Mapping, Cloning, Expression, and Sequencing of the rhaT Gene, Which Encodes a Novel L-Rhamnose-H+ Transport Protein in Salmonella typhimurium and Escherichia coli*

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A L-rhamnose transport-negative strain of Escherichia coli was generated by Mu d(Ap°, lac)I mutagenesis. This strain was used to isolate a clone of Salmonella typhimurium DNA that encoded L-rhamnose-H+ transport activity, the gene for which, rhaT, was sequenced. The rhaT gene was mapped on the E. coli chromosome between rhaR and sodA at 87.9 min, initially by Southern blot analysis and then by the isolation, expression, and sequencing of the rhaT gene. Both rhaT genes encoded a hydrophobic protein of 344 amino acids (91% identical) that contained 10 putative transmembrane regions. The RhaT protein represents a novel class of sugar transport protein.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank(TM)/EMBL Data Bank with accession number(s) M85157 and M85158.

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The RhaT transport system can transport L-mannose and L-lyxose in addition to L-rhamnose, but at reduced rates (Muir, 1989; Badia et al., 1991). It seems that RhaT catalyzes the uptake of L-rhamnose with the influx of protons i.e. it is a L-rhamnose-H+ symporter (Muir, 1989). Steady state kinetic analysis of 14C-labeled L-rhamnose transport gave apparent K_m values between 16 and 43 μM and apparent V_max, values between 11 and 17 nmol/min/mg dry mass (Muir, 1989). The kinetic data yielded only one set of kinetic constants, which suggested that there is only a single L-rhamnose transport system in E. coli. Apart from L-mannose and L-lyxose, no other L-sugar, nor any D-sugar, tested were inhibitors of L-rhamnose uptake. Thus, for a sugar to be transported by RhaT, the configuration at C2, C3, C4, and C5 of the sugar must be identical. Unlike most sugar/cation transport proteins, RhaT is not inhibited by N-ethylmaleimide, neither is it inhibited by cytochalasin B nor by forskolin, both of which are inhibitors of other sugar-H+ symporters (reviewed by Henderson, 1990).

This paper describes the isolation, characterization, and sequencing of rhaT clones from S. typhimurium and E. coli. An E. coli strain was constructed that was defective in L-rhamnose uptake by Mu d(Ap°, lac)I mutagenesis; this strain was used to screen a S. typhimurium C5 cosmid library by complementation. A single plasmid that complemented the lesion in 'the rhaT gene was obtained. A fragment of DNA that expressed a protein with L-rhamnose transport activity was subsequently isolated and sequenced. This clone was used to map the position of the rhaT gene between rhaR and sodA at 87.9 min on the E. coli chromosome by the isolation, expression, and sequencing of the E. coli rhaT gene.

EXPERIMENTAL PROCEDURES

Genetical Techniques—The E. coli strains used are listed in Table I. Mu d(Ap°, lac)I mutagenesis was performed as described by Casadaban and Cohen (1979). Samples of the infection mixture were spread onto minimal media plates (Miller, 1972) containing 10 mm

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L-rhamnose, 5 mM lactose, 80 µg/ml histidine, 100 µg/ml ampicillin to select for insertion of the Mu phage into the rhu operon. P1 transductions were carried out as described by Miller (1972). The E. coli strain used for the preparation of P1 phage to produce an fdp* strain was CSH25, and strain FB13 was used to generate a recA- strain.

β-Galactosidase Assays—Quantitative β-galactosidase assays were performed as described by Miller (1972); the method of Davis et al. (1984) was followed when performing plate assays for β-galactosidase.

Screening a Cosmid Library—The cosmid library was made by ligating a Sau3A partial digest of S. typhimurium C5 DNA into a BamHI-digested plasmid pH79 (Hohn and Collins, 1980) and was a kind gift from Dr. C. Hormaeche (Department of Pathology, University of Cambridge). The cosmid library was amplified using standard protocols (Maniatis et al., 1982).

