J. Microbiol. Biotechnol. (2008), **18**(6), 1024–1032

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Molecular Analysis of the *Salmonella* Typhimurium *tdc* Operon Regulation

Kim, Min-Jeong, Sangyong Lim[1], and Sangryeol Ryu*

Department of Food and Animal Biotechnology, School of Agricultural Biotechnology, Center for Agricultural Biomaterials, and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea
[1]Radiation Research Center for Biotechnology, Korea Atomic Energy Research Institute, Jeongeup 580-185, Korea

Received: October 8, 2007 / Accepted: February 12, 2008

Efficient expression of the *Salmonella* Typhimurium *tdcABCDEG* operon involved in the degradation of L-serine and L-threonine requires TdcA, the transcriptional activator of the *tdc* operon. We found that the *tdcA* gene was transiently activated when the bacterial growth condition was changed from aerobic to anaerobic, but this was not observed if *Salmonella* was grown anaerobically from the beginning of the culture. Expression kinetics of six *tdc* genes after anaerobic shock demonstrated by a real-time PCR assay showed that the *tdcCDEG* genes were not induced in the *tdcA* mutant but *tdcB* maintained its inducibility by anaerobic shock even in the absence of *tdcA*, suggesting that an additional unknown transcriptional regulation may be working for the *tdcB* expression. We also investigated the effects of nucleoid-associated proteins by primer extension analysis and found that H-NS repressed *tdcA* under anaerobic shock conditions, and *fis* mutation delayed the peak expression time of the *tdc* operon. DNA microarray analysis of genes regulated by TdcA revealed that the genes involved in *N*-acetylmannosamine, maltose, and propanediol utilization were significantly induced in a *tdcA* mutant. These findings suggest that Tdc enzymes may play a pivotal role in energy metabolism under a sudden change of oxygen tension.

Keywords: *Salmonella* Typhimurium, *tdcA*, *tdc* operon, anaerobic shock

The metabolism of hydroxyamino acids, L-serine and L-threonine, together forms a network of pathways linking several amino acids to central primary metabolites such as pyruvate, oxaloacetate, and 3-phosphoglycerate, and C_1 units in the form of 5,10-methylene tetrahydrofolate [37]. Of numerous enzymes involved in the metabolic pathway of both amino acids in *Escherichia coli*, proteins encoded by the polycistronic *tdcABCDEFG* operon form an anaerobic pathway that degrades L-serine and L-threonine to acetate and propionate, respectively [20, 37]. L-Serine dehydratases (TdcG) and L-threonine dehydratases (TdcB) catalyze the irreversible deaminations of L-serine to pyruvate and L-threonine to 2-ketobutyrate [9, 20]. TdcE functions as a 2-keto acid formate-lyase, converting pyruvate to acetyl-CoA and 2-ketobutyrate to propionyl-CoA [20]. The coenzyme A-dependent cleavage of these keto-acids can occur *via* acetyl- or propionyl-phosphate intermediates that are subsequently catalyzed by acetate or propionate kinase (TdcD) with the generation of ATP [44, 47]. In addition, TdcA is a *trans*-acting positive regulator for the autogenous regulation of the *tdc* operon [16], and TdcC is an integral membrane protein implicated in the transport of serine and threonine into the cell [46]. Although the function of TdcF protein, a member of the YjgF family, is not yet known, Burman *et al.* [5] recently suggested that TdcF may play a role in sensing the levels of 2-ketobutyrate, based on analysis of crystal structure. The importance of the Tdc enzymes for the metabolism of L-serine and L-threonine stems from their ability to provide the cell with energy-rich keto acids that are catabolized to produce ATP *via* substrate-level phosphorylation [20, 37].

Maximum induction of the *tdc* operon is seen when *E. coli* is grown under anaerobic condition in a medium containing an amino acid complex but no catabolite-repressing sugars, such as glucose [15, 50]. Previous works on the amino acid requirements for the synthesis of TdcB indicate that a combination of several amino acids, especially threonine, serine, valine, and isoleucine, is essential for multivalent induction of TdcB in the presence of cyclic AMP (cAMP) [21, 54]. The global transcription factor cAMP receptor protein (CRP) provides principal control of the operon expression by direct binding to the *tdcA* promoter between positions −55 and −26 with respect to the *tdcA* transcription start site (Fig. 1B) [43, 53]. The efficient expression of the *tdc* operon also requires a functional integration host factor (IHF) that binds to the *tdc* promoter region between positions −118 and −88 (Fig. 1B) [51, 53]. On the other hand, a small histone-

*Corresponding author
Phone: 82-2-880-4856; Fax: 82-2-873-5095;
E-mail: sangryu@snu.ac.kr

like protein, HU, which is known to bend and compact chromosomal DNA, drastically reduces *tdc* transcription [52]. Mutational analyses of the genes encoding DNA gyrase and topoisomerase I also revealed that relaxation of supercoiled DNA significantly enhanced *tdc* transcription *in vivo* [45, 52].

