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Cloning and Properties of the *Salmonella typhimurium* Tricarboxylate Transport Operon in *Escherichia coli*

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The tricarboxylate transport operon (*tctI*) was cloned in *Escherichia coli* as a 12-kilobase (kb) fragment from an *Eco*RI library of the *Salmonella typhimurium* chromosome in λgtWES. It was further subcloned as a 12-kb fragment into pACYC184 and as an 8-kb fragment into pBR322. By insertional mutagenesis mediated by λTn*5*, restriction mapping, and phenotypic testing, the *tctI* operon was localized to a 4.5-kb region. The *tctC* gene which encodes a periplasmic binding protein (C protein) was located near the center of the insert. *E. coli*/*tctI* clones on either multicopy or single-copy vectors grew on the same tricarboxylates as *S. typhimurium*, although unusually long growth lags were observed. *E. coli*/*tctI* clones exhibited similar [^{14}C]fluorocitrate transport kinetics to those of *S. typhimurium*, whereas *E. coli* alone was virtually impermeable to [^{14}C] fluorocitrate. The periplasmic C proteins (C1 and C2 isoelectric forms) were produced in prodigious quantities from the cloned strains. Motile *E. coli*/*tctI* clones were not chemotactic toward citrate, whereas *tctI* deletion mutants of *S. typhimurium* were. Taken together, these observations indicate that *tctI* is not an operon involved in chemotaxis.

Bacteria actively transport nutrients by a wide variety of membrane-associated systems. These can be functionally classified into three main distinct types: group translocation systems, which derive energy directly from phosphoenolpyruvate hydrolysis; cytoplasmic membrane systems, which derive energy from the electrochemical gradient of protons or other ions; and, in gram-negative bacteria, osmotic-shock-sensitive systems, the energetics of which is still obscure (3, 11).

Salmonella typhimurium shares with a wide range of bacteria of the family *Enterobacteriaceae* the ability to utilize citrate (Cit$^+$), one of several criteria which has been used to distinguish between the genera *Escherichia*, *Enterobacter*, and *Salmonella* (24). *S. typhimurium* has been shown to transport citrate, *cis*-aconitate, and isocitrate, the three tricarboxylates of the Krebs cycle (16). Tricarboxylate transport in *S. typhimurium* is a complex process mediated by at least three separate systems (17). The major system, TctI, has been mapped at 59 U (30). Fine-structure genetic mapping indicates the presence of an operon which is transcribed clockwise and consists of at least four genes (29). The *tctI* operon is inducible by tricarboxylates (16), and one component, a periplasmic binding protein (C protein), has been characterized extensively (32).

In contrast, *Escherichia coli* does not normally transport citrate nor does it grow on Krebs cycle tricarboxylates. However, rare isolates carrying antibiotic-resistant plasmids also encoding specific citrate transport systems have been investigated (14, 27, 33, 35). *E. coli* K-12 is also thought to harbor a vestigial tricarboxylate transport system activated only by rare double mutations (12).

In this report, we describe the cloning of the *tctI* system of *S. typhimurium* in *E. coli* and compare the properties of these two citrate-transporting strains.

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MATERIALS AND METHODS

Bacterial strains and growth conditions. Bacterial strains, bacteriophages, and plasmids used are listed in Table 1. The indicator medium for citrate-positive colonies was tetrazolium agar (5) containing 10 mM citrate. Cells for osmotic shock and transport assays were grown overnight in modified Davis minimal medium (DMM) (30), 10 mM citrate, and 0.4% (wt/vol) peptone if not indicated otherwise. Plasmid-containing strains were grown in the presence of the appropriate antibiotic. For chemotaxis assays, an overnight culture was grown in H1 minimal medium (23) and 0.5% glycerol, supplemented with 10 μg of thiamine per ml and 100 μg of each of the essential amino acids per ml. Overnight cultured cells were diluted 100-fold into fresh H1 medium containing 0.5% glycerol, 10 mM sodium citrate, and the required amino acids. Cells were grown to an optical density at 578 nm (OD_{578}) of 0.2 (0.07 mg [dry weight] per ml).

