacteriol. **180:**4406–4412.
41. **Mikulik, K., E. Zhulanova, M. Kratky, O. Kofronova, and O. Benada.** 2000. Isolation and characterization of *dcw* cluster from *Streptomyces collinus* producing kirromycin. Biochem. Biophys. Res. Commun. **268:**282–288.
42. **Ramos, A., M. P. Honrubia, D. Vega, J. A. Ayala, A. Bouhss, D. Mengin-Lecreulx, and J. A. Gil.** 2004. Characterization and chromosomal organization of the *murD-murC-ftsQ* region of *Corynebacterium glutamicum* ATCC 13869. Res. Microbiol. **155:**174–184.
43. **Roy, S., M. A. Mir, S. P. Anand, M. Niederweis, and P. Ajitkumar.** 2004. Identification and semi-quantitative analysis of *Mycobacterium tuberculosis* H37Rv *ftsZ* gene-specific promoter activity-containing regions. Res. Microbiol. **155:**817–826.
44. **Strohl, W. R.** 1992. Compilation and analysis of DNA sequence associated with apparent *Streptomyces* promoters. Nucleic Acids Res. **20:**961–974.
45. **Sun, R., P. J. Converse, C. Ko, S. Tyagi, N. E. Morrison, and W. R. Bishai.** 2004. Mycobacterium tuberculosis ECF sigma factor *sigC* is required for

lethality in mice and for the conditional expression of a defined gene set. Mol. Microbiol. **52:**25–38.
46. **Wang, X. D., P. A. de Boer, and L. I. Rothfield.** 1991. A factor that positively regulates cell division by activating transcription of the major cluster of essential cell division genes of *Escherichia coli*. EMBO J. **10:**3363–3372.
47. **Wayne, L. G., and L. G. Hayes.** 1996. An in vitro model for sequential study of shiftdown of *Mycobacterium tuberculosis* through two stages of nonreplicating persistence. Infect. Immun. **64:**2062–2069.
48. **Yaoi, T., P. Laksanalamai, A. Jiemjit, H. K. Kagawa, T. Alton, and J. D. Trent.** 2000. Cloning and characterization of *ftsZ* and *pyrF* from the archaeon *Thermoplasma acidophilum*. Biochem. Biophys. Res. Commun. **275:**936–945.