In addition to the global regulatory proteins mentioned above, the operon-specific transcriptional activators also influence *tdc* operon expression. The regulatory gene *tdcA*, proximal to the *tdc* promoter, encodes a protein homologous to the LysR family of transcriptional activators, which has the helix-turn-helix motif of typical DNA-binding proteins, and is required for maximal induction of the *tdc* operon [16]. Genetic and biochemical experiments revealed that TdcA occupies its unique binding sites on the *tdcA* promoter around position −175 [19, Fig. 1B]. A separate regulatory gene, *tdcR*, which is located immediately upstream from *tdcA* in the opposite transcriptional orientation, is also required for maximal expression of the *tdc* genes in *E. coli* [19, 42]. Sequence analysis shows that the nucleotide sequences of the *tdc* operon in *Salmonella enterica* serovar Typhimurium are about ~80% identical to those in *E. coli*, and the amino acid sequences of the product of *tdc* genes from *S.* Typhimurium are almost identical, ~95%, to *E. coli* without gap (Fig. 1A). Interestingly, however, the *Salmonella tdc* operon lacks the *tdcR* and *tdcF* genes (Fig. 1A). In the current study, we performed transcriptional analyses to investigate the *Salmonella tdc* operon expression, and a DNA microarray analysis to survey the effect of *tdcA* mutation.

MATERIALS AND METHODS

Growth Conditions

Bacteria were routinely cultivated at 37°C in Luria–Bertani (LB) broth containing 1% tryptone, 0.5% yeast extract, and 1% NaCl. A stationary-phase culture that had been grown overnight with shaking was inoculated into fresh LB broth at a 1:100 dilution and grown aerobically to the different growth phases. This culture was then subjected to an anaerobic shock (*i.e.*, static culture conditions) to maximally induce *tdcA* expression. Antibiotics at the following concentrations were used when necessary: tetracycline 15 μg/ml, kanamycin 50 μg/ml, chloramphenicol 25 μg/ml.

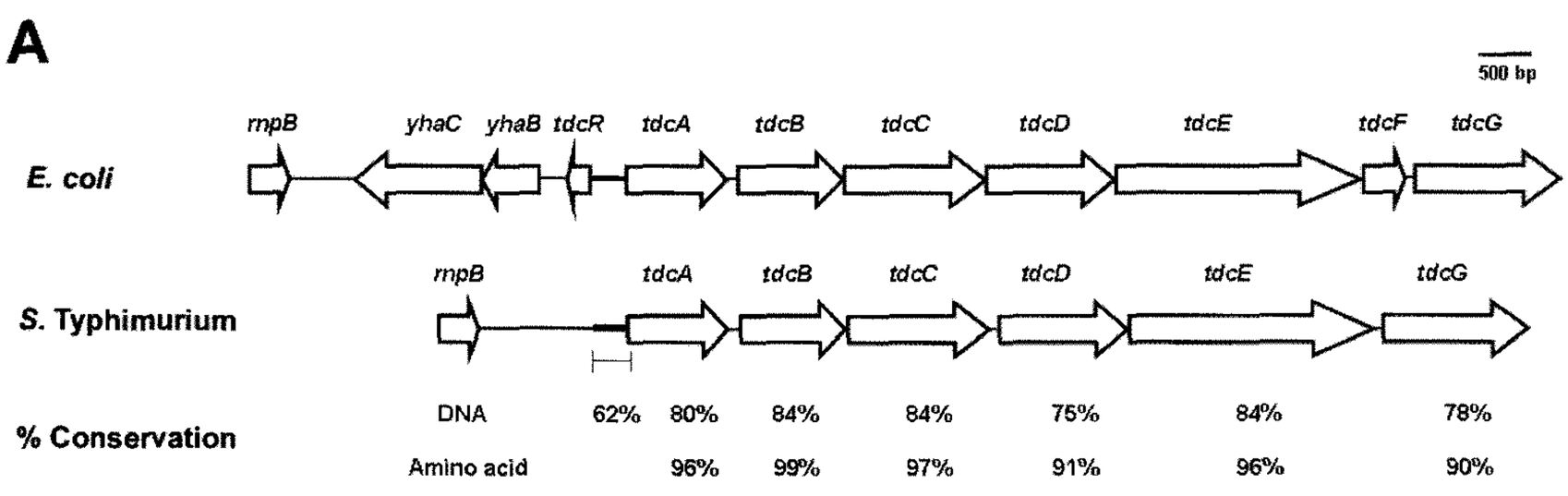

Fig. 1. Organization of the *tdc* operon and its regulatory region.
A. Schematic representation of the *tdc* operon region. The top line represents the *E. coli tdc* operon and upstream genes and the lower section represents the *S.* Typhimurium *tdc* operon. The percentages of conservation of nucleotide and deduced amino acid sequences between *S.* Typhimurium and *E. coli* are indicated for each *tdc* gene, and the *tdcA* promoter region. The map is drawn to scale. **B.** The nucleotide sequence of the *S.* Typhimurium *tdcA* promoter region. Nucleotides different in *E. coli* are shown directly below the *S.* Typhimurium sequences. Sequences protected by TdcA, IHF, and CRP [16, 52, 53] are boxed. The angled arrows designate transcriptional start sites of *E. coli* and *S.* Typhimurium *tdcA*, and the numbers on the left indicate the position relative to the +1 of *S.* Typhimurium *tdcA*. Translational start codons of *tdcA* and *tdcR* are boxed with dotted lines.

