JOURNAL OF BACTERIOLOGY, Sept. 1983, p. 1062–1070
0021-9193/83/091062-09$02.00/0

Vol. 155, No. 3

Molecular Cloning of the *uhp* Region and Evidence for a Positive Activator for Expression of the Hexose Phosphate Transport System of *Escherichia coli*

DONNA M. SHATTUCK-EIDENS† AND ROBERT J. KADNER*

Department of Microbiology, School of Medicine, The University of Virginia, Charlottesville, Virginia 22908

Received 20 January 1983/Accepted 7 June 1983

The *uhp* locus of *Escherichia coli* contains genes for the sugar phosphate transport system (*uhpT*) and the regulatory system which allows its induction by external glucose 6-phosphate (*uhpRA*). The *uhp* region was cloned onto high-copy-number plasmids, both from Uhp$^+$ plasmids of the Clarke-Carbon collection and from genetically characterized specialized transducing phages carrying *uhpT-lac* operon fusions. Two Clarke-Carbon plasmids and their Uhp$^+$ subclones in pBR322 shared restriction sites defining the *uhp* region, but exhibited different regulation of Uhp expression and dependence on chromosomal *uhp* genotype. Plasmid pLC17-47 and derivatives conferred constitutive glucose 6-phosphate uptake activity in all strains, even those with complete deletions of *uhp*. These plasmids also rendered constitutive the expression of a chromosomal *uhpT-lac* operon fusion. Plasmid pLC40-33 conferred inducible Uhp expression, which required the presence of the *uhpA*$^+$ gene on the chromosome. The induced transport levels in all strains carrying these plasmids were not appreciably amplified over haploid levels. Similar behavior was seen with the cloned operon fusions. A fusion-bearing plasmid that carried an intact regulatory system (*uhpR*$^+$*A*$^+$) exhibited *trans*-dominant constitutive expression of β-galactosidase, regardless of the chromosomal *uhp* genotype. In contrast, the cloned fusion carrying only *uhpR*$^+$ gave glucose 6-phosphate-inducible production of β-galactosidase that was dependent on the presence of chromosomal *uhpA*$^+$. Expression of both fusions in the haploid state was inducible. From these results, it was concluded that the *uhpA* product is necessary for *uhpT* transcription and that elevated dosage of *uhpA* results in at least partially constitutive expression of *uhpT*. A tentative model for *uhp* regulation is presented.

Utilization of a number of sugar phosphates in *Escherichia coli* requires the action of an inducible proton motive force-dependent active transport system. Induction of the transport system is in response to extracellular glucose 6-phosphate (G6P) and acts at the level of transcription (20). Both the transport system and its regulatory components are encoded in the *uhp* region located near min 82 on the *E. coli* linkage map (1, 9, 12, 14). The accompanying paper (13) describes genetic crosses using *uhp* point mutations, deletions, Tn*10* insertions, and specialized transducing phages. Map locations and the regulatory properties of Uhp$^+$ revertants suggested the existence of at least three genes necessary for Uhp expression. These were termed *uhpT*, for the transport system; *uhpR*, for a regulatory component necessary for response to G6P but whose loss could be compensated by second, linked mutations; and *uhpA*, another putative regulatory gene necessary for *uhpT* expression. The Uhp$^-$ phenotype of mutations in all three of these genes appeared to be recessive to the wild-type response.

To initiate studies of the structure and regulation of *uhp*, its molecular cloning was undertaken. Five plasmids from the Clarke-Carbon collection (3, 4) are capable of conferring Uhp$^+$ to a *uhp* recipient (10; R. C. Essenberg, personal communication). The location of the *uhp* sequences carried on two of those plasmids was defined by their regions of overlap and by subcloning into pBR322. Different Uhp$^+$ plasmids exhibited different regulatory behavior and dependences on the presence of chromosomal *uhp*$^+$ genes. Similar behavior was observed with

† Present address: Department of Biology, Washington University, St. Louis, MO 63130.

plasmids carrying *uhpT-lac* operon fusions and genetically defined *uhp* regulatory regions. It was concluded that elevated dosage of $uhpA^+$ results in the *trans*-acting constitutive expression of *uhpT*.

MATERIALS AND METHODS

Bacterial and phage strains. The *E. coli* K-12 strains and special phages used in this study are listed in Table 1. General techniques for bacterial growth and matings were as described by Miller (18) or Davis et al. (7).

The *recA56* allele was introduced into strains by conjugal mating with JC10240 (5) with selection for tetracycline (Tc) resistance. The $RecA^-$ phenotype was scored by sensitivity to UV light. The *srl*::Tn*10* insertion was eliminated by selection for Tc^s variants on fusaric acid medium (17).

