

SHORT COMMUNICATION

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Influence of DNA topology on expression of the *tdc* operon in *Escherichia coli* K-12

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Abstract TdcB activity expressed from the chromosomal gene and LacZ expression from single-copy *tdc-lacZ* transcriptional and translational fusions were measured in *Escherichia coli* strains harboring mutations in the genes encoding DNA gyrase, topoisomerase I and the HU protein. The pattern of *tdc* operon expression in these mutants suggests that relaxation of supercoiled DNA enhances *tdc* transcription in vivo.

Key words *tdc* operon · Gene regulation · DNA supercoiling · HU protein · Topoisomerases

The *tdc* operon of *Escherichia coli* K-12, which is involved in transport and metabolism of threonine and serine during anaerobic growth, consists of three genes, *tdcA*, *tdcB*, and *tdcC*, which encode, respectively, a regulatory protein, the enzyme biodegradative (catabolic) threonine dehydratase, and an L-threonine-L-serine permease (Goss et al. 1988; Sumantran et al. 1990). The TdcA polypeptide, with a helix-turn-helix motif typical of DNA-binding proteins, is required for autoregulation of the *tdcAp* (Ganduri et al. 1993). The efficient in vivo expression of *tdc* also requires a small polypeptide encoded by *tdcR*, located immediately upstream of *tdcABC* in opposite transcriptional orientation (Schweizer and Datta 1989b). Furthermore, both integration host factor (IHF) and the cyclic AMP (cAMP)–catabolite gene activator protein (Crp) complex, which bind to their specific DNA sequences in the *tdc* promoter, act in concert to activate *tdc* transcription (Wu and Datta 1992; Wu et al. 1992).

To establish whether the *tdc* operon is also regulated negatively, we searched for a *tdc* “repressor” by transforming MC4100(λ SH241) [λ SH241 ϕ (*tdcAB*'-'*lacZ*)] (Ganduri et al. 1993) with an *E. coli* genomic library, constructed in the expression vector pTrc99A (Pharma-

cia) containing a Kan^r cassette (Schweizer and Datta 1989b), and screening for Lac⁻ transformants on X-gal (5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside) plates (Miller 1992). From a few such transformants we selected one for isolation and characterization of the plasmid DNA. As shown in Fig. 1, the plasmid pGL3.7, containing a 3.7 kb *E. coli* DNA insert prevented TdcB expression when introduced into the wild-type DH5 α F' (Hagewood et al. 1994). TdcB expression in other *E. coli* strains including MC4100, W3110, C600R and NM522 was also severely depressed by pGL3.7. Subcloning of various restriction fragments of pGL3.7, followed by transformation of DH5 α F', indicated that a 1.3 kb *EcoRV* fragment in pGL1.3 encoded a repressor-like activity (Fig. 1).

DNA sequence analysis of the fragment and a GenBank database search revealed that the cloned insert contained, among other sequences, the 270-bp *hupA* coding region (Kano et al. 1987). That the *hupA* gene product,

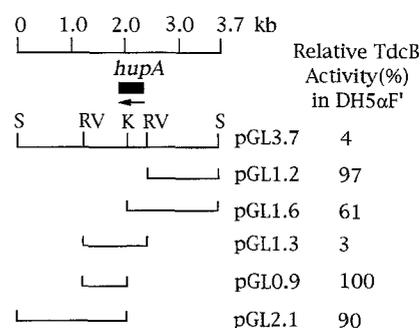


Fig. 1 Restriction map of the *hupA* region and TdcB expression in DH5 α F' harboring various restriction fragments cloned into the pTrc99A vector, yielding the pGL series of plasmids. Abbreviations: S, *Sau3A*; RV, *EcoRV*; K, *KpnI*. Threonine dehydratase activities of cells transformed with the indicated plasmids were assayed after anaerobic incubation in TYE medium as described (Hobert and Datta 1983). The specific activity is expressed relative to the value obtained with DH5 α F' harboring only the pTrc99A vector (3799 nmol of α -ketobutyrate formed per min per mg of protein)