DNA Sequencing—The S. typhimurium rhaT gene was sequenced by generating partial restriction digests of the 3.2-kbp EcoRV/PvuII fragment from plasmid pJAR6 with either AbuI, HaeIII, or Sau3A. In addition, a number of specific fragments were generated by digestion with combinations of HindIII, PvuII, EcoRV, and BglII. All the DNA fragments were ligated into bacteriophage M13mp18 and sequenced by the dideoxynucleotide chain termination method (Sanger et al., 1980). In addition, two oligonucleotides were synthesized to the rhaT gene sequence and were used as primers to obtain complete coverage of the DNA sequence. Single-stranded DNA was produced (Messing, 1983) and sequenced from universal primers using Sequenase (United States Biochemicals). The E. coli rhaT gene was sequenced by cloning the 1.6-kb Small/ XmnI fragment from plasmid pCGT6 into EcoRV-digested Bluescript plasmid (Stratagene), to make plasmid pCGT12. The sequencing strategy was to make unidirectional deletions in the fragment by using exonuclease III (Nestledon Kit, Pharmacia) followed by double-stranded sequencing of the product. To make deletions in the XmnI/XmnI fragment, to be sequenced with the M13 universal primer, plasmid pCGT12 was digested with BamHI and SacI prior to treatment with exonuclease III. Deletions were made in the opposite direction, to be sequenced using the M13 reverse primer, by digesting plasmid pCGT12 with HindIII and XmnI followed by digestion with exonuclease III. Preparations of double stranded plasmid DNA (Johnson, 1980) were sequenced using Sequenase (United States Biochemicals Ltd.). The double-stranded DNA sequencing protocol was exactly as described in the Sequenase protocol manual except that NaOH was removed after the denaturation step using a spun column (0.3 ml of Sepharose CL-6B 200 in a 0.5 ml microcentrifuge tube with a small hole in the bottom) rather than by precipitation. Additional DNA sequence was obtained from two oligonucleotide primers synthesized to the E. coli rhaT DNA sequence; oligonucleotides were synthesized on a Milligen "Cyclone" machine operated by M. Wheldon, Biochemistry Department, Cambridge University. DNA sequence was performed on a Pharmacia 2010 Macrophor sequencing apparatus at a constant voltage of 1200 V. DNA sequence data was analyzed using the Staden DNA sequencing programs (Bishop and Rawlings, 1987) processed by a DEC MicroVax 3100 operated by the University of Cambridge Department of Biological Sciences.

DNA Manipulations—Cosmid and plasmid DNA was prepared by the alkaline lysis method (Maniatis et al., 1982). Restriction digests were carried out according to the Manufacturers' recommendations (Amerham, Pharmacia, or New England Biolabs), and ligations were performed according to Maniatis et al. (1982). The CaCl2 method for preparing competent cells was routinely used (Maniatis et al., 1982). Restriction fragments were isolated from agarose gels either by electrophoresis onto Whatman DE81 ion exchange paper (Dretzen et al., 1981) or by excising the desired band and isolating the DNA by the "glass milk" method (Vogelstein and Gillespie, 1979).

Transport Assays—Transport of "C-labeled sugar and pH measurements of sugar-H+ symport activities were carried out as described elsewhere (Henderson et al., 1977; Henderson and Macpherson, 1986). Initial rates of sugar uptake were determined from the 15-s time point.

Southern Blots—Restriction enzyme-digested E. coli DNA was transferred by capillary blotting (Southern, 1975) onto Hybond N membranes (Amerham). A 400-bp HindII/BamHI restriction fragment (STRHAI) from the S. typhimurium rhaT gene was labeled with 32P using the random priming method (Feinberg and Vogelstein, 1983). Southern blots were probed with restriction fragment STRHAI (Maniatis et al., 1982) and washed at high stringency in 0.3 X SSC (20 X SSC: 3 M NaCl, 0.3 M tri-sodium citrate, pH 7) at 51 °C.

Restriction digests of a clone 4BG (Kohara et al., 1987) were probed with a gel-purified 32P-labeled oligonucleotide (Maniatis et al., 1982) with the 400-bp HindII/BamHI restriction fragment STRHAI. The oligonucleotide (ORHAI) corresponded to nucleotides 2185–2152 of the DNA sequence downstream from the rhaT gene sequence (Tobin and Schleif, 1987). The restriction-digested DNA was immobilized in an agarose gel and then it was probed with the 32P-labeled DNA. The preparation and drying of the agarose gel were as described by Tanen and Wallace (1986). The dried agarose gel probed with oligonucleotide ORHAI was washed in Tris (52 °C) in 5 X SSPE (20 X SSPE: 3 M NaCl, 180 mM NaH2PO4, 24H2O, 20 mM EDTA, pH 7.4). The dried agarose gel probed with restriction fragment STRHAI was washed at high stringency in 0.3 X SSC at 50 °C.

RESULTS AND DISCUSSION

Construction of a L-Rhamnose Transport-negative Strain of E. coli—The strategy employed to produce a L-rhamnose transport-negative strain of E. coli was to use Mu d(ApR,lac)I mutagenesis in a fdp host strain. The Mu d(ApR,lac)I phage inserts randomly into the host chromosome, inactivating the gene it inserts into. In addition, the lac operator in Mu d(ApR,lac)I, which lacks its own promoter, can be expressed from a suitably positioned promoter in the host chromosome. An E. coli host strain with the fdp lesion was used, because the presence of gluconecogenic carbon sources such as L-rhamnose or L-fucose results in the accumulation of toxic intermediates due to the absence of fructose 1,6-bisphosphatase, a key enzyme. Therefore a Mu d(ApR,lac)I insertion into a gene required for L-rhamnose utilization will make the fdp strain resistant to L-rhamnose.