Table 1. The bacterial strains and plasmids used in this study.

Strain or plasmid	Description	Reference or source
Strains		
SL1344	Wild-type serovar Typhimurium *xyl rpsL hisG*	[28]
RJ1827	LT2 *fis*::*kan*, null mutation	[26]
YK3064	UK1 *hns105*::Tn10, Tet^R	[27]
JE3999	TR6583 *ihfB*::*cat*	[33]
SR3501	SL1344 *ΔtdcA*	This study
SR3502	SL1344 *fis*::*kan*	This study
SR3503	SL1344 *hns105*::Tn10, Tet^R	This study
SR3504	SR3501 *ihfB*::*cat*	This study
Plasmids		
pKD46	*bla* P_{BAD} *gam beta exo* pSC101 oriTS	[14]
pKD13	*bla* FRT *ahp* FRT PS1 PS4 oriR6K	[14]
pCP20	*bla cat cI*857 λP_R *flp* pSC101 oriTS	[14]

Primer Extension Assay

Cells from 50-ml culture were collected by centrifugation, and total RNAs were isolated using a Trizol reagent (Life Tech., Inc.) according to the manufacturer's instructions. Primer extension analysis was carried out as described previously [28]. To study the transcription of a *tdcA* gene, a tdc3 primer (5'-TTG CGG CAG AAC CGA TAG AAC CAC TTC TAA-3') complementary to +56 to +86 relative to the translation start site was used.

Construction of Strains

Salmonella enterica serovar Typhimurium SL1344 was used as a wild-type strain in this study. Isogenic mutant strains were obtained through P22HT-mediated transduction of the mutation allele to wild type [7]. The bacterial strains and plasmids used in this study are listed in Table 1. The one-step gene inactivation method was used to construct a derivative of the wild-type *Salmonella*, deleting the *tdcA* gene [14]. Briefly, a kanamycin cassette from pKD13 was amplified using primers RedF (5'-CCT GCC GTT CAC AAT GTT ATT TCA GAG CGG TAG ATA TGA AGT GTA GGC TGG AGC TGC TTC-3') and RedR (5'-TCC ATA TCA TTC AAT TTC TAT TAA CTG CCT GCG TCT GCA TAT TCC GGG GAT CCG TCG ACC-3'), and the resulting PCR product was introduced into the C-terminal end of *tdcA* in the chromosome by homologous recombination. The correct strain containing the kanamycin resistance cassette was screened, and then the cassette region was removed using the plasmid pCP20 as described previously [14].

Real-Time PCR (RT-PCR) Analysis

Total RNA was prepared from bacterial cultures with the RNeasy mini kit (Qiagen) according to the manufacturer's instructions. For exclusion of contaminated genomic DNA, total RNA was treated with the TURBO DNA-free Kit (Ambion) and subsequently purified with the RNeasy mini kit (Qiagen). For RT-PCR analysis, cDNA was synthesized from 1 μg of DNase-treated total RNA using the Omniscript Reverse Transcription Kit (Qiagen) and random hexamers (Invitrogen) as recommended in the manufacturer's instructions. The SYBR green RT-PCR assay was performed in a 20-μl PCR mixture volume containing 10 μl of 2XiQ SYBR Green Supermix (Bio-Rad), 0.2 μM (each) specific primer sets, and 2 μl of cDNA sample. Amplification of the primers, data acquisition, and quantitative analysis were carried out with the iCycler real-time detection system. PCR reactions were performed as follows: one cycle of 94°C for 5 min, and then 40 cycles of 94°C for 20 s, 50°C for 20 s, and 72°C for 20 s. Following the amplification, melting temperature analysis of PCR products was performed to determine the specificity of the PCR. A standard curve was established using genomic DNA for each gene studied to confirm that the primers amplified at the same rate and to validate the experiment. The mRNA expression level of the target gene was normalized to the 16S rRNA expression level. The sequences of the primers used are presented in Table 2.

Probe Preparation and Hybridization

Total bacterial RNA was isolated from log-phase cells subjected to anaerobic shock, using an SV total RNA isolation kit (Promega) according to the manufacturer's instructions. The quality and integrity of the prepared total RNAs were confirmed with the use of an Agilent 2100 bioanalyzer (Agilent, Palo Alto, CA, U.S.A.), and by spectrophotometry. A total of 50 μg of RNA with 3 μg of random hexamer dissolved in 29.5 μl of nuclease-free water was denatured at 65°C for 10 min and then placed on ice. After addition of 6 μl of 0.1 M DTT, 12 μl of first-strand buffer (5×), 1.5 μl of dNTP mix (25 mM dATP, 25 mM dGTP, 25 mM dCTP, 10 mM dTTP), 4 μl of reverse transcriptase (Superscript II; Invitrogen), 2 μl of RNasin (Promega), and 4 μl of either Cy3- or Cy5-conjugated dUTP (Amersham Biosciences), the labeling mixture of 60 μl was incubated at 42°C for 2 h and then supplemented with 2 μl of Superscript II at the end of the first hour. Each probe was denatured with 10 μl of

Table 2. Primers for RT-PCR analysis.

