DNA Enzyme Generated by a Novel Single-Stranded DNA Expression Vector Inhibits Expression of the Essential Bacterial Cell Division Gene *ftsZ*

Xin-Xing Tan,[‡] Knesha Rose,[§] William Margolin,[§] and Yin Chen*,[‡]

CytoGenix, Incorporated, Suite 140, 3100 Wilcrest, Houston, Texas 77042, and Department of Microbiology and Molecular Genetics, University of Texas Medical School, 6431 Fannin Street, Houston, Texas 77030

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ABSTRACT: Rapid emergence of antibiotic-resistant bacterial pathogens has created urgent demand for the discovery and development of new antibacterial agents directed toward novel targets. Antisense oligodeoxynucleotides (AS-ODN) and their modified forms have been utilized to block gene expression in bacterial cells, showing potential for developing highly specific and efficacious antibacterial agents. In this study, a tetracycline-regulated expression vector was developed for generating single-stranded DNA (ssDNA) of a desired target sequence in bacterial cells. This inducible ssDNA expression vector was tested for producing a DNA enzyme designed to specifically cleave *ftsZ* mRNA. Our results indicate that the expressed DNA enzyme molecules not only repress *ftsZ* gene expression and but also inhibit bacterial cell proliferation. Although we believe that the cleavage of *fts*Z mRNA by the expressed DNA enzyme molecules is responsible for the inhibitory effects on *fts*Z gene expression and bacterial cell proliferation, the antisense mechanism could also be responsible for the biological effects. The ability of this ssDNA expression system to selectively modulate gene expression may provide a powerful strategy in determining the contribution of a given gene product to bacterial growth or pathogenesis and opens a new venue for developing antibacterial agents.

In recent years, the rapid emergence of bacterial pathogens with high resistance to antibiotic therapy constitutes a serious public health threat and has created an urgent demand for the discovery and development of new antibacterial agents directed toward novel targets (*1*, *2*). The primary tactics of the pharmaceutical industry in addressing this problem have been to seek incremental improvements in existing drugs (*3*). Although this approach has made a significant contribution in the fight against bacterial infections, difficulty remains in meeting the needs of the medical community in the face of increasing bacterial resistance to antibiotics.

The use of antisense oligodeoxyribonucleotides (AS-ODNs)[1] has been successful in a variety of applications to regulate gene expression in both prokaryotic and eukaryotic cells (*4*). AS-ODNs can bind to targeted mRNA and thus prevent the translation of the message into protein products, either by blocking the initiation of translation or by activating endogenous RNaseH that cleaves the RNA strand of the duplex (*4*). This technology has shown promise as a means for developing highly specific and efficacious antibacterial agents (*5*−*7*).

Recently, several different kinds of DNA enzymes (deoxyribozymes or DNAzymes) have been demonstrated to catalyze the cleavage of mRNA (*8*). One such enzyme, referred to as 10−23 DNA enzyme, has the potential to cleave any RNA targets containing a purine−pyrimidine junction (*9*, *10*). This 10−23 DNA enzyme consists of a 15 nucleotide catalytic domain, flanked by two RNA target-binding domains of seven to eight nucleotides each. In contrast to the complicated mechanisms of mRNA down-regulation by AS-ODNs (*11*), DNA enzymes are responsible for both mRNA targeting and cleavage (*9*, *10*).

The *ftsZ* gene is essential for bacterial division and viability (*12*). Bacteria such as *Escherichia coli* normally divide by binary fission, producing two daughter cells of equal size, each containing a nucleoid. The division process starts with the localization of FtsZ to the future division site and then the assembly of a septal structure called the Z ring. All other key cell division proteins are then recruited to the Z ring in an ordered manner, and the ring contracts as the ingrowing septum invaginates (*13*). Deletion or mutation of *ftsZ* gene blocks cell division at an early stage and causes the formation of long filamentous cells with multiple nucleoids (*13*). Homologues of *ftsZ* have been found in every free-living prokaryote cell examined to date (*14*−*16*). Its wide distribution and a high degree of sequence conservation make the *fts*Z gene a promising target for the development of broad-spectrum antibacterial agents.

In previous work, we developed a series of mammalian expression vectors for the purpose of generating ssDNA or ODN molecules intracellularly (*17*−*20*). These ssDNA expression vectors were demonstrated to be capable of generating (1) DNA enzymes for down-regulating gene expression and (2) triplex-forming oligos (TFO) for inducing genomic recombination. In the work reported here, we integrated all the key components of the mammalian expres-

* Corresponding author. Phone: (713) 789-0770. Fax: (713) 789-0702. E-mail: ychen@cytogenix.com.