Genetic techniques. For the initial cloning of the TctI system from *S. typhimurium*, lysogenic complementation (8) was used. After simultaneous infection of MC4100 with ligated λ*gt*WES/*Eco*RI digests of the *S. typhimurium* DNA and the λgt4*lac5* helper phage, cells were plated on Simmons citrate agar. After incubation for 3 days at 32°C, Cit$^+$ clones (blue colonies) were picked and phage lysates were prepared by heat induction. Hybrid phage was separated from helper phage by plating LE392 cells which had been infected with the mixed lysates onto LB-X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) plates. The colorless plaques were purified and retested for lysogenic complementation of MC4100 on citrate minimal plates. The preparation of λ lysates and the subsequent λ DNA isolation were carried out according to the method of Davis et al. (8). Plasmids were isolated according to the alkaline lysis method described by Maniatis et al. (22). DNA was digested with restriction enzymes as described elsewhere (10). DNA religations were carried out according to the method of Davis et al. (8).

Restriction analysis. All restriction enzymes were from

TABLE 1. Bacterial strains, phages, and plasmids

Strain, phage, or plasmid	Known genotype	Source or reference
Strains		
E. coli		
MC4100	F⁻ *araD139* Δ(*argF-lac*) *U169 relA1 rpsL150 flbB5301 deoCl ptsF25* λ⁻	CSH[a] genetics course
LE392	F⁻ *supF supE hsdR galK trpR mutB lacY*	CSH genetics course
DL291	F⁻ *gyrR* Δ(*glpT-glpA*) *593 recA glyR*	D. Ludtke
RP437	F⁻ *thr leu his met fam thi eda rpsL lac ara xyl mtl*	J. S. Parkinson
KW5	RP437 λgtWES-*tctI*⁺ (from *S. typhimurium* LT2) λgt4 *lac5*	This study
S. typhimurium		
LT2	*S. typhimurium* wild type	CSH genetics course
KS1070	*S. typhimurium hisF1009 trpB2 metA22 rpsL201 xyl-1 galE* Δ*tctI*	This laboratory
Phages		
λgtWES	*Eco*RI replacement vector; *W*am403 *E*am1100 *S*am100	20
λgt4*lac5*	*int*⁺ *att*⁺ λ*c*I857	CSH genetics course
λ467	λ *b*221 *rex*::Tn*5* λ *c*I857 *O*am29 *P*am80	N. Kleckner
Plasmids		
pACYC184	Cm[r] Tc[r]	P. C. Y. Chang (7)
pBR322	Ap[r] Tc[r]	H. W. Boyer (6)
pKW6	Tc[r] *tctI*⁺ (12 kb)	This study
pKW101	Ap[r] *tctI*⁺ (8 kb)	This study
pOU61	Ap[r] single copy number at 30°C	S. Molin (19)
pKW103	Ap[r] *tctI*⁺ (derivative of pOU61)	This study

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Pharmacia P-L Biochemicals or Boehringer Mannheim Biochemicals. Single, double, and triple digestions were carried out in the core buffer of Fuchs and Blakesley (10), adjusting the salt concentration by the addition of NaCl where necessary. Complete digestions were carried out for 1 to 2 h at 37°C and terminated by heating at 65°C for 10 min. Restriction fragments were separated in 0.7% agarose, with λDNA *Hin*dIII digests as size standards.

λTn*5* mutagenesis. DL291/pKW101 was infected with λ467 (9). Kanamycin- and ampicillin-resistant clones were pooled, and plasmids were isolated. The plasmid pool was used to transform LE392. Ampicillin- and kanamycin-resistant clones were selected and phenotypically tested on citrate-peptone-tetrazolium agar (5). Plasmid DNA from white Cit⁻ clones was prepared, and the position of the Tn*5* was mapped by using a *Bam*HI site.

Transport assays. Cells were grown overnight, washed three times, and suspended in DMM to an OD_{578} of 0.1 (35 μg/ml, dry weight). At 5 min before the addition of 2-fluoro-L-erythro-[3,4,5,6-^{14}C]citrate ([^{14}C]FC), 20 mM glucose was added to energize the cells (4, 16). Cells were maintained at 37°C with shaking. Transport was initiated by the addition of [^{14}C]FC. Samples were withdrawn at given times, filtered through membrane filters (0.45-μm pore size) and washed with 5 ml of DMM. Filters were solubilized and assayed for radioactivity in PCS (Amersham Corp.). For kinetic transport assays, cells were suspended to an OD_{578} of 0.5. Samples were taken at 30-s intervals for 2 min to establish transport rates.

