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Cloning of the Histidine Transport Genes From Salmonella typhimurium and Characterization of an Analogous Transport System in Escherichia coli

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The genes for the well-characterized high-affinity histidine transport system of S typhimurium have been cloned in λgt4. Genetic and physiological analyses of the analogous transport system of E coli were undertaken in order that available λ vectors, recombinant DNA techniques, and a genetic selection for transport function might be used to isolate the Salmonella genes. The presence of the transport genes on a 12.4 Kb cloned DNA fragment has been confirmed 1) genetically, by complementation studies; 2) physiologically, by the rates of histidine uptake by bacteria containing this DNA; and 3) by demonstrating that the cloned DNA codes for the previously identified transport proteins J and P. The isolated fragment carries the entire transport operon, the *argT* gene and the *ubiX* locus, but neither the *purF* gene nor the *ack/pta* loci.

Key words: S typhimurium histidine transport operon, cloning, E coli histidine transport, genetics, gene duplications

Salmonella typhimurium transports L-histidine through several systems with different patterns of specificity and affinity [1]. Transport of histidine through the high-affinity shock-sensitive system has been analyzed kinetically, biochemically, and genetically [1–5] and has been shown to require three components: 1) the J protein, a periplasmic histidine-binding protein that has been purified [6] and that is the product of the *hisJ* gene; 2) the P protein, the *hisP* gene product, a cytoplasmic membrane protein; and 3) the product of the *hisQ* gene, an as yet unidentified protein whose cellular location is unknown. The genes for these three proteins, together with a regulatory locus *dhuA*, constitute an operon located at 48.5 minutes on the S typhimurium linkage map [5].

The molecular mechanism by which these three proteins function together to transport histidine across the cytoplasmic membrane has not been fully elucidated. It is known that the histidine-binding protein J possesses a second site, besides its histidine-binding site, that is essential for transport [7] and is involved in an interaction with the P protein [8]. Interestingly, the P protein is required for the transport of substrates other than histidine, such as arginine (if used as a nitrogen source), and histidinol [9]. The transport of arginine as a nitrogen source utilizes a lysine-arginine-ornithine-binding protein (LAO protein) which presumably interacts with the P pro-

Abbreviations: SDS-PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis; HIPA, hydrazino imidazole propionic acid; RF, replicative form, double-stranded DNA.

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tein in the course of arginine transport as the J protein is proposed to do during histidine transport [10]. The *argT* gene, which encodes the LAO protein, is very closely linked to the histidine transport operon on the S typhimurium map, and the LAO protein and J protein cross-react immunologically [10]. The levels of the histidine transport proteins, the LAO protein, and two other amino acid transport systems are regulated in response to nitrogen availability [10].

We describe here the isolation, utilizing phage λ vectors, of a Salmonella DNA fragment that carries the entire transport operon. The use of such recombinant DNA techniques, combined with genetic methods, should enable us to identify the Q protein and should facilitate purification of the transport components through their overproduction in suitable vector-host systems. The isolation of this fragment will also allow us to study the physical structure and DNA sequence of the genes for the transport proteins and the regulation of their expression in vitro.

In order to use available λ vectors, we had to characterize genetically, biochemically, and physiologically the high-affinity histidine transport system of the λ host, E coli. Here we show that E coli has a system analogous in all respects to the well-characterized S typhimurium system.

METHODS

Strains and Media

Bacterial strains used are listed in Table I. Either M9 [12] or E [13] minimal salts medium with 0.4% glucose as carbon source was used. LB medium was used when growth in a rich medium was required.

Selection of Histidine Transport Mutants in E coli

The selection for elevated levels of the transport proteins involves the ability to grow on D-histidine as a source of L-histidine for a histidine auxotroph [14]. D-histidine utilizing mutations were selected in TA3472 (*his*Δ*461*) as fast-growing papillae on minimal medium plates, either by spreading 10 μmoles D-histidine per plate together with 2×10^7 cells, or by pouring 2×10^7 cells/plate in minimal soft agar and putting down discs containing from 0.5 to 3 μmoles D-histidine. D-histidine-utilizing colonies were purified twice and then screened for elevated levels of the *hisJ* protein by SDS-PAGE [15] of total cellular protein. Several mutations causing obviously increased levels of J protein were mapped by P1 phage transduction [16], for linkage to the *purF* locus using EM1407 as a recipient; two strains (TA3521 and TA3522) with the expected genetic linkage were saved. Mutants defective in histidine transport were isolated in D-histidine-utilizing strains as resistant colonies around a disc containing 5 μmoles of the analogue α-hydrazinoimidazole propionic acid (HIPA) on E medium plates with 10 μmoles of carnosine per plate as a source of histidine [17]. Larger zones of inhibition by HIPA were observed on media containing poorer nitrogen sources, together with carbon sources other than glucose. The combinations of 10 mM proline as nitrogen source and 0.2% succinate or acetate as carbon source were also used to select $HIPA^R$ derivatives. The $HIPA^R$ mutants were screened for the loss of their ability to grow on D-histidine. Several of the transport mutants and their parent strains were assayed for histidine transport by the incorporation of labeled histidine into protein [2].

Deletion mutants of the transport operon were isolated by simultaneously selecting for $HIPA^R$ and resistance to fluoroacetate (FAc^R) [18]. The fluoroacetate-resistant phenotype is conferred by mutations in either one of two genetic loci, *ack* and *pta* [19], which are located on the distal side of the transport operon relative to *purF* [18].