Gene	Primer sequence (5' to 3')	
	Forward	Reverse
tdcA	ccg caa aat cgt tag ggt ta	cat ttc gcg ggt gat aga tt
tdcB	aat atg cag cgt acg ggt tc	acg act ttg ccg tca ata cc
tdcC	ctt acg caa acc cga aca tt	agc aga cca atc agg gtg ac
tdcD	gtc agg tgg cgg tat ttg at	cat cga ggt caa gca gtt ca
tdcE	gcc gaa cct gac cat act gt	ctg cat ctg ctt acc gat ga
tdcG	ttg tcg aag agg agc gtt tt	cag cgt caa tct gct ctt tg
rrs	cgg gga gga agg tgt tgt g	cag ccc ggg gat ttc aca tc

1 M NaOH and neutralized with 10 μl of 1 M HCl, followed by purification with a PCR purification kit (Qiagen) and concentration with a speed vacuum drier. Each probe separately labeled with Cy3 or Cy5 was resuspended in 20 μl of distilled water, respectively. Equal volumes of labeled probes (each 20 μl) from wild type and the *tdcA* mutant strain were mixed with 40 μl of 2× hybridization solution consisting of 50% formamide, 10× SSC, and 0.2% SDS and denatured by boiling for 5 min. Probes were hybridized simultaneously to a chip at 58°C for 16 h in a hybridization chamber.

Scanning and Data Analysis

Salmonella DNA chips were kindly provided by Dr. McClelland. Details concerning the construction and characteristics of the *Salmonella* microarray used in this study have been described elsewhere [36]. Briefly, 97.5% of all 4,498 genetic elements annotated in *S.* Typhimurium LT2 are represented in triplicate on the array, as are 104 of the 109 annotated genetic elements of the virulence plasmid pSLT. Scans were performed with a GenePix 4000B scanner (Axon Instruments, Union City, CA, U.S.A.) by using the ScanArray 2.1 software (Packard BioChip Technologies). Signal intensities were quantified by using the GenePix 3.0 software (Axon Instruments, Union City, CA, U.S.A.). Normalization of gene expression by a LOWESS regression was applied for 6 data, which were obtained from two biological replicates. The genes were considered differentially expressed when the logarithmic gene expression ratios were more than a 2-fold difference in the expression level. Statistical significance of the data was determined by Student's *t* test. *P* values less than 0.001 were taken as statistically significant.

RESULTS AND DISCUSSION

Expression of the *tdcA* is Maximally Induced by Anaerobic Shock

Synthesis of threonine dehydratase (TdcB) is initiated 20–30 min after the culture is made anaerobic and continued until the cells reach the end of the exponential growth phase [34]. Because oxygen does not inactivate TdcB *in vivo* [13], the modulation of TdcB activity is known to be dependent mainly on the expression level of the gene. The promoter of the *tdcB* gene is located upstream of the *tdcA*, as shown on Fig. 1A. Above all, the transcription start site of the *S.* Typhimurium *tdcA* was determined by primer extension assay. The result in Fig. 2A shows one major transcript originating from nucleotide 31 relative to the translation start codon of *tdcA*, although the transcriptional start site of *Salmonella* was mapped 2 nucleotides downstream of that previously reported in *E. coli* (Fig. 1A). To investigate the expression of the *Salmonella tdc* operon under anaerobic growth conditions, wild-type cells (SL1344) were transferred into the static culture condition following aeration for 2 h, and the cultures subjected to the anaerobic shock were harvested for transcription analysis after 10, 30, 60, and 120 min. The expression of *tdcA* increased rapidly, peaked at 30 min after anaerobic shock, and decreased thereafter.