Preparation of periplasmic proteins. Periplasmic proteins were isolated from overnight cultures by cold osmotic shock (25). Protein concentrations of shock fluids were measured as described by Lowry et al. (21).

Analytical techniques. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was done in 12% gels according to the method of Ames (2). Slab isoelectric-focusing gels were run on a flatbed apparatus (FBE-3000, Pharmacia). The 5% acrylamide gels (0.75 by 10 by 210 mm) were run with a pH gradient of 5 to 7 according to the Bio-Rad instruction manual method except that riboflavin phosphate was omitted. Proteins were electrophoretically transferred to nitrocellulose paper for 3 h at 60 V (31) and subsequently stained by the immunoperoxidase method (34). Agarose gel electrophoresis for separating DNA fragments was performed in Tris-borate buffer as described by Davis et al. (8).

Chemotaxis assays. The chemotactic response of strains and clones was investigated by using the capillary assay developed by Adler (1). Cells were grown in H1 citrate minimal medium containing glycerol, washed twice with the chemotaxis buffer (10 mM potassium phosphate [pH 7], 0.1 mM EDTA, 0.01 mM methionine) and suspended in the same buffer to an OD_{578} of 1. The capillary assay was done at 29°C, and the capillaries containing different citrate concentrations were incubated in the cell suspensions for 45 min before dilution and plating.

Chemicals. [^{14}C]FC was synthesized by the method of Ashton et al. (4).

RESULTS

Since *E. coli* K-12 does not grow on citrate minimal medium, the cloning of the *tctI* locus into this host was relatively straightforward. By using a library constructed of an *Eco*RI digest of *Salmonella* DNA in λgtWES, a clone carrying a 12-kilobase (kb) fragment conferring citrate utilization (Cit⁺) was isolated (Fig. 1). DNA from a citrate-utilizing clone was used for further subcloning experiments. The 12-kb *Eco*RI-*Eco*RI insert was ligated into pACYC184, and on the basis of citrate utilization, an 8-kb *Eco*RI-*Bam*HI fragment was subcloned in pBR322 (pKW101).

Restriction map of the *tctI* operon. By using single, double, and triple digestions, a restriction map of the 8-kb insert in pKW101 was derived. Figure 2 shows the structure of the 12- and 8-kb plasmids, as well as a preliminary restriction map of the 8-kb fragment which contains two *Kpn*I, one *Bgl*II, and one *Sma*I site. No restriction sites were found for *Sst*I, *Xba*I, *Sph*I, *Pvu*II, *Hin*dIII, or *Cla*I.

λTn*5* mutagenesis and restriction. To define the location of the *tctI* operon within the 8-kb insert of pKW101, we inactivated the genes of the *tctI* operon by using Tn*5* insertion mutagenesis (9). pKW101 mutagenized with Tn*5* was screened on citrate-tetrazolium (5) agar for white, non-citrate-utilizing colonies. A single *Bam*HI site in Tn*5* and the single *Bam*HI site of the insert allowed us to localize the Tn*5* insertions on the various plasmids. After complete digestion with *Bam*HI, DNA fragments were sized by agarose electrophoresis and the location of the Tn*5* insertion was determined. Figure 3 describes the insertion sites of Tn*5*

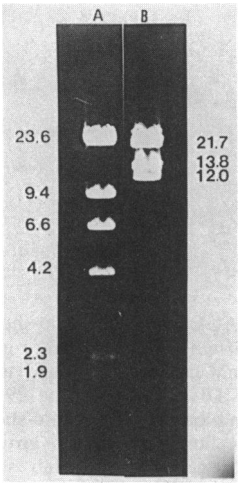

FIG. 1. Agarose gel electrophoresis of *Eco*RI-digested DNA of λgtWES *tctI* hybrid phage. Lanes: A, wild-type λ digested with *Hin*dIII; B, λgtWES containing the *S. typhimurium tctI* region.

in the 8-kb fragment and demonstrates the size of each fragment as well. Tn*5* insertions, inactivating the citrate-positive phenotype, limit the *tctI* operon to about 4.5 kb extending approximately from the *Bam*HI distal to the internal *Kpn*I site in pKW101.

