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Genetic Control of the Hexose Phosphate Transport System of *Escherichia coli*: Mapping of Deletion and Insertion Mutations in the *uhp* Region

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The *Escherichia coli* transport system responsible for the accumulation of a number of sugar phosphates is encoded by the *uhp* region and is induced by external, but not intracellular, glucose 6-phosphate. To delineate the genetic organization of the *uhp* region, a total of 225 independent point, deletion, and transposon Tn*10* insertion mutations were collected. Mutations conferring the Uhp$^-$ phenotype were obtained on the basis of their resistance to fosfomycin and their inability to use sugar phosphates as carbon source. Deletions of *uhp* sequences were obtained as a consequence of imprecise excision of Tn*10* insertions located on either side of *uhp*. Conjugal crosses between these deletions and the point of insertion mutations allowed determination of the relative order of the *uhp* alleles and of the deletion endpoints. Specialized λ transducing phages carrying a *uhpT-lac* operon fusion and various amounts of adjacent *uhp* material were isolated and used as genetic donors. Results from these crosses corroborated those obtained in the conjugal crosses. The locations of the mutant alleles were compared with the regulatory properties of Uhp$^+$ revertants of these alleles. This comparison suggested the existence of at least three genes in which mutation yields the Uhp$^-$ phenotype. Mapping experiments were consistent with the gene order *pyrE-gltS-uhpTRA-ilvB*, where *uhpT* encodes the transport system and *uhpR* and *uhpA* are regulatory genes whose products are necessary for proper *uhp* regulation.

The hexose phosphate uptake system of *Escherichia coli* mediates the active transport of a number of sugar phosphates (7, 18, 25). Full uptake activity is present in cytoplasmic membrane vesicles and the proton motive force is the energy source (26). Expression of this transport system is specifically induced by extracellular glucose 6-phosphate (G6P) (6, 29) and is also subject to catabolite repression (9). This unusual mode of regulation by external effector is termed exogenous induction and has been proposed for some other transport or catabolic systems whose substrates are common intracellular metabolites (11, 16).

Mutants lacking Uhp transport activity can be selected on the basis of resistance to fosfomycin, an antibiotic inhibitor of muramic acid biosynthesis which enters cells on either the glycerol phosphate (*glpT*) or hexose phosphate (*uhp*) transport system (14). Fosfomycin-resistant (Fos^r) strains specifically defective in growth on hexose phosphates, such as G6P or fructose 6-phosphate (F6P), carry mutations in the *uhp* region at 81.5 min on the *E. coli* chromosome map (1). In previous studies (12, 13), three-point transduction and conjugation crosses had been used to localize various *uhp* point mutations relative to one another and to the adjacent *pyrE* locus. The regulatory behavior of revertants of each of the Uhp$^-$ mutants was determined. Some mutations gave rise only to revertants with inducible expression of the transport activity. These mutations were found to map between *pyrE* and those *uhp* mutations which gave rise to revertants with altered regulatory behavior. It was proposed that the former group of mutations defined the gene(s) for the transport system (*uhpT*) and the latter defined regulatory components. Thus, the gene order was *pyrE-uhpT-uhpR*. Mutants with constitutive expression of the Uhp transport system were obtained by selection for growth on noninducing sugar phosphates (12, 18). These mutations were mapped to the *uhp* region, but were not localized more precisely relative to the mutations giving rise to the negative phenotype.

Analysis of this regulatory process was complicated by the fact that the transport system

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appears to be the only activity induced by this regulatory system. To investigate the requirement for a functional *uhpT* transport system in regulation, *uhpT-lac* operon fusion strains (3) were obtained in which the production of β-galactosidase was dependent on addition of G6P to the medium (27). Induction by exogenous G6P occurred as well or better in a strain with no detectable G6P uptake as in a strain proficient for uptake activity, showing that the accumulation of G6P by the transport system is not required for induction. This strongly suggests that the *uhp* regulatory system includes a component in the cytoplasmic membrane able to discriminate internal from external G6P.

The current study was undertaken to extend the fine-structure genetic mapping of the *uhp* region by the isolation of deletion and transposon insertion mutations and the isolation of transducing phages carrying portions of the *uhp* region. The results described in this paper confirm and extend the previous genetic conclusions. Deletion mutations entering *uhp* from either side were obtained as a consequence of the imprecise excision of transposon Tn*10* insertions flanking *uhp*. These mutations were used to obtain a map of approximately 40 point mutations. Excision of a λ prophage located adjacent to the *uhp-lac* operon fusion provided a complementary mapping technique, since each excision event resulted in the formation of specialized transducing phages carrying different amounts of *uhp* material. The results provide evidence for the presence of at least three linked genes whose functions are necessary for *uhp* expression. Two of them appear to play regulatory roles.

MATERIALS AND METHODS

Bacterial and phage strains. The *E. coli* K-12 strains used in this study are listed in Table 1. General techniques for growth of bacteria and for genetic crosses were as described by Miller (21).

The Tn*10* vehicle λNK370 (λ *b221 cI857 cI171*::Tn*10* *O*uga261) was obtained from N. Kleckner.