‡ CytoGenix, Incorporated.

§ University of Texas Medical School.

[1] Abbreviations: ssDNA, single-stranded DNA; AS-ODN, antisense oligodeoxyribonucleotides; RT, reverse transcriptase.

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sion vector into a tetracycline-inducible bacterial expression vector. The ability of this new vector to produce ssDNA was tested by generating an *ftsZ* mRNA-cleaving DNA enzyme in cells in a regulated manner. The biological effect of repressing *ftsZ* gene expression by the in vivo expressed DNA enzyme was also investigated.

MATERIALS AND METHODS

Oligodeoxyribonucleotides (ODNs) and Bacterial Strains. ODNs were synthesized by Integrated DNA Technologies (Coraville, IA) and suspended in H_2O to the final concentration of 100 nM. The bacterial strain used for constructing the inducible ssDNA expression system was DH5αpro (*deoR, endA1, yrA96, hsdR17*($r_k^-m_k^+$), *recA1, relA1, supE44, thi-1,* Δ*(lacZYA-argF)U169, ϕ80δlacZM15, F*$^-$, *λ*$^-$, P_{N25}/*tetR,* P_{laci}^q/*laci, Sp*^{r}), a derivative of DH5α capable of producing Tet repressor protein (Clontech, Palo Alto, CA). To maintain the selection of the ssDNA expression vector, DH5αpro cells were grown in LB culture medium containing 34 μg/mL chloramphenicol and 50 μg/mL spectinomycin.

Vector Construction. The ssDNA expression vector, pssXG, containing the hybrid $P_{LtetO-1}$ promoter was constructed by integrating all the key expression components from pssXE(CMV), a mammalian ssDNA expression vector (*20*), into the bacterial expression vector, pPROTet.E233 (Clontech, Palo Alto, CA) (Figure 1B). To do that, a minimal change had to be made with the pssXE(CMV) vector. A DNA fragment was generated from the pssXE(CMV) vector by PCR using a pair of primers, 5′NheIPvuIATG, 5′-d(CTAGCTAGCTAGCGATCGATGGGACCAATGGGGCAG)-3′ and 3′KpnI, 5′-d(CGGGGTACCAGTATTCCCTGGTC)-3′. The amplified DNA fragment was used to replace a portion of the pssXE(CMV) vector between the *Nhe*I and the *Kpn*I sites. This replacement was designed to remove the sequences unnecessary for bacterial gene expression and create a *Pvu*I site for subsequent subcloning. The resulting plasmid, pssXE(CMV)NK, was double-digested with *Pvu*I and *Xba*I, and the *Pvu*I-*Xba*I DNA fragment was then subcloned into the pPROTet.E233 vector. The newly created plasmid was designated as pssXGa. To construct a vector expressing the desired DNA enzyme and replace the original primer binding site (PBS) in the expression cassette of pssXE(CMV), the ODNs of sequence, 5′-d(TAACTGGATGATCGTTGTAGCTAGCCTTCGAAACTTGGTGGTGCGTCCGAGTGGACCGGGAGACCCCTGCTCGAGT)-3′ and 5′-d(CTAGACTCGAGCAGGGGTCTCCCGGTCCACTCGGACGCACCACCAAGTTTCGAAGGCTAGCTACAACGATCATCCAGTTAAT)-3′ were annealed to produce a synthetic duplex with 5′*Pac*I and 3′*Xba*I cohesive ends and ligated into the *Pac*I and *Xba*I sites of pssXGa. The resulting vector was designated as pssXGb(FtsZ-DZ).

Design of RNA-Cleaving DNA Enzyme. To identify the DNA enzyme-accessible region in the transcript of the *ftsZ* gene, a RNA folding analysis was performed using the mfold program as described by Zuker (*21*) (Figure 2A). The default parameters were used, and the secondary structure with the lowest free energy was exploited to identify the regions within the mRNA that are accessible to the DNA enzyme. On the basis of the predicted secondary structure of the full-length *ftsZ* mRNA, the DNA enzyme was designed according to the 10−23 model (*9*) (Figure 2B). DNA enzymes were synthesized by Integrated DNA Technologies (Coraville, IA).