TABLE 1. Bacterial and phage strains used in this study[a]

Strain	Genotype and source
E. coli K-12	
RE74	HfrC *relA1 pyrE40 uhp-40 gltS14 tna-6 metB1 tonA22* (CGSC 5569)
CH923	F^- *trpA46 lysA xyl* $glyS_H$ *argH polA1* (11; C. Hill)
JA200/17-47	F^+ Δ*trpE5 recA thr leu lacY*(pLC17-47 (4; via W. M. Holmes)
JA200/40-33	As above, but with plasmid pLC40-33
RK4353	F^- *araD139* Δ*(argF-lac)U169 relA1 rpsL150 thi gyrA219 non* (13)
RK5115	As RK4353, but *uhpT*::[*lacZ* λp1(209)] (20)
RK4929	As RK4353, but *metE70 pyrE40 gltC14 zib-615*::Tn*10* (13)
RK5396	As RK4929, but Δ(Tn*10-uhpTR2048*) *recA56*
RK5503	As RK4353, but also $\lambda\phi(uhpT\text{-}lac)_{1b}$
RK5504	As RK4353, but also $\lambda\phi(uhpT\text{-}lac)_{3a}$
RK5505	As RK4353, but also $\lambda\phi(uhpT\text{-}lac)_{3b}$
RK5506	As RK4353, but also $\lambda\phi(uhpT\text{-}lac)_{6b}$
RK5507	As RK4353, but also $\lambda\phi(uhpT\text{-}lac)_{6c}$
RK5508	As RK4353, but also $\lambda\phi(uhpT\text{-}lac)_{6j}$
RK5569	As RK5396, but carrying pDSE5
RK5570	As RK5396, but carrying pDSE6
RK5571	As RK5115, but *recA56*
RK5644	As RK4929, but Δ(Tn*10-uhpTRA2060*)
RK5723	As CH923, but carrying pDSE5
RK5724	As CH923, but carrying pDSE6
Plasmids	
pBR322	bla^+ tet^+
pMLB524	bla^+ (T. Silhavy)
Phages	
3a	$\lambda\phi(uhpT\text{-}lacR^+A^+)_{3a}$ (13)
6j	$\lambda\phi(uhpT\text{-}lacR^+)_{6j}$ (13)

[a] The source of previously described strains is given in parentheses after the genotype.

Chemicals and media. Growth media and conditions were as described previously (13). Tetracycline (15 μg/ml) or ampicillin (25 μg/ml) was added where indicated and was usually present to maintain presence of plasmids. Most chemicals were from Sigma Chemical Co., St. Louis, Mo. Radioisotopes were from New England Nuclear, Boston, Mass. Restriction endonucleases and T4 DNA ligase were obtained from Bethesda Research Laboratories, Bethesda, Md., and were used under the conditions recommended by the manufacturer or as described by Davis et al. (7).

Transport and enzyme assays. Preparation of cells and assay for rate of accumulation of [^{14}C]G6P were as described before (13). Induction was by addition of 300 μM G6P to a culture at least 30 min before harvest. Specific activities of uptake are expressed as nanomoles of G6P taken up per microliter of cell water per minute. Competition for uptake was measured by the addition of warmed cells to a mixture of [^{14}C]G6P and unlabeled inhibitor, each at a range of concentrations.

β-Galactosidase activity was assayed by measurement of the increase in absorbance at 420 nm of 2 mM *o*-nitrophenyl-β-D-galactopyranoside and toluene-treated cells in medium A containing 0.05 M 2-mercaptoethanol. Specific activity is expressed as nanomoles of *o*-nitrophenol formed per microliter of cell water per minute. It was previously determined that 1 μl of cell water corresponds to 0.27 mg of protein (14).

Restriction analysis of plasmids. Plasmid DNA was prepared by the alkaline extraction procedure of Birnboim and Doly (2), after amplification by growth overnight in the presence of chloramphenicol. Restriction endonuclease-generated fragments were analyzed by electrophoresis in 1% agarose gels with Tris-acetate or Tris-borate buffer (7, 19). Molecular weight standards included *Hin*dIII-generated fragments of bacteriophage λ DNA or *Hae*III-generated fragments of φX174 replicative form DNA.