All *uhp* mutations were isolated in strain RK4353 or its descendants, all of which were derived from strain MC4100 (3) by selection for resistance to nalidixate and introduction of a *non* (and for some, *metE*) mutation by cotransduction.

The conjugal donor was derived from Hfr strain PK3, which transfers the *uhp* region early and the remainder of the chromosome in a clockwise direction (15). The sex factor is the integrated Col V plasmid. The *pyrE60* allele was introduced by cotransduction with *zib-615*::Tn*10*. This strain, RK34, was transduced to PyrE$^+$ by P1 lysates grown on the RK4353 strains carrying *uhp* point mutations. Recombinants that were tetracycline sensitive (Tc^s) and Uhp$^-$ were tested for donor ability and other markers and then used in mating experiments. Where indicated, the *recA56* allele was introduced by conjugation with strain JC10240 and selection for the closely linked Tc^r. Tc^s derivatives were obtained on fusaric acid plates (19).

TABLE 1. Bacterial strains[a]

Strain	Genotype
RE74	HfrC *relA1 pyrE40 uhp-40 gltS14 tna-6 metB1 tonA22* (CGSC 5569)
PK3	HfrV(P0131) *thr-1 leuB6 thi-1 lacY1 azi-15 tonA21 supE44* (CGSC 5862)
RK34	As PK3, but *pyrE40 zib-615*::Tn*10*
RK4353	F$^-$ *araD139* Δ(*argF-lac*)*U169 relA1 rpsL150 thi gyrA219 non* (27)
RK5173	As RK4353, but *metE70*
RK4929	As RK5173, but *pyrE40 gltS15 zib-615*::Tn*10*
RK4952	As RK4353, but *zic-634*::Tn*10*
RK4953	As RK4353, but *zic-635*::Tn*10*
RK4954	As RK4353, but *zib-636*::Tn*10*
RK5115	As RK4353, but *uhpT*::[*lacZ* λp1(209)] (27)

[a] Standard genetic nomenclature was used. Allele numbers were as assigned by B. Bachmann. The source of previously described strains is in parentheses after the genotype, where CGSC represents the acquisition number of the *E. coli* Genetic Stock Center, Yale University. If this number or a literature citation is not provided, the strain was constructed in this study.

Chemicals and media. Growth media included medium A of Davis and Mingioli (4), MOPS (morpholinepropanesulfonic acid) medium (22), and L broth (5). The carbon sources for minimal media were glucose or glycerol at 0.5% or F6P or lactose at 0.2%. F6P was used to score the Uhp growth phenotype because it gave less background growth of Uhp$^-$ strains than G6P. Minimal media were supplemented with thiamine (1 μg/ml), required amino acids (100 μg/ml), and uracil (40 μg/ml). Where indicated, streptomycin was added to 100 μg/ml and tetracycline was added to 20 μg/ml. 5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside (Bachem, Inc., Torrance, Calif.) was dissolved in dimethyl sulfoxide and used in plates at a final concentration of 10 μg/ml. Fosfomycin was a gift from Merck Chemical Division, and freshly prepared solutions were added to plates at 50 μg/ml. Fosfomycin selection plates contained medium A, fosfomycin, required amino acids, 0.5% casein hydrolysate, and 0.01% G6P. Other chemicals were from Sigma Chemical Co., St. Louis, Mo. Radioisotopes were from New England Nuclear, Boston, Mass.

Isolation of EMS-induced mutations. Uhp mutants were obtained by mutagenesis with ethyl methane sulfonate (EMS) and selection on fosfomycin plates. Their Uhp phenotype was determined by growth response on F6P, glycerol, and glucose plates and by uptake of [^{14}C]G6P. In this and all other mutant

isolations, independent mutations were obtained by taking only one mutant from each mutagenesis of a single-colony isolate.

Uhp^+ revertants of the EMS-induced and *uhp*::Tn*10* mutants were obtained by spreading approximately 10^8 cells of each mutant onto minimal F6P plates. The spontaneous reversion frequency could be increased by spotting a small amount of EMS or 2-aminopurine onto the selection plate. Revertants were purified, and their basal and induced levels of G6P uptake activity were determined.

Assay of G6P transport activity. Transport of [^{14}C]G6P was assayed as previously described (27). Uptake activity is expressed as nanomoles of G6P accumulated per microliter of cell water per minute. Before assays, cells were grown in minimal medium with glycerol as carbon source and in the absence or presence of 300 μM G6P as inducer. Cells in mid-log phase were washed and suspended in MOPS medium containing glycerol and chloramphenicol (100 μg/ml) and kept at 4°C until assayed.

Isolation of λ transducing phages. Strain RK5115 carries a *uhpT-lac* operon fusion adjacent to integrated λ prophage (27). Early log-phase cultures of single-colony isolates of this strain in L broth were treated with mitomycin C (1 μg/ml) in the dark for 4 h. Dilutions of the resultant phage lysates were plated with strain RK4353 on L-agar plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside and G6P in the top layer. From 0.1 to 1% of the plaques were blue on this medium. One blue plaque and one white plaque from each isolate were purified by two cycles of single-plaque isolation. A stock of each fusion-bearing phage was prepared by the confluent plate lysis technique (5). The phage that gave white plaques did not carry the intact operon fusion.