Synthesis of Template ftsZ RNA. The *ftsZ* gene was amplified by PCR directly from *E. coli* cells using the MasterTag kit (Eppendorf, Germany). Briefly, a single DH5α colony was picked and suspended in 500 μL of H_2O. A portion of the suspension (20 μL) was added to the 50 μL PCR reaction to supply the template DNA, and PCR was carried out for 40 cycles: 92 °C, 1 min; 56 °C, 1 min; and 72 °C, 1 min. The sequences of the PCR primers are FtsZ(SacIBamHI)5′, 5−d(TTACGAGCTCAGGCGACAGGCACAAATCGGAG)-3′ and FtsZ(SacIBamHI)3′, 5′-d(AACCCAGGATCCAGTCAATTCTTAATCAGC)-3′. The PCR products were then subcloned into the pTRI-amp-18 vector (Ambion, Austin, TX) between the *Sac*I and the *BamH*I sites. The resulting plasmid, pTRI-amp-18(FstZ), was linearized by *BamH*I digestion. The RNA for *ftsZ* (1225 nt) was synthesized using the MEGAscript kit purchased from Ambion (Austin, TX) according to the manufacturer's instructions.

In Vitro RNA Cleavage Assay. In vitro *ftsZ* RNA cleavage by the DNA enzyme was assayed following the procedure described by Chen et al. (*20*) with some modifications. Briefly, the cleavage assay was carried out at 37 °C in a 10 μL reaction containing 10 mM $MgCl_2$, 50 mM Tris-HCl, pH 7.5, 100 nM template *ftsZ* RNA, 100 μM DNA enzyme, and 10 units of RNasin (Promega, Madison, WI). The reaction was stopped at different time points by adding 2 μL of 500 μM EDTA and then analyzed on a 10% denaturing polyacrylamide gel containing 8 M urea.

Induction of Reverse Transcriptase (RT) Expression in DH5αpro Cells. The expression of RT was induced by anhydrotetracycline (aTc), a derivative of tetracycline, according to the manufacturer's instruction (Clontech, Palo Alto, CA). Briefly, DH5αpro cells carrying pssXGb(FtsZ-DZ) vector were grown in LB media with 34 μg/mL chloramphenicol and 50 μg/mL spectinomycin. When the OD_{600} reading of the culture reached 0.5, different amounts of aTc were added. Cells were then collected at different time points by centrifugation and stored at −20 °C until use.

Reverse Transcriptase (RT) Activity Assay. To measure the RT activity, DH5αpro cells carrying pssXGb(FtsZ-DZ), treated with various amount of aTc, were lysed using the B-PER II Bacterial Protein Extraction Reagent following the mini-scale protocol supplied by the manufacturer (Pierce, Rochford, IL). The soluble fractions were then collected by centrifugation, and the RT activity assay was performed following the protocol described by Chen et al. (*17*).

Measurements of Cell Growth. Overnight cell culture (200 μL) of DH5αpro cells carrying pssXGb(FtsZ-DZ) vector were inoculated into 10 mL of LB media with 34 μg/mL chloramphenicol and 50 μg/mL spectinomycin. aTc was added to final concentration of 0, 200, 400, and 800 ng/mL, respectively. Cell cultures were incubated at 42 °C with shaking (*22*−*24*), and 1 mL of sample was removed at either 1 or 2 h for cell growth assay by measuring viable cell count. Viable cell count was done by diluting the cultures and plating them in triplicate on LB plates with the appropriate antibiotics. The plates were then incubated overnight at 37 °C, and the number of colonies was enumerated by visual inspection.

Western Blot Analysis. DH5αpro cells carrying pssXGb-(FtsZ-DZ) vector were incubated with aTc as described