Mapping of chromosomal location of plasmid insert. The procedure described by Greener and Hill (11) was used to map the original chromosomal location of genetic material carried on a recombinant plasmid. Homologous integration of the plasmid into the chromosome is necessary for the maintenance of plasmid antibiotic resistance determinants in a *polA* strain, which lacks the DNA polymerase I activity required for replication of plasmid ColE1 and its derivatives. Plasmids pDSE5 and pDSE6 were transformed into *polA1* strain CH923, provided by C. Hill. Tc^r transformants were grown for 4 days by repeatedly streaking onto L-tetracycline plates. P1 lysates were prepared from each strain and used to infect RE74. Tc^r recombinants were purified and tested for inheritance of donor uhp^+ and $pyrE^+$ markers.

Transformation. Cells to be transformed were grown in L broth and then made competent by suspension in 0.10 volume of cold 0.5 M $CaCl_2$, as described by Dagert and Ehrlich (6). Competent cells were usually stored overnight at 4°C. Plasmid DNA (5 ng) was added, and the cells were incubated on ice for 10 min. L broth at 42°C was added and incubation was continued at 37°C for 1 h. Transformed cells were then plated on L plates containing the appropriate antibiotic. Antibiotic-resistant transformants were purified, tested for relevant growth responses, and assayed for G6P uptake activity and, when appropriate, β-galactosidase activity. Cells to be assayed were grown with-

out and with 300 μM G6P as inducer and with maintenance of antibiotic selection. Each strain was grown and assayed at least twice. After G6P uptake activity had been assayed, plasmid DNA was extracted from each culture by a "mini-Birnboim" procedure (2). The DNA was transformed into strain RK5396 and shown to confer the expected Uhp$^+$ phenotype.

RESULTS

Cloning of the *uhp* region. Plasmids pLC17-47 and pLC40-33 from the Clarke-Carbon collection gave rise to Uhp$^+$ transconjugants when transferred by conjugation into the Δ(*uhpTR*) *recA* strain RK5396. The plasmids were isolated and the cleavage sites for several restriction endonucleases were determined (Fig. 1). The location of the pColE1 vector was determined from the *Sma*I and *Pst*I sites in the *cea* gene adjacent to the *Eco*RI site used for insertion of the bacterial DNA (3, 24). The insert in pLC17-47 was approximately 19 kilobase pairs (kbp) and that in pLC40-33 was approximately 11 kbp.

To localize the *uhp* region, portions of the plasmids were cloned into pBR322, with selection for Uhp$^+$ activity. Plasmid pDSE1 carried the 19-kbp *Bam*HI fragment from pLC17-47. Ligation after treatment with *Hin*dIII or *Sma*I or both yielded smaller Uhp$^+$ plasmids. Plasmid pDSE5 carried the 11-kbp *Pst*I fragment from pLC17-47. The restriction sites on these five Uhp$^+$ plasmids were compared with those of pLC17-47 (Fig. 1). The only common region was the 7-kbp segment from the terminal *Pst*I site in pColE1 to the single *Hin*dIII site. The 9.5-kbp *Pst*I fragment from pLC40-33 carried most of its insert and, when cloned in pBR322, conferred the Uhp$^+$ response to strain RK5396.

The location and extent of overlap between the two families of Uhp$^+$ plasmids were found by determination of the cleavage sites for *Cla*I, *Hpa*I, and *Pvu*I on plasmids pDSE4, -5, and -6 (Fig. 2). Common restriction fragments defined the region of overlap of pLC17-47 and pLC40-33 as 4.5 kbp in length and including the single *Bgl*II site. At least the *uhpT* gene must be carried on that segment.

Each of the Uhp$^+$ subclones was introduced into strain RK5644, which carries a deletion removing all of *uhp* and the adjacent *ilvB* (13). The phenotypes of transformants for Ilv, Uhp, and colicin El immunity were determined (Fig. 1). Plasmid pLC17-47 conferred IlvB$^+$, a marker located to the right of *uhp* (clockwise on the standard genetic map). The Ilv$^+$ phenotype required maintenance of the *Hin*dIII-*Bam*HI region. Insertion of pBR322 into the *Hin*dIII site resulted in loss of Ilv$^+$, but retention of Uhp$^+$ and colicin El immunity (not shown). Thus, the