Isolation of Tn*10* insertions in and near *uhp*. Transposon Tn*10* insertions in *uhp* were obtained. Tetracycline-resistant colonies arising after infection of strain RK5173 with λNK370 were replicated onto fosfomycin (Fos) selection plates. Cases in which an entire Tc^r colony appeared Fos^r upon replica-plating were tested for the linkage of Uhp^-, Tc^r, and PyrE responses by P1-mediated transduction. The *uhp*::Tn*10* insertions were those in which Uhp^- and Tc^r were 100% linked during selection for either Tc^r or Uhp^+.

Transposon Tn*10* insertions near *uhp* were also obtained. A collection of random insertions of Tn*10* in strain RK4353 (obtained after infection with λNK370) was used as donor in P1 transductions. The *uhp* mutant RE74 was transduced to Uhp^+ and Tc^r. The site of the Tn*10* insertion in these recombinants was confirmed by linkage of Uhp^+ and Tc^r in transduction backcrosses. Precise localization used multifactor genetic crosses scoring various markers in the region (*pyrE*, *gltS*, *uhp*, and *tna*). In this way, insertions lying on either side of *uhp* were obtained.

Generation of deletions from Tn*10* insertions. Excision of Tn*10* can be accompanied by local deletions or chromosomal rearrangements (17). Single-colony isolates of strains with Tn*10* insertions lying on either side of *uhp* (see Fig. 1 for location) were spread on the fusaric acid-containing medium (2, 19) selective for Tc^s cells. After incubation at 37°C for 24 h, the selection plates were replicated onto fosfomycin selection plates to identify those variants which had simultaneously become Tc^s and Fos^r. These strains were purified and tested for the revertibility of the Uhp^- phenotype, acquisition of a requirement for isoleucine-valine, and their ability to recombine with *uhp* point mutations. Over 100 independent variants were analyzed to characterize the nature of the Tn*10*-induced alteration.

Genetic mapping. Two types of crosses were used for genetic mapping. The first used as genetic donor derivatives of the Hfr strain RK34 into which *uhp* point mutations and *uhp*::Tn*10* insertions had been transferred by P1-mediated cotransduction with *pyrE*. Logarithmically growing cells of the donor strains (10^7 cells) were spread onto minimal F6P plates containing the auxotrophic requirements of the recipients and streptomycin to prevent further growth of the donor. The recipient strains containing presumptive *uhp* deletions were grown in L broth and were transferred onto the plate from a microtiter plate with the aid of a 48-pin replicating block. This transfer was made shortly after the donor cells had been applied. Control plates showed that neither donor nor recipient cells grew or gave rise to revertants on these plates. In these crosses, the appearance of any Uhp^+ recombinants in replicate matings indicated that the mutation on the donor chromosome and the deletion in the recipient did not overlap.

In the second type of cross, the *uhpT-lac* operon fusion-bearing λ phages were crossed against *uhp* point mutations, with selection for the Uhp^+ phenotype. As in the previous crosses, the phage suspensions (usually 10^9 PFU/plate) were spread onto the surface of minimal F6P plates. The recipient strains, grown in L broth, were transferred onto the plate from a microtiter plate. Plates were incubated at 37°C for 24 h. If solid growth occurred in the area where any one strain was transferred, it was considered that the phage carried the entire gene affected by the mutation in that recipient, i.e., complementation. If only a limited number of colonies arose (above the frequency of revertants), then the phage carried only a portion of the gene affected by the mutation, so that a specific recombination event was required for restoration of Uhp function.

In all genetic crosses, each mating was carried out at least twice. The linear maps of the order of point mutations and of deletion endpoints were consistent in the two mating methods.

RESULTS

Isolation of Uhp^- mutants. Independent EMS-induced Fos^r mutants were collected. Roughly half (41%) were specifically defective in the utilization of hexose phosphates as carbon source and showed normal growth with glucose or glycerol. All of these mutants lacked G6P uptake activity (<10% of induced wild-type levels) and carried mutations which gave approximately 40% cotransduction with *pyrE*. The remaining mutants were also defective in growth on hexose phosphates but, in addition, were altered in their growth on glycerol or glucose at various temperatures. These strains had pleiotropic defects in carbohydrate utilization and were not studied further.