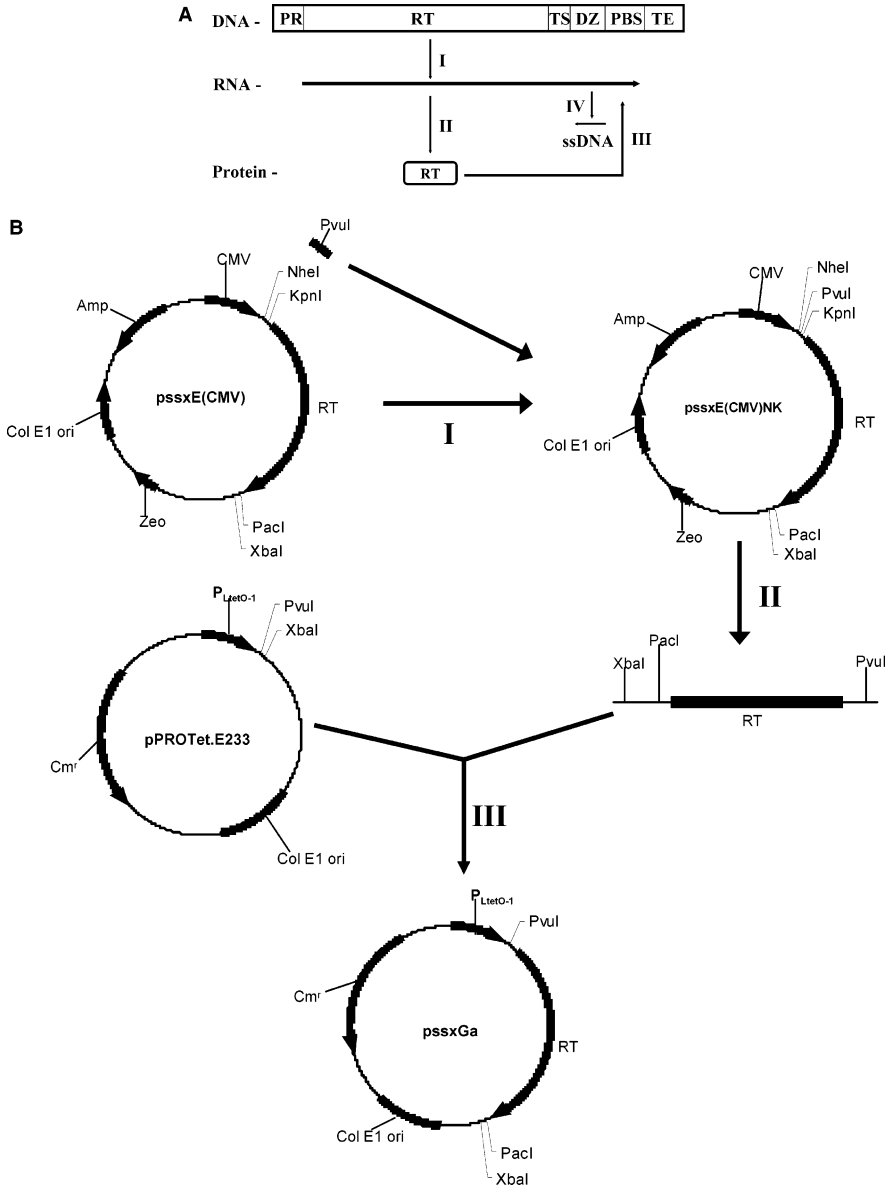

FIGURE 1: (A) Schematic representation of prokaryotic inducible single-stranded DNA expression system. PR: inducible promoter, P_{LtetO}-1; RT: reverse transcriptase; TS: reverse transcription termination sequence; DZ: DNA enzyme coding sequence; PBS: RT primer binding sequence; and TE: terminator sequence. I: transcription; II: translation; III and IV: reverse transcription. (B) Construction of the single-stranded DNA expression vector. (I) DNA fragment was generated from the pssXE(CMV) vector by PCR using a pair of primers, 5′NheIPvuIATG, 5′-d(CTAGCTAGCTAGCGATCGATGGGACCAATGGGGCAG)-3′ and 3′KpnI, 5′-d(CGGGGTACCAGTATTCCCTG-GTC)-3′. The amplified DNA fragment was used to replace a portion of the pssXE(CMV) vector between the *Nhe*I and the *Kpn*I sites. The resulting plasmid was designated as pssXE(CMV)NK. (II) pssXE(CMV)NK plasmid was double-digested with *Pvu*I and *Xba*I. (III) *Pvu*I-*Xba*I DNA fragment was subcloned into the pPROTet.E233 vector. The newly created plasmid was designated as pssXGa. pssXGb(FtsZ-DZ) was constructed by subcloning double-stranded ODNs into the *Pac*I and *Xba*I sites of pssXGa. The double-stranded ODNs were formed by annealing two ODNs of sequence, 5′-d(TAACTGGATGATCGTTGTAGCTAGCCTTCGAAACTTGGTGGTGCGTCCGA-GTGGACCGGGAGACCCCTGCTCGAGT)-3′ and 5′-d(CTAGACTCGAGCAGGGGTCTCCCGGTCCACTCGGACGCACCACCAA-GTTTCGAAGGCTAGCTACAACGATCATCCAGTTAAT)-3′.

previously. Cells were then collected by centrifugation and lysed. The lysates were resolved by 10% denaturing polyacrylamide gel, transferred onto nitrocellulose membrane (Amersham Pharmacia Biotech, Buckinghamshire, UK), and probed using either anti-histidine polyclonal antibody (1:1000) (Clontech, Palo Alto, CA) or anti-FtsZ antibody (*25*) (1:1000) as primary antibodies for the detection of RT (RT was expressed as a fusion protein with histidine tag) and FtsZ, respectively. The proteins were visualized using a chemiluminescence detection system from Pierce (Rockford, IL) according to the manufacturer's instructions.