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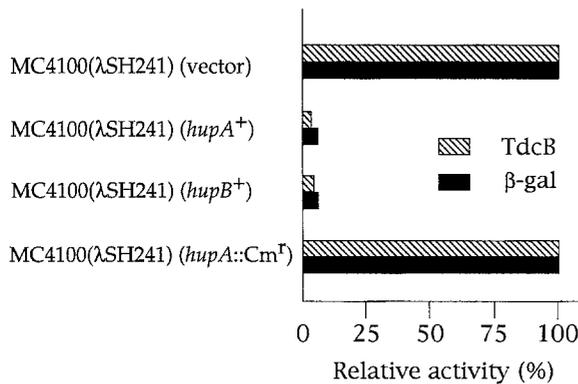


Fig. 2 Effects of multicopy *hup* genes on *tdc* expression. β -Galactosidase and threonine dehydratase activities of strain MC4100(λ SH241) transformed separately with the plasmids pYW409, pYW410 and pYW411 bearing, respectively, the cloned *hupA*, *hupB*, and *hupA::Cm^r* and the vector (pTrc99A) were measured as described (Miller 1992; Hobert and Datta 1983). Enzyme activities are normalized with respect to the values obtained with the vector (1787 units and 3914 units of β -galactosidase and threonine dehydratase, respectively)

and not the flanking DNA, prevented *tdc* expression was confirmed by cloning into pTrc99AKan^r a 551-bp fragment encompassing *hupA* with its own promoter and terminator sequences (from nucleotide -57 to +494, Kano et al. 1987), which was amplified by polymerase chain reaction (PCR) employing appropriate primers. When this *hupA*⁺ gene fragment (pYW409) was introduced into MC4100(λ SH241), both TdcB activity from the chromosome and *lacZ* expression from the fusion were reduced by about 90% as compared with the vector control (Fig. 2). A control experiment showed that transformation of MC4100(λ SH241) with the plasmid pYW411 containing the PCR-amplified *hupA::Cm^r* gene from OHP109 (Huisman et al. 1989) had no effect on TdcB or LacZ levels. Therefore, a mutation that insertionally inactivates the *hupA* gene abolishes the *hupA*-mediated repression of *tdc*, and the DNA sequence cloned into the multicopy plasmid did not titrate a factor that is required for transcriptional activation of the *tdc* promoter.

Figure 2 also shows that the plasmid pYW410, containing the PCR-amplified *hupB*⁺ gene (from nucleotide -122 to +477, see Kano et al. 1985) also repressed *tdc* gene expression. Published reports indicate that the *hupA* and *hupB* genes encode, respectively, HU2 and HU1 subunits of the 20-kDa, heterodimeric, basic, histone-like protein HU, (Kano et al. 1985, 1987), which affects a variety of cellular processes including replication of DNA, integrative recombination of λ and μ phages, and transcription of certain *E. coli* genes (Drlica and Rouvière-Yaniv 1987; Flashner and Gralla 1988; and references therein).

It is difficult to assess the physiological role of a protein in gene regulation when it is produced in excess in the cell from multicopy plasmids. To circumvent this problem, we compared expression of chromosomal *tdcB* in individual *hup* mutants with that in their isogenic counterparts, because the results described above suggested that *tdc* expression would be partially repressed in strains harboring single-copy *hup* genes, and that mutants lacking *hupA* and/or *hupB* would have derepressed levels of *tdc* gene products. For this experiment the mutant *hupA* and *hupB* genes from strains OHP109 and OHP96 (Huisman et al. 1989) were transferred into MC4100 by P1 transduction, yielding, respectively, DW109 and DW96. The *hupA hupB* double mutant (DW111) was constructed by P1 transduction from OHP96 into DW109. Representative results of enzyme assays given in Table 1, Expt. I show 2 to 3-fold higher TdcB activities in the *hupA* strain and in the *hupA hupB* double mutant, relative to the isogenic parent. Both the protein and operon fusions of *tdc* with *lacZ* (Expts. II and III, respectively) showed similar extents of derepression in the *hupA* strain. These results clearly suggest that a single-copy *hupA* gene significantly regulates *tdc* operon expression *in vivo* at the level of transcription.