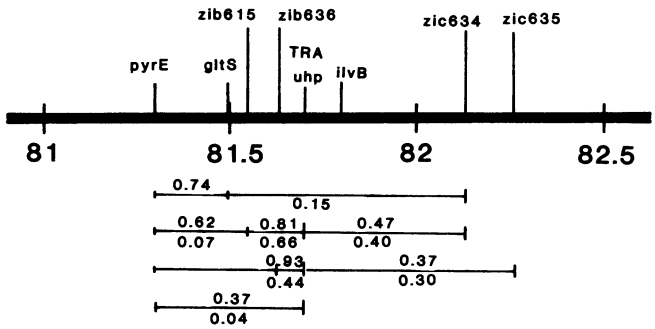

FIG. 1. Genetic map of the *uhp* region. Various markers and the location of four Tn*10* insertions isolated in this study are displayed above the line, which is calibrated in time units according to the genetic map (1). The location of the insertions is based on the transduction frequencies, assuming that frequencies obtained when the selected marker lies to the left of the unselected marker are reflections of physical distance. Below the thick line are the cotransduction frequencies between pairs of markers. The frequency above each line is that observed when the leftward gene of the two was the selected marker. The frequency below the line was observed when the rightward marker was selected.

Transposon Tn*10* insertions in *uhp* were found among 0.4% of Tc^r colonies after infection with the Tn*10* vehicle, λNK370. Transduction crosses showed that the Tc^r phenotype was 100% associated with Uhp^- response, and both were linked to *pyrE*.

Isolation of *uhp* deletion mutations. Transposon Tn*10* insertions lying near *uhp* were obtained and mapped to determine their location relative to *pyrE*, *gltS*, *uhp*, and *tna*. The analysis of recombinant classes confirmed the deduction by Essenberg and Kornberg (8) of the gene order *pyrE-gltS-uhp-tna*. Several Tn*10* insertions were mapped to the *gltS-uhp* interval, and two were obtained lying to the right of *uhp* (Fig. 1).

Transduction crosses in this region revealed a marked asymmetry of cotransduction frequencies, as previously described (20, 23). For example, when $PyrE^+$ was selected, approximately 40% acquired the donor *uhp* allele. However, only 4% of Uhp^+ recombinants acquired the donor *pyrE* allele. This asymmetry appeared to reflect the location of the markers and not their nature, since both point and insertion mutations displayed this effect. The asymmetry occurred whether the donor or the recipient carried a Tn*10* insertion. In all crosses in this area, cotransduction frequencies were markedly higher if the selected marker lay to the left of the unselected marker, relative to the genetic map in Fig. 1. The degree of asymmetry did not remain constant throughout this region. For example, in the case of insertion *zib-636*::Tn*10*, the cotransduction frequencies between Tc^r and *uhp* were 93% when Tc^r was selected but 44% when Uhp^+ was selected. For insertion *zic-634*::Tn*10*, lying on the other side of *uhp*, the analogous cotransduction frequencies were 40 and 47%, respectively. The results are consistent with those of Newman and Levinthal (23). The basis for this asymmetry is not known. Proximity to the origin of chromosome replication could be involved (20). However, the results obtained here showed diminution of the transductional asymmetry with markers that were all on the same side of the origin. Of practical importance, the existence of this effect renders suspect the estimation of distances from cotransduction frequencies in this region.

Tn*10* insertions flanking *uhp* gave rise to Tc^s variants that were Uhp^-. When the Tc^s strains arising on a fusaric acid plate were replicated onto fosfomycin plates, a fraction (0.5 to 4%) appeared (as a "patch") in which all of the Tc^s cells were Fos^r. Many of the Tc^s Fos^r isolates required isoleucine and valine. This new auxotrophic requirement could result from an alteration in *ilvB*, previously shown to be located near *uhp* (23). The *ilvB*-coded acetohydroxy acid synthetase activity was shown by C. Berg (personal communication) to be missing from these strains. The Uhp^- Ilv^- strains did not revert to Uhp^+, but did form Ilv^+ revertants, some of which remained valine sensitive whereas others were partially valine resistant. Presumably, these resulted from alterations at *ilvHI* and *ilvG*, respectively, to provide increased expression of their isozyme of acetohydroxy acid synthetase. It is not understood why, in this strain background, deletion of *ilvB* results in Ilv^- auxotrophy.

Tn*10* insertions lying to the left of *uhp*, including *zib-615*::Tn*10* and *zib-636*::Tn*10*, and also *zic-634*::Tn*10*, lying to the right of *uhp*, gave rise to some Ilv^- variants among the Tc^s Fos^r offspring. The *zic-635*::Tn*10* insertion, apparently lying a little further to the right, did not. The explanation for this behavior and of the location

of *ilvB* relative to *uhp* was provided by the mapping experiments showing what *uhp* material remained in these putative deletion mutations.

Mapping of the *uhp* region. A collection of Tc^s Fos^r strains were crossed with a series of Hfr strains carrying *uhp* point or Tn*10* insertion mutations. A total of 184 Tc^s Fos^r variants were mapped against 41 donor mutations. Selection was for growth on F6P as sole carbon source.

All of 61 Tc^s Fos^r Ilv^- variants derived from strains carrying insertions *zib-615* and *zib-636*, which are insertions to the left of *uhp*, did not give Uhp^+ recombinants with any *uhp* point mutation, although they did give recombinants with a uhp^+ donor. Thus, loss of *ilvB* by deletions coming from the left was accompanied by loss of all detectable *uhp* material.

