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Genetic Fine Structure of the Tricarboxylate Transport (*tct*) Locus of *Salmonella typhimurium*

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Summary. The *tct* (tricarboxylate transport) locus of *Salmonella typhimurium* is found at 59 units between *nalB* and *pheA* (Somers et al. 1981). This locus was further resolved by fine structure genetic mapping and by analysis of one of the gene products, the tricarboxylate binding protein (C protein). 135 independent fluorocitrate resistant clones were isolated and 12 point mutants were ordered by 3 point reciprocal crosses using an adjacent Tn*10* insertion. Eight spontaneous deletions as well as 17 deletions arising from imprecise excisions of internal and flanking Tn*10* elements were used to construct a deletion map comprising 21 deletion segments. 115 strains were then assigned to these segments to complete the fine-structure map.

Using the expression of the C protein as a guide, an analysis of a variety of mutant strains indicated: that the *tct* locus is composed of at least four genes and transcription is clockwise; the C protein structural gene (*tctC*) resides in the centre of the region and codes for two isoelectric forms of the C gene product; *tctC* is flanked by two regions which are involved in transport but whose gene products are not yet identified.

Introduction

A wide range of Enterobacteriaceae are able to utilize citrate, a criterion which has been used to distinguish between the genera *Escherichia, Enterobacter*, and *Salmonella*. *Salmonella typhimurium* is able to transport and metabolize citrate, *cis*-aconitate and isocitrate, the three tricarboxylates of the Krebs cycle (Kay 1978) as well as tricarballylate (Imai et al. 1977, Somers et al. 1981). *S. typhimurium* also possesses citrate and isocitrate chemoreceptors (Kihira and Macnab 1979) as well as divalent cation-citrate chemoreceptors (Ingolia and Koshland 1979).

Citrate transport appears to be a complex process mediated by several systems (Imai et al. 1977, Ashton et al. 1980), one of which is represented by the *tct* locus (Somers et al. 1981) which codes for the only identified transport component, a periplasmic tricarboxylate binding protein (Sweet et al. 1979). This protein, the C protein, also binds the toxic analogue 2-fluoro-L-*erythro*-citrate (Ashton et al. 1980) and is at least partly responsible for the toxicity of this lethal analogue.

In this study we isolated a large number and variety of fluorocitrate resistant (FC^R) mutants in the *tct* locus. These were used to construct a fine structure genetic map of this region. Through the characterization of many of these mutants it was demonstrated that the *tct* locus is a complex transport operon composed of at least four genes, one of which is *tctC* coding for the C protein.

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Materials and Methods

Strains. The strains of *Salmonella typhimurium* LT2 used are listed in Table 1. All *tct* mutants were derived from parent strain SU453.

Media. Nutrient medium was LB broth (Miller 1972). The minimal medium (MDM) (modified Davis) has been described previously (Somers et al. 1981). Carbon sources were sterilized by filtration and added separately to a final concentration of 10 mM. Amino acid supplements were added as required at 20 μg/ml, and tetracycline at 25 μg/ml. All incubations were at 37° C.

Chemicals. DL-fluorocitric acid (barium salt) and tricarboxylic acids were obtained from Sigma Chemical Company. [1,5-^{14}C]-Citrate was from Amersham.

Isolation of tct Mutants. Spontaneous *tct* mutants of SU453 were isolated as fluorocitrate-resistant colonies (Somers et al. 1981). Deletion mutants arising by imprecise excision of Tn*10* insertions were selected as fusaric acid-resistant colonies (Maloy and Nunn 1981).

Transductional Analysis and Deletion Mapping. Transducing phage P22 *int3* HT12/4 were grown on mutant strains as previously described (Somers et al. 1981). Tct^+ transductants were selected by growth on isocitrate as the carbon source. For deletion mapping, unmapped point mutants were first approximately mapped by spot tests; 0.1 ml of P22 phage grown on *tct* deletion strains were spread as backgrounds on isocitrate plates, over which 0.01 ml recipient point mutant cells were spotted at a multiplicity of infection (MOI) of ~1. Those spot crosses giving no Tct^+ recombinants were repeated as whole plate tests by co-plating 0.1 ml phage and 0.1 ml cells (MOI ~1) on triplicate isocitrate selective plates. These conditions yield approximately 3,000 Tct^+ recombinants per plate when donor phage has been grown on wild type strain SU453 and a *tct* point mutant is used as recipient.