E. coli strain JM2463 (Table I) was used as the parent strain for mutagenesis; the strain was fdp and could not grow on L-rhamnose, L-fucose, or glycerol. The ability to grow on L-rhamnose and L-fucose was restored by P1 phage-mediated transduction to fdp+ (strain JAR3, Table I), which showed that strain JM2463 contained functional rha and fuc operons. A Mu d(ApR,lac)I phage lysate was prepared from the Mu lysogen E. coli strain MAL103 (Table I) and was used immediately to infect strain JM2463. Ampicillin-resistant colonies were screened for L-rhamnose inducible growth on lactose. A single isolate, strain JAR1 (Table I) had the phenotypes Ap, Rha-, and Lac+. Plate assays for β-galactosidase activity indicated that the rhaT gene is transcribed from a suitably positioned promoter in the host chromosome. The rhaT genes from S. typhimurium and E. coli-The strategy employed to produce a L-rhamnose transport-negative strain of E. coli was to use Mu d(ApR,lac)I mutagenesis in a fdp host strain. The Mu d(ApR,lac)I phage inserts randomly into the host chromosome, inactivating the gene it inserts into. In addition, the lac operator in Mu d(ApR,lac)I, which lacks its own promoter, can be expressed from a suitably positioned promoter in the host chromosome. An E. coli host strain with the fdp lesion was used, because the presence of gluconecogenic carbon sources such as L-rhamnose or L-fucose results in the accumulation of toxic intermediates due to the absence of fructose 1,6-bisphosphatase, a key enzyme. Therefore a Mu d(ApR,lac)I insertion into a gene required for L-rhamnose utilization will make the fdp strain resistant to L-rhamnose. Our results were as described by Tanen and Wallace (1986). The dried agarose gel probed with restriction fragment STRHAI was washed at high stringency in 0.3 X SSC at 50 °C.

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erol as carbon source and were induced with either L-rhamnose or L-fucose, or not induced. The positive control  

Additional evidence that the Mu d(Ap$^+$,lac)I phage in strain JAR2 was inserted in the L-rhamnose-H$^+$ symporter gene was obtained by measuring the sugar-R$^+$ symporter activity of strain JAR2 after induction with L-rhamnose (Fig. 1). On addition of L-rhamnose to de-energized JAR3 cells (positive control) a rapid alkalization of the external medium was observed followed by a rapid acidification (Fig. 1). This is characteristic of a sugar being imported into the cell via a sugar-H$^+$ symporter followed by metabolism of the sugar to acidic end products that are excreted into the external medium (Henderson and Macpherson, 1986). In contrast, L-rhamnose-induced JAR2 cells did not elicit a rapid alkalization on addition of L-rhamnose, but only a slow acidification. This apparent metabolism was at a reduced rate compared with that observed for strain JAR3 and would have been unlikely to obscure sugar-H$^+$ symporter activity if it had been present. L-rhamnose induction of the rha operon in strain JAR2 could be observed by measuring the activity of LacY, encoded by the Mu phage and expressed from a rha promoter. On addition of isopropyl-1-thio-β-D-galactopyranoside (a substrate for LacY) to JAR2 cells induced with L-rhamnose, a rapid alkalization of the medium was observed (Fig. 1). This is not followed by acidification because isopropyl-1-thio-β-D-galactopyranoside cannot be metabolized (Fig. 1). This experiment showed that the absence of L-rhamnose-H$^+$ symporter activity from strain JAR2 was not due to leakiness of the membrane.

Additional manipulations were required before strain JAR2 could be used to screen a genomic library to isolate the rhaT gene. It was first made recA$^-$ by phage P1-mediated transduction; the P1 lysate was obtained from P1-infected strain PB13 (Table I). The derived recA$^-$ strain JAR62 subsequently had the Mu phage excised from the L-rhamnose transport gene to produce strain JAR66 with a stable genetic background for the screening of a genomic library using ampicillin for plasmid selection. During excision of the Mu phage there is often selection of genomic DNA which was adjacent to the phage, thus producing a stable deletion in the E. coli chromosome.