A marginal derepression of *tdc* in the *hupB* mutant (DW96) relative to MC4100 (Table 1), seen consistently in all experiments, was not surprising. It has been previously shown that *hupA* cells contain HU1 homodimers and *hupB* strain accumulates HU2 homodimers; furthermore, the *hupA* gene appears to be dominant in its effect

Table 1 *tdc* gene expression in *hup* mutants

Experiment	Strain	Relevant genotype	TdcB activity ^a	LacZ activity ^a
I	MC4100	<i>hupA</i> ⁺ <i>hupB</i> ⁺	2,892 (1.0)	ND
	DW109	<i>hupA hupB</i> ⁺	8,250 (2.9)	ND
	DW96	<i>hupA</i> ⁺ <i>hupB</i>	4,137 (1.4)	ND
	DW111	<i>hupA hupB</i>	6,607 (2.3)	ND
II	MC4100(λ SH241)	<i>hupA</i> ⁺ λ <i>tdcAB</i> '-' <i>lacZ</i>	ND	915 (1.0)
	DW109(λ SH241)	<i>hupA</i> λ <i>tdcAB</i> '-' <i>lacZ</i>	ND	3,593 (3.9)
III	MC4100(λ YW245) ^b	<i>hupA</i> ⁺ λ <i>tdcP-lacZ</i>	3,314 (1.0)	623 (1.0)
	DW109(λ YW245)	<i>hupA</i> λ <i>tdcP-lacZ</i>	10,133 (3.1)	2,109 (3.4)

^a Cells were incubated anaerobically in TYE medium for 8 h and enzyme assays were performed according to Hobert and Datta (1983) and Miller (1992). TdcB activity is expressed as nmol of α -ketobutyrate formed per min per mg of protein, and LacZ activity in Miller units. Values in parenthesis represent the activity relative to the control (1.0). ND, not determined

^b The *tdc* operon fusion was constructed by cloning the *tdcP* DNA fragment (nucleotides 1335 to 1889; Schweizer and Datta 1989a) into pRS415 (Simons et al. 1987)

over *hupB* (Bonney et al. 1989; Huisman et al. 1989; Hsieh et al. 1991). In view of this, it is likely that HU2 homodimers encoded by *hupA*⁺ in DW96 repressed *tdc* expression to the same extent as that seen in MC4100.

How does HU protein influence the transcriptional efficiency of the *tdc* promoter? It has been reported that HU binds DNA non-specifically in a sequence-independent manner (Drlica and Rouvière-Yaniv 1987; Bonney and Rouvière-Yaniv 1991) and bends and coils DNA into higher order structures. For example, in vitro experiments with purified HU showed that the pitch of the DNA helix in the HU-DNA complex is shortened from a repeat length of 10.5 to 8.5 bp per helical turn, due to DNA compaction (Broyles and Pettijohn 1986); also, estimates of DNA supercoil densities by gel electrophoresis of reporter plasmids isolated from *hup* mutants revealed reduced DNA supercoiling in cells lacking HU protein (Hsieh et al. 1991; Yuzuzawa et al. 1992). Thus, it is reasonable to assume that expression of the *tdc* operon might be sensitive to HU-mediated topological changes in the chromosomal DNA.