Table 1. Strain list

Strain[a]	Genotype or origin	Source or reference
SU453	*hisF1009 trpB2 metA22 rpsL201 xyl-1*	K.E. Sanderson
KS177	Tn*10* insertion in SU453 cotransducible with *tct* (*zfi1*::Tn*10*	Somers et al. (1981)
KS182	Tn*10* insertion in SU453 cotransducible with *tct* (*zfi2*::Tn*10*)	Somers et al. (1981)
KS202	*tct*::Tn*10* insertion in KS1 transduced into SU453	Somers et al. (1981)
KS203	*tct*::Tn*10* insertion in KS2 transduced into SU453	Somers et al. (1981)
KS204	*tct*::Tn*10* insertion in KS3 transduced into SU453	Somers et al. (1981)
KS205	*tct*::Tn*10* insertion in KS6 transduced into SU453	Somers et al. (1981)
KS481	Spontaneous deletion of *tct* in SU453	This study
KS484	Spontaneous deletion of *tct* in SU453	This study
KS512	Spontaneous deletion of *tct* in SU453	This study
KS515	Spontaneous deletion of *tct* in SU453	This study
KS722	Spontaneous deletion of *tct* in SU453	This study
KS723	Spontaneous deletion of *tct* in SU453	This study
KS724	Spontaneous deletion of *tct* in SU453	This study
KS725	Spontaneous deletion of *tct* in SU453	This study
KS387	*Δtct* by Tn*10* excision in KS177	This study
KS621	*Δtct* by Tn*10* excision in KS177	This study
KS622	*Δtct* by Tn*10* excision in KS177	This study
KS623	*Δtct* by Tn*10* excision in KS177	This study
KS630	*Δtct* by Tn*10* excision in KS182	This study
KS624	*Δtct* by Tn*10* excision in KS182	This study
KS686–689	*Δtct* by Tn*10* excision in KS202	This study
KS691	*Δtct* by Tn*10* excision in KS202	This study
KS681–683	*Δtct* by Tn*10* excision in KS203	This study
KS684, 685	*Δtct* by Tn*10* excision in KS204	This study
KS456	*Δtct* by Tn*10* excision in KS205	This study

[a] All other KS strains cited in this study are spontaneous *tct* mutants of SU453

Periplasmic proteins. To isolate periplasmic proteins prior to electrophoresis and for binding assays, cells were grown for 18–20 h in 50 ml of MDM containing 0.4% Difco proteose peptone #3, 10 mM citrate and amino acid supplements (PC medium). This media was devised to ensure good growth and periplasmic protein production either in the presence or absence of citrate. Osmotic shock was carried out as previously described (Willis et al. 1974). For rapid production of periplasmic proteins from mutants prior to ELISA analysis cells were grown in 10 ml of inducing PC medium as above and shocked into 1 ml of H_2O. [1,5-^{14}C]-Citrate binding was measured as previously described (Sweet et al. 1979). Shock fluids were prepared from uninduced (PC medium) and induced (PC plus citrate) cells. Background binding from uninduced cells from each strain used was subtracted from induced values.

Enzyme Linked Immunoadsorbent Assay (ELISA) for the C Protein. An ELISA assay was set up for the C protein in the usual way (Engvall 1980). The second antibody was goat anti-rabbit IgG to which alkaline phosphatase had been coupled and the substrate was *p*-nitrophenylpyrophosphate. Optical densities at 400 were read using Microelisa minireader MR590 (Dynatech). The assay was quantitated against purified C protein and was accurate to approximately 0.1 μg/ml.

Electrophoresis and Isoelectric Focussing. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out on 12% slab gels (0.7 mm) essentially as previously described (Ames 1974). Tube isoelectric focussing (IEF) gels were run according to the Biorad manual instructions using pH 3–10 ampholines. Both gels were stained with Coomassie blue. 2-D Gel electrophoresis was carried out essentially by the method of O'Farrell 1975 (O'Farrell et al. 1977), with the modifications of Copeland (Copeland et al. 1982). Gels were silver stained using the modifications of the procedure of Oakley (Oakley et al. 1980) according to Copeland.

Iodination of Periplasmic Proteins. Crude shock proteins were iodinated with ^{125}I using the Iodogen procedure (Salacinski et al. 1981). 100 μl of shock fluid was iodinated with 100 μCi ^{125}I at 0° C for 15 min and the reaction terminated by the addition of the SDS sample buffer containing mercaptoethanol. Samples (10 μl) were applied directly to the SDS gels and the gels run until the ion front ran off the gel to eliminate unreacted ^{125}I. Gels were acid fixed and washed using the Fairbanks procedure (Fairbanks 1971) without the stain, dried and autoradiographed overnight.

Autoradiographic Transport Assay. A rapid method for the estimation of transport defects in mutants was developed. Single clones from a fresh overnight culture on PC agar were rinsed into 0.1 ml of MDM in a multiple well holder. Using a multiple constant inoculating device a sample was deposited onto a Millipore filter on a PC agar plate previously soaked with 1 μCi of [1,5-^{14}C]-citrate at 5×10^{-7} M. Plates were incubated at 37° C for 6 h, the

Table 2. Three-point reciprocal crosses between *tct* point mutants of *S. typhimurium*