TABLE I. Bacterial Strains

	Genotype	Source
E coli		
TA3472	*hisΔ(OGDCBHAFIE)461*	*P. Hartman*
TA3474	*hisΔ(OGDCBHAFIE)461 (λgt4-sa1)*	*This lab*
TA3475	*hisΔ(OGDCBHAFIE)461 (λgt4-sa2)*	"
TA3476	*hisΔ(OGDCBHAFIE)461 hisΔ(JQP ack/pta)700*	"
TA3478	*hisΔ(OGDCBHAFIE)461 hisΔ(JQP ack/pta)700 (λgt4-sa1)*	"
TA3479	*hisΔ(OGDCBHAFIE)461 hisΔ(JQP ack/pta)700 (λgt4-sa2)*	" "
TA3521	*hisΔ(OGDCBHAFIE)461 dhuA2*	"
TA3522	*hisΔ(OGDCBHAFIE)461 dhuA1*	"
TA3516	*hisΔ(OGDCBHAFIE)461 dhuA2 ackΔ(ack pta hisP)201*	"
EM1407	*dsdA⁻ purF⁻his⁻ supF trp⁻*	*E. McFall*
SF8	*thr-1 leu-6 thi-1 supE44 lacY1 ton A21 lop11, lig⁺ suII galΔ strR recBC⁻ r_K^- m_K^-*	*R. Davis*
TA3456[a]	*thr-1 leu-6 thi-1 supE44 lacY1 tonA21 r_K^- m_K^+*	"
NO1406	*uvrA⁻ sup⁻ gal⁻ str^R (λ papa)*	*A. DeFranco*
71-18	*lacΔ/F' lac I^qZΔM15 pro⁺*	*J. Messing*
S typhimurium		
ST314	*zea-2*::Tn10	A. DeFranco
TT317	*purF1741*::Tn10	J. Roth
TR5696[b]	*hisΔ712 (ΔhisDCBHAFIE)/F'128 lac⁺ pro⁺*	"
TA3412[b]	*hisF645 galE503 hisΔ(Q)6711 dhuA1/F'128 lac⁺ pro⁺*	*This lab*
TA3413[b]	*hisF645 galE503 hisΔ(QP)6712 dhuA1/F'128 lac⁺ pro⁺*	"
TA3415	*hisF645 hisΔ(J)6776 dhuA1/F'128 lac⁺ pro⁺*	"
TA3416	*hisF645 hisΔ(dhuA J)5638/F'128 lac⁺ pro⁺*	"
TA3417	*hisF645 hisΔ(P)5503 dhuA1/F'128 lac⁺ pro⁺*	"
TA3419	*hisF645 hisΔ(QP)6712 dhuA1/F'128 lac⁺ pro⁺*	"
TA3078	*hisF645 hisΔ (dhuA J ubiX)8907*[c]	"
TA3190	*dhuA1 hisF645 hisJ8918*::Tn10	"
TA3193	*dhuA1 hisF645 hisP6641*::Tn10	"
TA3194	*dhuA1 hisF645 hisQ6642*::Tn10	"
TA1852	*purF145 dhuA1 hisF645*	"
TA3292	*dhuA1 gln-139*	"
TA1014	*dhuA1*	"
TA271	*dhuA1 hisF645*	"

[a]Also known as $C600_r^-m^+$.
[b]The parent of these strains has been cured of the S typhimurium fi^+ cryptic plasmid [11] and was obtained from H. Whitfield.
[c]Deletion produced by excision of Tn10, the strain remains tetracycline resistant.

Growth and Purification of λ Phage

High titer phage lysates were grown on LB medium plates using 10^9 cells of TA3472 and 10^6 phage [20] per plate. Titers varied from 5×10^9 to 10^{11} pfu/ml. When purified phage were required, the phage lysate from 10 to 20 plates was concentrated by centrifugation at 48,000g for 3 hours. The pellet was gently resuspended in 4 ml 4 M CsCl, 10 mM $MgSO_4$, 10 mM Tris-Cl pH 8.0, and 0.1 mM EDTA, and this suspension was centrifuged to equilibrium at 36,000 rpm for 18 hours at 5°C in a Beckman SW 50.1 rotor. The phage

band was removed from the gradient with a syringe, in a volume of less than 0.5 ml, and dialyzed against a large volume of λ diluent (10 mM Tris-Cl pH 7.5, 10 mM $MgSO_4$). Phage yields were 20–30% of the crude lysate yields.

In Vitro Construction of a Bank of Clones of S typhimuirum DNA Fragments

The phage vector used was λgt4 [21], which carries the temperature-sensitive repressor mutation cI857. The bank was constructed by Anthony DeFranco [22], by using endonuclease *Eco*RI to digest DNA from the S typhimurium strain ST314 and λgt4 DNA, ligating the two digested DNA preparations, transfecting SF8, pooling the resultant plaques, and growing them as phage lysates on TA3456. The phage banks so obtained were calculated to contain approximately 500–1,000 independently cloned fragments of S typhimurium DNA, enough to represent the entire genome.

Screening for Clones Carrying the Histidine Transport Genes

Phages carrying the histidine transport operon were selected from the bank by their ability to impart D-histidine-growing capacity to TA3516 and TA3472. Two modes of complementation were attempted.

