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Genetic Control of the Transport of Hexose Phosphates in *Escherichia coli*: Mapping of the *uhp* Locus

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A number of mutations affecting the transport of hexose phosphates in *Escherichia coli* were ordered within the *uhp* locus. Three-point crosses by transduction or conjugation allowed the ordering of the alleles relative to the adjacent *pyrE* marker. The same linear map was obtained by both methods. This, combined with the regulatory properties of revertants of these mutants, allowed a tentative identification of two genes, one presumably coding for the transport system (*uhpT*) and the other(s) specifying a regulatory element (*uhpR*). The order of these is *pyrE-uhpT-uhpR*. Mutants exhibiting constitutive expression of the transport system were isolated. This behavior is genetically linked to the *uhp* locus, but more precise localization was not possible.

Cells of *Escherichia coli* possess an inducible transport system allowing the active accumulation of hexose phosphates within the cell (4, 8, 9). This transport system is responsible for the ability of these cells to utilize hexose phosphates as carbon sources, apparently without their extracellular hydrolysis to the free hexose. Perhaps the most interesting feature of this transport system is its regulation, which has been termed exogenous induction (1, 10, 11). Thus, induction requires the presence of extracellular glucose-6-phosphate (G6P). Neither intracellular G6P alone nor other extracellular hexose phosphates that are substrates for the transport system directly result in induction. Winkler (11) has shown that the induction process is different from the transport process in that the former is specific for G6P, has a lower apparent K_m, and is not subject to competition by substrates for the transport system. Thus, there would appear to be separate structural genes encoding the transport system and the regulatory system, both of which may be membrane associated.

The previous report (6) on this system showed that all of the mutations found to eliminate the activity of this system were located in the same region of the chromosome, near *pyrE*. All of these *uhp* alleles gave very similar co-transduction frequencies with *pyrE*. Some of the mutations gave rise to Uhp$^+$ revertants with inducible regulation, whereas other mutations yielded some revertants with constitutive expression. This might imply that the former mutations affected the structural gene for the transport system, whereas the latter affected the regulatory system. At present, there is no biochemical method to differentiate between the two. The genetic order of these mutations was investigated as a step in the characterization of the *uhp* system.

In addition, mutants exhibiting constitutive expression of the *uhp* system have been described by Ferenci et al. (2). These mutations were also mapped near *pyrE*. This paper reports the isolation of similar constitutive mutants by a different procedure and mapping experiments with them.

MATERIALS AND METHODS

Media and genetic crosses. The conditions for the maintenance and growth of bacterial strains have been described (6). The techniques for phage P1-mediated transductions were as described except that the recipient bacteria were concentrated to 10^{10}/ml and the phage lysates were added at a multiplicity of 1.

Conjugation crosses employed a donor-recipient ratio of 1:10, and the mating mixture was plated without interruption after 30 min of incubation at 37 C with gentle aeration. The donor cells were counter-selected by the absence of adenine from the selective plates.

Strains. The *E. coli* strains used in this study are listed in Table 1. The recipient strains carrying the *uhp* alleles to be ordered were constructed as follows. Strain RK1041 (*cysE pyrE60 uhp*$^+$) was transduced to