The Ilv^+ variants derived from the same insertions fell into two classes. Of 57 Tc^s Fos^r Ilv^+ derivatives, 24 recombined with all or almost all *uhp* point mutations. These may result from inversions of the region between the transposon and a site within *uhp*, thereby inactivating *uhp* function. It is also possible that some of these mutants arose by two independent events, i.e, loss of the transposon and a point mutation in *uhp*. Of the remaining 33 Ilv^+ variants, 6 did not recombine with any *uhp* point mutations, and the rest recombined with some *uhp* point mutations. The pattern of recombinations given by these partial *uhp* deletions entering from the left allowed construction of a linear map of the location of the point mutations. Since partial *uhp* deletions, or even a few complete deletions of *uhp*, remained $ilvB^+$, *ilvB* must lie to the right of *uhp*, consistent with results of Newman and Levinthal (23).

Insertion *zic-635*::Tn*10* gave rise to Tc^s Fos^r variants, but none of them were Ilv^-. Furthermore, none contained deletions of *uhp* material, since they recombined with most or all *uhp* point mutations. Perhaps some essential gene lies between *zic-635*::Tn*10* and *uhp*, such that only inversions could give rise to Tc^s Fos^r variants. Essential genes in the vicinity of this insertion include *rnpA*, *gyrB*, and *dnaA*. The location of this insertion with respect to these markers is not known.

The *zic-634*::Tn*10* insertion, also lying to the right of *uhp*, gave rise to Tc^s Fos^r variants with either Ilv^+ or Ilv^- phenotype. Of 50 Tc^s Fos^r variants examined, 15 were Ilv^+; these gave Uhp^+ recombinants with most *uhp* point mutations and probably resulted from an inversion event. Of the 35 Ilv^- strains, 14 carried deletions of all *uhp* material. The remaining 21 Ilv^- strains recombined with some *uhp* mutations. The pattern of recombinational activity indicated that these strains contained partial deletions of *uhp* entering from the end opposite to that from which the previous deletions entered. Examination of 50 Tc^s Ilv^+ variants derived from the *zic-634*::Tn*10* insertion gave no strains which were Uhp^-. These results confirm that *ilvB* is located to the right of *uhp*.

Although excision of Tn*10* insertions near *uhp* did give rise to deletions extending from the site of the transposon and ending in *uhp*, examination of the genetic map so derived (Fig. 2) revealed marked clustering of the deletion endpoints. Of 48 independent deletions ending in *uhp* from either side, three sites represented 31 (65%) of the total events. Most of the remaining endpoints were close to these sites. Most of the Tn*10* insertions within *uhp* were in proximity to the preferred sites for deletion formation. Both insertion and deletion formations are thought to be alternative outcomes of the same initial transposition event (17, 24), and transposition by Tn*10* exhibits specificity for target DNA sequences (10). Thus, both products of transposition are likely to involve the same sequences on the chromosome and show the same nonrandom distribution.

Mapping with *uhpT-lac* operon fusion-bearing phages. Another genetic mapping procedure made use of specialized transducing phages carrying portions of the *uhp* region. We had previously isolated *uhpT-lac* operon fusions in which prophage λ was integrated next to the fusion (27). Excision of this prophage from the chromosome requires an "illegitimate" recombination event, often between regions of bacterial DNA on either side of the prophage. The majority of the phages released from the fusion-bearing lysogen RK5115 gave white plaques when plated on a Δ*lac* strain on minimal plates containing G6P and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside. Blue plaques were formed by phages which carry the intact fusion extending at least to the *uhpT* promoter site.

Several phages were crossed with recipients carrying representative *uhp* point mutations, with selection for Uhp^+. Lysogenization requires integration of the phage into the *uhp* region of the chromosome. Three outcomes were observed. The presence of no Uhp^+ progeny showed that the phage did not contain the region altered in the recipient. Many crosses gave rise to a limited number of Uhp^+ progeny, indicating that a specific recombination event(s) was required to restore the wild-type phenotype. Finally, other crosses revealed confluent growth on the selection plate, such that all lysogens were Uhp^+. This may indicate that the phage carried a functional copy of the gene altered in the recipient. Since the strains are Rec^+ and integration must occur in the *uhp* region, the results which indicate complementation must be interpreted with caution, since it is possible that