Lysogenic complementation. The recipient strains were grown in LB medium and 0.2% maltose; 4×10^8 phage from the bank of S typhimurium DNA and 2×10^8 cells were mixed in 0.5 ml of LB medium containing 10 mM $MgCl_2$. After 15 minutes at room temperature, 1 ml of LB medium containing 0.4% glucose was added, and the culture was grown overnight at 30°C. The cells were then pelleted, washed in E medium, plated on E medium with 10 μmoles of D-histidine per plate, and incubated at 30°C on D-histidine medium. They were then checked for the temperature sensitivity expected of lysogens at 42°C on both D-histidine medium and LB medium plates.

Phages produced after induction of these lysogens at 42°C were used to reselect D-histidine-utilizing lysogens in TA3516. The resulting lysogens were taken through a second cycle of phage induction, single plaque purification and lysogen selection on D-histidine medium, before further analysis as putative clones of the transport operon.

Lytic complementation. Expression of genes on DNA fragments cloned in λgt4 can occur by read-through transcription from the λ promoter pL under lytic growth conditions, possibly resulting in high levels of the gene products selected for [23]. 10^5 or 10^6 phages were plated on a lawn of the recipient bacteria, with a limiting supply of L-histidine (concentrations ranging from 0.02 μmole to 0.003 μmole per plate), enough to permit only the initial events of phage infection, and with 10 μmoles per plate of D-histidine. The growth of a background lawn cannot occur under these conditions, but cells carrying hybrid phages expressing the histidine transport genes can grow because they can utilize the D-histidine. Because of the ability to grow, these cells suport multiplication of the phage, resulting in a "plaque without a lawn" [23]. Plaques thus obtained were picked, inoculated into cultures of TA3472, and incubated overnight at 37°C. The phages produced were used to select lysogens in TA3472, which were then purified as single colonies on D-histidine medium at 30°C. Cultures from these single colonies were induced for phage lysis at 42°C, and the recombinant phages were repurified, first as single plaques, and then as single colony lysogens from the plaque centers.

Production of Transport Proteins by Lysogen Induction

Heat induction of λ lysogens for the purpose of overproducing transport proteins was performed by growing cultures in either LB or minimal medium (supplemented with

either L- or D-histidine) to about 5–8 × 10^7 cells/ml at 30°C and then placing them at 42°C for 10 min with occasional shaking. Cultures were then incubated at 37°C with shaking, and 0.5 ml aliquots were centrifuged at appropriate time intervals. The supernatants were assayed for the presence of phage and the pelleted cells were examined by SDS-PAGE. To assay for the level of J activity, samples were sonicated, the debris spun out, and the histidine-binding activity of the supernatant was quantitated by the dialysis binding assay [24].

Identification of Phage Encoded Proteins

UV irradiation and labeling of proteins was done as described in Jaskunas et al [25], using strain NO1406 as a recipient for the purified phage; 20 μCi of ^{35}S-methionine was used to label 5 × 10^8 cells. At the end of the labeling reaction, portions of the reaction mixture were subjected to electrophoresis either on SDS gels (7 × 10^7 cells per well), or on a basic two-dimensional gel system [4] (1.8 × 10^8 cells per gel). Samples were prepared for the SDS-PAGE by resuspending the cells at a concentration of 3 × 10^9 cells/ml in Laemmli solubilization buffer [26] and boiling for three minutes. For the two-dimensional gel electrophoresis, cells were resuspended in lysis buffer A [27] at 6 × 10^9 cells/ml, and frozen and thawed six times. The gels were exposed to Kodak XRP-1 x-ray film.

Preparation of λ DNA

Phage DNA was obtained by extraction of the purified phage suspensions with an equal volume of phenol equilibrated with 10 mM Tris-Cl pH 8, 1 mM EDTA, 50 mM NaCl. The aqueous phase was then extracted 3 or 4 times with diethyl ether, and the DNA precipitated by adding Na acetate to a final concentration of 0.3 M, together with three volumes of ethanol. DNA was stored in 10 mM Tris-Cl, 1 mM EDTA pH 8.0 at 4°C. The DNA was digested with endonuclease *Eco*RI and the digest resolved by electrophoresis on 0.8% agarose gels [28] to determine the size of the fragments of S typhimurium DNA inserted between the two arms of λgt4.

Restriction Digests

All restriction endonucleases were obtained from New England BioLabs, with the exception of *Eco*RI, which was a gift from Daisy Roulland-Dussoix. The conditions recommended by the manufacturer were used for the nuclease reactions.

Cloning the Transport Operon DNA into Ml3mp2

λgt4-*sal* DNA was used as the source of transport operon DNA. The vector, Ml3mp2, a male-specific phage [29], was obtained from J. Messing. The Ml3mp2 replicative form (RF) DNA was prepared as described in Messing et al [30]: 1.1 μg of Ml3mp2 RF DNA and 3.2 μg of λgt4-*sal* DNA were digested with *Eco*RI, then mixed and ligated with 0.7 Weiss units T4 DNA ligase (P-L Biochemicals), in 0.2 ml of 50 mM Tris-Cl pH 7.6, 50 mM NaCl, 10 mM DTT, 10 mM $MgCl_2$, 1 mM ATP, 100 μg/ml BSA, for one hour at 15°C. The ligation mixture was then diluted to 1 ml with the same buffer, an equal amount of ligase added, and the reaction was continued overnight. The ligated DNA was used to transform cells of 71-18, essentially as described by Lederberg and Cohen [31], and the mixture was plated on indicator medium as described by Messing et al [30]. Several white plaques were picked and used to produce phages, which were tested for their ability to complement transport mutations in the S typhimurium strains TA3412 and TA3415. Two of the 24 original white plaques with this ability were saved.

