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Physical linkage and transcriptional orientation of the *tdc* operon on the *Escherichia coli* chromosome**

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Summary. The physical and genetic structure of 37 kilobases of DNA encopassing the *tdc* region at 68.3 min of the *Escherichia coli* chromosome was determined by DNA sequence analysis and restriction mapping. Reexamination of new data concerning the direction of transcription of the *tdc* operon revealed that in strain W3110 the *tdc* region is located on a transposable segment of DNA.

Key words: *Escherichia coli* – *tdc* operon – Mapping – Restriction analysis – Transposable element

The anaerobically-induced *tdc* operon of *Escherichia coli* (Goss and Datta 1985; Goss et al. 1988) consists of three genes, *tdcA*, *tdcB* and *tdcC*, encoding, respectively, a regulatory protein involved in inducer synthesis (S.R. Sadda and P. Datta, unpublished data), the biodegradative threonine dehydratase (EC 4.2.1.16) (Datta et al. 1987) and a threonine-serine permease (Sumantran et al. 1990). The operon was previously mapped at 68 min on the *E. coli* K-12 chromosome and, by using a *tdcB-lacZ* fusion, the direction of transcription was determined to be clockwise (Schweizer and Datta 1988). A recent genetic analysis indicated that a separate regulatory gene, *tdcR*, located immediately upstream of the *tdc* operon in opposite transcriptional orientation, is needed for efficient expression of *tdc* (Schweizer and Datta 1989a). These four genes are found within a 6295 bp *Eco*RI fragment. Complete nucleotide sequence of the cloned DNA (Schweizer and Datta 1989b) revealed three additional open reading frames (ORFs), flanking *tdcABC* and *tdcR*, which remain uncharacterized.

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To establish the physical linkage of the 6.3 kilobase pairs (kb) fragment to adjacent regions of the *E. coli* chromosome, we compared the restriction patterns of *tdc* DNA with those of lambda clones 514, 515 and 516 derived from coordinates 3320 kb to 3350 kb on the chromosome of strain W3110 (Kohara et al. 1987). The results presented in Fig. 1 show that the 6.3 kb *Eco*RI fragment was located in its entirety within the lambda clone 515 and in part on lambda clones 514 and 516. Restriction patterns of the latter lambda clones determined using *Bam*HI, *Eco*RI and *Hin*dIII confirmed the identity of the regions flanking the 6.3 kb segment with

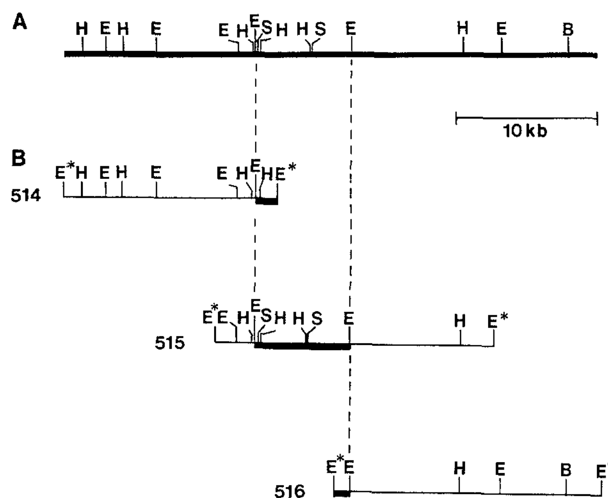

Fig. 1 A, B. Restriction map of the *tdc* region (**A**) and extent of DNA carried by individual lambda phages from the region. Lysates of lambda clones 514, 515 and 516 were prepared as described by Silhavy et al. (1984) using LE392 as the host. Phage DNA was prepared from 50 ml lysates as described by Kaslow (1986). Phage DNA was digested with *Bam*HI (B), *Eco*RI (E), *Hin*dIII (H) and *Sal*I (S). Restriction fragments were separated on 0.8% agarose gels. Only phage 515 DNA was analyzed with *Sal*I. E* indicates *Eco*RI sites from the polylinker region of EMBL4 (Frischauf et al. 1983). The extent of DNA of the 6.3 kb *Eco*RI fragment containing *tdc* carried by the individual phages is indicated by the bold-faced line