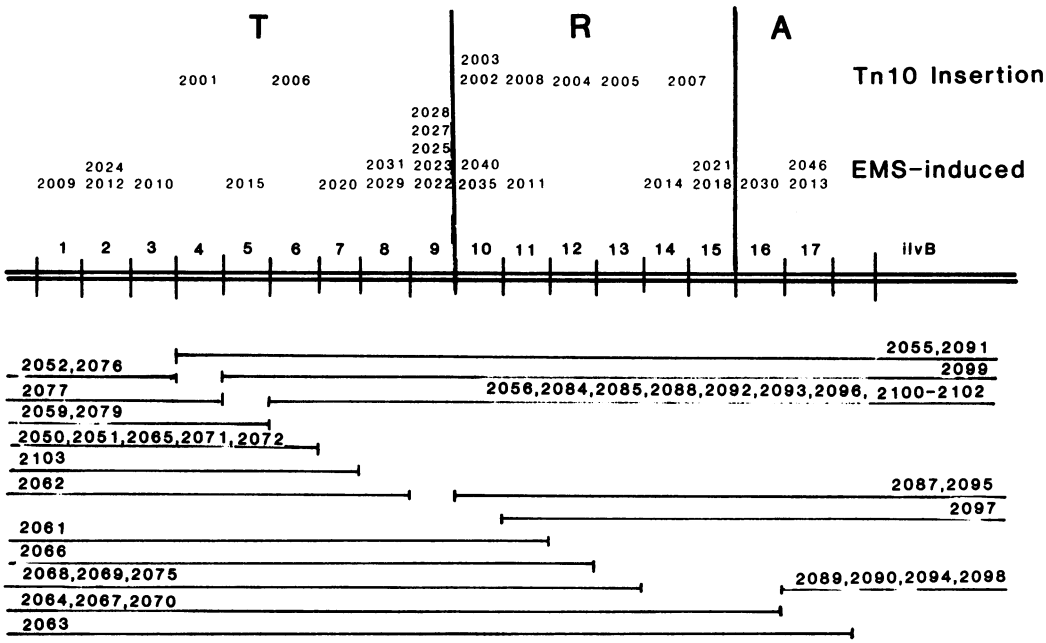

FIG. 2. Deletion map of the *uhp* region. The double central line is marked off into 17 deletion groups within the *uhp* region. Below the line is shown the material deleted in various strains carrying deletions generated upon excision of flanking Tn*10* insertions. Not listed are deletions which removed all *uhp* material. Above the line are presented allele numbers for EMS-induced point mutations and Tn*10* insertions. The division of the *uhp* region into T, R, and A genes is based on the properties of Uhp$^+$ revertants and complementation results with specialized transducing phages. All of the mutations designated as *uhpR* gave rise to revertants with constitutive expression of G6P uptake activity.

the integration event reconstructed the wild-type allele.

Several of the fusion-bearing phages (1a, 1b, 3a, 3b) recombined with most or all of the *uhp* alleles on the left side of the map in Fig. 2 (Table 2), but complemented mutations on the right side. Phage 6j also recombined with all alleles on the left side. It exhibited complementation of several of the mutations on the right end, but did not carry the material represented by the two alleles at the rightmost end. Although alternative explanations are possible, this result suggests the presence of at least two genes on the right side of the *uhp* region. These are designated

TABLE 2. Genetic properties of λ(*uhpT-lac*) operon fusion transducing phages[a]

Phage isolate	Fusion activity	Ability to confer Uhp$^+$ phenotype to *uhp* recipients								
		T2009	T2012	T2010	T2015	T2022 T2023	R2011	R2014	R2018 R2021	A2013 A2046
1a, 2b	+	R	R	R	R	R	C	C	C	C
3a	+	—	—	R	R	R	C	C	C	C
3b	+	R	R	R	R	R	C	C	C	C
6j	+	R	R	R	R	R	C	C	C	—
6c	+	R	R	R		R	R	R	—	—
6i	+	—	—	R		R	R	—	—	—
6b	−	—	R	R	—	—	—	—	—	—
6a	−	—	R	—	—	—	—	—	—	—
1c, 1d	−	—	—	—	—	—	—	—	—	—

[a] Matings were conducted by spotting droplets of recipient bacteria, grown in L broth, onto the surface of an F6P-minimal selective plate on which a lawn of the indicated phage had been spread. After incubation for 24 h at 37°C, the presence of Uhp$^+$ recombinants was scored. C, Confluent growth of spot, indicating complementation; R, individual colonies indicating need for specific recombination events to restore Uhp$^+$ function; —, no Uhp$^+$ recombinants.

uhpR and *uhpA*. Both are needed for Uhp expression, and their mutant phenotypes appear to be recessive to the wild-type alleles.

Other phages contained progressively less *uhp* material from the right-hand end and were unable to complement *uhpR* mutants, but did give recombinants. Phages lacking the intact fusion either carried no detectable *uhp* material or were able to recombine with only a few alleles near the left end. These results suggested that the fusion was in the gene at the left end, in the region between the *T2012* and *T2010* alleles. These mapping results with specialized transducing phages were fully consistent with those from deletion mapping.

Regulatory properties of Uhp$^+$ revertants. A correlation was found between the properties of Uhp$^+$ revertants and the map position of the original mutation. Most of the EMS-induced mutants and Tn*10* insertions did revert to Uhp$^+$ (Table 3). Some *uhp* mutants reverted infrequently (10^{-7} to 10^{-8}), and these revertants exhibited inducible regulation of G6P uptake activity. Other mutants reverted more frequently (10^{-5} to 10^{-7}) and often to an altered regulatory phenotype. Revertants of the latter class exhibited a range of regulatory behavior, including low-level constitutive, high-level constitutive, constitutive but further inducible, and even constitutive but repressible by G6P.