Complementation by Ml3 Clones of Salmonella Transport Mutations

Fifty microliters of phage suspensions of the Ml3mp2 derivatives were spotted on E medium plates spread with 10 μmoles D-histidine and 10^8 cells of the following strains, which carry defined mutations in the transport genes: TA3415, TA3412, TA3413, TA3416, TA3417, and TA3419. Male derivatives of TT317, TA1852, TA3078, TA3190, TA3193, and TA3194 were constructed by conjugation with TR5696 as donor and used as recipients in the complementation assay. Some of the resulting D-histidine utilizers were screened by SDS-PAGE for the presence of J protein, or by basic two-dimensional gel electrophoresis for the presence of P protein.

RESULTS

Characterization of High-Affinity Histidine Transport in E coli

Previous work [17] indicated that the introduction into S typhimurium of an episome, F'32, carrying the region of the E coli chromosome analogous to the Salmonella transport region, does indeed confer all the phenotypic properties consistent with the introduction of genes coding for histidine transport function. Hence we proceeded with the assumption that the E coli high-affinity histidine transport system was located near the *purF* gene.

Wild-type S typhimurium is sensitive to the inhibitory histidine analogue α-hydrazino imidazole propionic acid (HIPA), which is transported by the high-affinity transport system. S typhimurium mutants defective in *hisP* or *hisQ* functions are isolated as resistant to HIPA ($HIPA^R$). However, several strains of E coli were found to be already partially resistant to this analogue (on E medium with glucose), making it difficult to select transport mutants by this method. To obviate this problem, we first obtained mutant strains of E coli with increased levels of expression of the transport genes by selecting for the ability to use D-histidine (see Methods). These strains possess increased sensitivity to HIPA, therby allowing the selection of $HIPA^R$ derivatives.

The D-histidine utilizing isolates of TA3472 were screened by SDS-polyacrylamide gel electrophoresis of total cellular protein. Among them, several had elevated levels of the J protein. Six of these mutations were mapped and found to be 95% linked to the *purF* locus by phage Pl mediated transduction. They are presumed to be regulatory mutations (*dhuA*) of the histidine transport operon. None of them appear to grow on D-histidine as well as the best D-histidine-utilizing S typhimurium strain (TA271); nor do they produce as much J protein, as determined by inspection of electrophoretic patterns of whole cell protein. Two strains (TA3521 and TA3522) were saved for further study.

These two strains did indeed possess slightly greater sensitivity to HIPA than TA3472, as measured by the diameters of zones of inhibition around paper discs containing 1–5 μmoles of HIPA. Most of the $HIPA^R$ colonies selected in these two strains had lost their ability to grow on D-histidine and were presumed to be histidine transport mutants. Some of the $HIPA^R$ mutants were still able to grow on D-histidine: therefore, we assumed these were not mutants of the histidine uptake system.

When the $HIPA^R$ transport mutants were tested for their response to the analogue, they appeared to have lost the extra sensitivity to HIPA attained by acquisition of the D-histidine-utilizing ability; however, none of them was more resistant than the original wild type, TA3472. It was possible to select for additional HIPA resistance in the partially HIPA-resistant transport mutants and obtain strains completely resistant to the analogue. Based on these results we feel that E coli K12 strains, despite their relative

insensitivity to HIPA, and in contrast to S typhimurium strains, must possess a second efficient uptake pathway for HIPA other than the high-affinity histidine permease.

Histidine transport assays were performed on the parent strain, the two D-histidine-utilizing strains, and two selected $HIPA^R$ isolates from each D-histidine-utilizing strain (Table II). The *dhuA* mutations caused about a twofold increase in the rate of incorporation of histidine into protein. Two of the $HIPA^R$ mutants have rates of uptake one-fifth or one-tenth the wild-type rate, whereas the other two mutants transport histidine at the same rate as wild type, and could have arisen by the reversion of the *dhuA* mutations.

The histidine-binding protein J is a component of histidine transport in S typhimurium. E coli produces a very closely related periplasmic protein, which, by analogy, also has been designated as J protein and which has been characterized electrophoretically. Antibody to the Salmonella J protein precipitates the E coli J protein. Immunoprecipitates of labeled cell extracts from E coli strain TA3521 and S typhimurium strain TA271 were compared by two-dimensional gel electrophoresis and autoradiography: the E coli J protein has the same molecular weight but a slightly more acidic pI than the S typhimurium J protein (data not shown). The E coli J protein is genetically analogous to the S typhimurium one, as is demonstrated by the elevation of J protein levels in *dhuA* mutant strains, and confirmed by the finding that deletion mutants of the E coli transport operon exist that lack the J protein [18]. Deletion mutants also exist at the same locus which lack transport function but do produce J protein [18], indicating that, as in Salmonella, other genes located at this site are necessary for transport: presumably these are equivalent to the *hisP* and *hisQ* genes of Salmonella. E coli does produce a protein with the same electrophoretic mobility in a basic two-dimensional system as the Salmonella P protein. This protein is missing in E coli transport deletion strains and is probably the *hisP* gene product in E coli.