Donor strain (Tet^R)	Percentage of Tct^+ transductants that were tetracycline resistant[a]											
	Recipient strain (tet^S)											
	KS500	KS522	KS471	KS502	KS509	KS493	KS467	KS486	KS476	KS520	KS514	KS523
KS500	–[b]	12.4 (10.4)[c]	16.2 (21.3)	9.0 (14.0)	3.5 (13.6)	2.0 (18.9)	1.2 (25.6)	0.5 (25.8)	0 (51.7)	0 (40.9)	0 (26.8)	0.7 (36.4)
KS522	10.4 (12.4)	–	–	–	7.6 (13.6)	7.5 (14.5)	6.3 (14.0)	1.3 (29.1)	0 (39.3)	1.2 (28.9)	0 (28.5)	0.7 (35.4)
KS471	21.3 (16.2)	–	–	–	10.5 (8.4)	7.2 (13.1)	8.3 (10.4)	0.6 (24.7)	0.4 (35.6)	4.4 (25.3)	0 (23.3)	0.3 (30.9)
KS502	14.0 (9.0)	–	–	–	11.8 (17.0)	8.6 (11.9)	8.9 (18.4)	0.8 (30.9)	0.7 (42.7)	0.8 (31.3)	1.7 (32.5)	0.4 (30.3)
KS509	13.6 (3.5)	13.6 (7.6)	8.4 (10.5)	17.0 (11.8)	–	–	8.8 (20.5)	3.0 (19.0)	2.1 (31.8)	3.3 (16.5)	0.8 (16.1)	1.3 (22.0)
KS493	18.9 (2.0)	14.5 (7.5)	13.1 (7.2)	11.9 (8.6)	–	–	9.3 (15.0)	3.5 (16.7)	2.7 (41.4)	12.2 (10.7)	0.7 (16.6)	1.9 (27.8)
KS467	25.6 (1.2)	14.0 (6.3)	10.4 (8.3)	18.4 (8.9)	20.5 (8.8)	15.0 (9.3)	–	4.7 (18.5)	0.9 (41.2)	5.2 (10.5)	1.5 (14.1)	1.4 (25.2)
KS486	25.8 (0.5)	29.1 (1.3)	24.7 (0.6)	30.9 (0.8)	19.0 (3.0)	16.7 (3.5)	18.5 (4.7)	–	0 (37.8)	0 (28.3)	0.2 (14.3)	0.3 (25.3)
KS476	51.7 (0)	39.3 (0)	35.6 (0.4)	42.7 (0.7)	31.8 (2.1)	41.4 (2.7)	41.2 (0.9)	37.8 (0)	–	0 (11.1)	0.6 (5.3)	1.4 (10.4)
KS520	40.9 (0)	28.9 (1.2)	25.3 (4.4)	31.3 (0.8)	16.5 (3.3)	10.7 (12.2)	10.5 (5.2)	28.3 (0)	11.1 (0)	–	0.2 (26.7)	0 (34.2)
KS514	26.8 (0)	28.5 (0)	23.3 (0)	32.5 (1.7)	16.1 (0.8)	16.6 (0.7)	14.1 (1.5)	14.3 (0.2)	5.3 (0.6)	26.7 (0.2)	–	0 (3.8)
KS523	36.4 (0.7)	35.4 (0.7)	30.9 (0.3)	30.3 (0.4)	22.0 (1.3)	27.8 (1.9)	25.2 (1.4)	25.3 (0.3)	10.4 (1.4)	340 (0)	3.8 (0)	–

[a] Total number of Tct^+ transductants per cross varied from 50–1,000. (Closely linked sites giving fewer total Tct^+ transductants)
[b] No Tct^+ transductants
[c] Percentage of Tet Tct^+ transductants from the reciprocal cross are shown below in brackets for convenience of comparison

filters dried at 37° C for 30 min and exposed to X-ray film overnight. Citrate incorporation could also be accurately quantitated by merely punching out the dried clones on the filters and assaying for radioactivity by scintillation spectrometry.

Results

Fine Structure Mapping

135 independently isolated spontaneous FC^R mutants were studied. All showed the normal growth characteristics of *tct* mutants (Somers et al. 1981) including the inability to grow on isocitrate as a carbon source. Twelve mutants with low spontaneous reversion frequency were chosen at random and the linear order of these mutant sites was determined by 3-point reciprocal crosses, using the Tn*10* insertion of KS182 (*zfi2::*Tn*10*) as the closely linked outside marker (Somers et al. 1981). Selection was made for Tct^+ recombinants on isocitrate medium, and the number of Tct^+ transductants which were also tetracycline resistant was determined by replica plating. Table 2 shows the results of these crosses, and the linear order of sites is shown in Fig. 1.

Deletion mutants (Fig. 1) were identified by their inability to revert and by their failure to give Tct^+ recombinants on whole plate tests with two or more adjacent mapped point mutants. Eight spontaneous FC^R mutants were identified as deletions (KS481, 484, 512, 515, 722, 723, 724, 725). KS725 is a double deletion strain. The remaining deletion mutants were obtained by fusaric acid selection of tetracycline sensitive derivatives of Tn*10* insertion strains (Maloy and Nunn 1981) arising by imprecise excision of transposon Tn*10* (Kleckner et al. 1979). 'Right-hand' external Tn*10* insertion KS177 yielded four deletion strains (KS387, 621, 622, and 623) and 'left-hand' external Tn*10* insertion KS182 yielded two deletion strains (KS630 and 624). The deletions of strains KS623 and 624 covered the entire *tct* region. The remaining deletions were obtained from the four internal *tct*::Tn*10* insertions in strains KS202–205. Mapping of the internal end-points of these deletions allowed us to locate the position of the original internal Tn*10* insertions on the linear map (Kleckner et al. 1979).

The entire *tct* region is subdivided into 21 deletion segments using all available deletion strains above. The remaining 115 unmapped spontaneous FC^R mutants were assigned to 20 of these deletion segments (Fig. 1) on the basis of spot tests and whole plate transduction tests.