In order to decide whether the HU-promoted DNA structural changes indirectly influence *tdc* expression by regulating the synthesis of Crp and/or IHF which activate transcription, we compared chromosomally encoded β -galactosidase and acetohydroxy acid synthetase (the *ilvB* gene product) activities in W3110 to those in the same strain transformed with the cloned *hupA*⁺ gene. If overexpression of HU depleted the cells of Crp and/or IHF, it should also reduce the levels of these enzymes, because *lacZ* expression requires Crp (Reznikoff 1992) and the *ilvBN* operon is subject to regulation by both Crp and IHF (cited in Wu et al. 1992). Our results revealed no significant difference in β -galactosidase and acetohydroxy acid synthetase activities in W3110 harboring the *hupA*⁺ gene, the *hupA*::Cm^r plasmid and the control vector. We assume therefore that *hupA*⁺ gene product does not influence Crp and IHF levels; however, we cannot completely rule out the unlikely possibility that high concentrations of HU might deplete some, as yet unknown, regulatory factor(s) essential for *tdc* expression. It should be noted however that single-copy *hupA* regulates *tdc* expression in vivo (see Table 1).

To test whether DNA topology influences *tdc* expression, we assayed TdcB levels in *E. coli* strains harboring a combination of mutations in the genes encoding the HU protein, DNA gyrase and topoisomerase I. Reports in the literature indicate that in topological terms bacterial chromosomal DNA appears to exist in the negative supercoiled state, and the extent of supercoiling depends, at least partially, on the ratio of two enzyme activities, namely DNA gyrase, which introduces negative supercoils, and topoisomerase I, which relaxes the supercoiled form (reviewed by Wang 1991). Figure 3 shows that MC4100 harboring one copy each of *gyrAB*⁺, *hupAB*⁺, and *topA*⁺ genes exhibited low dehydratase activity, whereas, DW1093 harboring mutations in *gyrB* and *hupA* but containing *topA*⁺ showed an increase in TdcB level. Mutations in *gyrB* (DW4222) or in *hupA* (DW109)

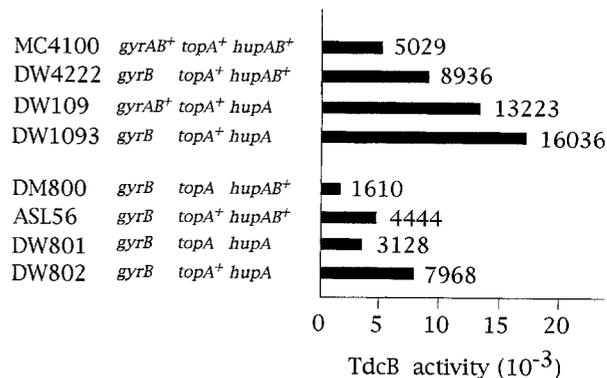


Fig. 3 TdcB activities in strains harboring various mutations in the *gyrB*, *topA*, and *hupA* genes after anaerobic incubation for 8 h in TYE medium (Hobert and Datta 1983). Enzyme specific activities (nmoles of α -ketobutyrate formed per min per mg protein) are shown next to the solid bars. DW4222 (Cou^r) and DW1093 (Cou^r) are spontaneous coumermycin-resistant (50 μ g/ml) derivatives of MC4100 and DW109, respectively. DM800 [Δ (*topA-cysB*)204 *gyrB*225 (Cou^r)] and its derivative ASL56 [*topA*⁺ *gyrB*225 (Cou^r)] were constructed in W3110 (Sternglanz et al. 1981). DM801 (DM800 *hupA*) was isolated by P1 transduction from DW109, and DW802 [(DW801(λ *topA*⁺))] was constructed by cloning pJW249 *topA*⁺ (Wang and Becherer 1983) into λ RZ5 and then recombining onto the DW801 chromosome as described (Hagewood et al. 1994). The four strains shown at the top are derivatives of MC4100, whereas the four strains shown at the bottom are derivatives of W3110

alone led to intermediate levels of TdcB. Similarly, in a different strain background a 5-fold higher dehydratase activity was seen in DW802 *topA*⁺ *gyrB* *hupA* as compared with DM800 *topA* *gyrB* *hupA*⁺, whereas mutations in all three genes, *gyrB*, *topA* and *hupA* (DW801), caused a decrease in TdcB level. The same pattern of enzyme levels was seen using single-copy *lacZ* transcriptional fusions of MC4100 derivatives (data not shown). These results are consistent with the previous report (Sumantran et al. 1989) that inhibition of DNA gyrase activity by nalidixic acid and coumermycin increases TdcB expression in *E. coli* K37.