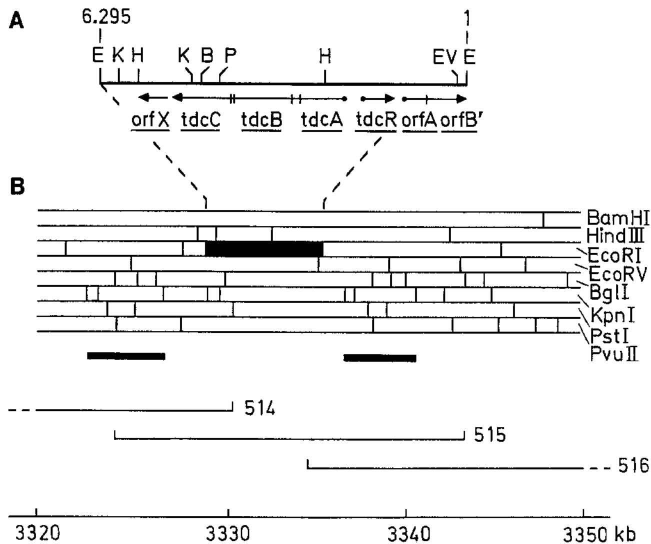

Fig. 2A, B. Localization of the *tdc* genes on the *E. coli* physical map. A restriction map of a 6295 bp *Eco*RI restriction fragment harboring the *tdc* genes and adjacent reading frames (**A**). Location of restriction sites is based on DNA sequence analysis and was verified by restriction mapping. The gene products of all open reading frames have been identified in a minicell system (Goss and Datta (1985), Goss et al. (1988), Schweizer and Datta (1989a)). Abbreviations: B, *Bgl*I; E, *Eco*RI; EV, *Eco*RV; H, *Hind*III; K, *Kpn*I. An *E. coli* restriction map of the *tdc*-containing region (**B**) based on a revised version (Y. Kohara, personal communication) of the published map (Kohara et al. 1987). Horizontal lines below the map indicate the extent of the chromosomal fragments carried by transducing phages designated on the right side of each fragment (see Fig. 1). The location of the 6.3 kb *Eco*RI fragment harboring *tdc* is indicated in black. The extent of a 4 kb repeated region is indicated by solid bars

the published physical map of *E. coli* W3110 DNA (Kohara et al. 1987). The 6.3 kb *Eco*RI fragment harboring the *tdc* operon maps approximately at coordinate 3330 kb, or at 68.3 min, on the *E. coli* chromosome. This result is in excellent agreement with the previously reported map position of 68 min established by genetic experiments (Goss and Datta 1984; Schweizer and Datta 1988).

Alignment of the 6.3 kb *tdc* DNA on the physical map, based on the restriction patterns indicated above, however, yielded a surprising finding: counterclockwise transcription of the *tdcABC* operon and *orfX*, and clockwise transcription of *tdcR* and *orfA* and *orfB'* (Fig. 2). This is in obvious contrast to the clockwise direction of transcription of *tdcB* established by Hfr-mediated chromosome transfer in strain MC4100 (Schweizer and Datta 1988), and the counterclockwise direction of transcription of *tdcR* from DNA sequence data and deletion analysis (Schweizer and Datta 1989a), and from primer extension experiments to determine the transcription start site (HP Schweizer and P Datta, unpublished data). In view of this, we re-examined the direction of transcription of the *tdcB* gene fused to *lacZ* by the procedure of Eckhardt (1977). A lysogen of MC4100 was isolated that had a *tdcB-lacZ* fusion, carried by λpSH202, integrated in the *tdc* region (TH9) (Schweizer and Datta 1988). Two possible orientations of the prophage and the fusion on the chromosome are shown in Fig. 3. Depending on the direction of transcription of the *tdcB* gene, the lysogenization would place the *tdcB-lacZ* fusion on either side of *tdc*. To distinguish between the two possible arrangements the order of the markers *tdc*, *tdc-lac* and *zgi-1*::Tn*10* was determined by P1 transduction. In order to obtain a suitable recipient, a *tdcA* mutation was introduced into TH9 by transduction with P1 grown on TG426 (*tdcA*::Tn*5*) with selection of kanamycin resistance. (For a list of bacterial strains and phages, see Table 1). LacY was introduced into the resulting strain TH19 by transduction with P1 grown on NK5587 (*lacY*::Tn*9*) and selection for chloramphenicol resistance. The *zgi-1*::Tn*10* insertion was introduced into TH9 by transduction with P1 grown on TH1 and selection for tetracycline resistance. Since the crosses involved a prophage in both donor and recipient, care was taken to ensure lambda lysogeny in each step of strain construction. The result of the three-factor crosses is shown