Location of the *tctC* gene. Citrate-negative clones for which the position of the Tn*5* was mapped were grown overnight in DMM–0.4% (wt/vol) peptone–10 mM citrate. Periplasmic proteins were isolated by the cold osmotic-shock procedure and analyzed on 12% SDS-polyacrylamide gels (Fig. 4A). The production of C protein under these conditions and its absence in various mutants was clearly evident from the Coomassie-stained gels. When shock fluids from the same mutants were analyzed for proteins cross-reacting with anti-C-protein antisera, one could easily observe that inserts I23, 110, and 102 were devoid of cross-reacting material. However, mutants 28, 228, 124, and 122 showed the presence of a small amount of C protein, especially when antibody staining was used (Fig. 4B). Thus, the structural gene for C protein was inactivated by the Tn*5* insertions in I23, 110, and 102. It is likely that the insertions III7 to 57 affect genes downstream of C protein involved in citrate transport. Insertions 122 to 28 result in a reduced amount of C protein, suggesting either that they are polar mutations affecting the downstream *tctC* gene or that they are inactivating a regulatory region of the operon. Thus, it can be concluded that the C-protein gene coding for a periplasmic binding protein resides near the center of the 4.5-kb *tctI* operon in the region encompassing approximately 1 kb.

Growth properties of strains harboring the plasmid *tctI* operon. Since *S. typhimurium* contains at least two other

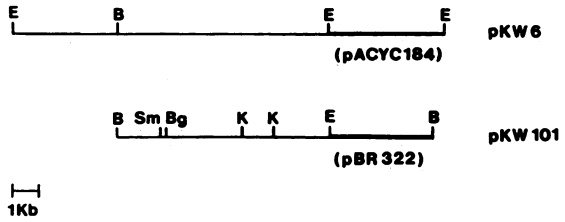

FIG. 2. Plasmids carrying the *tctI* region from *S. typhimurium*. The relevant restriction sites are B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; K, *Kpn*I; Sm, *Sma*I.

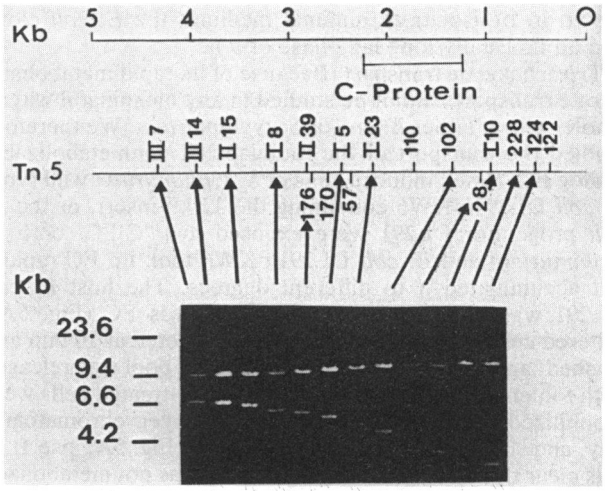

FIG. 3. Tn*5* mutagenesis of pKW101. The upper part shows the position of mapped Tn*5* insertions, limiting *tctI* genes to a 4.5-kb fragment cloned in M13 (26). The position of *tctC* is indicated. The lower part shows the agarose gel electrophoresis of Tn*5* insertions in pKW101. Plasmid DNA was digested with *Bam*HI and run on a 0.7% agarose gel. From the size of the low-molecular-weight *Bam*HI restriction fragment, the position of the Tn*5* in pKW101 was determined.

tricarboxylate transport systems, we compared the growth properties of both *S. typhimurium* and *E. coli* strains containing a plasmid-borne *tctI* operon. The 8-kb *Eco*RI-*Bam*HI Tct$^+$ fragment was subcloned into pOU61, a single-copy plasmid, to yield pKW103, which was subsequently transformed into both *E. coli* DL291 and *S. typhimurium* KS1070 Δ*tct*. On minimal agar, *E. coli*/pKW103 utilized the same range of growth substrates as wild-type *S. typhimurium*, i.e., citrate, DL-isocitrate, and *cis*-aconitate. *S. typhimurium* KS1070/pKW103 behaved as wild type with respect to growth rate, yield, and lag time in citrate minimal medium. However, when it was compared with *E. coli* DL291/pKW103, a difference was apparent when strains were