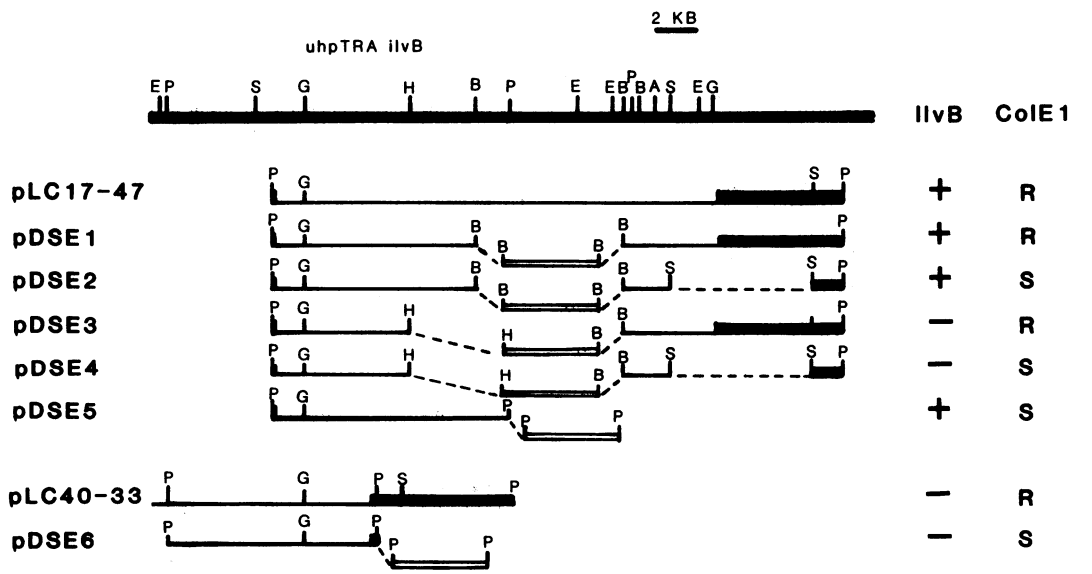

FIG. 1. Restriction map of Uhp$^+$ plasmids. Sites for restriction enzymes: A, *Ava*I; B, *Bam*HI; E, *Eco*RI; G, *Bgl*II; H, *Hin*dIII; P, *Pst*I; S, *Sma*I. The thick upper line represents the chromosome. Below it is portrayed the material carried on the Uhp$^+$ plasmids described in the text. The plasmid maps have been made linear and are lined up relative to the map of the chromosome. The *Bgl*II site (G) is shown for each plasmid to emphasize the common segment. The dashed lines represent material deleted by treatment with a restriction enzyme and ligation. The double line represents pBR322; the thick line on each plasmid shows sequences from the pColE1 vector. To the right are shown the IlvB and ColE1 immunity phenotypes conferred by each plasmid. At the top are the approximate locations of *uhp* and *ilvB* and a length marker of 2 kbp.

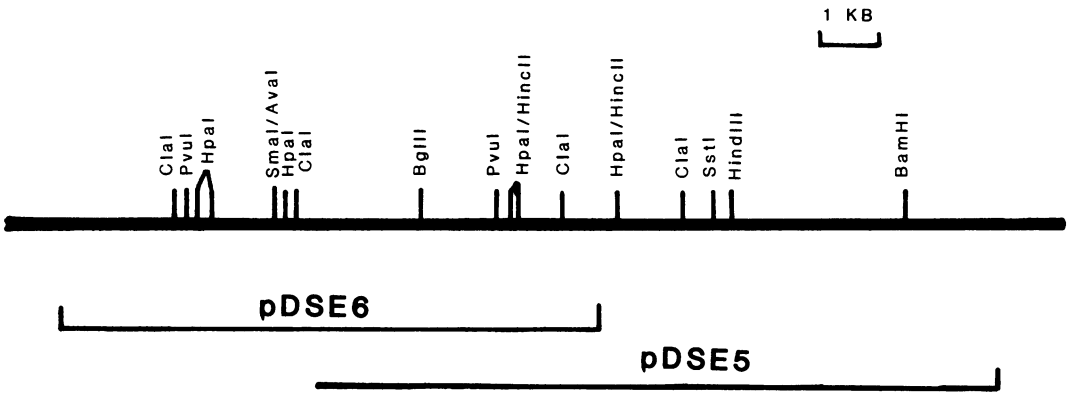

FIG. 2. Restriction map of the *uhp* region and the overlap between the two Clarke-Carbon plasmids. The sites for cleavage by the indicated restriction enzymes on the bacterial DNA carried by plasmids pDSE4, -5, and -6 are shown. Plasmids pDSE5 and -6 show the region of overlap of plasmids pLC17-47 and pLC40-33, respectively. Plasmid pDSE4 has the same sites as pDSE5 from its left end to the *Hin*dIII site.

*Hin*dIII site lies within *ilvB*. The *Bgl*II site is probably in *uhp*, since most Tc^r transformants that received plasmid pDSE5 cut with *Bgl*II but not ligated were Uhp^-. Plasmids pLC40-33 and pDSE6 in strain RK5644 did not confer Ilv^+ or, surprisingly, Uhp^+.