TABLE 1. *Bacterial strains*

Strain	Relevant genotype
E15[a]	Hfr *thi* (Δ *phoS*)
Lin 8[a]	Hfr *thi* (Δ *phoS*) *glpT*
DF40[b]	Hfr, *thi pgi*
RK21[c]	Hfr *thi purC metB pyrE60 strA uhp*$^+$
RK1042	F$^-$ *ilv argH his metB pyrE60 bgl*$^+$ *mtl strA uhp*$^+$
RK1412	As E15, but *uhp-12*
RK1414	As E15, but *uhp-14*
RK1415	As E15, but *uhp-15*
RK1417	As E15, but *uhp-17*
RK1419	As E15, but *uhp-19*
RK1420	As E15, but *uhp-20*
RK1421	As Lin 8, but *uhp-21*
RK1423	As Lin 8, but *uhp-23*
RK1424	As Lin 8, but *uhp-24*
RK1425	As Lin 8, but *uhp-25*
RK1426	As Lin 8, but *uhp-26*
RK1427	As Lin 8, but *uhp-27*
RK1428	As Lin 8, but *uhp-28*
RK1042-12	As RK1042, but *uhp-12*
RK1042-14	As RK1042, but *uhp-14*
RK1042-15	As RK1042, but *uhp-15*
RK1042-17	As RK1042, but *uhp-17*
RK1042-19	As RK1042, but *uhp-19*
RK1042-20	As RK1042, but *uhp-20*
RK1042-21	As RK1042, but *uhp-21*
RK1042-23	As RK1042, but *uhp-23*
RK1042-24	As RK1042, but *uhp-24*
RK1042-25	As RK1042, but *uhp-25*
RK1042-26	As RK1042, but *uhp-26*
RK1042-27	As RK1042, but *uhp-27*
RK1042-28	As RK1042, but *uhp-28*
RK21-12	As RK21, but *uhp-12*
RK21-14	As RK21, but *uhp-14*
RK21-15	As RK21, but *uhp-15*
RK21-17	As RK21, but *uhp-17*
RK21-19	As RK21, but *uhp-19*
RK21-20	As RK21, but *uhp-20*
RK21-21	As RK21, but *uhp-21*
RK21-23	As RK21, but *uhp-23*
RK21-24	As RK21, but *uhp-24*
RK21-25	As RK21, but *uhp-25*
RK21-26	As RK21, but *uhp-26*
RK21-27	As RK21, but *uhp-27*
RK21-28	As RK21, but *uhp-28*

[a] From E. Lin.
[b] From D. Fraenkel (3).
[c] From R. Kadner and H. Winkler (6).

pyrE$^+$ with phage lysates grown on the prototrophic strains derived from strain E15 or Lin 6 in which the *uhp* alleles had been selected. *pyrE*$^+$ transductants that had received each of the *uhp* alleles were then transduced with phage prepared on strain RK1034 (*cysE*$^+$ *pyrE60 uhp*$^+$), selecting for *cysE*$^+$. The *pyrE60 uhp* derivatives thus obtained were designated as strains RK1042 *uhp-11*, etc. The derivation of the Hfr strains carrying these *uhp* alleles from strain JC12 (O-argG-strA----thyA) has been described (6).

Isolation of constitutive mutants. Mutants exhibiting constitutive expression of the hexose phosphate transport system were selected in strain DF40 (*pgi*) by selection for the utilization of fructose-6-phosphate (F6P) as sole carbon source (3, 9). A culture of strain DF40 was inoculated into minimal medium supplemented with 1 mM F6P and incubated at 37 C for several days. After growth became evident, single-colony isolates were tested for their *pgi* character and the level of the hexose phosphate transport system. The mutations in seven independent mutants with constitutive expression were designated *uhp 31-37*.

Transport assays. The assay for the transport of G6P has been described (6). For testing the uptake activity of various recombinants, cells were grown in medium supplemented with 1% Casamino Acids with and without G6P. Samples were assayed directly from the growth medium with 1 min of incubation at room temperature with labeled G6P prior to filtration. Radioactivity retained on the filter was measured on planchets with a Nuclear-Chicago gas-flow counter.

RESULTS

Mapping by transduction. Three-point transduction crosses were employed to determine the sequence of the *uhp* alleles with respect to the outside marker, *pyrE60*. The donor strains were derived from strain E15 (*pyr*$^+$ *uhp*). The *uhp* recipient strains were isogenic with strain RK1042 (*ilv pyrE60 uhp*). After transduction, selection was made for *pyrE*$^+$, *uhp*$^+$, and *pyrE*$^+$ *uhp*$^+$ recombinants. The number of *uhp*$^+$ recombinants, normalized to the number of *pyrE*$^+$ recombinants, was used to estimate the genetic distance between the *uhp* alleles tested. The relative ratio of the number of *pyr*$^+$ *uhp*$^+$ recombinants derived from reciprocal crosses, normalized to the number of *uhp*$^+$ recombinants, was used to determine the order of the alleles with respect to *pyrE*.