RESULTS

Construction of a Tetracycline-Inducible ssDNA Expression Vector. Key components of the tetracycline-inducible ssDNA expression vector designed to produce ssDNA in bacterial cells are shown in Figure 1A. This ssDNA expression vector contains coding sequences for (1) the hybrid $P_{LtetO-1}$ promoter; (2) the moloney murine leukemia virus (MoMuLV) RT gene; (3) a reverse transcription termination structure; (4) a DNA enzyme; (5) a primer binding site (PBS); and (6) a terminator. As described in

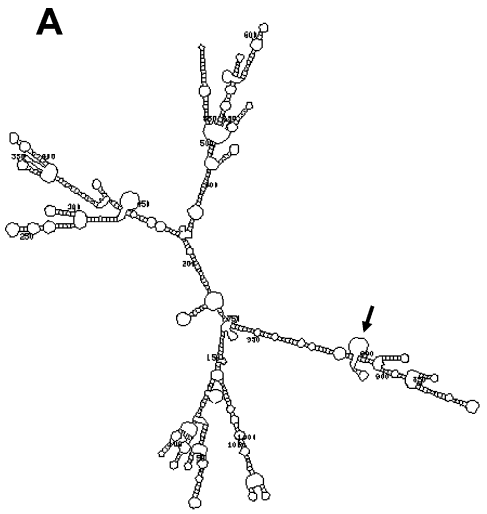

FIGURE 2: Design of the *ftsZ* mRNA-cleaving DNA enzyme. (A) Secondary structure of *ftsZ* mRNA predicted using mfold program (*21*). The selected DNA enzyme-targeting site was marked by the arrow. (B) Sequences of DNA enzyme and targeting RNA. The DNA enzyme cleavage site was marked by the arrow.

Figure 1A, the resulting transcript (I) contains the MoMuLV RT coding sequence, a reverse transcription termination structure, the coding sequence for the desired ssDNA, and a primer binding site. The expressed RT (II) would then synthesize the ssDNA of the desired sequence using endogenous tRNA(Val) as a primer that binds to the PBS (III). The reverse transcription reaction catalyzed by the RT would be stopped by the reverse transcription termination structure (IV).

To produce ssDNA intracellularly, RT usually uses an endogenous tRNA as primer that can bind the PBS located at the 3′ end of the RNA transcript. In this bacterial expression vector, all the key components including the RT gene and PBS were integrated from the mammalian expression vector, pssXE(CMV) (*20*). The original PBS, designed to bind mammalian tRNA(Pro), had to be replaced because bacterial tRNA(Pro) shares limited similarity with mammalian tRNA(Pro) (www.medlib.med.utah.edu/RNAmods). Since bacterial tRNA(Val) has been used as a primer for MoMuLV RT (*26*), a new PBS was designed to bind the 3′ end of bacterial tRNA(Val) (Figure 1B).

$P_{LtetO-1}$ is a novel tetracycline-regulated promoter that takes advantage of the high expression levels from the P_L promoter of phage and the tight control provided by operator 2 of the Tn10 tetracycline resistance operon (*27*). $P_{LtetO-1}$ is thus tightly repressed by the highly specific Tet repressor protein and induced in response to aTc, allowing the expression vector to precisely control the RT expression and thus ssDNA production.

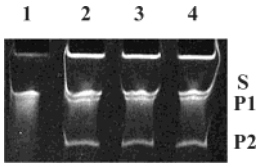

FIGURE 3: In vitro cleavage of *ftsZ* RNA. The 1225 nt *ftsZ* RNA template was synthesized by in vitro transcription. The in vitro cleavage assay was carried out at 37 °C in a 10 μL reaction containing 10 mM $MgCl_2$, 50 mM Tris-HCl, pH 7.5, 100 nM template *ftsZ* RNA, 100 μM DNA enzyme, and 10 units of RNasin for 0.5 h (lane 2), 1 h (lane 3), or 2 h (lane 4). The reactions were stopped by adding 83 mM EDTA and then analyzed on 10% polyacrylamide gels containing 8 M urea. Lane 1 is a control reaction incubated for 2 h in the absence of DNA enzyme. S: substrate; P1 (857nt) and P2 (368nt): DNA enzyme digestion products.