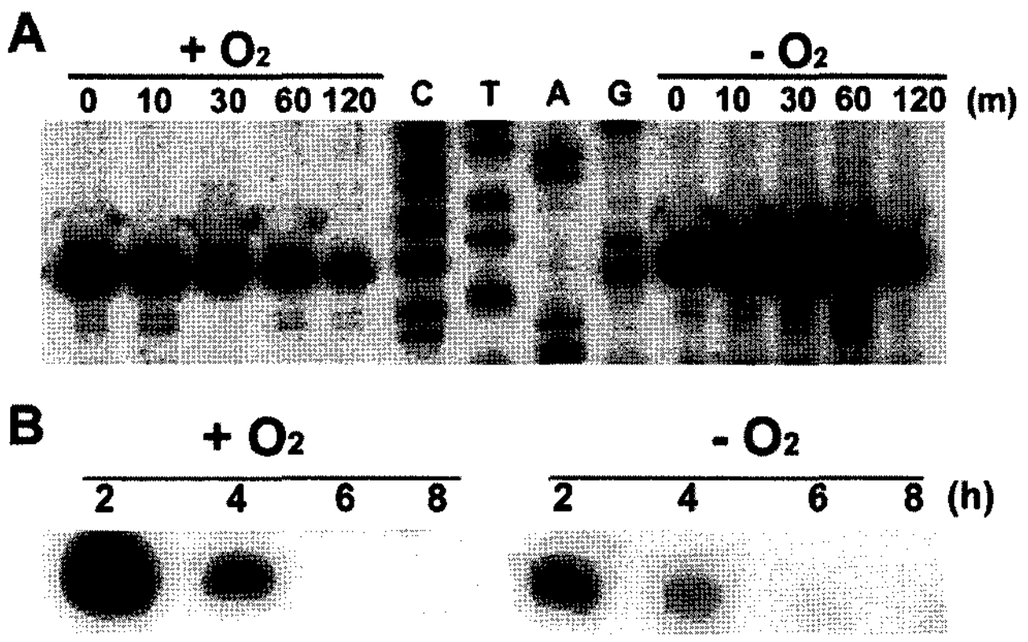

Fig. 2. Effect of anaerobiosis on *tdcA* gene expression as analyzed by primer extension analysis. Aliquots of 30 μg of total RNA were co-precipitated and annealed with end-labeled tdc3 primer. **A**. *S.* Typhimurium SL1344 was grown aerobically to the mid-exponential phase (~ 2 h), transferred into static culture conditions ($-O_2$), and harvested at the indicated times. Total RNA was also isolated from cultures maintaining aerobic conditions ($+O_2$). The initial time, 2 h post-inoculation, was designated the reference time point (0 m). **B**. After inoculation of stock cultures, cells (SL1344) were cultivated aerobically with shaking ($+O_2$) or anaerobically ($-O_2$) and total RNA was isolated at 2 h intervals from 2 to 8 h after inoculations and subjected to primer extension analysis.

When cells were grown for 2 h under anaerobic growth, the level of *tdcA* transcription returned to the initial level (Fig. 2A). However, when cells were grown aerobically with shaking, *tdcA* expression was decreased gradually during the same growth period monitored (Fig. 2A). In addition, *tdcA* expression was not induced unless cultures grown aerobically were transferred into static culture conditions (Fig. 2B). These results suggest that TdcR may not play a role in the anaerobic induction of *tdc* expression because the *tdc* operon of *Salmonella* lacks the *tdcR* gene. TdcR specifies a small protein essential for efficient transcription of the *E. coli tdc* operon. A multicopy $tdcA^+$ plasmid introduced into the *tdcR* mutant strain completely restores *tdc* expression in *E. coli* [19].

Requirement for an anaerobic environment is presumably linked to intracellular levels of cyclic AMP. When an aerobically growing culture is made anaerobic, internal cAMP levels extensively increase and subsequently lead to an activation of *tdc* expression [34]. Although the promoter region (~300 bp) of *tdcA* was only 62% conserved between *E. coli* and *S.* Typhimurium (darker line in Fig. 1A), the location and nucleotide sequences of the CRP binding site of *S.* Typhimurium were almost the same as that of *E. coli* (Fig. 1B). These results suggest that the regulatory role of the cAMP·CRP complex in the *Salmonella tdc* operon is not much different from *E. coli*.

Peak Expression of *tdcA* was Delayed in Fis Mutant

Bacteria harbor several abundant small DNA-binding proteins collectively called nucleoid-associated proteins (NAPs) such as Fis, H-NS, HU, and IHF, which can affect

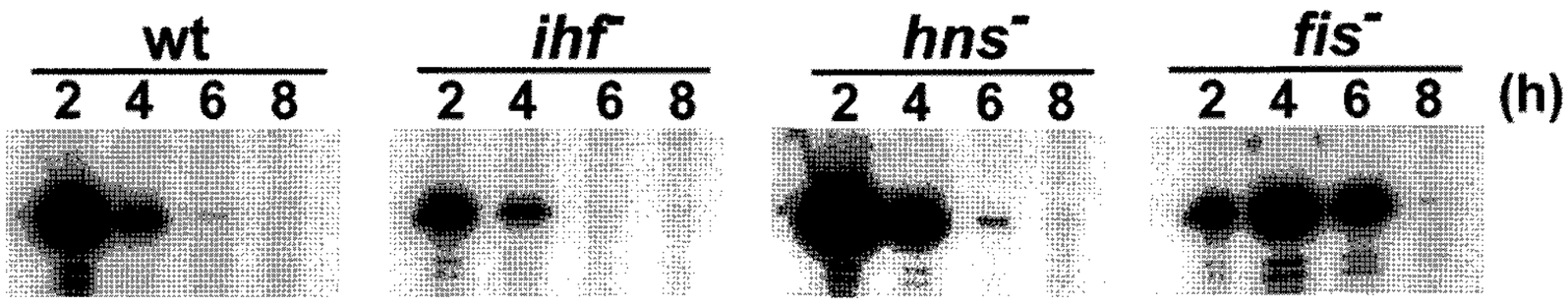

Fig. 3. Effects of IHF, H-NS, and FIS on the anaerobic induction of the *tdcA* gene analyzed by primer extension analysis.
Salmonella cultures were grown aerobically in LB medium for 2, 4, 6, and 8 h after inoculation and then each culture was transferred into static culture conditions for 30 min. Total RNA was isolated from aliquots of cells subjected to this anaerobic shock. Thirty μg of total RNA from the indicated strains was co-precipitated and annealed with the end-labeled tdc3 primer. The products were resolved on a 6% sequencing gel.