Inducing Conditions and Characterization of the C Protein

The C protein is a periplasmic tricarboxylate binding protein (Sweet et al. 1979) of *S. typhimurium* and defective mutants were previously shown to map in this region (Somers et al. 1981). The best comparative growth conditions were sought to demonstrate the expression of the C protein gene. Of a variety of media and culture conditions the PC medium was found to be optimal for growth of *tct* mutants and for the expression of the C gene. Figure 2

demonstrates the production of C protein under inducing (+citrate) and non-inducing conditions. This medium permits the demonstration of a wide variety of periplasmic proteins (Fig. 2). Superimposed on these are two well represented spots of approximately 30 kdal and corresponding to pIs of 6.1 and 5.5. Both of these represent the C protein, and bind citrate equally (Sweet et al. 1979, Sweet unpublished results). The presence of citrate also precludes the expression of a low molecular weight periplasmic protein of unknown function.

Expression of the C Gene in tct Mutants

SDS-PAGE was used to detect the presence of C protein in osmotic shock fluids of the twelve point mutants mapped by 3 point crosses, and the four internal Tn*10* insertion mutants shown in Fig. 1. Strains were grown under both non-inducing and inducing (+citrate) conditions. To enhance the sensitivity of the method the shock fluids were first idodinated with ^{125}I. Figure 3 illustrates that part of the gel in which the C protein is found.

These results demonstrate firstly that the C protein gene does indeed reside in this region. Mutations in two regions, covered by deletion groups 1–9, and by 12 (Fig. 1), preclude expression. Furthermore the four Tn*10* insertion strains KS202–205, the position of which span almost the entire region, describe the polarity of this operon, suggesting the direction of transcription from right to left (Fig. 1). If a single transcription unit is assumed, KS205 (and 202) would define the downstream limit of the C gene. The results also indicate that two other regions exist which result in the FC^R phenotype but which apparently do not code for the C protein (represented by deletion groups 10, and by 16–21 in Fig. 1).

Shock fluids from induced cells were further examined for C protein by isoelectric focussing (Fig. 4). IEF is somewhat more resolving than SDS-PAGE for periplasmic proteins in the pI 5–6 range. Wild type SU453 and C protein-producing point mutant strains KS500, 509, 493, and 467 show both isoelectric forms of the C protein (C_1 and C_2), as does the 'downstream' insertion strain KS202. 'Upstream' insertion mutants KS203, 204 and 205 have lost both isoelectric forms, as have all the deletion strains examined, with the exception of KS515 which retained both. In all mutants examined, C_1 and C_2 are either both retained, or both equally depressed or disappear.

Citrate Binding Analyses of Shock Fluids from tct Mutants

The activity of C protein in shockates of the above point, insertion and deletion mutants was measured by the ability to bind [1,5-^{14}C]-citrate. The results are listed in Table 3. In general, those mutants showing C protein on gels also showed citrate binding activity while those with no C protein showed no significant binding activity. The exception to this is deletion strain KS515 which shows normal C protein on gels but lacks binding activity. It is possible that this mutant may have a small deletion in *tctC* which destroys the C protein citrate binding site since the molecular weight and pIs of C_1 and C_2 appear to be unaltered.

ELISA Assay for C Protein

In order to rapidly screen a larger number of mutants for the presence of C protein we set up an ELISA assay system.