Two lines of evidence showed that these plasmids conferred the same Uhp activity. First, the substrate specificities of the transport activity conferred by plasmids pDSE5 and pDSE6 were identical to those of a wild-type strain with respect to inhibition by fosfomycin, fructose 6-phosphate, and mannose 6-phosphate and with respect to the K_m for G6P. Second, plasmids pDSE5 and pDSE6 were forced to integrate into the bacterial chromosome by the method of Greener and Hill (11). The site of insertion of both plasmids was in *uhp*, as shown by the 6 to 15% cotransduction of *pyrE* and the tetracycline resistance of the integrated plasmid.

Expression of the cloned *uhp* region. The G6P transport system was expressed constitutively in strain RK5396 carrying plasmid pLC17-47, but was inducible when that strain carried pLC40-33. This finding and the fact that strain RK5644 carrying pLC40-33 was Uhp^- prompted the measurement of the level and regulation of G6P uptake activity conferred by the Uhp^+ plasmids. The plasmids were introduced by transformation into strains carrying *uhp* deletions or into *recA* strains with *uhp* point mutations (Tables 2 and 3). The genetic location of the mutations are summarized in Fig. 3 (13). Acquisition of the plasmids was selected by appropriate drug resistance. Transformants were grown under inducing and noninducing conditions and were

TABLE 2. G6P uptake activity in Δ*uhp* strains carrying Uhp^+ plasmids[a]

Recipient genotype	G6P uptake activity (nmol min^{-1} μl of cell $water^{-1}$)											
	pDSE1		pDSE2		pDSE3		pDSE4		pDSE5		pDSE6	
	−	+	−	+	−	+	−	+	−	+	−	+
uhp$^+$	10	13	17	11	10	13	15	10	0.6	12	0.9	12
Δ*uhpT2052*	22	16	13	14	16	13	21	10	17	10	0.5	9
Δ*uhpT2059*	13	21	9	25	17	24	23	16	0.4	3	0.7	6.4
Δ*uhpT2050*	17	17	16	16	8	17	22	15	17	10	0.3	12
Δ*uhpTRA2055*	10	14	20	15	9	21	22	26	19	13	0.2	0.2
Δ*uhpTRA2056*	10	13	17	16	8	24	20	16	12	9	0.2	0.3
Δ*uhpTRA2054*	10	14	3	4	10	10	9	8	9	3	0.5	0.1
Δ*uhpTRA2060*	9	12	17	15	12	19	20	16	0.4	0.5	0.9	0.3

[a] Cells were grown in the absence (−) or presence (+) of 300 μM G6P as inducer in MOPS (morpholinepropanesulfonic acid) medium with glycerol as carbon source and required amino acids. Values presented are the average of duplicate determinations. The activity in haploid *uhp*$^+$ strains ranged from 0.3 to 0.7 U, uninduced, and 8 to 14 U for induced cultures. Uptake in *uhp* mutants ranged from 0.1 to 0.5 U whether grown with or without inducer.

TABLE 3. G6P uptake activity in *uhp* point mutants carrying Uhp$^+$ plasmids[a]

Recipient *uhp* genotype	G6P uptake activity (nmol min^{-1} μl of cell water^{-1})			
	pDSE5		pDSE6	
	−G6P	+G6P	−G6P	+G6P
uhp$^+$	0.6	12	0.9	12
uhpT2009	11	11	0.5	13
uhpT2012	10	8	ND	ND
uhpT2016	24	16	2	21
uhpT2024	14	17	0.8	21
uhpT2010	18	17	0.7	16
uhpT2015	10	11	ND	ND
uhpT2020	14	12	0.8	20
uhpT2022	9	13	ND	ND
uhpT2023	0.2	12	0.6	15
uhpT2025	11	10	0.6	20
uhpR2011	12	12	1	12
uhpR2014	0.1	10	0.3	13
uhpR2018	13	12	2	3
uhpA2013	15	12	0.1	0.3

[a] Uptake activity in all *uhp* mutant strains without plasmid was <0.5 nmol min^{-1} μl of cell water^{-1}. ND, Not determined.

assayed for G6P uptake at least twice.

Four of the five Uhp$^+$ plasmids which carried portions of pLC17-47 conferred constitutive Uhp expression in all recipient strains, including *uhp*$^+$ and those with a complete deletion of *uhp*. Plasmid pDSE5 was unusual in that it conferred constitutive expression in some *uhp* recipients, but inducible expression in *uhp*$^+$ and inducible or negative expression in other recipients. In all cases, the maximal level of G6P uptake activity was not appreciably greater than that of a haploid strain.