various biological processes involved in site-specific DNA recombination, DNA replication, and transcription [12]. Among the NAPs, IHF and HU have been shown to exert regulatory effects on *tdcA* expression in *E. coli*; IHF activates *tdcA* expression by binding directly to the promoter [51, 53], whereas HU reduces *tdcA* expression indirectly by altering overall DNA topology [52]. To test whether other NAPs, FIS and H-NS, are involved in the anaerobic induction of the *tdc* operon in *S.* Typhimurium, we isolated total RNAs from *fis* (SR3502) and *hns* (SR3503) mutant strains subjected to anaerobic shock for 30 min and analyzed these using primer extension assay. When an exponentially growing aerobic culture (2 h after inoculation) was made anaerobic, wild-type cells (SL1344) showed the highest induction of *tdcA* expression (Fig. 3). In addition, IHF was required for positive regulation of the *tdcA* gene as in *E. coli*, although the IHF-binding sequences of *S.* Typhimurium were significantly deviated from those of *E. coli* (Fig. 1B). However, H-NS inhibits *tdcA* transcription in *Salmonella* because *tdcA* expression was increased slightly in the *hns* mutant (Fig. 3). H-NS can almost always silence promoters by interacting with distal sequences and has been shown to counteract transcriptional activators [11].

Interestingly, Fis appeared to delay the peak expression of *tdcA* after transition to anaerobic conditions, as shown in Fig. 3. Fis can influence transcription through its ability to affect the level of DNA supercoiling in the cell. The *fis* expression is maximal at high levels of negative supercoiling [41], and acts as a repressor of both *gyrA* and *gyrB* [23, 39] but as an activator of *topA* [49], allowing Fis to relax overall DNA topology. Moreover, Fis affects growth phase-specific alterations in the supercoiling level of DNA that keeps DNA in a moderately supercoiled state [30, 40]. Considering that *tdc* expression is also modulated by the DNA supercoiling [45, 52], Fis may be required to adjust optimum DNA supercoiling for the maximum expression of *tdcA*. However, we also cannot rule out the possibility that Fis may influence *tdcA* transcription directly by specific binding to the *tdcA* promoter.

The *tdcA* Promoter Can Be Induced by Anaerobic Shock in the Absence of TdcA

Anaerobic regulation of the *tdc* operon is mediated by the FNR and ArcA proteins, but they appear to act indirectly [8]. Thus, to know the role of TdcA in an anaeorobic induction of the *tdc* operon, the mRNA level of each gene of the *tdc* operon was determined by RT-PCR analysis using RNA prepared from wild-type and *tdcA* mutant strains. *Salmonella* was grown aerobically for 2 h in LB medium, shifted to anaerobic condition, and total RNA was isolated from aliquots of cells obtained after 30 min. Expression levels of the target genes were normalized to that of the 16S rRNA gene. As expected, although the fold increases were somewhat different between genes, the expression of all *tdc* genes was activated by a 30-min anaerobic shock (Table 3). Interestingly, *tdcA* mutation abolished the anaerobic induction of the *tdcC*, *D*, *E*, and *G* genes, but the *tdcB* gene was still induced, nine-fold, by anaerobic shock, although the fold increase was lower by about ten-fold than that of the wild type. Because another promoter-like element of *tdcB*, which is located within the coding region of *tdcA* near the 3' end [17], was absent in our *tdcA* mutant strain (SR3501), the *tdcA* promoter was likely solely responsible for the anaerobic induction of *tdcB* in the absence of TdcA (Fig. 1B). This result suggests that TdcA is not a sufficient but a necessary factor for anaerobic induction. Our results add another line of complex and redundant regulatory pathways in the anaerobic regulation of *tdc* genes, reported to be mediated by ArcA/FNR, TdcA, CRP, and/or DNA supercoiling [8, 16, 34, 52].

Another feature noted in the RT-PCR results is the higher induction of *tdcCDEG* than *tdcAB* when the wild-type culture was made anaerobic (Table 3). Because the C_T values of all *tdc* genes were around ~16 after anaerobic shock, the higher induction of *tdcC* to *G* may be caused by the low mRNA level under aerobic growth condition; the C_T values for the *tdcC* to *G* genes were about 26, which corresponds to the 16-fold reduction of the mRNA level compared to the *tdcA* and *B*. Furthermore, in the *tdcA* mutant strain, the transcription initiated from the *tdcA* promoter by anaerobic shock appeared to be blocked in front of *tdcC*; the difference of C_T values between *tdcB* and *tdcC* was also equivalent to about 16-fold (Table 3). This result suggests that an additional unknown transcriptional regulation may be working for *tdcB*. A similar case was reported in the *E. coli mreBCD* operon. Most of the transcripts from three promoters of the *mreBCD* operon are expressed as a

Table 3. RT-PCR analysis of *tdc* gene expression by anaerobic shock.