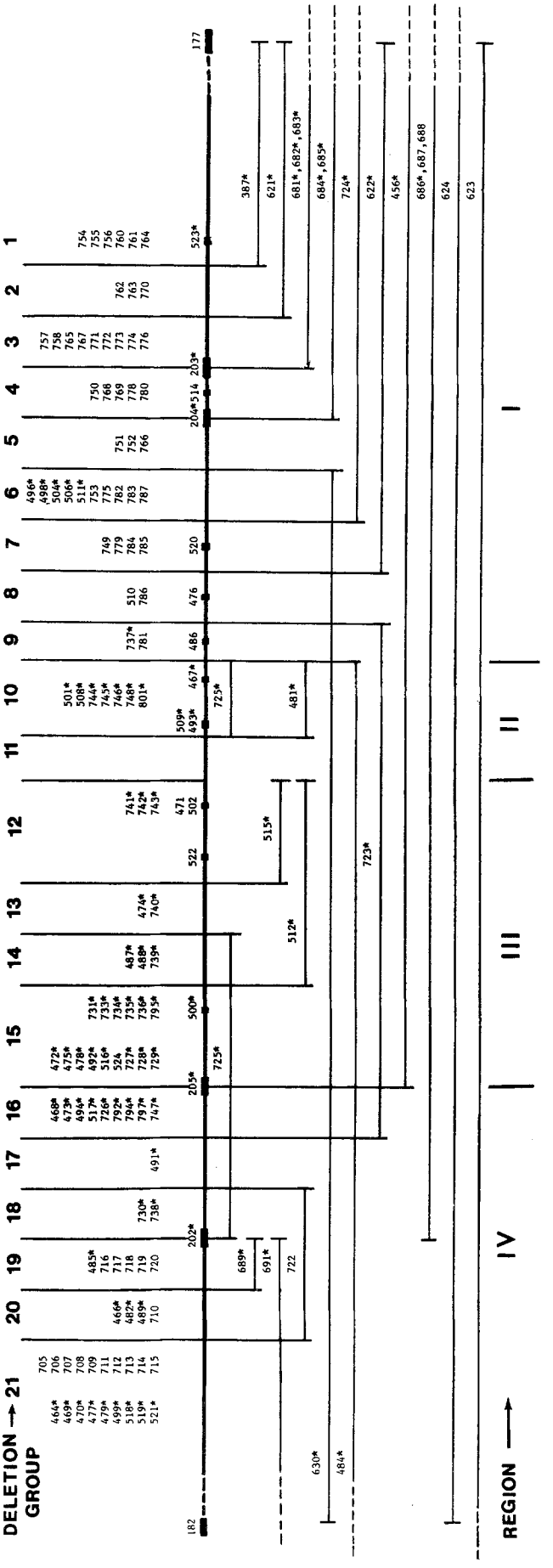

Fig. 1. Fine structure genetic map of the *tct* locus of *S. typhimurium*. Twelve point mutants used for the initial reciprocal crosses are indicated by squares on the chromosome; Tn*10* insertions are indicated by bars. The whole region is divided into 21 deletion groups. Mutants which have been analyzed for C protein by ELISA are indicated by asterisks. The proposed genetic regions are indicated below the map

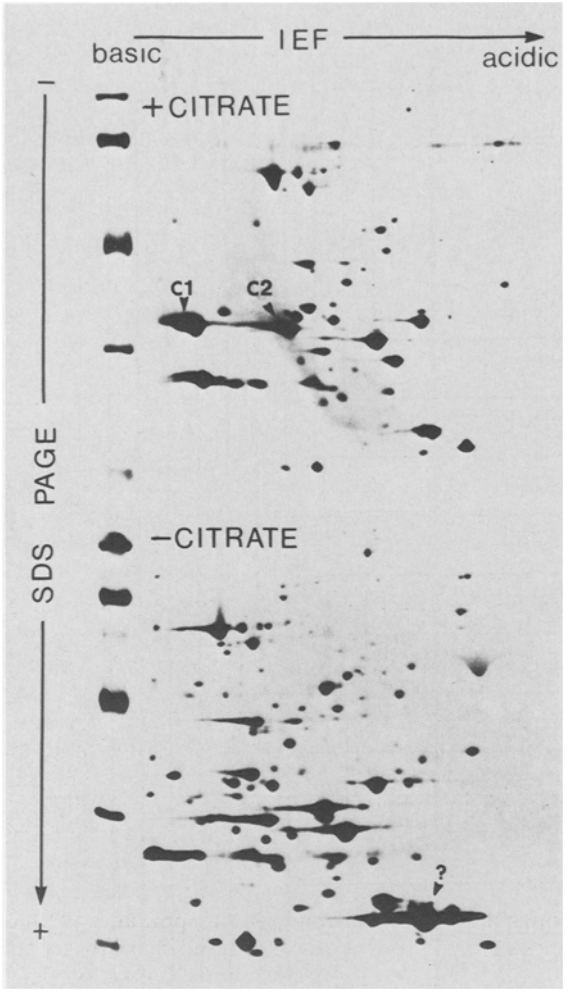

Fig. 2. 2-D Gel electrophoresis of the periplasmic fraction from induced and uninduced wild-type cells. Citrate induced fraction is in the upper gel. Molecular weight markers (92.5, 66.2, 45, 31, 21.5, and 14.4 kdal) are on the left. The gels were silver stained (Copeland 1982)

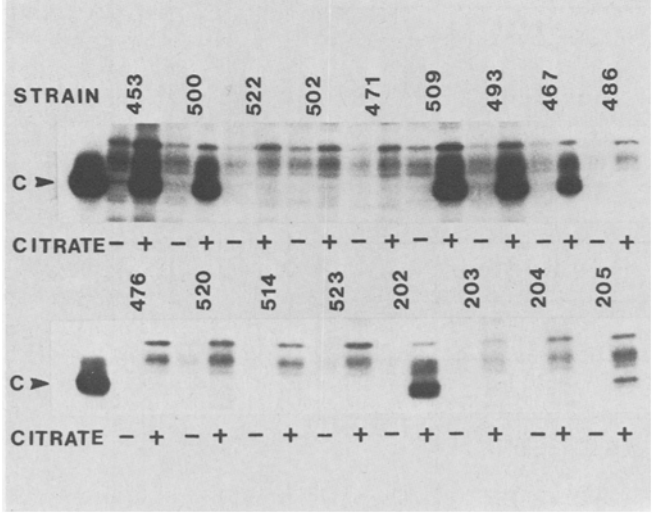

Fig. 3. SDS-PAGE of ^{125}I-iodinated periplasmic proteins from induced and uninduced cells. Strain SU453 is indicated as the wild-type control. The position of purified C protein is shown as is the induction condition (+or−citrate). The figure represents an autoradiograph of the 30 kdal region of the gel.