The results obtained for six representative pairs of crosses are presented in Table 2. The order of the *uhp* alleles was determined from the data of the final column. The basis for this was the assumption that, of the reciprocal pair of crosses, the cross yielding the smaller proportion of *uhp*$^+$ *pyr*$^+$ recombinants required a minimum of four crossover events. Hence the *uhp* allele of the donor strain in this "minority" cross lies between the other *uhp* allele and *pyrE*. All transduction crosses were performed at least twice. All recombinants were found to retain the *ilv*, *his*, and *str* markers of the recipient. The number of spontaneous revertants was determined for each recipient and subtracted from the transduction data. In the "majority" cross, that requiring two crossover events, the linkage of *uhp* to *pyrE* is on the order of 40 to 50%, which is the co-transduction frequency previously described for these genes.

TABLE 2. *Ordering of uhp alleles by reciprocal three-point transduction crosses*[a]

Donor[b] *uhp* allele × Recipient[b] *uhp* allele	No. of pyrE+ recombinants/ml	No. of *uhp*+/no. of *pyr*+	No. of *uhp*+ *pyr*+/no. of *pyr*+	No. of *uhp*+ *pyr*+/no. of *uhp*+
uhp 15 × *uhp 26*	1,620	0.181	0.023	0.127
uhp 26 × *uhp 15*	3,630	0.122	0.056	0.460
uhp 17 × *uhp 21*	685	0.047	0.0028	0.059
uhp 21 × *uhp 17*	1,360	0.045	0.0267	0.591
uhp 17 × *uhp 23*	620	0.055	0.043	0.773
uhp 23 × *uhp 17*	1,035	0.051	0.015	0.286
uhp 21 × *uhp 25*	1,000	0.055	0.013	0.240
uhp 25 × *uhp 21*	1,420	0.083	0.044	0.533
uhp 23 × *uhp 25*	1,225	0.104	0.005	0.052
uhp 25 × *uhp 23*	1,790	0.089	0.028	0.312
uhp 23 × *uhp 26*	435	0.143	0.016	0.113
uhp 26 × *uhp 23*	2,145	0.098	0.042	0.423

[a] Several (4 to 6) 0.1-ml portions of each transduction mixture were spread onto plates selective for *pyr*+, *uhp*+, or *pyr*+ *uhp*+ recombinants. The number of recombinants was counted, and the number of *uhp*+ or *pyr*+ *uhp*+ recombinants, normalized to the number of *pyr*+, was corrected for reversion from the cross in which each donor *uhp* allele was transduced into a recipient carrying the same *uhp* allele. Each cross was done twice.

[b] Donor genotype: *pyrE*+ *uhp*; recipient genotype: *pyrE uhp*. The phage lysates were prepared on strains RK1412 through RK1428. The recipients were strains RK1042-12 through RK1042-28.

Table 3 summarizes the data from a similar analysis of nine other pairs of crosses. The first column of figures presents the ratio of the normalized number of *uhp*+ *pyr*+ recombinants to the number of *uhp*+ recombinants for the cross in which the first allele is from the donor. The second column presents the same data for the cross in the reciprocal orientation. For example, from the first row of data, it was determined that *uhp15* lies between *pyrE* and *uhp21*. By employing this analysis, a linear map was generated, yielding the order *pyrE-uhp15-23-17-21-25-20-26*.