Gene	TdcA[a]	Cycle threshold (C_T±SD)[b]		Fold increase[c]
		Aerobic condition	Anaerobic shock	
tdcA	+	20.99±0.82	16.62±0.92	20
	–	NA[d]	NA	
tdcB	+	21.62±1.01	14.79±0.99	114
	–	26.09±1.05	22.88±1.46	9
tdcC	+	25.99±2.80	17.74±3.13	304
	–	26.93±4.13	27.45±2.09	0.7
tdcD	+	25.40±1.75	17.40±0.93	255
	–	26.61±1.50	27.01±3.37	0.7
tdcE	+	26.29±1.11	16.71±0.46	767
	–	24.87±0.19	27.05±1.27	0.2
tdcG	+	25.95±3.15	17.22±2.35	426
	–	25.32±2.72	27.08±2.04	0.3

[a]+, Wild-type strain (SL1344); –, *tdcA* mutant strain (SR3501).
[b]Values are the mean±standard deviation of RT-PCR analysis performed three times in duplicate.
[c]Using the modified comparative method [22, 25, 29] $2^{-\Delta CT}$.
[d]Not amplified.

monocistronic *mreB* mRNA, and only 1–2% of the transcripts are expressed as a polycistronic *mreBCD* mRNA [48].

tdcA Mutation Caused an Induction of Genes for Some Sugar Metabolism.

To define the repertoire of *Salmonella* genes whose expressions are altered by *tdcA* mutation, we used a DNA microarray that includes 4,498 ORFs from *Salmonella* Typhimurium LT2 and 104 ORFs from the pSLT virulence plasmid [36]. Total RNA was extracted from an exponentially growing culture subjected to anaerobic shock for 30 min, and used to make cDNA that was labeled and hybridized to a microarray (see Materials and Methods). Among genes showing at least a 2-fold change relative to wild type with a statistically significant expression ratio ($p<0.001$), 17 genes were reduced and 51 genes were induced in the *tdcA* mutant under anaerobic shock conditions (Tables 4 and 5). Except fir the *tdcA* regulon, the expression of genes involved in flagellar biosynthesis such as *fljA*, *fljB*, and *flgN* were reduced by *tdcA* mutation (Table 4). Flagella play a role in attachment to and invasion of various cultured cells, as *S.* Typhimurium strains carrying a null mutation in the flagellar genes were less able to enter cultured epithelial cells [6, 10, 38], suggesting that the *tdc* operon may be involved in the virulence of *Salmonella*.

Over 50% of 51 genes induced by *tdcA* mutation are associated with energy metabolism pathways including sugar transport and utilization (Table 5). Among them, all five genes of the *nan* operon, *nanAT*, *nanEK*, and *yhcH*, encoding proteins for utilization of *N*-acetylmannosamine (ManNAc), were induced more than 5-fold in the *tdcA* mutant strain. The amino sugars are particularly useful to bacteria as energy sources since they supply both carbon and nitrogen. However, unlike the two common amino sugars, *N*-acetylglucosamine (GlcNAc) and *N*-acetylneuraminic acid (NANA), ManNAc may not be used efficiently as a carbon source by wild-type *E. coli* [35]. Therefore, *tdcA* mutation, which enhanced utilization of ManNAc, allows bacteria to activate pathways for dissimilation of the three amino sugars GlcNAc, ManNAc, and NANA, all converging at the step of GlcNAc-6-P. Genomic analysis using the microarray demonstrated a higher expression of the maltose system in the *tdcA* mutant (Table 5). Maltose and maltodextrins are present in high concentrations in the intestinal tracts of animals as by-products of starch metabolism. Both transport

Table 4. List of the genes reduced by *tdcA* mutation.

Common name	Systematic name	Function	Ratio (*tdcA*/wt)
tdcG	STM3240	L-Serine deaminase	0.11
tdcD	STM3242	Propionate kinase/acetate kinase II, anaerobic	0.14
tdcE	STM3241	Pyruvate formate-lyase 4/2-ketobutyrate formate-lyase	0.14
zraP	STM4172	Zinc-resistance-associated protein	0.21
tdcC	STM3243	HAAAP family, L-threonine/L-serine permease, anaerobically inducible	0.28
yifE	STM3898	Putative LysR type transcriptional regulator with *pssR*	0.29
fljA	STM2770	Flagellar synthesis: repressor of *fliC*	0.32
tdcB	STM3244	Threonine dehydratase, catabolic	0.32
ydfZ	STM1509	Putative cytoplasmic protein	0.33
fljB	STM2771	Flagellar synthesis: phase 2 flagellin (filament structural protein)	0.39
rplP	STM3433	50S ribosomal subunit protein L16	0.40
rpsQ	STM3431	30S ribosomal subunit protein S17	0.46
rpsO	STM3283	30S ribosomal subunit protein S15	0.47
yhhK	STM3565	Putative acetyltransferase	0.48
flgN	STM1171	Flagellar biosynthesis: believed to be export chaperone for FlgK and FlgL	0.48
rplQ	STM3414	50S ribosomal subunit protein L17	0.49
lpp	STM1377	Murein lipoprotein, links outer and inner membranes	0.50

Table 5. List of the genes induced by *tdcA* mutation.