| Strain   | Genotype                                                                 | Derivation                      |
|---------|---------------------------------------------------------------------------|--------------------------------|
| AD5827  | F-, ilv, his, sup$, strA, proC::Tn10, gal OP151, Δbiu (X BamN)$             | A. Das$                         |
| AR120   | ΛN99, cl$, Δ-gal, nac::Tn10, Tn11 (ΔIlluvB)                                  | I. Miller                      |
| CSH28   | supF, tyrT, thi, pro                                                      | P. Oliver$                      |
| HU835   | F-, ara-, leu, pro, lacY1, gln, galK, recA, rpsL, rpsL, yx, mtl, thi, hadS, αIacO, b2, redβ3, st | M. C. Jones-Mortimer$           |
| JM2418  | Δhis-arg, Δlac, araDC, rpsL, ptsF, ptsM, ptsM, fdp                          | T. Britton$                    |
| JM2463  | Δhis-gnd, Δlac, araD, rpsL, ptsF, ptsM, fdp (P1, 100 Cm)                   | T. Gibbon$                     |
| JM2513  | As JM2463 but rhaΔpλacMulg                                                  | T. Gibbon$                     |
| MAL103  | F-, Muc$^+$ d(Ap$^+$,lac), Muc$^+$, (proA, lacIPOZYA)XIII, str                | T. Gibbon$                     |
| PB13    | Δ lac-pro, supE, thi, hadD5 [F' traD36, pro A$^+$, lacI$, lacZΔM15]        | T. Gibbon$                     |
| TG1     | Δ lac-pro, supE, thi, hadD5 [F' traD36, pro A$^+$, lacI$, lacZΔM15]         | T. Gibbon$                     |
| JAR1    | Δhis-gnd, Δlac, araD, rpsL, ptsF, ptsM, fdp, (P1, 100 Cm)                  | T. Gibbon$                     |
| JAR2    | As JAR1, but Δpλac                                                        | This work                      |
| JAR3    | As JAR1, but Δpλac                                                        | This work                      |
| JAR62   | As JAR2, but ΔrhaΔMu d(Ap$^+$, lac$^+$), Ap$^+$                             | This work                      |
| JAR66   | As JAR62, but rsl-recA::Tn10, Tc$^+$                                      | This work                      |

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TABLE II
Sugar transport assays in a control strain (JAR3) and two Mu lysogen strains (JM2513 and JAR2)

Uninduced cultures were grown on minimal medium with glycerol as carbon source. Cultures induced with either L-rhamnose or L-fucose were grown in minimal medium with glycerol and 10 mM inducer. Cultures were grown overnight at 30 °C; preparation of the cell suspension for the uptake experiments and β-galactosidase assays are as described in Henderson et al. (1977) and Miller (1972). The results are averages of duplicate measurements from three different experiments.

| Strain | Inducer | Sugar uptake | β-Galactosidase activity |
|--------|---------|--------------|--------------------------|
|        |         | L-Rhamnose   | L-Fucose                  |
|        |         | nmol/min/mg dry mass | Miller units |
| JAR3   | None    | 2.4          | 0.5                      | 0.0                     |
|        | L-Rhamnose | 14.6        | 25.3                     | 0.1                     |
|        | L-Fucose   | 0.4          | 35.5                     | 0.1                     |
| JM2513 | None    | 6.4          | 0.4                      | 0.3                     |
|        | L-Rhamnose | 0.8         | 6.8                      | 0.8                     |
|        | L-Fucose   | 0.4          | 2.0                      | 1.6                     |
| JAR2   | None    | 0.4          | 0.8                      | 10.8                    |

The rhaT genes of S. typhimurium and E. coli
Strain JAR66 was Ap<sup>R</sup>, Rha<sup>−</sup>, Lac<sup>−</sup> and no longer had L-rhamnose-inducible β-galactosidase activity. Interestingly, strain JAR66 would not grow at even high concentrations (100 mM) of L-rhamnose, whereas strain JAR2 could grow on L-rhamnose as sole carbon source if the concentration was greater than 40 mM. This suggested that additional genes had been deleted during the excision of Mu d(ApR<sup>R</sup>, lac<sup>−</sup>).

Screening a S. typhimurium Genomic Library to Isolate the rhaT Gene—A genomic library prepared from S. typhimurium C5 DNA (see “Experimental Procedures”) was amplified in strain HU835 and then used to infect the L-rhamnose transport-negative strain JAR66. 3000 Ap<sup>R</sup> colonies were screened by replica plating for growth on 10 mM L-rhamnose as sole carbon source. Three colonies grew on L-rhamnose. Restriction analysis of the plasmids from the three Rha<sup>+</sup> colonies indicated that they were identical (pJAR1).

Plasmid pJAR1 (Fig. 2) fully compensated for the deletion at the rha locus in strain JAR66. Strain JAR66(pJAR1) grew on minimal medium with L-rhamnose as sole carbon source and effected the uptake of 14C-labeled L-rhamnose following induction by L-rhamnose. Plasmid pJAR2 (SalI-digested pBR322 ligated to the 9.8-kb SalI fragment from plasmid pJAR1) fully complemented the deletion in pJAR66; L-rhamnose-H<sup>+</sup> symport activity was also observed (Fig. 1). Plasmid pJAR2 was used as a source of DNA for further subcloning of the rhaT gene.