The approximate genetic distance between the various *uhp* alleles could be estimated from the frequency of *uhp*+ recombinants, normalized to the frequency of *pyr*+ recombinants from these transductions. Figure 1 summarizes the map of the *uhp* locus and the approximate genetic distance between the *uhp* alleles as determined by transduction and expressed as the normalized average frequency of *uhp*+ recombinants. The two extreme *uhp* alleles, *uhp15* and *26*, yielded *uhp*+ recombinants with a frequency of approximately 15% relative to the number of *pyr*+ recombinants. Since *pyr*+ *uhp*+ recombinants represented 46% of the number of *pyr*+ recombinants, this implies that the *uhp* locus is approximately 33% as long as the distance between *pyrE* and *uhp*.

Mapping by conjugation. Although transduction crosses provided a satisfactory mapping technique, it required the use of a large number of expensive G6P plates to obtain significant numbers of *uhp*+ recombinants. Hence, another procedure employing conjugation was used to order other *uhp* alleles. A series of Hfr strains with the origin of Hfr JC12 (near min 58) was prepared, carrying each of the *uhp* alleles to be mapped. The recipients were the same as used in the transduction mapping. As before, reciprocal crosses were performed and selection was made for *pyr*+, *ilv*+, *uhp*+, *pyr*+ *uhp*+, and *ilv*+ *uhp*+ recombinants. The frequency of recombinants was about a 1,000-fold greater than in the transduction crosses. However, there was no significant difference between the reciprocal crosses in the number of *pyr*+ *uhp*+ or *ilv*+ *uhp*+ recombinants relative to the number of *uhp*+ recombinants. A *uhp*+ donor strain yielded about 70 *uhp*+ recombinants per 100 *pyr*+ recombinants; the *uhp* donors gave less than 2 *uhp*+ per 100 *pyr*+ recombinants (Table 4).

However, differences were observed when the *uhp*+ recombinants were tested for their inheritance of the donor *pyr*+ or *ilv*+ markers. In all crosses, an average of 85% of the *uhp*+ recombinants had inherited *ilv*+, with no significant difference between any two reciprocal crosses. In most pairs of crosses, there was a statistically

TABLE 3. *Summarized data ordering uhp alleles by reciprocal three-point transduction crosses*

Parental *uhp* alleles[a]	Ratio of *uhp*+ *pyr*+ recombinants per *uhp*+ recombinant[b]		*uhp*+/*pyr*+
	Cross as written	Reciprocal cross	
15 × 21	0.053	0.719	0.152
15 × 23	0.019	0.474	0.047
17 × 20	<0.005	0.227	0.044
17 × 25	0.039	0.514	0.049
20 × 21	0.071	0.209	0.025
20 × 23	0.269	<0.01	0.090
20 × 25	0.529	0.144	0.056
21 × 23	0.245	0.099	0.069
21 × 26	0.526	0.719	0.035

[a] The two crosses are presented such that in the first cross (cross as written), the first parental allele mentioned was carried on the donor and the second on the recipient. In the reciprocal cross, this arrangement was reversed.

[b] These two columns present the data obtained in the same manner as the data in the last column of Table 2.

significant difference in the number of *uhp*$^+$ recombinants inheriting the unselected donor *pyr*$^+$ marker. Assuming that the donor *uhp* allele in the cross giving the fewer *pyr*$^+$ recombinants is located between *pyr* and the other *uhp* allele, a linear map can be constructed (Fig. 2). Distances between some of the *uhp* alleles were estimated from the number of *uhp*$^+$ recombinants obtained. It can be seen that the order obtained by the conjugation crosses is identical to that obtained by transduction.