Common name	Systematic name	Function	Ratio (*tdcA*/wt)
yhcH	STM3335	Putative cytoplasmic protein	5.82
nanK	STM3336	Putative ManNAc kinase	5.81
nanA	STM3339	*N*-Acetylneuraminate lyase (aldolase)	5.60
nanT	STM3338	MFS family, sialic acid transport protein	5.42
	STM1132	Putative sugar transport protein	5.31
lamB	STM4231	Maltose high-affinity receptor	5.06
nanE	STM3337	Putative ManNAc-6P epimerase	4.87
cspB	STM1996	Putative cold-shock protein	4.73
	STM1133	Putative dehydrogenases and related proteins	4.44
malE	STM4229	Maltose transport protein	4.34
	STM1130	Putative inner membrane protein	4.16
	STM1128	Putative sodium/glucose cotransporter	4.15
	STM1131	Putative outer membrane protein	4.01
ansB	STM3106	Periplasmic L-asparaginase II	3.89
galP	STM3091	MFS family, galactose:proton symporter	3.77
	STM0650	Putative hydrolase C-terminus	3.75
malK	STM4230	Maltose transport protein; phenotypic repressor of *mal* operon	3.68
	STM0649	Putative hydrolase *N*-terminus	3.52
malM	STM4232	Periplasmic protein of *mal* regulon	3.35
malF	STM4228	ABC superfamily (membrane), maltose transport protein	3.16
sbmC	STM2061	DNA gyrase inhibitor	3.00
	STM2343	Putative cytoplasmic protein	2.93
mglB	STM2190	ABC superfamily (peri_perm), galactose transport protein	2.83
	STM4540	Putative glucosamine-fructose-6-phosphate aminotransferase	2.79
	STM0651	Putative permease	2.73
yjfO	STM4379	Putative lipoprotein	2.69
lysA	STM3013	Diaminopimelate decarboxylase	2.68
	STM1129	Putative inner membrane protein	2.60
	STM2342	Putative inner membrane protein	2.58
phsA	STM2065	Hydrogen sulfide production: membrane anchoring protein	2.56
pduB	STM2039	Propanediol utilization: polyhedral bodies	2.53
pduF	STM2037	Propanediol utilization: propanediol diffusion facilitator	2.52
allD	STM0528	Ureidoglycolate dehydrogenase	2.50
celC	STM1314	PTS family, sugar-specific enzyme III for cellobiose, arbutin, and salicin	2.48
celF	STM1316	Phospho-beta-glucosidase (cellobiose-6-phosphate hydrolase)	2.47
manY	STM1831	Sugar-specific PTS family, mannose-specific enzyme IIC	2.41
allC	STM0527	Allantoate amidohydrolase	2.40
glpF	STM4087	MIP channel, glycerol diffusion	2.40
phsC	STM2063	Hydrogen sulfide production: membrane anchoring protein	2.37
sulA	STM1071	Suppressor of lon; inhibitor of cell division and FtsZ ring formation	2.30
pduD	STM2041	Propanediol utilization: dehydratase, medium subunit	2.29
yneA	STM4077	Putative ABC superfamily (peri_perm), sugar transport protein	2.28
sdaC	STM2970	Putative HAAAP family, serine transport protein	2.27
pduM	STM2048	Propanediol utilization	2.25
pduC	STM2040	Propanediol utilization: dehydratase, large subunit	2.24
malP	STM3514	Maltodextrin phosphorylase	2.19
pduO	STM2050	Propanediol utilization: B_{12} related	2.19
cbiC	STM2033	Synthesis of vitamin B_{12} adenosyl cobalamide precursor	2.19
rbsA	STM3883	ABC superfamily (atp_bind), D-ribose high-affinity transport protein	2.18
pduL	STM2047	Propanediol utilization	2.14
yjfN	STM4378	Putative inner membrane protein	2.14

and utilization of these compounds are regulated by MalT, a regulator required for transcription at *mal* promoters [4]. Although the expression of *malT* in the *tdcA* mutant compared with wild type was increased only 1.5-fold (data not shown), the expression of genes under the control of MalT was induced approximately three to four-fold above the levels of the wild type in the *tdcA* mutant (Table 5). We also found that genes required for the degradation of propanediol were more expressed in the *tdcA* mutant. Propanediol is a by-product of catabolism of rhamnose and fucose [1, 31], and the *pdu* operon encodes enzymes responsible for propionate production from propanediol [3]. Collectively, *tdcA* mutation may enable *Salmonella* to utilize versatile carbon sources. Considering that Tdc enzymes formed under anaerobic conditions are involved in energy metabolism, rather than in threonine or serine degradation *per se* [20, 37], when the *tdc* operon cannot operate efficiently, the activation of carbon catabolism genes could meet the cellular need for energy production before the full development of ATP synthesis from anaerobic electron transport-mediated processes linked to the ultimate reduction of fumarate, nitrate, or protons *via* anaerobic cytochrome systems [18].

Acknowledgments

This work was supported by a Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MOST) (No. 2007-04213). M. Kim was the recipient of a graduate fellowship provided by the Ministry of Education through the Brain Korea 21 Project.

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