Expression of the rhaT Gene from a λ Promoter and the Localization of the rhaT Gene—Plasmid pAD284<sup>+</sup> contains the λ P<sub>λ</sub> promoter on a 2.4-kb HindIII/BamHI DNA fragment ligated into plasmid pBR322. Plasmid constructs derived from pAD284 can be maintained in either of two λ lysogenic E. coli strains which produce CI repressor to prevent potentially lethal levels of expression from λ P<sub>λ</sub> during routine growth of strains. E. coli strain AR120 contained wild type λ as lysogen; the λ P<sub>λ</sub> promoter could be induced by nalidixic acid (Mott et al., 1985). E. coli strain AD5827 was a λ lysogen that expressed the cI<sub>57</sub> gene product; increasing the cell culture temperature from 33 to 42 °C resulted in the inactivation of the thermolabile cI<sub>57</sub> protein, thus allowing expression from the λ P<sub>λ</sub> promoter. The 2.4-kb HindIII/BamHI fragment from plasmid pAD284 containing the λ P<sub>λ</sub> promoter was ligated into HindIII/BamHI-digested plasmid pBR322. Only one orientation of the 9.8-kb SalI insert in relation to the λ P<sub>λ</sub> promoter was obtained with this method. To obtain the opposite orientation (pJAR4), plasmid pJAR3 was digested with SalI to remove the 9.8-kb SalI insert in relation to the λ P<sub>λ</sub> promoter.

* A. Das, unpublished data.
The rhaT genes of S. typhimurium and E. coli

TRANSPORT

RHAMNOSE

a. pJAR1 positive
b. pJAR2 positive
c. pJAR3 positive
d. pJAR5 positive
e. pJAR6 positive
f. pJAR7 negative
g. pJAR9 negative
h. pJAR10 positive

A Pv

P H B Se Bi A S Bl S E P A S E P N E B Hc A S

(S/N)

(B/B) A Pv P H B Bi A Bse Bi A E B Hc A A

1000bp

FIG. 2. Identification of a DNA fragment encoding the rhaT gene. Plasmid constructs that contained different restriction fragments of the cloned S. typhimurium genomic DNA were tested for their ability to complement the lesion in JAR66 as measured by uptake of [14C]-rhamnose and sugar-H+ symport experiments. Plasmids pJAR1 and pJAR2 (a and b) both complemented the rha- lesion in JAR66, as indicated by growth of strains JAR66(pJAR1) and JAR66(pJAR2) on minimal medium that contained L-rhamnose as sole carbon source. Uptake activity and L-rhamnose-H+ symport assays were performed on strains JAR66(pJAR1) and JAR66(pJAR2) after growth in minimal media that contained L-rhamnose as sole carbon source. Plasmids pJAR3–10 (c–h) contained partial deletions of the cloned S. typhimurium genomic DNA downstream from the λ promoter P1 in plasmid pAD284. Induction of the P1 promoter upstream from the rhaT gene was achieved using both nalidixic acid induction for the plasmids in E. coli strain AR120 and by heat shock for the plasmids in E. coli strain AD5827. The ability of induced strains to exhibit uptake activity and L-rhamnose-H+ symport activity is indicated by "positive" or "negative" in the column labeled "rhamnose transport." The rate of [14C]-rhamnose uptake in positive strains varied between 1.7 and 14.7 nmol/min/mg dry mass, whereas negative strains typically showed uptake rates between 0.0 and 1.1 nmol/min/mg dry mass. a and b, linear diagrams of plasmid constructs pJAR1 and pJAR2. The hatched box in the diagram of plasmid pJAR1 represents part of plasmid pHC79, and the stippled box in plasmid pJAR2 represents part of plasmid pBR322. c–h, linear diagrams of plasmid constructs pJAR3 to pJAR10. Filled boxes represent part of plasmid pAD284 (Fig. 5). The arrow beneath the filled boxes represents the position and direction of transcription of the rhaT gene (filled box) as deduced from c–h shown below. Transcription of the rhaT gene should start between the EcoRV and BgII sites (open box) and end between the HincII and AosI sites (hatched box).

Nalidixic acid induction of strains AR120(pJAR3) and AR120(pJAR4) resulted in expression of L-rhamnose transport activity (4.2 ± 0.3 nmol/mg/min) only in strain AR120 (pJAR3). The L-rhamnose transport activity in nalidixic acid induced strain AR120(pJAR4) was 0.5 ± 0.4 nmol/mg/min. This defined the direction of the transcription of the rhaT gene as BgII to AosI (Fig. 2). To locate the rhaT gene within the 9.8-kb SauI fragment, a series of plasmids were constructed from plasmid pJAR3 with deletions in the insert DNA. Each construct was transformed into E. coli strains AR120 and AD5827 and tested for induction of L-rhamnose transport activity. The results (Fig. 2) suggested the presence of the rhaT gene between the EcoRV site (proximal to a BgII site) and the AosI site, with transcription starting somewhere between the EcoRV site and the BgII site. Thus, we have located the rhaT gene to a 2.4-kb portion of DNA within the original 9.8-kb fragment (Fig. 2i).