In contrast, plasmid pDSE6, derived from pLC40-33, conferred inducible Uhp expression, with the maximal induced level in the range of the haploid value. Some recipients were not converted to Uhp$^+$ by this plasmid. Whereas chromosomal deletions in *uhpT* did not affect expression of the plasmid *uhpT* locus, deletions entering *uhpR* and *uhpA* prevented expression. When the plasmid was introduced into strains with *uhp* point mutations, inducible expression occurred in all cases except the *uhpA* mutant, which remained Uhp$^-$ for both growth and transport activity (Table 3). The plasmid could be recovered from all strains with the Uhp$^-$ phenotype and still conferred the Uhp$^+$ phenotype when introduced into a Δ*uhpT* strain. These results suggested that pDSE6 lacks an intact *uhpA* gene and is dependent on the presence of a functional *uhpA* on the chromosome for expression of the *uhpT* gene.

Effect of *uhp* plasmids on expression of chromosomal *uhpT*. To examine whether the different patterns of regulation exhibited by the Uhp$^+$ plasmids also affect expression of the chromosomal *uhpT* gene, each plasmid was introduced into a *recA uhpT-lac* operon fusion-bearing strain, RK5571. In this strain, G6P uptake is plasmid mediated and β-galactosidase production is a monitor of chromosomal *uhpT* expression. As above, all of the plasmids derived from

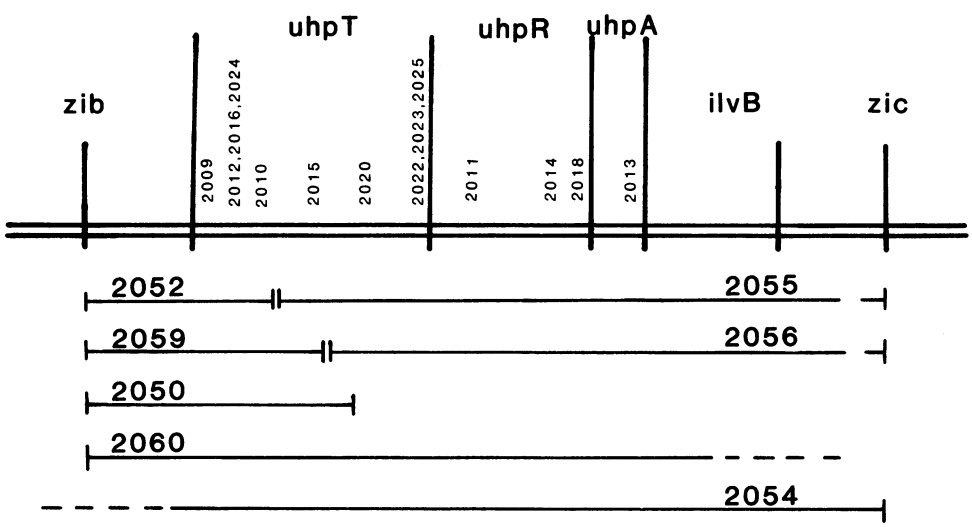

FIG. 3. Location of *uhp* point and deletion mutations used in this study. All mutations are in derivatives of strain RK4353, and their mapping was described in the preceding paper (13). The deletions were generated during excision of Tn*10* insertions designated here as *zib* and *zic*. The dashed lines indicate that all *uhp* material was removed by the deletion, but the endpoint outside *uhp* is not known.

TABLE 4. Effect of Uhp$^+$ plasmids on expression of chromosomal *uhpT-lac* fusion

Plasmid	G6P uptake (nmol min^{-1} μl of cell water^{-1})		β-Galactosidase (nmol of *o*-nitrophenol formed min^{-1} μl of cell water^{-1})	
	−G6P[a]	+G6P	−G6P	+G6P
None	0.7	0.8	0.3	39
pDSE1	16	18	7	10
pDSE2	17	15	20	17
pDSE3	15	17	8	10
pDSE4	15	19	9	11
pDSE5	19	14	17	8
pDSE6	0.9	15	0.1	5

[a] Designates whether cells were grown in the absence or presence of 300 μM G6P as inducer before harvest and assay.

pLC17-47 carrying the entire *uhp* region conferred high-level constitutive G6P uptake activity (Table 4). Expression of β-galactosidase was also constitutive, although lower than in the induced plasmid-free parent. It is possible that this decreased expression represents autogenous repression by the *uhpT* product. Both G6P transport and β-galactosidase activities were inducible in the strain carrying plasmid pDSE6. The low induced level of β-galactosidase in the strain could represent autogenous regulation combined with competition for *uhpA* product. This strain carries only a single copy of *uhpA*, and its product may be limiting in the face of multiple copies of *uhpT*. Whatever the explanation for the quantitative levels of expression in these strains, it was apparent that the constitutive expression from pLC17-47 was *trans* acting.