The cumulative genetic evidence summarized above clearly shows that the transcriptional efficiency of the *tdc* promoter in various mutants follows a common pattern: the more relaxed the DNA, the higher the level of the expression of the *tdc* operon. These results, however, do not distinguish between changes in the global DNA topology and perturbation of local DNA structure at the *tdc* promoter, because the HU protein and topoisomerases affect DNA structure in a sequence-independent manner. Nevertheless, it is reasonable to conclude that DNA supercoiling plays an important part in the steady-state expression of the *tdc* operon, and that genetic and/or environmental factors that influence DNA topology are likely to modulate its transcription pattern significantly.

We showed earlier (Schweizer and Datta 1989b; Wu and Datta 1992; Wu et al. 1992; Ganduri et al. 1993) that four proteins, TdcA, TdcR, Crp and IHF, are required for *tdc* gene expression. IHF and Crp occupy unique binding sites on *tdcP* at positions -104 and -41, respectively

(with respect to transcription-start site at +1). These proteins, individually, induce DNA bending at *tdcP* but cannot activate transcription. However, simultaneous binding of both proteins bends DNA into a new conformation to allow transcription to proceed. Recent experiments suggest that TdcA binds upstream from IHF at around -175, and the IHF- and Crp-mediated DNA bending allows looping of the promoter DNA to juxtapose TdcR, TdcA, and Crp into a favorable configuration, with respect to each other for transcription initiation by RNA polymerase (Hagewood et al. 1994). It is plausible then that relaxation of supercoiled DNA near the *tdc* operon might promote an optimal configuration of the protein-DNA complex by adjusting the phasing and/or relative spacing of the protein binding sites on the DNA for enhanced transcription. On the other hand, compaction of DNA adjacent to the *tdc* region might disrupt the correct placement of the protein-DNA complex to an extent that prevents transcription. Alternatively, changes in global DNA topology might somehow indirectly regulate *tdc* transcription. Whatever the exact mechanism may be, it is evident that regulation of *tdc* gene expression by the operon-specific transcriptional factors, TdcR and TdcA, is greatly influenced by the global regulatory proteins HU and topoisomerases, which alter overall DNA topology, and by IHF and Crp, which promote bending and/or looping of the *tdc* promoter region. In view of this, the roles of transcription factors in gene expression in general must be viewed in the larger context of DNA structure and dynamics at or near the site of the target operon.