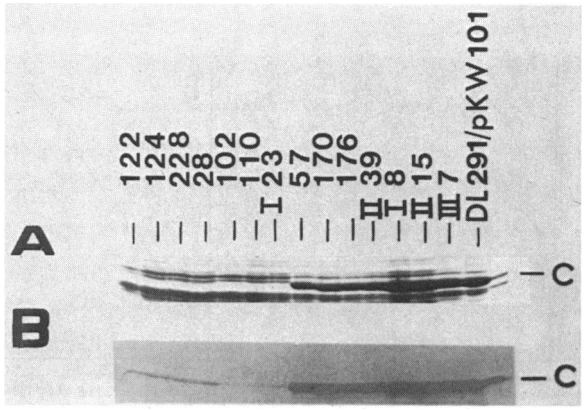

FIG. 4. (A) SDS-PAGE of cold osmotic-shock fluids of Tn*5* insertions inactivating the TctI$^+$ phenotype of pKW101. Cells were grown in DMM–0.4% (wt/vol) peptone–10 mM citrate. Strain numbers correspond to Fig. 3. Only the part of the SDS-PAGE relevant for C protein (29,000 M_r) is shown. (B) Western blot (immunoblot) of cold osmotic-shock fluids of Tn*5* insertions inactivating the TctI$^+$ phenotype of pKW101. After SDS-PAGE, proteins were transferred and cross-reacted with anti-C-protein antiserum. The cutout portions represent approximately 13% of the gel length.

grown in DL-isocitrate minimal medium; the *E. coli* clone had an unusually long lag phase of 9 h.

Tricarboxylate transport. Because of its rapid metabolism, citrate transport cannot be studied in any meaningful way in whole cells of either *E. coli* or *S. typhimurium*. We therefore studied the transport of the radiolabeled nonmetabolizable analog FC. When induced cells of *S. typhimurium* wild type, *E. coli* DL291/pKW6 containing the 12-kb insert, or the *E. coli* progenitor DL291 were exposed to [^{14}C]FC, both *S. typhimurium* and *E. coli* DL291/pKW6 took up FC rapidly but accumulated it to different degrees. The host *E. coli* DL291 was virtually impermeable towards FC (Fig. 5A). Filtered cells from both strains were collected at 10 min and washed, and the intracellular radiolabeled pool was released with toluene-saturated water. The toluene-treated cells were lyophilized, chromatographed by thin-layer chromatography, and subjected to radioautography (4; Fig. 5A, insert). It was clear that in each strain, the analog was not metabolized for the duration of the experiment.

When the kinetics of [^{14}C]FC transport were studied, both *S. typhimurium* and *E. coli* DL291/pKW6 exhibited similarly high-affinity kinetics for FC transport. The K_m values for transport, 11 μM for KW6 (Fig. 5B) and 15 μM for *S. typhimurium* (not shown), were of the order measured previously in other strains of *S. typhimurium* (4). In addition, the V_{max} for transport was similar in both strains (8.3 nm/min per mg for *E. coli* DL291/pKW6 and 10 nm/min per mg for *S. typhimurium*). The transport values of [^{14}C]FC for *S. typhimurium* LT2 and *E. coli* DL291/pKW6 were also similar in that they were both stimulated by sodium and potassium ions, but differences were noted in the efficiency of the carbon source used for transport energization. For instance, α-glycerolphosphate served as a better energy source for [^{14}C]FC transport in *E. coli* DL291/pKW6 than in *S. typhimurium* (data not shown).

Citrate chemotaxis. Binding-protein-dependent transport systems in bacteria fall into two classes: those in which the binding proteins also serve as chemoreceptors and those systems in which they do not (23). Citrate chemotaxis in *S. typhimurium* has been described previously (13, 18), and we felt it was of interest to discern whether the TctI system was chemotactic. When the capillary assay technique of Adler (1) was used, *E. coli* KW5 (12-kb insert in a motile *E. coli* background) was nonchemotactic towards citrate at concentrations up to 75 mM. However, *S. typhimurium* LT2 showed a gradient of chemotactic response up to a maximum of approximately 20 mM citrate. In a separate set of experiments, *S. typhimurium* Δ*tctI* strains were found to be chemotactic towards citrate (data not shown). Thus, it appeared that the *tctI* operon and its periplasmic binding protein were not components of the tricarboxylate chemoreceptors of *S. typhimurium*.