Sequencing of the S. typhimurium rhaT Gene—A 3-kb EcoRV-PvuII fragment from plasmids pJAR6 and pJAR10, which contained the complete rhaT gene, was sequenced (see "Experimental Procedures"). A contiguous sequence of 3052 bp was assembled; this comprised 2377 bp of S. typhimurium DNA (Fig. 3) between the EcoRV and AosI sites in plasmid pJAR10 (Fig. 2i) and 675 bp of plasmid pBR322 DNA sequence (data not shown) between the AosI and PvuII restriction sites. The S. typhimurium DNA sequence was determined insert followed by re-ligation of the insert with phosphatase-treated vector.

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**The rhaT genes of S. typhimurium and E. coli**

**a.**

The rhaT gene was sequenced directly from genomic DNA of E. coli K12 from pJAR3 and pJAR4 (Fig. 2). The DNA sequence was determined on both strands of the DNA gene from E. coli K12. A single open reading frame was identified by measuring the codon usage in all of the possible reading frames. The single open reading frame is in the 5' to 3' orientation, as predicted from the Shine-Dalgarno sequence and sequencing strategy of the DNA sequence. Possible -10 and -35 regions are indicated by arrows. A partial CRP binding site is shown by a box. DNA sequences predicted to form hairpin loop structures, or palindromic repeats, are shown by asterisks. The putative Shine-Dalgarno sequence is shown by a line above the DNA sequence. The Shine-Dalgarno sequence is typical of bacterial genes whose expression is controlled by a positive transcriptional activator, such as Schleif, 1987, 1990b). In addition, there was a short palindromic repeat (nucleotides 920-926 and 944-950) adjacent to the termination codon, there was an inverted repeat (nucleotides 920-926 and 944-950) adjacent to the termination codon.

**b.**

The rhaT protein sequence is given in the single-letter amino acid code above the DNA sequence. A putative Shine-Dalgarno sequence was obtained more than once, this is indicated by a filled box. The Delgarno sequence is shown by a line above the DNA sequence. The Delgarno sequence is typical of bacterial genes whose expression is controlled by a positive transcriptional activator, such as Schleif, 1987, 1990b). In addition, there was a short palindromic repeat (nucleotides 920-926 and 944-950) adjacent to the termination codon, there was an inverted repeat (nucleotides 920-926 and 944-950) adjacent to the termination codon.

**Fig. 3.** The DNA sequence and sequencing strategy of the rhaT gene from S. typhimurium. **a.** The RhaT protein sequence is given in the single-letter amino acid code above the DNA sequence. A putative Shine-Dalgarno sequence was obtained more than once, this is indicated by a filled box. The Delgarno sequence is shown by a line above the DNA sequence. The Delgarno sequence is typical of bacterial genes whose expression is controlled by a positive transcriptional activator, such as Schleif, 1987, 1990b). In addition, there was a short palindromic repeat (nucleotides 920-926 and 944-950) adjacent to the termination codon, there was an inverted repeat (nucleotides 920-926 and 944-950) adjacent to the termination codon.

**b.** The DNA sequence was determined on both strands of the DNA gene from E. coli K12. A single open reading frame was identified by measuring the codon usage in all of the possible reading frames. The single open reading frame is in the 5' to 3' orientation, as predicted from the Shine-Dalgarno sequence and sequencing strategy of the DNA sequence. Possible -10 and -35 regions are indicated by arrows. A DNA sequence predicted to form hairpin loop structures, or palindromic repeats, are shown by asterisks. The putative Shine-Dalgarno sequence is shown by a line above the DNA sequence. The Shine-Dalgarno sequence is typical of bacterial genes whose expression is controlled by a positive transcriptional activator, such as Schleif, 1987, 1990b). In addition, there was a short palindromic repeat (nucleotides 920-926 and 944-950) adjacent to the potential ribosome and CRP binding sites. A partial restriction map is shown below. Arrows represent a DNA sequence derived from one sequencing reaction. If a particular sequence was obtained more than once, this is indicated by a number at the start of the arrow.