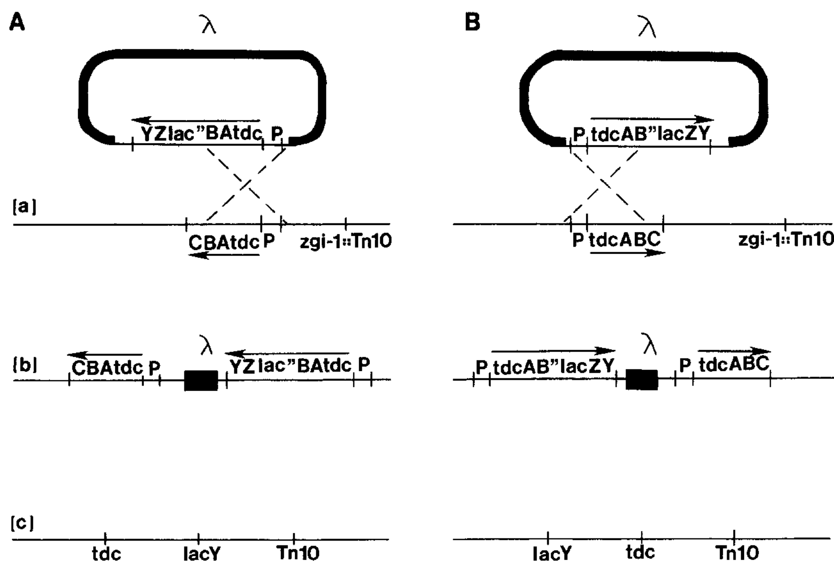

Fig. 3A, B. Integration of a λ phage carrying a *tdc-lac* fusion into the *tdc* region. Depending on the direction of transcription of *tdc* two possibilities (A and B) arise. (a) Orientation of *tdc* on the chromosome; (b) integration of λp(*tdcB-lacZ*); (c) orientation of markers in P1 cross

Table 1. *Escherichia coli* K-12 strains and phages

	Relevant genotype	Source or reference
Strains		
TH1	Hfr *metB1 relA1 zgi-1*::Tn*10*	Schweizer and Datta (1988)
TH9	F^- *araD139 Δ(argF-lac)169 rpsL150 deoC1 relA1 rbsR ptsF25 flbB5301* (λpSH202)	Schweizer and Datta (1988)
TH19	TH9 *tdcA*::Tn*5*	This study
TH20	TH19 *lacY*::Tn*9*	This study
TH21	TH9 *zgi-1*::Tn*10*	This study
LE392	F^- *hsdR514 supE44 supF58 Δ(lacIZY)6 galK2 galT22 metB1 trpR55*	Maniatis et al. (1982)
NK5587	*Δ(lac-pro)XIII thiA rha trkA trkB*/F′ *lacZ*$^+$ *lacY*::Tn*9*	N. Kleckner
TG426	F^- *thyA thi tdcA*::Tn*5*	Goss and Datta (1984)
Phages		
λpSH202	λ(*tdcB-lacZ*)hyb *bla*$^+$	Schweizer and Datta (1988)
λ514	λEMBL4 16.0 kb *Sau*3A partial *E. coli* fragment	Kohara et al. (1987)
λ515	λEMBL4 19.5 kb *Sau*3A partial *E. coli* fragment	Kohara et al. (1987)
λ516	λEMBL4 18.7 kb *Sau*3A partial *E. coli* fragment	Kohara et al. (1987)