Figure 2 also summarizes data presented in the previous paper (6) concerning the regulatory behavior of some *uhp*$^+$ revertants of each of the mutations. The mutations *uhp 15*, *23*, *12*, *28*, and *14* yielded revertants all of which exhibited normal, inducible regulation. In contrast, *uhp 17*, *21*, *25*, *20*, *24*, and *26* gave some revertants with constitutive expression of G6P transport. It was assumed that the former class of mutations might have affected the structural gene of the transport system, whereas the second type (those yielding constitutive revertants) might have affected a regulatory element. The correlation of reversion pattern with map order implies that mutations *uhp 23* to *uhp 14* define the structural gene for the transport system, whereas *uhp 17* to *uhp 26* define a regulatory locus. The assignment of mutation *uhp 27* is in question. Previous data showed that 9 of 13 revertants were inducible. However, 10 other revertants from this strain all exhibited normal inducibility. The mapping experiments showed that *uhp 27* was very close to *uhp 14* (less than 0.001% recombination). Thus, these mapping data and the analysis of revertants allow the tentative identification of two distinct regions within the *uhp* locus.

Isolation of constitutive mutants. Ferenci et al. (2) have described the isolation of mutants exhibiting constitutive expression of the G6P transport system by selection for growth on fructose-1-phosphate. This behavior was genetically linked to *pyrE* (2). Similar mutants have been obtained by a somewhat different selection procedure. F6P is a substrate of the hexose phosphate transport system (9). F6P does induce this system in wild-type cells, but not in a strain deficient in phosphoglucoisomerase (*pgi*). This is due to the fact that a portion of this enzymatic activity is located in the periplasmic space (5). Thus, a *pgi* strain can utilize F6P as sole carbon source only if G6P is added as inducer or if the *uhp* system is expressed constitutively.

Mutants of strain DF40 (*pgi*) which could utilize F6P as sole carbon source were selected. A number of these were tested and found to exhibit constitutive expression of the *uhp* system. Several of these were chosen for further study, and their mutations were designated *uhp 31* through *37*. The rates of G6P transport

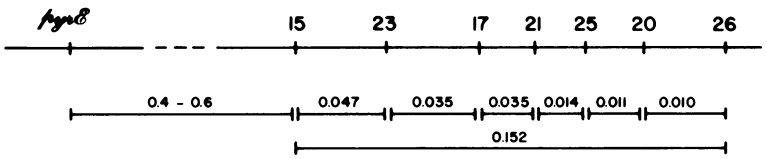

FIG. 1. *Ordering of uhp alleles by three-point transduction crosses. The order of the uhp alleles is determined from the data of Tables 2 and 3. The approximate genetic distances between alleles was estimated by averaging the number of uhp*$^+$ *recombinants obtained from each pair of crosses and is expressed as the number of uhp*$^+$ *recombinants per pyr*$^+$ *recombinant. This is not drawn to scale.*

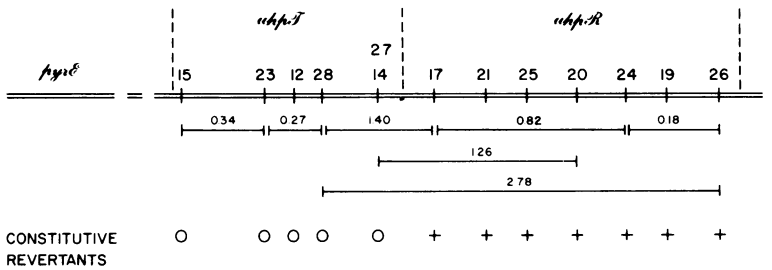

FIG. 2. *Ordering of uhp alleles by three-point conjugation crosses. The order of the uhp alleles is determined from the data of Table 4. The approximate genetic distances between alleles was estimated by averaging the number of uhp*$^+$ *recombinants from each pair of crosses and assuming that these frequencies are additive. These frequencies are expressed as the number of uhp*$^+$ *recombinants per 100 pyrE*$^+$ *recombinants. The bottom line indicates whether uhp*$^+$ *revertants of each allele exhibited constitutive expression (+), or whether they were all inducible (O).*

TABLE 4. *Mapping of uhp alleles by three-point conjugation crosses*[a]