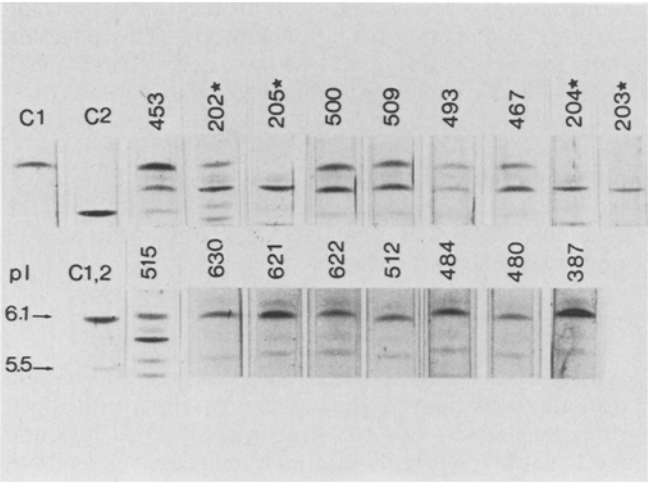

Fig. 4. IEF of periplasmic proteins from induced and uninduced cells. Both isoelectric forms of C protein, C1 and C2 are shown and the wild-type control is SU453. All strains were grown under inducing conditions. The pH gradient was 3–10 but only the C1 and C2 regions are shown. The gels were stained with Coomassie blue. The top line are points and insertions, the bottom are deletions

All strains in Fig. 1 analyzed for C protein are indicated with an asterisk, and from these (data not shown) the four distinct map regions could be more clearly resolved. Proximal mutations (from right to left in Fig. 1) falling in deletion segments 1–9 inclusive do not make C protein; segment 10 mutants make C protein; segments 12–15 inclusive are devoid of C protein (with the exception of KS500); segments 16–21 make the C protein.

FC Mutants as Transport Mutants

We devised a rapid screening system to assess the citrate transport activity of mutants defined as falling into the *tct* region (Fig. 1). Cells deposited on Millipore filters were allowed to grow for 6 h on PC medium in the presence of low concentrations of [1,5-^{14}C]-citrate. The results of an autoradiograph of such an experiment are shown in Fig. 5. Clearly, all mutants were defective in the uptake of citrate but to varying degrees. However, the severity of the defect could not be ascribed to any particular region of the *tct* locus. Thus all mutants defined by the FC^{R} phenotype appear to harbor transport defects. A variety of point, deletion and insertion mutants (25) representative of the breadth of the *tct* locus were also analyzed for the activities of enzymes concerned with citrate metabolism (aconitase, EC 4.2.1.3; isocitrate lyase, EC 4.1.3.1; malate synthase, EC 4.1.3.2; citrate lyase, EC 4.2.1.4; oxalacetate decarboxylase, EC 4.1.1.3) under inducing conditions (+citrate) as previously described (Somers et al. 1981). No enzyme activities were significantly changed (data not shown). Tricarboxylate cycle and gluconeogenic enzymes were assumed to be normal since all mutants grew normally on other TCA cycle carboxylates. These data strengthen the premise that the *tct* locus is a transport operon not involved directly in metabolism.

Table 3. Citrate binding activity in osmotic shock fluids from mutants in the *tct* locus

Strain	Deletion segment	Citrate binding (pmoles ml^{-1})	Strain	Deletion segment	Citrate binding (pmoles ml^{-1})
SU453	wildtype	15.5	*Tn10 insertions*		
Point mutants			KS203	3/4	0.5
KS523	1	0	KS204	4/5	0.1
KS514	4	3.3	KS205	15/16	2.9
KS520	7	0	KS202	18/19	16.3
KS476	8	0	*Deletion strains*		
KS486	9	0.1	KS387	1	2.9
KS467	10	16.5	KS621	1–2	0.8
KS509	10	15.6	KS622	1–7	1.3
KS493	10	23.7	KS515	12	1.1
KLS471	12	0	KS512	12–14	0.2
KS502	12	0.2	KS630	6–21	0.4
KS522	12	0	KS484	10–12	0.6
KS500	15	17.2	KS623	1–21	0.7
			KS624	1–21	0