Expression and cloning of *uhpT-lac* operon fusion. To correlate regulatory behavior with the genetic content of cloned *uhp* regions, *uhpT-lac* operon fusions were cloned from specialized transducing phages of known *uhp* content (13, 20). To examine *uhp* regulation in the haploid state, six of the phages were transduced into a Δ*lac uhp*$^+$ recipient, with selection for growth on lactose in the presence of inducing levels of G6P. All lysogens exhibited G6P-inducible β-galactosidase production (Table 5). The induced level in several lysogens was only 30% of that in the haploid parent. However, the different levels of expression did not correlate in any obvious way with the genetic constitution of the input phage. Five of the lysogens were Uhp$^+$, with inducible transport activities. Strain RK5506 was constructed from a phage which lacks an intact fusion, but did carry some *uhpT* material adjacent to the original site of insertion of the phage. For this phage to integrate by homologous recombination to generate an inducible fusion, a nonfunctional (deleted) *uhpT* gene would result.

The fusion and flanking *uhp* regulatory materials from phages 3a (*uhpR*$^+$*A*$^+$) and 6j (*uhpR*$^+$) were cloned into the *Eco*RI site of plasmid pMLB524. This plasmid, constructed by M. Berman, was derived from pBR322 and contains the distal portion of *lacZ* from its *Eco*RI site, allowing transfer of a fusion from a transducing phage to the plasmid. Selection was for ampicillin resistance transformants that were blue on 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside + G6P plates. The plasmids from appropriate transformants were isolated and introduced into various recipients.

Expression of the cloned fusions. The level and regulation of β-galactosidase production from the two fusion-bearing plasmids were determined in several *recA uhp* recipients (Table 6). Plasmid pDSE8, from phage 3a (*uhpR*$^+$*A*$^+$), conferred very high levels of β-galactosidase in all recipients, including *uhp*$^+$ and a complete deletion of *uhp*. In most cases, the level of expression was increased two- to threefold by growth with inducing levels of G6P. The maximal induced specific activity was approximately 20 times higher than that of the haploid fusion,

TABLE 5. Uhp expression in Uhp$^+$ strains lysogenized with various λ(*uhpT-lac*) transducing phages[a]

Strain	λ	Genotype	G6P uptake activity (nmol min^{-1} μl of cell water^{-1})		β-Galactosidase (nmol of *o*-nitrophenol formed min^{-1} μl of cell water^{-1})	
			−G6P	+G6P	−G6P	+G6P
RK5571		T$^\Phi$R$^+$A$^+$	0.7	0.8	0.3	39
RK5503	1b	T$^\Phi$R$^+$A$^+$/Uhp$^+$	0.5	6	0.9	11
RK5504	3a	T$^\Phi$R$^+$A$^+$/Uhp$^+$	0.5	8	0.9	30
RK5505	3b	T$^\Phi$R$^+$A$^+$/Uhp$^+$	0.4	3	0.9	13
RK5506	6b	T$^\Phi$/Uhp$^+$	0.4	1	0.9	14
RK5507	6c	T$^\Phi$R′/Uhp$^+$	0.6	8	0.9	39
RK5508	6j	T$^\Phi$R$^+$/Uhp$^+$	0.4	8	0.9	29

[a] The strains were constructed by lysogenizing the *uhp*$^+$ strain RK4353 with the indicated fusion-bearing phage. All strains except RK5571 and RK5506 were Uhp$^+$ in phenotype.

TABLE 6. Expression of cloned *uhpT-lac* operon fusions in *uhp* recipients[a]

Recipient *uhp* genotype	Plasmid pDSE7			Plasmid pDSE8	
	Uhp response	β-Galactosidase activity		β-Galactosidase activity	
		−G6P	+G6P	−G6P	+G6P
uhp$^+$	+	2.6	281	390	638
Δ*uhpT2052*		1.8	677	247	733
Δ*uhpT2059*		0.9	196	201	792
Δ*uhpT2050*		0.7	195	183	603
Δ*uhpTRA2055*		1.1	1.4	448	900
Δ*uhpTRA2056*		1.3	1.8	378	653
Δ*uhpTRA2054*		1.5	1.9	436	497
Δ*uhpTRA2060*		1.3	1.8	377	823
uhpT2012	−	1	389		
uhpT2010	−	1	403		
uhpT2024	−	1	206		
uhpT2022	−	1	88		
uhpR2011	+	1	42		
uhpR2014	+	1	129		
uhpA2013	−	1	1		