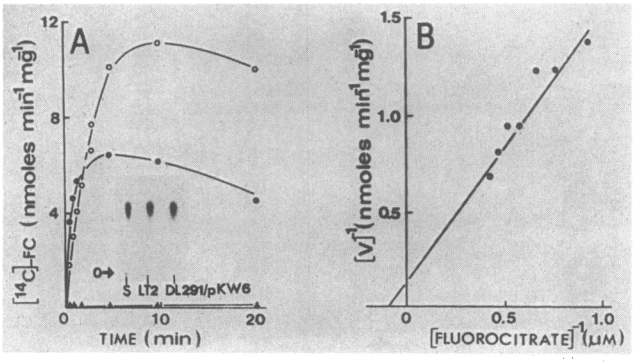

FIG. 5. (A) Transport of [^{14}C]FC in *E. coli* DL291/pKW6 and *S. typhimurium*. Citrate-grown cells were incubated with 20 mM glucose for 5 min before the addition of 21.7 M [^{14}C]FC, 63.6 mCi/mM. ●, *E. coli* DL291/pKW6; ▲, *E. coli* DL291; ○, *S. typhimurium* LT2. Inset: Thin-layer chromatography and autoradiography of the intracellular label collected after toluene treatment of cells (O, origin; S, FC standard). (B) Double reciprocal plot of *tctI*-dependent citrate uptake in *E. coli* DL291/pKW6. Cells were induced with citrate and energized with glucose as described in Materials and Methods.

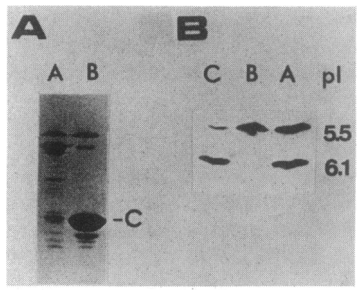

FIG. 6. (A) SDS-PAGE of cold osmotic-shock fluids from *E. coli* TctI$^+$ and *S. typhimurium* LT2. Cells were grown in DMM–0.4% (wt/vol) peptone–10 mM citrate. Lanes: A, wild-type *S. typhimurium* LT2; B, *E. coli* DL291/pKW6. The 29,000-M_r C protein is indicated. (B) Western blot of cold osmotic-shock fluid from *E. coli* TctI$^+$. Osmotic-shock fluids from cells grown in 0.4% (wt/vol) peptone–10 mM citrate were run on a slab isoelectric-focusing gel with a pH gradient from 5 to 7. Proteins were then transferred to nitrocellulose paper and cross-reacted with anti-C-protein antiserum. Lanes: A, *E. coli* DL291/pKW6; B, C2 protein; C, C1 protein isolated from *S. typhimurium* LT2.

Expression of *tctC*. Expression of *tctC* in *S. typhimurium* is known to be under the control of citrate (16). Two isoelectric forms, one with a blocked N terminal (32), appeared in the periplasmic fraction of *S. typhimurium* on citrate induction. *E. coli* strains with cloned *tctI* genes such as DL291/pKW6 were also found to express the C protein in prodigious quantities in the periplasmic fraction of induced cells up to a level at which the C protein accounted for approximately 70 to 80% of the proteins in the periplasmic space (Fig. 6A). When these periplasmic proteins were also subjected to isoelectric focusing, transferred to nitrocellulose, and cross-reacted with anti-C-protein antibody, the presence of both isoelectric forms of C protein, C1 and C2, could be demonstrated in *E. coli* transformed with the *tctI* operon (Fig. 6B). Thus, the cyclization of the N-terminal glutamine residue of the C protein is not a phenomenon peculiar to *S. typhimurium*.