Design of an RNA-Cleaving DNA Enzyme. The 10−23 DNA enzyme, a ssDNA with sequence-specific cleavage activity, has been recently demonstrated as a powerful tool to inhibit gene expression (*8*, *17*, *19*, *20*). Like many other agents that function by hybridization with targeted mRNA, the DNA enzyme must compete with the target's own intramolecular base pairing that forms its characteristic secondary structure. Fortunately, DNA enzyme cleavage sites exist abundantly in most target mRNAs and thus provide a host of opportunities to achieve maximum cleavage efficiency. As noted previously, to find sites in the *ftsZ* mRNA that are accessible to the DNA enzyme, a free energy-based RNA folding analysis program, mfold (*21*) was used to predict a secondary structure of the full-length *ftsZ* mRNA (1225 nt). As shown in Figure 2A, the predicted secondary structure contains multiple loop regions. Position 857 was selected as an optimal DNA enzyme target site since it was presented as one of the largest loop regions predicted.

In Vitro Cleavage of ftsZ RNA by the DNA Enzyme. Before testing the ability of the ssDNA expression vector to produce DNA enzyme molecules in bacterial cells, we evaluated the cleavage activity of the designed DNA enzyme in a cell-free system. The 1225 nt *ftsZ* RNA, produced by in vitro transcription, was used as a substrate, and the cleavage assay was carried out at 37 °C for a various period of time as indicated in a 10 μL reaction containing 10 mM $MgCl_2$, 50 mM Tris-HCl, pH 7.5, 100 nM template *ftsZ* RNA, 100 μM DNA enzyme, and 10 units of RNasin. As shown in Figure 3, the synthetic DNA enzyme can effectively cleave the *ftsZ* RNA in the time as short as 0.5 h, producing the products with expected sizes (368 and 857 nt).

Induction of RT Expression in DH5αpro Cells. To assess the capability of the ssDNA expression vector to produce active RT in bacterial cells under the induction condition, both RT protein expression and its biological activity were investigated. DH5αpro cells carrying pssXGb(FtsZ-DZ) were grown in the presence of 0, 100, or 200 ng/mL of aTc for either 1, 2, or 3 h. As shown in Figure 4A, in the absence of aTc, no expression of RT was detected in cells. Upon addition of aTc to the cultures, RT expression was induced significantly in both aTc dose- and time-dependent manners (Figure 4A). Using the RT-PCR assay described by Chen et

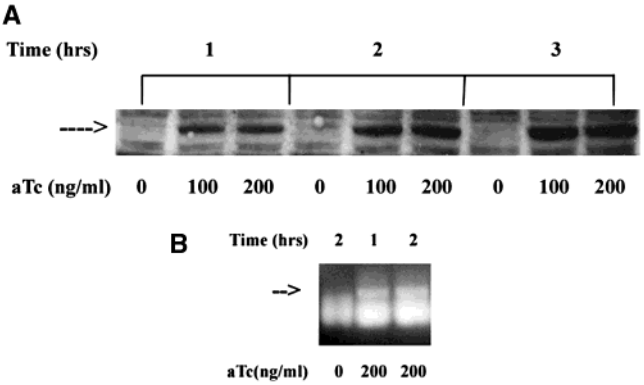

FIGURE 4: Expression of reverse transcriptase (RT) in DH5αpro cells. (A) DH5αpro cells carrying the pssXGb(FtsZ-DZ) vector were grown in the presence of 0, 100, or 200 ng/mL of aTc for 1 h (lanes 1−3), 2 h (lanes 4−6), or 3 h (lanes 7−9). The aTc treated cells were then lysed, and the RT expression was determined by Western blot analysis. (B) Activity of the expressed RT in aTc-treated cells was assayed using RT-PCR as described by Chen et al. (*17*). RT-PCR products were marked by the arrow.

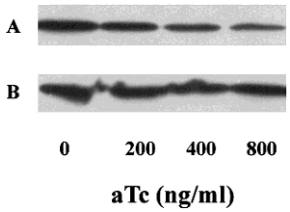

FIGURE 5: DNA enzyme-mediated repression of *ftsZ* gene expression. DH5αpro cells carrying the pssXGb(FtsZ-DZ) vector, or cells carrying pssXGa vector, were incubated in LB media with 0, 200, 400, or 800 ng/mL of aTc for 3 h. The cells were then lysed, and the FtsZ protein expression was determined by Western blot analysis. (A) DH5αpro cells carrying the pssXGb(FtsZ-DZ) vector. (B) DH5αpro cells carrying the control pssXGa vector.

al. (*17*), RT activity was detected in cells in the presence but not in the absence of aTc, as indicated by generating a PCR product of the expected size (Figure 4B).