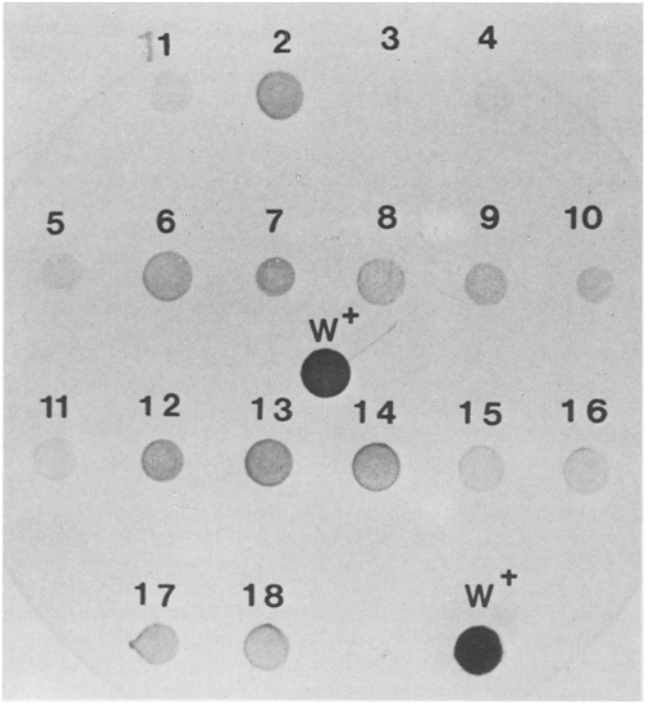

Fig. 5. [1,5-^{14}C]-Citrate uptake by *tct* mutant strains of *S. typhimurium*. Constant inocula from induced cells were exposed to 5×10^{-7} M [1,5-^{14}C]-citrate for 6 h. The figure represents an autoradiogram of the dried Millipore filter. The wild-type strain (W^+) was SU453 and the FC^R mutants were: 1-KS523, 2-KS574, 3-KS520, 4-KS486, 5-KS467, 6-KS509, 7-KS493, 8-KS502, 9-KS522, 10-KS500, 11-KS480, 12-KS481, 13-KS723, 14-KS515, 15-KS512, 16-KS725, 17-KS689, 18-KS691

Discussion

The transport of citrate and other tricarboxylates appears to be a complicated process in *S. typhimurium*. At least three distinct transport systems have been suggested at various times (Imai et al. 1977; Ashton et al. 1980; Somers et al. 1981). One of these systems is characterized by high affinity active transport of citrate, isocitrate and citrate analogues (Ashton et al. 1980), and was accurately mapped by us to a rather silent region (59 units) of the *Salmonella* chromosome (Somers et al. 1981). The only gene product found to date in this locus has been the high affinity C protein (Sweet et al. 1979).

We attempted to resolve this region further by fine-structure genetic mapping in order to elucidate the exact position of the C gene (C protein) and to get some feel as to the complexity of this locus and the possible number of genes comprising *tct*. Using a series of revertable point mutants and 'internal' Tn*10* insertions we were able to construct a linear order of mutations by three point crosses against outside Tn*10* insertions. Both spontaneous and Tn*10* excision deletions were isolated and used to set up the deletion map (Fig. 1); finally 115 independent FC^R isolates were mapped to twenty various deletion segments.

The expression of this *tct* locus in the various mutants was monitored using the C protein as an indicator and SDS-PAGE, IEF, and the ELISA immunoassay technique. The results herein indicate several things about this region: first, the region is complex consisting of at least four genetic regions; second, the region coding for the C protein (*tctC*) was clearly identified since some mutants in this gene such as KS515 had inactive binding protein; third, the direction of transcription was inferred to be clockwise from the polar effects of Tn*10* insertions; fourth, the C gene codes for the two isoelectric forms of the C protein since both forms appear and disappear together in the various mutants. Recent evidence indicates that C2 is a post-translational modification of C1 and we have been unable to find any functional differences between C_1 and C_2 (Sweet unpublished results). Recently the periplasmic glutamine binding protein from *Escherichia coli* has also been shown to exist in two isoelectric forms (Copeland et al. manuscript in press). The most proximal region of the *tct* locus is likely an operator-promotor region since the majority of mutants in this region do not produce C protein and appear to be strongly polar.

The tentative construction of this region into a tricarboxylate transport operon raises two important questions: what are the functions of the other two *tctC* flanking genetic regions, and why would the C protein be found in the middle of these genes? It is possible that these regions code for other transport components such as inner and outer

membrane proteins similar to that found in the maltose transport system (Hofnung 1974; Szmelcman and Schwartz 1976) and the phosphate transport system (Overbuke and Lugtenberg 1980; Tommassen et al. 1982). We have recently found both outer and inner membrane proteins inducible by citrate; however we do not as yet know their genetic location (unpublished data). Like other periplasmic proteins the C protein is produced in abundance (Sweet et al. 1981). Given the natural polar effects of bacterial operons one would expect to find the C gene as the first gene of an operon, such as for the *hisJ* gene in the histidine transport operon (Ames et al. 1977), otherwise the preceeding gene would be expressed in even greater abundance. Another possible role for these regions is that they in fact represent not substrate binding sites but interaction regions on the C protein for either outer membrane proteins such as postulated for the maltose binding protein (Ferenci et al. 1977; Heuzenroeder 1980) and as demonstrated for the histidine binding protein (Ames and Spudich 1976; Higgins and Ames 1981) and the maltose binding protein (Richarme 1982). However this seems unlikely since none of the C proteins from mutants in this region had either altered molecular weights or isoelectric points especially since it has been reported that even single amino acid substitutions in some binding proteins can cause an easily discernable displacement on SDS-PAGE (Noel et al. 1979). One mutant, KS500, mapped within the C gene but retained normal binding activity and was apparently unaltered with respect to molecular weight and isoelectric point as defined by our methods. Such a strain is clearly a good candidate for an "interaction site" defect with either outer or inner membrane transport components. A confirmation of the number of genes in the *tct* locus must await a complementation analysis which is now in progress. The identity of the other regions postulated here should result from a closer analysis of mutants in these regions.