The mutations that reverted infrequently and to inducible behavior were clustered in two regions of the *uhp* map. Most of them lay to the left end of the map and were not complemented by the *uhpT-lac* fusion-bearing transducing phages. These mutations define the *uhpT* locus, but the number of genes cannot be defined on the basis of current evidence. Three mutations giving inducible revertants lay at the opposite end of the *uhp* region. The complementation results suggested that they represent a discrete gene, termed *uhpA*. Between these two loci lay all of the mutations whose revertants had altered regulatory behavior. These mutations were listed as defining the *uhpR* region, but these may represent more than one gene.

Strains carrying the *uhp*::Tn*10* insertions gave rise to Uhp$^+$ revertants at different frequencies and having different regulatory properties (Table 3). Two (*T2001*, *T2006*) reverted only at very low

TABLE 3. Regulatory properties of Uhp$^+$ revertants[a]

uhp allele	Revertants/ 10^8 cells	No. of revertants having the indicated regulatory phenotype				
		Inducible	Inducible, <30% WT	Constitutive, <30% WT	Constitutive	Other
T2009	<10	5				
T2010	<10	4				
T2012	<10	5				
T2015		4				
T2016	<10	4	3			
T2020		2	1			
T2022	<10	3	5			
T2029	<10	3				
T2032		4				
T2001::Tn*10*	<1					
T2006::Tn*10*		1 (Tc^s)				
R2011	50			1	5	2
R2014	100			1	5	3
R2018			2	3	1	
R2019			4	2		
R2021					1	
R2035				4	1	
R2040		2	2	1		
R2003::Tn*10*	10				2 (Tc^s)	
R2004::Tn*10*	100			6	1	
R2005::Tn*10*	200			11	1	
R2007::Tn*10*	100			4	2	
R2008::Tn*10*	200			6	5	1
A2013	2	1				
A2046	5	4				
A2030	1	1				

[a] Regulatory phenotype was determined from the induced and uninduced levels of G6P uptake activities. The second and third categories had induced levels that were <30% of induced wild-type (WT) strains.

frequency ($<10^{-7}$). All of these revertants exhibited inducible regulation and were Tc^s, i.e., had lost the Tn*10* insertion. These insertions were mapped within the *uhpT* locus (Fig. 2). Other insertions yielded higher frequencies of Uhp$^+$ revertants, most of which retained the *uhp*::Tn*10* insertion. These revertants exhibited aberrant regulatory behavior, including low- or high-level constitutive expression. These insertions were mapped within the *uhpR* region.

The isolation of revertants which still retained the *uhpR*::Tn*10* insertions showed that alterations at a second site could overcome the block in *uhp* expression caused by the loss of *uhpR*. Genetic crosses with the revertants as recipient showed that all of the second mutations were linked to the *uhp* region (Table 4). When the donor P1 lysate was grown on a strain carrying the same *uhp*::Tn*10* as in the revertant, 30 to 40% of the *pyrE*$^+$ recombinants became Uhp$^-$. All of these recombinants were still Tc^r. Thus, some of the recombinants had inherited the wild-type allele of the second mutation, thereby restoring the Uhp$^-$ phenotype. Linkage to the *gltS* locus showed that the second mutations lay in or near *uhp*.

In addition, when the P1 lysate was grown on the wild-type strain, RK4353, and used to transduce the revertants, 50 to 70% of the *pyrE*$^+$ recombinants were Tc^s and Uhp$^+$, showing that the revertants retained *uhp*::Tn*10*. In some cases, Uhp$^-$ recombinants were observed; all of these were Tc^r. These could arise only by inheritance of the wild-type allele of the second mutation but without replacement of the insertion. In these cases, the second mutation must lie in *uhp* between the insertion and *pyrE*. The cases in which no Uhp$^-$ recombinants were recovered could indicate either that the second mutation was distal to the insertion or that it was too closely linked to the insertion to be separated in the number of recombinants examined.

DISCUSSION

A genetic map of the *uhp* region was constructed on the basis of genetic crosses between point mutation, transposon Tn*10* insertions, deletions entering from either side, and specialized transducing phages. The genetic location of various alleles correlated with the regulatory properties of Uhp$^+$ revertants. The results supported the existence of at least three *uhp* genes in this area, although detailed complementation analysis and identification of polypeptide products will be necessary for any definite conclusions concerning the number of genes involved in transport or regulation. However, it is possible to predict certain properties of the three gene products on the basis of information provided in this paper, as well as data not presented here.