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References

- Bonnefoy E, Rouvière-Yaniv J (1991) HU and IHF, two homologous histone-like proteins of *Escherichia coli*, form different protein-DNA complexes with short DNA fragments. *EMBO J* 10:687-696
- Bonnefoy E, Almeida A, Rouvière-Yaniv J (1989) Lon-dependent regulation of the DNA binding protein HU in *Escherichia coli*. *Proc Natl Acad Sci USA* 86:7691-7695
- Broyles SS, Pettijohn DE (1986) Interaction of the *Escherichia coli* HU protein with DNA. Evidence for formation of nucleosome-like structures with altered DNA helical pitch. *J Mol Biol* 187:47-60
- Drlica K, Rouvière-Yaniv J (1987) Histone-like proteins of bacteria. *Microbiol Rev* 51:301-319
- Flashner Y, Gralla JD (1988) DNA dynamic flexibility and protein recognition: differential stimulation by bacterial histone-like protein HU. *Cell* 54:713-721
- Ganduri YL, Sada SR, Datta MW, Jambukeswaran RK, Datta P (1993) TdcA, a transcriptional activator of the *tdcABC* operon of *Escherichia coli*, is a member of the LysR family of proteins. *Mol Gen Genet* 240:395-402
- Goss TJ, Schweizer HP, Datta P (1988) Molecular characterization of the *tdc* operon of *Escherichia coli* K-12. *J Bacteriol* 170:5352-5359
- Hagewood BT, Ganduri YL, Datta P (1994) Functional analysis of the *tdcABC* promoter of *Escherichia coli*: roles of TdcA and TdcR. *J Bacteriol* 176:6214-6220
- Hobert EH, Datta P (1983) Synthesis of biodegradative threonine dehydratase of *Escherichia coli*: role of amino acids, electron acceptors, and certain intermediary metabolites. *J Bacteriol* 155:586-592
- Hsieh L-S, Rouvière-Yaniv J, Drlica K (1991) Bacterial DNA supercoiling and [ATP]/[ADP] ratio: changes associated with salt shock. *J Bacteriol* 173:3914-3917
- Huisman O, Faellen M, Girard D, Jaffe A, Toussant A, Rouvière-Yaniv J (1989) Multiple defects in *Escherichia coli* mutants lacking HU protein. *J Bacteriol* 171:3704-3712
- Kano Y, Yoshino S, Wada M, Yokoyama K, Nobuhara M, Imamoto F (1985) Molecular cloning and nucleotide sequence of the HU-1 gene of *Escherichia coli*. *Mol Gen Genet* 201:360-362
- Kano Y, Osato K, Wada M, Imamoto F (1987) Cloning and sequencing of the HU-2 gene of *Escherichia coli*. *Mol Gen Genet* 209:408-410
- Miller JH (1992) A short course in bacterial genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York
- Reznikoff WS (1992) Catabolite gene activator protein activation of *lac* transcription. *J Bacteriol* 174:655-658
- Schweizer HP, Datta P (1989a) The complete nucleotide sequence of the *tdc* region of *Escherichia coli*. *Nucleic Acids Res* 17:3994
- Schweizer HP, Datta P (1989b) Identification and DNA sequence of *tdcR*, a positive regulatory gene of the *tdc* operon of *Escherichia coli*. *Mol Gen Genet* 218:516-522
- Simons RW, Houman F, Kleckner N (1987) Improved single and multicopy *lac*-based cloning vectors for protein and operon fusions. *Gene* 53:85-96
- Sternglanz R, DiNardo S, Voelkel KA, Nishimura Y, Hirota Y, Becherer K, Zumstein L, Wang JC (1981) Mutations in the gene coding for *Escherichia coli* DNA topoisomerase I affect transcription and transposition. *Proc Natl Acad Sci USA* 78:2747-2751
- Sumantran VN, Schweizer HP, Datta P (1990) A novel membrane-associated threonine permease encoded by the *tdcC* gene of *Escherichia coli*. *J Bacteriol* 172:4288-4294
- Sumantran VN, Tranguch AJ, Datta P (1989) Increased expression of biodegradative threonine dehydratase of *Escherichia coli* by DNA gyrase inhibitors. *FEMS Microbiol Lett* 65:37-40
- Wang JC (1991) DNA topoisomerases: Why so many? *J Biol Chem* 266:6659-6662
- Wang JC, Becherer K (1983) Cloning of the gene *topA* encoding for DNA topoisomerase I and the physical mapping of the *cysB-topA-trp* region of *Escherichia coli*. *Nucleic Acids Res* 11:1773-1790
- Wu Y, Datta P (1992) Integration host factor is required for positive regulation of the *tdc* operon of *Escherichia coli*. *J Bacteriol* 174:233-240
- Wu Y, Patil RV, Datta P (1992) Catabolite gene activator protein and integration host factor act in concert to regulate *tdc* operon expression in *Escherichia coli*. *J Bacteriol* 174:6918-6927
- Yusuzawa K, Hayashi N, Goshima N, Kohno K, Imamoto F, Kano Y (1992) Histone-like proteins are required for cell growth and constraint of supercoils in DNA. *Gene* 122:9-15