Based on these data we conclude that E coli has a high-affinity transport system analogous to the Salmonella one, both in physiological properties (D-histidine and HIPA transport), genetic properties (map location, regulatory *dhuA* site, and multiple cistrons), and biochemical components (periplasmic J protein, P protein).

Identification of Clones Carrying the Transport Genes

Upon screening the bank of clones of S typhimurium DNA, D-histidine utilizers were obtained in TA3516 by both the lytic and the lysogenic modes of complementa-

TABLE II. Rates of Histidine Uptake in E coli Transport Mutants

Strain[a]	D-histidine utilization[b]	Rate of histidine uptake[c]
TA3472	–	0.42
TA3521	+	0.72
TA3522	+	0.69
TA3521–$HIPA^R$ 1	–	0.48
TA3521–$HIPA^R$ 2	–	0.03
TA3522–$HIPA^R$ 1	–	0.09
TA3522–$HIPA^R$ 2	–	0.48

[a] His^+ derivatives of the strains indicated were constructed by P1 transduction with a histidine prototroph as donor and used in these assays.
[b] The ability to grow on D-histidine is an indication of elevated levels of the high-affinity transport system.
[c] μmoles histidine per g dry weight of cells, per min.

tion. Five putative clones were purified (as described in the Methods section), three of which were selected as lysogens and two of which were selected as "plaques without lawns" [23]. The resulting purified phages were still able to complement the transport mutation in TA3516. All five strains produced rather small plaques on LB medium plates, with large plaques (the same size as λgt4 plaques) appearing at a frequency of about one in a thousand. We have evidence that the large plaque-forming phages are derived from the small plaque formers by deletion of Salmonella and λ DNA (unpublished observations). These five phage strains routinely produce lysates with 5- to 10-fold lower titers than λgt4 lysates grown under identical conditions, and the viability of the phages produced is poorer.

Phage DNA was prepared from four of these λ clones, and *Eco*RI digests of these DNA preparations were compared by electrophoresis on 0.8% agarose gels. All four of the putative transport operon clones in λgt4 were found to contain the same 12.4 Kb *Eco*RI fragment of S typhimurium DNA. These phages are identical, as shown by detailed restriction analysis of their DNA (to be published elsewhere). The cloned *Eco*RI fragment of S typhimurium DNA is designated *sa1*, and the λgt4 derivative carrying it is called λgt4-*sa1*. The insertion of *sa1* into λgt4 DNA results in a phage DNA molecule larger than wild-type λ by 2.5 Kb.

Identification of Protein Products of λgt4-*sa2*

UV irradiation of E coli cells, followed by infection with phages, results in the selective synthesis of phage-encoded proteins, with a slight background synthesis of major cell proteins [25]. Labeled proteins, synthesized in UV-irradiated cells of N01406 following infection with λgt4-*sa2*, were resolved by two-dimensional gel electrophoresis and subjected to autoradiography (Fig. 1). λgt4-*sa2* is a derivative of λgt4-*sa1*, which carries a mutation, *dhuA1*, known to cause elevated synthesis of the transport proteins [14]. Its construction will be described elsewhere. An autoradiogram of λgt4-infected cells was used as a control. λgt4-*sa2* is found to encode between 10 and 15 polypeptides not specified by λgt4. Of these, three proteins, the Salmonella J, P, and LAO proteins, were identified by comparison of their electrophoretic mobilities with the mobilities of unlabeled marker proteins run on the same gel. A membrane preparation from TA1014 was used to provide a marker for the P protein, and an osmotic shock fluid from TA3292 provided markers for the J and LAO proteins. Thus λgt4-*sa2* DNA encodes the two previously identified histidine transport proteins. Presumably one of the other proteins specified by λgt4-*sa2* is the Q protein. Studies are in progress to identify this component.

Complementation of Neighboring Genetic Markers by λgt4-*sa1*

When λgt4-*sa1* is lysogenized in EM1407, the cells do not lose their purine auxotrophy, indicating that λgt4-*sa1* DNA does not contain a functional *purF* gene. Also, λgt4-*sa1* does not complement the *ack/pta* lesion causing the FAc^R phenotype of the strain TA3516, as lysogens of this phage in TA3516 are not FAc^S. λgt4-*sa1* appears to carry the site of the *zei102*::Tn10 insertion (Fig. 2). This is demonstrated by the transduction of an E coli strain lysogenic for λgt4-*sa1* to tetracycline resistance with P1 phage grown on the Salmonella strain TA3078, which carries *zei102*::Tn10 (details to be published elsewhere).