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References

Ames GF (1974) Resolution of bacterial proteins by polyacrylamide gel electrophoresis on slabs. Membrane, soluble and periplasmic fractions. J Biol Chem 249:634–644

Ames GF-L, Spudich AN (1976) Protein-protein interaction in transport: Periplasmic histidine-binding protein J interacts with P protein. Proc Natl Acad Sci USA 73:1877–1881

Ames GF-L, Noel KD, Taber H, Spudich EN, Nikaido K, Afong J, Adeshir F (1977) Fine structure map of the histidine transport genes in *Salmonella typhimurium*. J Bacteriol 129:1289–1297

Ashton DM, Sweet GD, Somers JM, Kay WW (1980) Citrate transport in *Salmonella typhimurium*: studies with 2-fluoro-L-*erythro*-citrate as a substrate. Can J Biochem 58:797–803

Copeland BR, Todd SA, Furlong CE (1982) High resolution two dimensional gel electrophoresis of human erythrocyte membrane proteins. Amer J Hum Genet 34:15–31

Engvall E (1980) Enzyme immunoassay ELISA and EMIT. In: Vunakis HV and Langone JJ (eds) Methods in enzymology. Academic Press, Toronto, p 419

Fairbanks G, Steck TL, Wallach DFH (1971) Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. Biochemistry 10:2606–2617

Ferenci T, Boos W, Schwartz M, Szmelcman S (1977) Energy-coupling of the transport system of *Escherichia coli* dependent on the maltose-binding protein. Eur J Biochem 75:187–193

Higgins CF, Ames GF-L (1981) Two periplasmic transport proteins which interact with a common membrane receptor show extensive homoloy: complete nucleotide sequences. Proc Natl Acad Sci USA 78:6038–6042

Heuzenroeder MW, Reeves P (1980) Periplasmic maltose-binding protein confers specificity on the outer membrane maltose pore of *Escherichia coli*. J Bacteriol 141:431–435

Hofnung M (1974) Divergent operons and the genetic structure of the maltose B region in *Escherichia coli* K12. Genetics 76:169–184

Ingolia TJ, Koshland Jr, DE (1979) Response to a metal ion citrate complex in bacterial sensing. J Bacteriol 140:798–804

Imai K, Iijima T, Banno I (1977) Location of tct (tricarboxylic acid transport) genes on the chromosome of *Salmonella typhimurium*. Inst Ferment Res Commun (Osaka) 8:63–68

Kihara M, Macnab RM (1979) Chemotaxis of *Salmonella typhimurium* toward citrate. J Bacteriol 140:297–300

Kleckner N, Reichardt K, Botstein D (1979) Inversions and deletions of the *Salmonella* chromosome generated by the translocatable tetracycline resistance element Tn*10*. J Mol Biol 127:89–115

Maloy SR, Nunn WD (1981) Selection for loss of tetracycline resistance by *Escherichia coli*. J Bacteriol 145:1110–1112

Miller JH (1972) Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Noel D, Nikaido K, Ames GF-L (1979) A single amino acid substitution in a histidine-transport protein drastically alters its mobility in sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Biochem 18:4159–4165

Oakley BR, Kirsch DR, Morris NR (1980) A simplified ultrasensitive silver stain for detecting proteins in polyacrylamide gels. Anal Biochem 105:361–363

O'Farrell PH (1975) High resolution two-dimensional electrophoresis of proteins. J Biol Chem 250:4007–4021

O'Farrell PZ, Goodman HM, O'Farrell PH (1977) High resolution two-dimensional electrophoresis of basic as well as acidic proteins. Cell 12:1133–1142

Overbuke N, Lugtenberg B (1980) Expression of outer membrane protein of *Escherichia coli* K12 by phosphate limitation. FEBS Lett 112:299–232

Richarme G (1982) Interaction of the maltose-binding protein with membrane vesicles of *Escherichia coli*. J Bacteriol 149:662–667

Salacinski PRP, McLean C, Sykes JEC, Clement-Jones VV, Lowry PJ (1981) Iodination of proteins, glycoproteins and peptides using a solid-phase oxidizing agent, 1,3,4,6-tetrachloro-3α,6α-diphenyl glycoluril (Iodogen). Anal Biochem 117:136–146

Somers JM, Sweet GD, Kay WW (1981) Fluorocitrate resistant tricarboxylate transport mutants of *Salmonella typhimurium*. Mol Gen Genet 181:338–345

Sweet GD, Somers JM, Kay WW (1979) Purification and properties of a citrate-binding transport component, the C protein of *Salmonella typhimurium*. Can J Biochem 57:710–715

Szmelcman S, Schwartz M (1976) Maltose transport in *Escherichia coli* K12. A comparison of transport kinetics in wild-type and λ resistant mutants with the dissociation constants of the maltose-binding protein as measured by fluorescence quenching. Eur J Biochem 65:13–19

Tommassen J, Overduin P, Lugtenberg B, Bergmans H (1982) Cloning of *phoE*, the structural gene for the *Escherichia coli* phosphate limitation-inducible outer membrane pore protein. J Bacteriol 149:668–672

Willis RE, Morris RG, Cirakoglu C, Schellenberg GD, Gerber NH, Furlong CE (1975) Preparation of the periplasmic binding proteins from *Salmonella typhimurium* and *Escherichia coli*. Arch Biochem Biophys 161:64–75

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