Parental *uhp* alleles	Cross as written		Reciprocal cross	
	No. of *uhp*$^+$ tested	*pyr*$^+$ (%)	No. of *uhp*$^+$ tested	*pyr*$^+$ (%)
12 × 14	300	18.3	435	51.3
12 × 15	168	45.2	162	38.3
12 × 19	100	12.0	431	45.2
12 × 20	185	37.3	57	49.1
12 × 23	67	41.8	291	41.4
12 × 26			219	49.3
12 × 28			280	60.0
14 × 15	149	40.9	100	33.0
14 × 17	400	46.5	200	71.5
14 × 19	241	45.2	150	62.0
14 × 21	540	39.3	431	47.6
14 × 23	869	41.6	400	24.3
14 × 26	500	35.6	300	72.0
14 × 28	205	59.5	300	48.3
15 × 17	130	40.5	197	71.6
15 × 23			195	60.5
17 × 19	389	29.0	600	57.2
17 × 21			167	74.2
17 × 26	254	46.9	181	66.3
17 × 27	297	58.6	64	43.7
19 × 21	378	38.6	292	21.6
19 × 23	396	43.4	276	25.4
19 × 26	569	36.7	72	46.7
20 × 24	170	57.6	174	73.6
20 × 25	186	55.4	87	29.9
20 × 26	77	29.9	33	39.4
20 × 28	91	53.9	147	37.4
21 × 28	299	92.0	579	64.3
23 × 28	214	56.6	220	61.4
24 × 25	236	60.2	107	45.4
24 × 26	112	45.6	297	56.3
24 × 28	271	55.3	276	35.5
25 × 26	127	32.3	216	62.0
25 × 28	71	56.3	258	45.3
26 × 28	312	70.5	397	56.7
+ × 26	292	88.7		
+ × 28	264	96.9		

[a] Conjugation crosses were performed in reciprocal fashion, and *uhp*$^+$ recombinants were selected. A number of these were tested by replica plating for auxotrophy for pyrimidines and isoleucine-valine. The percentage of *pyr*$^+$ among the *uhp*$^+$ recombinants is reported. The two crosses are presented such that in the first cross (cross as written), the first parental allele mentioned was carried on the donor and the second on the recipient. In the reciprocal cross, this arrangement was reversed.

following several conditions of growth of a derivative of strain RK1042 carrying *uhp 32* were compared to those of an isogenic *uhp*$^+$ strain (Fig. 3). The constitutive expression in the *uhp 32* strain is apparent. The rates of uptake in both strains grown in the presence of G6P are quite similar. Further, growth in the presence of G6P plus glucose results in a similar decrease in the rate of uptake for both strains.

Mapping of mutations to constitutivity. It was expected that these mutations resulting in constitutive expression would be located in the *uhp* locus and probably within the regulatory gene. An attempt was made to localize them relative to some of the mapped alleles. Crosses between these constitutive alleles were not attempted owing to the inability to select directly for *uhp*$^+$ recombinants. Hence, crosses were performed in which the donor strains were *pyr*$^+$ and constitutive, and the recipients were *pyrE uhp20, 21, 23, 25,* or *26*. The *pyr*$^+$, *uhp*$^+$ recombinants obtained were purified, and their