DNA Enzyme-Mediated Repression of ftsZ Gene Expression. Given that the designed DNA enzyme was shown to be capable of cleaving *ftsZ* RNA in vitro (Figure 3) and an aTc regulated expression vector can produce active RT (Figure 4A and B), we wanted to exploit this ssDNA expression vector to generate the DNA enzyme in cells and determine the effects of the DNA enzyme on the expression of the *ftsZ* gene. DH5αpro cells carrying the pssXGb(FtsZ-DZ) vector (A) as well as negative control cells carrying the pssXGa vector (B) were grown in the presence of various amount of aTc (0, 200, 400, and 800 ng/mL) for 3 h. As shown in Figure 5, compared to the cells grown in the absence of aTc, the FtsZ expression level in the DH5apro carrying pssXGb(FtsZ-DZ) was reduced significantly upon the addition of aTc (Figure 5A). This reduction was not observed in the control cells (Figure 5B).

Inhibition of Bacterial Cell Proliferaion by in Vivo Expressed DNA Enzyme. Because the *ftsZ* gene is essential for bacterial division and viability (*12*) and our result as shown in Figure 5 indicates that the intracellularly generated DNA enzyme can significantly repress the *ftsZ* expression, we investigated the effect of the expressed DNA on bacterial cell proliferation. DH5αpro cells carrying the pssXGb(FtsZ-DZ) vector were grown in the presence of various amounts of aTc (0, 200, 400, and 800 ng/mL) for either 1 or 2 h, and viable cells were enumerated as described in the Materials and Methods. As shown in Figure 6A, cell growth inhibition was in a time- and aTc concentration-dependent manners. However, there is no significant inhibition of control cell (carrying the pssXGa vector) growth when various amounts of aTc were present, indicating that the growth inhibition is not due to an indirect effect of either aTc or expressed RT (data not shown). Furthermore, long filamentous cells were formed as a result of the cell growth inhibition (Figure 6B) (*13*).

DISCUSSION

We have constructed a tetracycline-regulated ssDNA expression vector by integrating the key components from our earlier version of a mammalian expression vector, pssXE(CMV), into the bacterial expression vector, pPROTet.E233. The original PBS in the mammalian expression cassette was replaced so that in vivo expressed RT can utilize bacterial tRNA(Val) as a primer for ssDNA synthesis. We chose the *ftsZ* gene, critical for bacterial division and viability, as our testing target and demonstrated that this novel inducible ssDNA expression vector can effectively produce *ftsZ* RNA-cleaving DNA enzyme. More importantly, the DNA enzyme generated in cells was shown to effectively inhibit both *ftsZ* gene expression and bacterial proliferation. Previous studies have shown that the endogenous genes *DicF* and *stfZ* encode antisense RNA, and the produced RNA molecules can block cell division in *E. coli* by inhibiting the translation of the *ftsZ* gene (*22*−*24*, *28*). Our results further confirm the essential role played by the *ftsZ* gene in cell proliferation. 10−23 DNA enzyme consists of a 15 nucleotide catalytic domain and two RNA target-binding domains (*9*, *10*). Similar to antisense ODNs, 10−23′ DNA enzyme binds to the RNA substrate through Watson−Crick base pairing. Although we believe that the cleavage of *ftsZ* mRNA by the expressed DNA enzyme molecules is responsible for the inhibitory effects on *ftsZ* gene expression and bacterial cell proliferation, other mechanisms such as the antisense mechanism could also be responsible for the biological effects.

Some investigators have explored the use of intracellularly generated RNA for regulating gene expression in bacterial cells (*29*−*31*). However, instability of antisense RNA, resulting from degradation by RNases in vivo, limits its potential application. Inouye et al. have utilized a bacterial retron system to generate ssDNA as a form of multicopy single-stranded DNA (msDNA), a complex of DNA and RNA (*32*−*35*). Antisense ODNs generated as form of msDNA in bacteria have been shown to inhibit the expression of a lipoprotein gene in *E. coli* (*32*).

In conclusion, the results presented here demonstrate that ssDNA can be effectively generated inside bacterial cells for the purpose of gene knock-down. Although we examined the production of the designed DNA enzyme, it should be feasible using this expression system to produce ssDNA of any desired sequence. More importantly, the ability of the expression system to produce ssDNA in response to induction allows the creation of conditional lethal phenotypes when genes are essential, which offers an important novel research tool for a screening library on a large scale for new antibiotic agents.