Overproduction of Transport Proteins

One of the objectives of our recombinant DNA work is the identification and overproduction of all components of the histidine transport operon, thus facilitating their

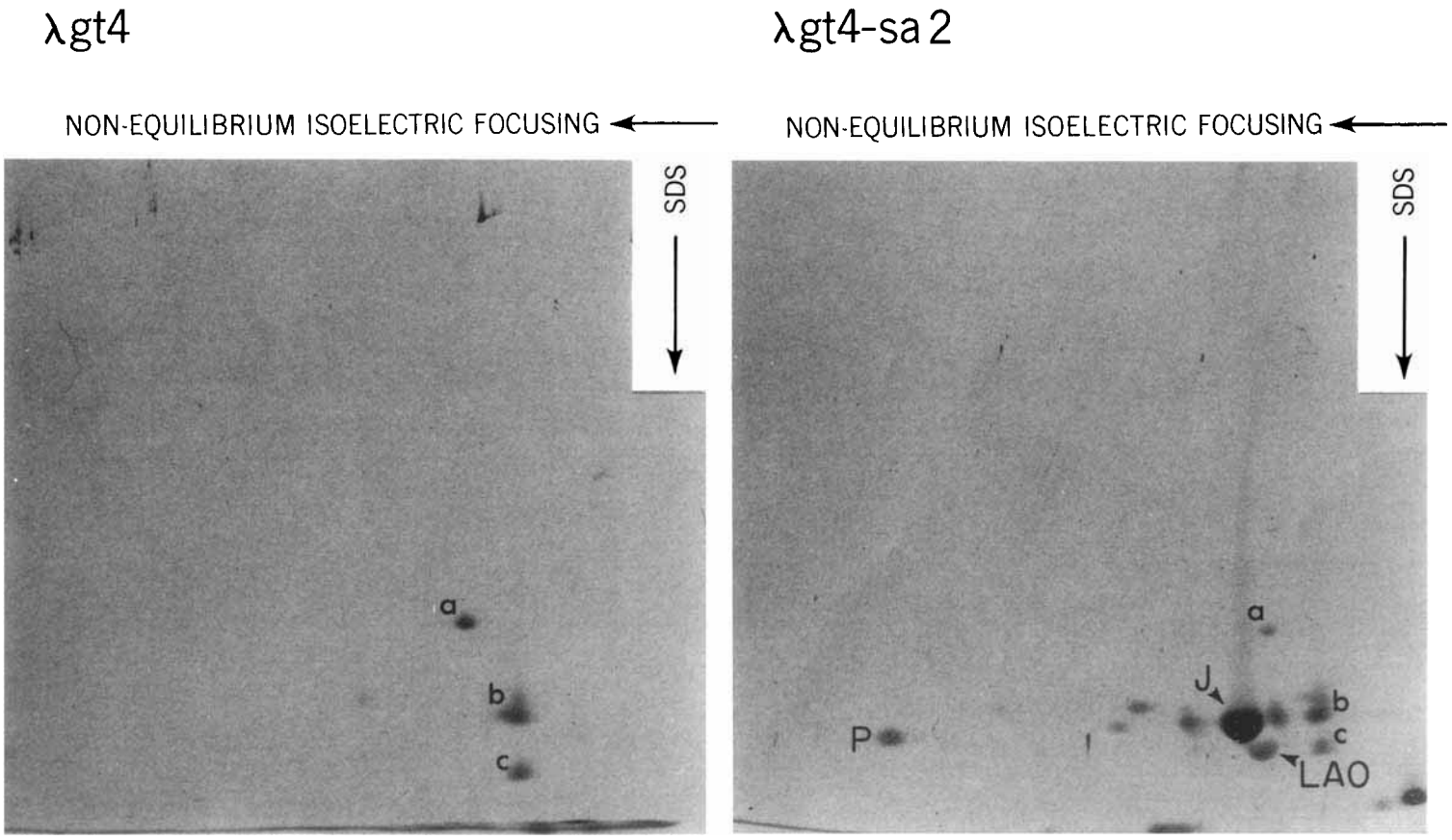

Fig. 1. Autoradiograms of two-dimensional gels of the labeled proteins synthesized in UV-irradiated cells of NO1406 following infection with λgt4 (left panel) and λgt4-*sa2* (right panel). Spots a, b, and c correspond to proteins synthesized in the control cells infected with λgt4.

purification. To estimate the level of synthesis of the transport proteins, the amount of J protein produced in heat-induced lysogens was examined by SDS-PAGE of whole cell extracts at various intervals after the heat induction at 42°C (data not shown). Lysogens of λgt4-*sa1*, or its derivative λgt4-*sa2*, either in the wild-type, TA3472, or a transport mutant, TA3476, were used in these studies.

The lysogens produce a visible amount of J protein after heat induction at 42°C and 5 min of incubation at 37°C. Levels of J protein reach a maximum after 30 min, and lysis of the cultures occurs 70 to 90 min after heat shock. The amount of J produced is not appreciably changed if the cells are grown in LB medium, M-9 medium supplemented with D- or L-histidine, or with arginine as a nitrogen source. As expected, lysogens of λgt4-*sa2* produce considerably higher amounts of J protein than λgt4-*sa1* lysogens of the same strain.

The amount of J protein produced in the four different lysogens was quantitated by histidine-binding assays of cell extracts of the heat-induced lysogens at 0 and 45 min after induction (see Table III). The λgt4-*sa2* lysogen of TA3472 produced 36 times as much J protein as the non-lysogenic parent, whereas the λgt4-*sa2* lysogen of TA3476 produced at least 1,000 times more J protein than its parent, a transport mutant that completely lacks the E coli J protein and therefore has no baseline binding activity.

A comparison of the highest value of histidine-binding activity from the heat-induced lysogen with those routinely obtained in a S typhimurium strain TA271 (an overproducer by virtue of the *dhuA1* mutation) indicates that the lysogens are, at best, producing about 5- to 10-fold more J protein than TA271.