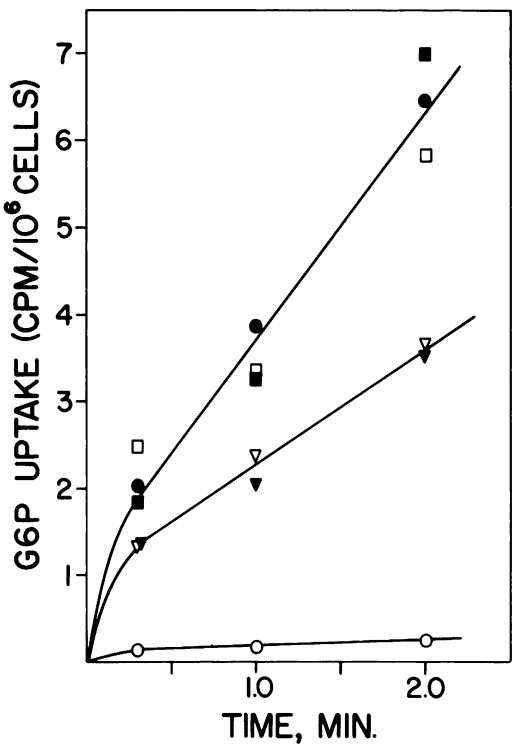

FIG. 3. *Rates of G6P uptake in wild-type and constitutive strains. Cells were grown into the logarithmic phase of growth in medium A supplemented with 1% Casamino Acids and other supplements as listed below. Cells were harvested, washed, and assayed for G6P uptake in medium A. The uptake medium contained* ^{14}*C-G6P at a concentration of 0.625 mM (0.125 μCi/ml). Approximately 8 × 10^7 cells were collected on each filter. Each point represents the average of duplicate determinations. The additions to the Casamino Acids for growth were none* (○, ●)*; G6P, 1 mM* (□, ■)*; or G6P, 1 mM, + glucose, 28 mM* (▽, ▼)*. The strains used were derivatives of RK1042 carrying either the wild-type uhp*$^+$ *allele (open symbols) or the constitutive uhp32 allele (filled symbols).*

regulatory behavior was tested. Linkage of constitutivity to the *uhp* locus would be demonstrated by the constitutive behavior of the recombinants. Table 5 presents the number of *pyr*$^+$ recombinants that became *uhp*$^+$ and the regulatory behavior of some of the *pyr*$^+$ *uhp*$^+$ recombinants when each constitutive allele was crossed into a *uhp26* recipient. Constitutivity is closely linked to the *uhp* locus, as evidenced by the constitutive behavior of the majority of the *pyr*$^+$ *uhp*$^+$ recombinants. Similar recombinants derived from wild-type donor strain all exhibited normal, inducible regulation. It was hoped that, if the constitutive allele was distal to the *uhp* allele of the recipient, a significant proportion of the *pyr*$^+$ *uhp*$^+$ recombinants would not have inherited the constitutive allele of the donor. But the close linkage prevented the determination of whether constitutive alleles were proximal or distal to the *uhp* alleles of the recipient, as demonstrated in Table 6 in which either *uhp32*, *35*, or *37* was crossed into the five *uhp* recipients. There was no apparent difference between the donor alleles in this regard. Ordering was further prevented by the inability to score for constitutivity in a strain lacking the transport system.

TABLE 5. *Genetic linkage of constitutivity to the uhp locus*

Donor[a] *uhp* allele × Recipient[a] *uhp* allele	No. of *pyr*$^+$ tested	*pyr*$^+$ that are *uhp*$^+$ (%)	No. of *pyr*$^+$ *uhp*$^+$ assayed	No. constitutive
uhp31 × *uhp26*	200	48.0	36	34
uhp32 × *uhp26*	484	49.8	66	66
uhp33 × *uhp26*	200	56.0	12	12
uhp34 × *uhp26*	200	54.0	11	11
uhp35 × *uhp26*	392	43.1	11	11
uhp37 × *uhp26*	304	51.0	36	35

[a] Donor genotype: *pyrE*$^+$ *uhp*$^+$, *uhp*^{c}; recipient genotype: *pyrE uhp26*. The phage lysates were prepared on strains DF40 uhp31 through DF40 uhp37. The recipient strain was strain RK1042-26.