TABLE 4. Linkage to *uhp* of the second mutation in Uhp$^+$ revertants of *uhp*::Tn*10*[a]

uhp::Tn*10* allele	Uhp$^+$ revertant	G6P uptake activity		Cotransduction frequency		
		−G6P	+G6P	P1(*uhp*::Tn*10*) (Uhp$^-$/Ura$^+$)	P1(*uhp*$^+$) Tc^s/Ura$^+$	P1(*uhp*$^+$) No. Uhp$^-$[b]
R2003	R1	5.7	7.1	36/90 (0.40)	50/96 (0.52)	14
R2004	R1	1.1	0.9	27/93 (0.29)	33/60 (0.55)	0
	R4	7.0	3.5	29/110 (0.26)	59/96 (0.52)	0
R2005	R1	0.8	0.8	27/83 (0.33)	63/108 (0.58)	0
	R12	0.8	0.7	26/83 (0.31)	55/96 (0.57)	4
R2007	R3	1.1	1.1	15/46 (0.33)	37/48 (0.77)	0
	R4	3.0	1.9	25/65 (0.39)	81/156 (0.52)	1
	R6	6.8	6.9	18/98 (0.18)	65/96 (0.68)	2
R2008	R1	0.5	0.6	30/143 (0.21)	64/96 (0.67)	0
	R4	1.9	1.8	20/70 (0.29)	33/60 (0.55)	
	R6	8.0	9.6	29/103 (0.28)	39/72 (0.54)	0
	R10	11.8	4.3	30/123 (0.24)	55/96 (0.57)	4

[a] Uhp$^+$ revertants were selected by spreading 10^8 cells of the *uhp*::Tn*10* strains on F6P-minimal plates. All of these revertants retained tetracycline resistance. Their G6P uptake activity was assayed as usual after growth in the absence or presence of 0.1% G6P as inducer. Each revertant was transduced with a P1 lysate growth on either the original *uhp*::Tn*10* mutant or the wild-type parent. Selection was for Ura$^+$, and the Tc and Uhp phenotypes were scored on purified recombinants. The first cross shows the linkage of *pyrE* to the second mutation that conferred the Uhp$^+$ phenotype. The second cross confirmed that the Tn*10* remained linked to *pyrE* and suggests that the second mutation lies between *pyrE* and *uhpR*::Tn*10*.

[b] All of the Ura$^+$ Uhp$^-$ recombinants in these crosses were Tc^r.

The putative gene for the transport system, *uhpT*, lies at the left end of the region and is transcribed from right to left (27), relative to the map in Fig. 1 and 2. Point mutations in this region can revert at low frequency to Uhp^+ forms which exhibit normal inducibility. Deletions removing this region did give rise to rare small colonies on F6P plates, but these did not possess detectable G6P uptake activity. Tn*10* insertions in this gene gave rise to Uhp^+ revertants very rarely, and all were tetracycline sensitive. These must have arisen by precise excision of the insertion. Finally, this gene is the site of the insertion of the *uhpT-lac* operon fusion which exhibits inducible regulation that exactly parallels the regulation of the Uhp transport system.

Three point mutations that gave rise to inducible Uhp^+ revertants were localized by deletion mapping to the opposite end of the *uhp* region. Deletions that remove this region did not yield Uhp^+ revertants, except for rare forms having very low uninducible levels of G6P uptake. It is not known whether the low activity in these revertants is encoded by *uhpT*. The product of this gene, termed *uhpA*, thus plays a positive role in *uhpT* expression.

The *uhp* mutations which gave rise to frequent constitutive revertants lie between *uhpT* and *uhpA*. The constitutive revertants resulted from a second mutation in the *uhp* region. Constitutive revertants of *uhpR*::Tn*10* insertions retained the tetracycline resistance of the transposon. When the donor lysate in a transduction cross was prepared from the starting Uhp^- *uhpR*::Tn*10* strain, 20 to 40% of the $pyrE^+$ recombinants were Uhp^-. This resulted from replacement of the second mutation in *uhp* (which allowed constitutive Uhp^+ expression) with the wild-type form, restoring the Uhp^- phenotype. The site of this second mutation affecting regulation has not been determined precisely within the *uhp* region. However, it is possible that there are two regulatory genes in the region between *uhpT* and *uhpA* and that the loss of one of them gives rise to the Uhp^- phenotype and loss of the other confers Uhp^c constitutive expression. Current studies are directed toward this question.

The use of Tn*10* insertions to generate deletions into the *uhp* region seemed an attractive and powerful approach, owing to the ease with which Tc^s variants can be selected. However, only a limited number of deletion endpoints were observed, no matter from which direction the deletion entered the *uhp* region. It is possible that the deletion endpoints were randomly distributed, but that the point mutations only occurred in local hot spots. This is being determined by the use of restriction endonuclease mapping to determine the physical extent of these deletions. However, marked clustering of Tn*10*-induced deletion endpoints had been previously observed in the histidine transport genes (24). Furthermore, the sites of Tn*10* insertions in the *uhp* region were usually very close to the preferred positions of deletion endpoints. The preference for Tn*10* insertion into target DNA sequences having homology to the Tn*10* ends has been documented (10). Since deletion formation probably results from the same processes that can lead to transposition, it is not surprising that preferred sites for insertion and deletion should be identical (24).

In addition, comparison of the genetic and physical maps of the *uhp* region may help document the peculiar asymmetry of cotransduction frequencies in this area by defining the exact distance of the external Tn*10* insertions from *uhp*. The accompanying paper (28) provides an initial characterization of the physical location of the *uhp* region on the chromosome.

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