DISCUSSION

S. typhimurium possesses a Krebs cycle tricarboxylate transport system which belongs to the periplasmic binding-protein-dependent class (3, 11). This system is not present in *E. coli*, which is therefore unable to grow on a tricarboxylate such as citrate. In this study, DNA derived from *S. typhimurium* endowed citrate utilization properties on *E. coli*. This is not a surprising finding, since we have been able to demonstrate that both transconjugants and transductants carrying the *tctI* operon of *S. typhimurium* were Cit$^+$ in *E. coli* (17). Additionally, naturally occurring plasmids harboring other Cit$^+$ genes conveyed this phenotype on *E. coli* hosts (27, 33, 35). The location and expression of C protein agrees with the fine-structure genetic map of the *tctI* operon

(29). The *tctC* gene lies near the center of the operon and probably is under the control of a gene product located proximal to it, since Tn*5* insertions in this region greatly reduced the expression of the binding protein.

Mutations downstream of *tctC* continued to express the C protein in abundance. It is not surprising that the *tctI* locus comprises several genes, since other binding-protein-dependent systems which have been analyzed in greater detail embody four to six genes (3). So far we have been unable to discern the nature of the other gene products either in membranes or whole cells. Neither silver-stained two-dimensional gels, [^{35}S]methionine- or [^{14}C]leucine-labeled proteins in minicell or maxicell expression systems, nor in vitro transcription-translation systems allowed the unambiguous identification of gene products other than C protein (unpublished observations). Since periplasmic-binding-protein-coding operons exhibit a high degree of polarity, a more powerful expression system will be needed for an analysis of the operon.

The presence of the *tctI* operon in an *E. coli* background either in multi- or single-copy vectors conveyed the ability to grow on citrate or isocitrate only after a significantly long (9-h) growth lag. Even longer induction lags have been reported for the plasmid-borne citrate transport genes when cloned in an *E. coli* background (27). Perhaps these genes are not well expressed in the *E. coli* background. An alternative explanation is that the other two tricarboxylate transport systems in the *S. typhimurium* genetic background (TctII and TctIII) (17) significantly shortened the induction lag by enhancing the permeability for the inducer in *S. typhimurium*. *E. coli* is virtually impermeable to fluorocitrate (Fig. 5A). *E. coli* K-12 has also been shown to harbor an anaerobic tricarboxylate transport system which apparently differs from the TctI system of *S. typhimurium* in substrate range and by the absence of periplasmic binding proteins (M. Gauglitz and G. Gottschalk, personal communication), yet this system does not suffice to shorten the induction lag. A more obscure *E. coli* cryptic citrate transport system has been reported which requires a mutation in each of two genes to function (12), but our strains do not carry these mutations.

With respect to the transport of [^{14}C]FC, *S. typhimurium* and *E. coli* containing the cloned genes of interest exhibited similar kinetic parameters for transport. In addition, both systems were also stimulated by monovalent cations (sodium and potassium) but differed with respect to the efficiency of energization by external carbon sources. The requirement for the addition of an external carbon or energy source to measure effective active transport is characteristic of *S. typhimurium* (4, 16) and is also seen when cloned in the *E. coli* background. We have also assayed all of the Krebs cycle enzymes in the *E. coli tctI* clones, including citrate synthase (EC 4.1.3.7), aconitase (EC 4.2.1.3), isocitrate dehydrogenase (EC 1.1.1.41), citrate lyase (EC 4.1.3.6), isocitrate lyase (EC 4.1.3.1), and malate synthase (EC 4.1.3.2), and none exhibited elevated enzyme levels (unpublished results), suggesting that these enzymes are not directly involved in the TctI operon.

Although some binding-protein-dependent operons such as the *mal*B operon (23) elicit a chemotactic function, this did not appear to be true for TctI. It appears that other receptors are involved in the citrate chemotaxis systems of *S. typhimurium*, in agreement with previous citrate and isocitrate chemoreceptor studies (13, 18).

The tricarboxylate transport operon of *S. typhimurium* has unique features worthy of further study. For example, it is the only tricarboxylic transport system so far which has a demonstrated binding protein component, and it has complex energetics and ion requirements (unpublished results) (15). The regulation of this system is also of interest since the substrates are directed into a highly active metabolic cycle in which they are also intermediates. Since tricarboxylates are the inducers for transport (16, 29), TctI would appear to be a good candidate for the "exogenous induction" phenomenon (28).

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