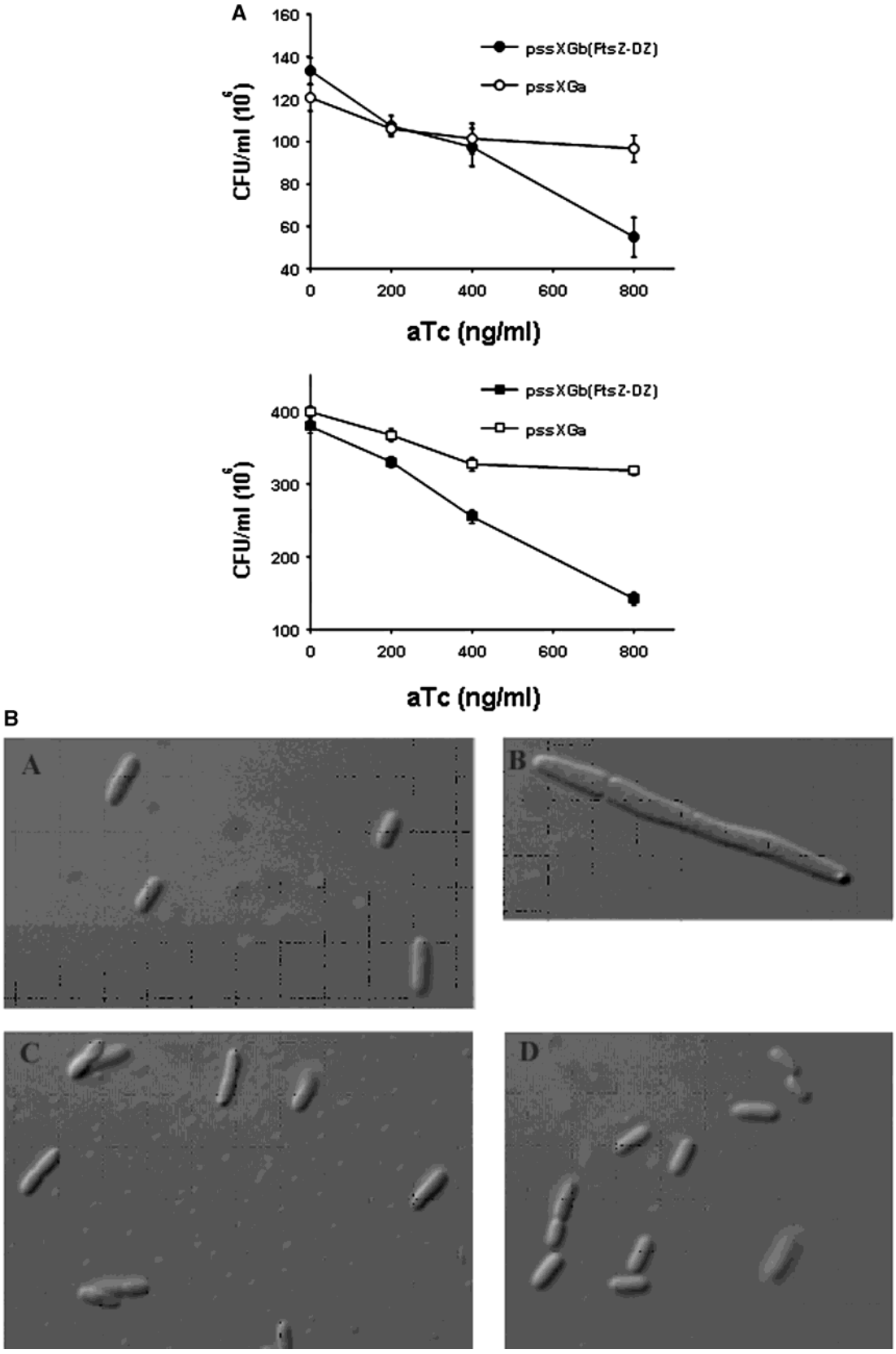

FIGURE 6: (A) Inhibition of bacterial proliferation by in vivo expressed DNA enzyme. Inhibition of bacterial proliferation by DNA enzyme expressed in cells was determined by measuring the viable cell count. DH5αpro cells carrying the pssXGb(FtsZ-DZ) vector were grown in the presence of 0, 200, 400, or 800 ng/mL of aTc for 1 (top panel) or 2 h (bottom panel). One milliliter of sample was removed at either 1 or 2 h for cell growth assay by measuring viable cell count. Viable cell count was done by diluting the cultures and plating them in triplicate on LB plates with the appropriate antibiotics. The plates were then incubated overnight at 37 °C, and the number of colonies was enumerated by visual inspection. (B) Morphology of DH5αpro cells carrying pssXGb(FtsZ-DZ). (A) DH5αpro cells carrying pssXGb(FtsZ-DZ), without aTc; (B) DH5αpro cells carrying pssXGb(FtsZ-DZ), with 800 ng/mL aTc; (C) DH5αpro cells carrying vector pssXGa, without aTc; and (D) DH5αpro cells carrying pssXGa, with 800 ng/mL.

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