TABLE 6. *Linkage of constitutivity to various uhp alleles*[a]

Donor *uhp* allele	Recipient *uhp* allele	No. *pyr*$^+$ scored	% of *pyr*$^+$ that are *uhp*$^+$	No. *pyr*$^+$ *uhp*$^+$ assayed	No. constitutive
32	26	484	50.0	66	66 (100%)
35	26	511	39.3	11	11 (100%)
37	26	174	40.2	36	35 (97%)
32	23	443	55.3	34	32 (94%)
35	23	398	58.3	48	44 (92%)
37	23	321	52.3	24	22 (92%)
32	25	114	42.1	32	28 (88%)
35	25				
37	25	226	45.1	24	23 (96%)
32	21	312	50.5	36	34 (94%)
35	21	327	57.2	12	12 (100%)
37	21	191	59.7		
32	20	289	58.1	30	29 (97%)
35	20	196	54.1	24	24 (100%)
37	20	100	54.0		

[a] Donor genotype: *pyrE*$^+$ *uhp 32*, *35*, *37* (constitutives); recipient genotype: *pyrE uhp*. The phage lysates were prepared on strains DF40 *uhp32*, *35*, or *37*. The recipients were strains RK1042-26, 23, 25, 21, or 20.

DISCUSSION

The investigation of the regulatory control of the hexose phosphate transport system must rely heavily on genetic analyses. Biochemical studies are complicated by the difficulties in assaying for the activity of this system and by the inability to detect the presence of a nonfunctional carrier. A difficulty in the genetic analysis is that apparently only one activity, that of the transport system itself, is controlled by the *uhp* regulatory system. This prevents a clear demonstration that mutants with a negative phenotype are altered in the gene for the transport system or in some regulatory element.

The first step in the genetic characterization of the *uhp* system involved the mapping of a number of mutations which gave a negative phenotype. These were ordered relative to the adjacent *pyrE* marker by three-point transduction and conjugation crosses. The same linear map was constructed from the data obtained by either type of cross. Although the absolute genetic distances between alleles (based on the number of *uhp*$^+$ recombinants relative to the number of *pyr*$^+$ recombinants) were different for the two mating procedures (15% for transduction versus 2% for conjugation), the relative distances were similar. For example, the distance between the *uhp15* and *uhp17* alleles, as a fraction of the total distance between the *uhp15* and *uhp26* alleles, was found to be 54% by transduction crosses and 59% by conjugation crosses.

There is a strong correlation between the map position of an allele and the regulatory behavior of *uhp*$^+$ revertants obtained from it. The previous paper (6) described the regulatory behavior of a number of these revertants. Some alleles yielded revertants all of which had normal inducible regulation. The assumption was made that these mutations might have affected the structural gene for the transport system. Other *uhp* mutations yielded revertants, some of which exhibited constitutive expression of the transport activity. It was assumed that these

mutations might have affected a regulatory element. Unpublished experiments have shown that some revertants of *uhpR* mutations were temperature sensitive for utilization of G6P and that this temperature sensitivity was a property of the regulatory system and not the transport system. So far, no mutants with a temperature-sensitive transport system have been obtained.

All of the presumed regulatory mutations were localized distal to the structural gene mutations, relative to *pyrE*. This was interpreted as tentatively indicating the presence of two functional regions within the *uhp* locus, termed *uhpR* (regulatory) and *uhpT* (transporter). On the basis of the recombination frequencies, it was calculated that the *uhpR* region was approximately equal in size to the *uhpT* region, although there is no basis for assuming that the alleles that were mapped represent the ends of the genes. Further, this study does not imply that there are only two genetic regions within the *uhp* locus or that there is only one regulatory element. It is obvious that further characterizations are required to define these genes and their interactions. Work in progress includes deletion mapping of the *uhp* locus and dominance studies with stable merodiploid strains. These should allow definition of the type of regulation, whether positive or negative control.

Mutants with constitutive expression of this transport system have also been obtained. These mutations were shown to lie within the *uhp* locus, but their exact location could not be defined owing to the close linkage of these markers and the lack of appropriate selective procedures to allow more precise mapping. The constitutive mutants exhibited approximately the same rate of G6P uptake, whether grown in the presence or absence of G6P, as that of an induced *uhp*$^+$ strain. Furthermore, growth of either the inducible or constitutive strain in the presence of glucose resulted in a 40% decrease in the rate of G6P uptake. Whether this represents catabolite repression is being investigated.

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