Duplication of Transport Genes Carried on λgt4-*sa1*

We observed that TA3474 (λgt4-*sa1*) gave a variety of colony sizes when growing on a D-histidine plate. We suspected that duplications of genes coding for limiting components of transport could be responsible for the variety of sizes. Duplications are extremely common [32] in the bacterial chromosome (of which phage DNA is an integral part during lysogenic growth) and, in particular, have been shown to occur in the histidine transport region when cells are required to transport a poor histidine source [33]. We studied as prototypes some of the largest and some of the smallest colonies in order to investigate the hypothesis that the transport operon is duplicated in these lysogens. The large colony phenotype is unstable, and upon growing the large colony formers on non-selective medium (such as LB), they rapidly yield small colonies (30% upon 10^{-6} dilution and subculturing in LB medium). The small colonies so obtained are extremely poor growers on D-histidine, and, if maintained on D-histidine, rapidly accumulate larger (better growing) colonies. Both small and large colonies are temeprature sensitive, indicating that they are lysogenic. The large colonies have an increased sensitivity to HIPA compared to the small ones, an additional indication (Table IV) that transport is elevated in these strains. All these properties (high frequency of occurrence, instability, and elevation of function) are characteristic of strains carrying duplications of genetic material [32]. Phage λ is known to harbor duplications [34], and defective derivatives of λ have been suspected of containing duplications [35].

Recloning of the Salmonella Transport Operon DNA in Ml3mp2

Ml3mp2 was used as a cloning vector for two reasons. First, Ml3 is a single-stranded DNA phage (with a double-stranded RF), so the single-stranded form of an Ml3 clone provides an easily obtainable supply of DNA template for sequencing by the chain termina-

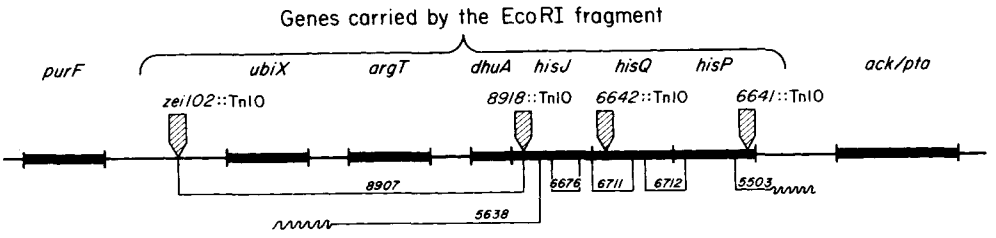

Fig. 2. Deletion and insertion mutations of the S typhimurium histidine transport operon which are complemented by the Ml3mp2 clone carrying the 12.4 Kb S typhimurium DNA fragment. Cross-hatched markers indicate insertions of Tn10.

TABLE III. Histidine-Binding Activity After Induction of Lysogens*

		Minutes after heat induction	
Strain	Phage	0	45
TA3472	Uninfected	0.4	0.5[a]
TA3474	λgt4-*sa1*	0.9	3.1
TA3475	λgt4-*sa2*	Not assayed	17.9
TA3476	Uninfected	<0.01	<0.01[a]
TA3478	λgt4-*sa1*	1.8	10.8
TA3479	λgt4-*sa2*	8.6	54.3

*Lysogenic cultures were grown in LB medium and heat-shocked; samples were sonicated and analyzed as described in Methods. Binding activity is expressed in units (pmoles histidine-bound) per mg protein.
[a]30-min incubation.

tion method of Sanger [30, 36]. Second, unlike λ, Ml3 and its derivatives can be made to infect S typhimurium, the organism in which the genetics of histidine transport is best characterized. The 12.4 Kb *Eco*RI fragment was moved from λgt4-*sa1* into the *Eco*RI site of Ml3mp2 as described in the Methods section. An Ml3 clone was obtained that was able to complement the defined deletion and insertion mutations of each gene of the transport operon in S typhimurium shown in Figure 2, thus, confirming genetically the presence of the operon DNA on the cloned fragment. The Ml3 clone also complements the *ubiX* lesion in TA3078: the lesion causes the strain to form small colonies on rich medium and the complementation results in normal-sized colonies.

The presence of the J and P genes on the fragment cloned in M13 was further confirmed by electrophoretic analysis of *hisJ* (TA3415) and *hisP* (TA3413) deletion strains after their complementation by the Ml3 transport clone. These strains were found to be producing S typhimurium J protein and P protein, respectively, following infection with the Ml3 transport clones. However, all attempts to purify the RF DNA of these Ml3 clones of the transport genes have proved fruitless. The hybrid Ml3 appears to be very unstable, and its ability to complement transport function is readily lost upon growth in the absence of selective pressure. In S typhimurium such loss may be due in part to the ability of the cloned portion of the hybrid phage to recombine into the chromosome. We have recently found that the transport genes can be maintained on the hybrid phage by propagation in

TABLE IV. HIPA-Sensitivity of Large and Small Colonies of Lysogens of λgt4-*sa1* and λgt4-*sa2**

Strain	Lysogenic for	Diameter of inhibition zone (in mm)	
		0.2 mg HIPA[a]	0.5 mg HIPA[a]
TA3473	λgt4	No inhibition	10 (turbid)
TA3474 large colony		15	17
TA3474 small colony	λgt4-*sa1*		
		No inhibition	12
TA3475 large colony		15 (very clear)	19 (very clear)
TA3475 small colony	λgt4-*sa2*		
		13	17

*LB medium cultures of a large and a small colony of TA3474 and TA3475 were obtained by inoculating heavily from a D-histidine master plate. The test was performed at 30°C on a minimal plate containing carnosine (3×10^{-5} M).
[a]HIPA was applied to a filter paper disc in amounts indicated.