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**Fig. 3.** The DNA sequence and sequencing strategy of the rhaT gene from S. typhimurium. **a.** The RhaT protein sequence is given in the single-letter amino acid code above the DNA sequence. A putative Shine-Dalgarno sequence was obtained more than once, this is indicated by a filled box. The Delgarno sequence is shown by a line above the DNA sequence. The Delgarno sequence is typical of bacterial genes whose expression is controlled by a positive transcriptional activator, such as Schleif, 1987, 1990b). In addition, there was a short palindromic repeat (nucleotides 920-926 and 944-950) adjacent to the termination codon, there was an inverted repeat (nucleotides 920-926 and 944-950) adjacent to the termination codon.
structure comprising a 9-bp stem and eight-nucleotide loop. It is unlikely that this stem loop represents a rho-independent terminator (reviewed by Platt, 1986). Identification of the rhaT Gene in E. coli—With the publication of a complete restriction map for the E. coli K12 chromosome (Kohara et al., 1987), it is comparatively easy to map the position of the rhaT gene. E. coli K10 genomic DNA was digested singly and in combination with various restriction enzymes. The DNA fragments were separated by agarose gel electrophoresis and blotted onto a membrane. The Southern blots were probed with a 32P-labeled 400-bp HindII/BalI restriction fragment (STRHA1) corresponding to the C-terminal portion of the RhaT protein. A single band in each lane was seen on autoradiography of the Southern blot (results not shown). The sizes of the restriction fragments produced by BamHI, EcoRI, PstI, HindIII, PvuII, and KpnI digestions were consistent with only one region of the E. coli DNA restriction map (Fig. 4), adjacent to the rha locus. However, fragments derived by EcoRV digestion did not concur with the published data. The λ clone 4B6 which encompassed this region was obtained from Dr. Y. Kohara, Nagoya University, Japan. To define the position of the rhaT gene in relation to the sequenced rhaS/R genes (Tobin and Schleif, 1987), an oligonucleotide (ORHA2) was synthesized to a region downstream of the rhaR gene. A dried agarose gel containing restriction digests of the bacteriophage clone was probed with 32P-labeled oligonucleotide ORHA2 and the S. typhimurium rhaT gene fragment, STRHA1, also labeled with 32P (see “Experimental Procedures”). Autoradiography of the probed gel indicated that the rhaT gene was on the same 3-kb PvuII fragment as the region downstream from the rhaR gene. Computerized analyses of the DNA sequences for S. typhimurium rhaT and E. coli rhaR genes identified the region downstream from the E. coli rhaR gene as the C terminus of RhaT. However, there was not a single open reading frame in the region downstream from the rhaR gene (Tobin and Schleif, 1987) that might encode RhaT; this was ascribed to a sequencing error in this region of DNA (see below).

Expression of the E. coli rhaT Gene under Control of a λ Promoter—To confirm the presence of the rhaT gene on the 3-kb PvuII fragment, a restriction fragment that contained the rhaT gene was ligated into plasmid pAD284 downstream of the λ P1 promoter (Fig. 5). The 3-kb E. coli genomic DNA PvuII fragment from λ clone 4B6 (Kohara et al., 1987) was first subcloned into plasmid pBR322 (pCGT6); a derived 1.6-kb XmnI/SmaI restriction fragment predicted to contain the rhaT gene was subsequently cloned from plasmid pCGT6 into the HpaI site of plasmid pAD284 (Fig. 5). The SmaI site was predicted to be at the end of the rhaR gene (Tobin and Schleif, 1987), whereas the XmnI site was in the PBR322 DNA, 35 nucleotides from the PvuII site (Fig. 5). Two orientations of the insert were obtained; plasmid pCGT10 was predicted to contain the insert in the right orientation for its expression by the λ P1 promoter, whereas in plasmid pCGT11 the rhaT gene was predicted to be in the wrong orientation for expression (Fig. 5). Plasmids pCGT10 and pCGT11 were transferred into E. coli strain TG2 (pLAV5), which expressed the temperature-sensitive cI gene product, cI857 (Remaut et al., 1983). Thus, at 33 °C the λ P1 promoter was inactive due to the cI857 protein binding at the operator sites, whereas at 42 °C the cI857 protein was nonfunctional and the λ P1 promoter expressed the DNA inserted downstream from it. After induction at 42 °C, only strain TG2 (pCGT10/pLAV5) produced a protein which could effect the uptake of radiolabeled L-rhamnose into cells (Fig. 5). This confirmed that there was a functional open reading frame in the XmnI/SmaI fragment, despite the lack of an open reading frame in the region downstream from the rhaR gene (Tobin and Schleif, 1987). The XmnI/SmaI fragment was also cloned into EcoRV-digested Bluescript plasmid (plasmid pCGT12) so that the E. coli rhaT gene could be sequenced.

DNA Sequence of the E. coli rhaT Gene—The 1.6-kb XmnI/SmaI fragment containing the E. coli rhaT gene was sequenced directly from plasmid pCGT12 (see “Experimental Procedures”). The DNA was sequenced at least once on both strands; Fig. 6 depicts the 1560 nucleotides of DNA sequenced between the PvuII and SmaI restriction sites. Three open reading frames were identified in the sequence. A single complete open reading frame that encoded a 344-amino acid protein was found; the orientation of the open reading frame corresponded to the orientation of the rhaT gene deduced from the induction of the λ P1 promoter in plasmid pCGT10. The protein was 91% identical to the RhaT protein predicted from the S. typhimurium gene sequence. Upstream from the putative initiator Met codon of the E. coli rhaT gene was a good match to the consensus Shine-Delgarno sequence (nucleotides 462–466), possible —35 and —10 regions (nucleotides...
Comparison of the RhuT Protein and Gene Sequences

A palindromic repeat downstream from the rhaT gene (nucleotides 1508-1533) and its homologs in S. typhimurium and E. coli.