Table 2. Transductional analysis of the position of the *tdcB-lacZ* fusion with respect to *tdc* and *zgi-1*::Tn*10*

Strains (relevant genotype)		Recombinant class	Percentage of total
Donor	Recipient		
TH20	TH21	Lac$^+$Tdc$^+$	0 (0/194)
(*tdc*$^+$ *lacY*$^+$	(*tdcA*::Tn*5*	Lac$^+$Tdc$^-$	0 (0/194)
zgi-1::Tn*10*)	*lacY*::Tn*9*)	Lac$^-$Tdc$^-$	88.7 (172/194)
		Lac$^-$Tdc$^+$	11.3 (22/194)

A P1 lysate grown on TH20 was used to transduce TH21 to tetracycline resistance (Tc^r) according to Silhavy et al. (1984). Transductants were selected on LB medium containing 20 mM sodium-citrate and 5 μg/ml tetracycline. Tdc was scored as kanamycin resistance (Tdc$^-$) or sensitivity (Tdc$^+$) on LB medium containing 20 mM sodium citrate and 50 μg/ml kanamycin. Lac was scored as growth (Lac$^+$) or no growth (Lac$^-$) on M9 medium (Miller 1972) containing 0.2% lactose, 20 mM sodium citrate and 5 μg/ml tetracycline

in Table 2. Selection was for tetracycline-resistance (Tc^r). As can be seen, the Tdc$^+$ phenotype cotransduces with Tc^r at a frequency of 11% whereas no co-transduction of Lac$^+$ and Tc^r is observed. *tdc* and *zgi-1*::Tn*10* normally co-transduce at a frequency of 8–18% (Schweizer and Datta 1988). Thus, the deduced gene order is *tdcAB″lacZY-tdc-zgi-1*::Tn*10*, indicating the direction of transcription of *tdcB-lacZ* towards *zgi-1*::Tn*10* or clockwise on the *E. coli* chromosome, according to the established linkage of *tdc* to *zgi-1*::Tn*10* (Schweizer and Datta 1988). Clearly, this finding corroborates the previously established direction of transcription using the same *lacZ* fusion (Schweizer and Datta 1988) but is not consistent with the physical map of *E. coli* shown in Fig. 2.

How do we reconcile the apparent discrepancy in the transcriptional orientation of the *tdc* genes between strains MC4100 and W3110? A plausible explanation is that this segment of the chromosome in certain *E. coli* strains may be a likely target site for transposon elements resulting in chromosomal rearrangements through translocation, duplication and inversion-like events. Indeed in strain W3110, Muramatsu et al. (1988) found at least four copies of IS*5*-related sequences at coordinates 3144 kb, 3158 kb, 3172 kb, and 3186 kb, and a separate one at coordinate 3328 kb, each with a 14 kb flanking sequence exhibiting essentially identical restriction patterns. In addition, there appear to be two 4 kb regions, flanking the 6.3 kb *Eco*RI fragment located at coordinate 3330 kb, which are completely homologous with each other (Fig. 2B; Y. Kohara, personal communication). The authors (Muramatsu et al. 1988) proposed that the 14 kb DNA segments with IS*5*-related sequences may represent transposable elements and, over time, may have caused translocation and duplication of this region of the W3110 chromosome. Interestingly, these events appear to be specific for the particular W3110 strain used by this group of investigators (Kohara et al. 1987; Muramatsu et al. 1988) since the original *E. coli* K-12 strain does not have a translocation at the 3160 kb region (Y. Kohara, personal communication). This notion is also consistent with the results reported from our laboratory (Goss and Datta 1984; Schweizer and Datta 1988) that a Tn*5* insertion isolated in a W3110 strain, and plasmid-borne mutations introduced by allelic exchange into strain MC4100 always mapped to a single location at 68 min. Thus, it is most likely that the observed orientation of the *tdc* DNA on the physical map of the W3110 strain of Kohara et al. (1987), shown in Fig. 2, is fortuitous, and that the clockwise direction of transcription of *tdcABC* reported here and in a previous study (Schweizer and Datta 1988) reflects the correct orientation of the *tdc* region on the *E. coli* chromosome.

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