S typhimurium strains containing deletions of the chromosome that extend beyond the cloned segment, thereby preventing homologous recombination of the cloned fragment into the chromosome.

DISCUSSION

We have isolated a fragment of S typhimurium DNA carrying the histidine transport operon by using recombinant DNA techniques. The presence of the genes of the histidine transport operon on the cloned material has been demonstrated by a variety of methods. These include complementation of known defective transport genes, recombination into chromosomal transport genes, identification of the protein products produced by the cloned DNA, and the physiological characterization of histidine transport in bacteria containing this DNA fragment. Recently, the use of recombinant DNA techniques has yielded valuable information on the structure of genes of other bacterial transport systems [37–39] and of the proteins coded for by these genes. The availability of the DNA encoding transport proteins should greatly facilitate the characterization of membrane-bound transport components, which have previously been difficult to analyze biochemically.

The isolated fragment of S typhimurium DNA is 12.4 Kb in length. Of these 12.4 Kb, the transport operon should occupy no more than 3.5 Kb. This estimate is reached from a knowledge of the size of two of the transport gene products (25,000 and 24,000 daltons, respectively, for the J and P proteins), an estimate of 1 Kb for the size of the third gene (*hisQ*) in the operon (data to be published elsewhere), and a maximum length estimate of 0.5 Kb for the regulatory region *dhuA*. Thus, the isolated fragment contains an additional 9 Kb of material, sufficient to code for eight or nine other proteins of average size. We have shown that one of these other proteins encoded by the cloned DNA is the LAO protein, the product of the *argT* gene. Also, from complementation studies, we know that the *ubiX* locus is carried on the cloned fragment. However, nothing is known about the nature or the number of the gene products of the *ubiX* site. There are no clues as to the functions of the gene products encoded by the DNA on either side of the transport operon, because deletions extending outside the transport operon in either direction have no obvious effects on cell growth under the conditions we use.

We have observed that λgt4 carrying the cloned fragment grows poorly and yields lysates of lower titer than lysates of λgt4. It is possible that this is due to overproduction from this fragment of uncharacterized gene products that are deleterious to the cell. Alternatively, the increased size of λgt4-*sa1* DNA may be responsible for the poor yields and viability of these phages by interfering with normal packaging and stability of the DNA in phage heads. Subclones of smaller DNA fragments carrying the transport genes have been obtained (data not shown) and should allow the resolution of this problem.

We have demonstrated that thermal induction of lysogens of the λgt4-*sa2* hybrid leads to a 36-fold increase in the production of the J protein over a non-lysogenic parental strain. Approximately one-sixth of this increase is due to the introduction of the *dhuA1* mutation into λgt4-*sa1* (Table III, lines 2 and 3). The remaining 6-fold increase in production is presumably due to an increase in the gene dosage by the replication of λ upon thermal induction. These values are consistent with reports in the literature of 3- to 4-fold gene amplification upon λ induction [40], although they are somewhat lower than the 20-fold increase reported in one case for the products of genes cloned in the same vector [19].

Earlier studies indicated that D-histidine utilization by S typhimurium requires an elevation of the transport gene products [14]. It is likely that in E coli strains lysogenic for λgt4-*sa1* (such as TA3474), the transport of D-histidine is not efficient enough for normal growth, as the cloned transport operon is not derepressed and duplications (or amplifications) elevating the level of transport can occur at a frequency high enough to overcome this problem. The DNA length of λgt4-*sa1* is already greater than the length of wild-type λ DNA. Therefore, the phage produced upon heat induction of a lysogen carrying duplications in the λgt4-*sa1* region would probably not be able to package DNA large enough to include the duplication of the transport operon. It is not known whether the presumed duplication(s) cover only bacterial DNA (in which case they could be identical in extent to previously characterized duplications in that area [33]) or whether they extend into neighboring λ DNA and E coli chromosomal DNA.

We have characterized histidine transport in E coli in order to be able to utilize available λ vectors for the isolation of the well-understood Salmonella transport genes. It is clear from our data for the high-affinity histidine transport in E coli that this bacterium has a system analogous to that of S typhimurium in physiological, biochemical, and genetic properties. We encountered several problems due to the multiplicity of histidine transport systems in E coli. The analogue HIPA can efficiently enter E coli through a system other than the high-affinity histidine transport system, as evidenced by our inability to select in a single step mutants completely resistant to HIPA. Also, we found that different strains of E coli exhibited different levels of HIPA sensitivity, which implies different levels of transport. The possibility that genetic differences may affect levels of uptake should be taken into consideration whenever rates of uptake are compared between nonisogenic strains. A multiplicity of transport systems for the same substrate may be responsible for the observed variability in levels of transport between strains. In fact, a strain defective in one system may compensate for the defect by elevating one of the other systems, thus altering the kinetic and physiological properties of the overall uptake.

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