Another open reading frame (nucleotides 1-189) at the start of the DNA sequence encoded the N terminus of a predicted protein identical to the published sequences of the manganese-containing superoxide dismutase SodA (Steinman, 1978). The DNA sequence of the sodA gene has also been published (Takeda and Avila, 1986); nucleotides 1-329 are identical to the published sequence except for a base change at nucleotide 16.

Nucleotides 1508-1560 were part of an open reading frame that encode the C terminus of RhaR identical to that published by Tobin and Schleif (1987). The DNA sequence of their rhaR gene included 325 nucleotides of sequence downstream from the rhaT gene, which should have encoded the rhaT gene. However, the presence of a compression resulted in the omission of a single base (nucleotide 1306, Fig. 6) thereby disrupting the coding sequence of the rhaT gene.
The *rhaT* genes of *S. typhimurium* and *E. coli*

changes, and 4 conservative changes. The majority of the differences seem to be in the hydrophilic regions and not in the putative transmembrane domains. The codon usage tables (Fig. 8) show that many non-preferred codons are used in the *rhaT* genes. The frequency of optimal codon usage (Ikemura, 1981) for RhaT is 0.59, compared with 0.61 for LacY, 0.57 for MelB, 0.65 for XylE, and 0.74 for AraE. Highly expressed proteins tend to have a higher frequency of optimal codon usage; for example, OmpA has an optimal codon usage frequency of 0.92 (Ikemura, 1981). The RhaT protein is extremely hydrophobic; the *E. coli* RhaT protein contains 73.3% hydrophobic amino acids with a hydropathic index of 0.82 (Kyte and Doolittle, 1982).

Alignment of the DNA sequences from the two organisms revealed a region of 1236 nucleotides with 81% identity (nucleotides 328–1560 of the *E. coli* sequence aligned with nucleotides 888–2123 of the *S. typhimurium* DNA sequence). The sequence upstream from the *rhaT* gene in *S. typhimurium* did not contain a sequence which corresponded to the *sodA* gene and was not homologous to any DNA sequence in computer databases. The region downstream from the *rhaT* gene in *S. typhimurium* was homologous to the *rhaT* gene from *E. coli*. However, two nucleotides in different locations were absent from the *S. typhimurium* *rhaT* gene, which resulted in the disruption of the reading frame for RhaT. This region was sequenced adequately on both strands of the DNA (Fig. 3), so it is unlikely that the differences are due to a sequencing error. Therefore these changes may be a cloning artifact or may represent the true *rhaT* gene sequence in *S. typhimurium* C5. This strain of *S. typhimurium* cannot grow on L-rhamnose as sole carbon source and does not possess a L-rhamnose-inducible RhaT activity, despite having a fully functional *rhaT* gene (see Figs. 1 and 2); a nonfunctional *rhaT* gene in *S. typhimurium* C5 could explain these observations.

**CONCLUSIONS**

We have isolated and sequenced the *rhaT* genes from *S. typhimurium* and *E. coli*. Expression of the *rhaT* genes from a λ P, promoter resulted in an *E. coli* strain that could effect the uptake of "C-labeled L-rhamnose and showed sugar-"symport activity. The *rhaT* gene maps between the *sodA* and *rhaR* genes at 87.9 min (3605 kb) on the *E. coli* chromosome restriction map (Kohara *et al.*, 1987). It encodes an extremely hydrophobic protein of 344 amino acids; the *S. typhimurium* and *E. coli* RhaT proteins are 91% identical. The RhaT protein sequence has been compared with protein sequences in computer databases, but no significant homology was detected. The model for RhaT in Fig. 9b was deduced from a
hydropathy plot (Fig. 9a) which identified 10 clearly defined hydrophobic regions in the protein that could span a lipid bilayer. The orientation of RhaT in the membrane was deduced from the “positive inside” rule (von Heijne, 1986), which predicts that hydrophilic loops in the cytoplasm generally have a net positive charge. A model of RhaT (Fig. 9) with the N and C terminus on the periplasmic face of the membrane conforms to the positive inside rule; all the hydrophilic loops in the cytoplasm have a net positive charge, whereas all the loops in the periplasm have a net negative charge or are uncharged. The proposed topology of the RhaT protein is therefore completely different to the 12 transmembrane domain models proposed for other sugar-H+ symporters (reviewed by Henderson, 1990). In addition, the amino acid sequence of the RhaT protein is not homologous to any other protein. Thus the L-rhamnose-H+ symporter represents a novel type of sugar transport protein. Current work is focusing on the overexpression of rhaT and the use of β-lactamase as a topological reporter to define the structure of the RhaT protein.

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