[a] Recipient strains carried *recA56* and the indicated *uhp* deletion or point mutations. Cells were grown in minimal medium in the absence (−) and presence (+) of 300 μM G6P as inducer and were harvested in mid-log phase. Harvested cells were treated with toluene and freeze-thaw steps, and replicate β-galactosidase assays were made with 2 mM *o*-nitrophenyl-β-D-galactopyranoside. The Uhp response is measured by growth on fructose 6-phosphate as sole carbon source. β-Galactosidase activity is given in nanomoles of *o*-nitrophenol formed per minute per microliter of cell water.

reflecting the amplified gene dosage. The constitutive expression of the plasmid-borne *uhpT-lac* was *trans* dominant, as shown by the constitutive G6P uptake activity of the *uhpT*$^+$ strain carrying this plasmid (data not shown). Growth of cells carrying this plasmid was poor and depressed further by addition of 300 μM G6P, even though most strains were deficient in G6P uptake.

Expression from pDSE7, from phage 6j (*uhpR*$^+$), was similar to that of plasmid pLC40-33. This fusion-bearing plasmid conferred β-galactosidase activity that was induced about 100-fold by growth in the presence of G6P. The fully induced level in a *uhp*$^+$ strain was seven times higher than in the induced haploid strain lacking the plasmid. Production of β-galactosidase was strictly dependent on the presence of *uhpA*$^+$ on the chromosome, since point or deletion mutations which affected *uhpA* prevented *uhpT-lac* expression. Consistent with the genetic analysis of the parental phage, this plasmid was able to complement chromosomal *uhpR*, but not *uhpT* or *uhpA*, mutants for G6P transport activity.

Thus, the expression of the cloned *uhp-lac* fusion was essentially identical to that of the *uhp* region cloned from the Clarke-Carbon plasmids. Both studies point to the requirement for the *trans*-acting *uhpA* product for *uhpT* transcription, and that carriage of *uhpA* on multicopy plasmids results in partially constitutive *uhpT* expression.

DISCUSSION

Restriction mapping and subcloning of two plasmids from the Clarke-Carbon collection allowed localization of *uhp* to within a 7-kbp region. The location and extent of the coding regions for *uhp* products within this 7 kbp are the subjects of current investigations. However, the two Clarke-Carbon Uhp$^+$ plasmids differed in their regulatory behavior, which led to a tentative model for this regulatory system. Plasmid pLC17-47 carries *uhpT* and *ilvB* and, presumably, the *uhp* regulatory genes lying between them (13). This was corroborated by the Uhp$^+$ phenotype of strains carrying these plasmids and complete deletion of the chromosomal *uhp* locus. However, strains carrying these plasmids showed aberrant constitutive Uhp expression in all recipients. The same behavior was shown by the operon fusion-bearing plasmid, pDSE8, which also carries *uhpA*$^+$. Although the fusion was expressed constitutively when on the plasmid, it exhibited normal inducibility when in the haploid state.

In contrast, plasmids pDSE7, which lacks *uhpA*, and pLC40-33, whose insert terminates in the region where *uhpA* is expected to lie, showed inducible regulation of *uhpT*. This transcription was dependent on the presence of a

uhpA$^+$ allele on the chromosome, leading to the view that these two plasmids lack *uhpA* and that *uhpA*$^+$ functions in a *trans*-dominant manner. Thus, elevated dosage of *uhpA*$^+$ results in at least partially constitutive *uhpT* expression.

The one exception to this behavior was given by plasmid pDSE5, the *Pst*I subclone of pLC17-47. Its expression was constitutive in many recipients, but inducible in *uhp*$^+$ and certain *uhp* mutants. The basis for this behavior is not yet understood. Since hexose phosphates are inhibitory for strains carrying Uhp$^+$ plasmids, it is possible that this plasmid acquired a mutation which affects the regulatory response.

It was striking that the maximal G6P uptake activity exhibited by strains bearing Uhp$^+$ plasmids was not markedly elevated relative to the haploid level. A lack of proportionality to gene dosage has been observed with other cloned transport systems (16, 21). Since the level of β-galactosidase from the cloned operon fusions greatly elevated over the haploid fusion, the potential for *uhpT* transcription does not seem to be the limiting factor. It remains to be seen whether the reduced transport activity results from autogenous regulation of *uhpT* transcription or translation, or limitation of the insertion of the transport protein into the membrane, or the existence of some control over the activity of the transport system which prevents elevated levels of substrate accumulation or excessive rates of proton entry.

The following model can account for the major experimental observations and points out future lines of study. The model invokes three regulatory genes in addition to *uhpT*, the gene(s) for the transport system. The regulatory genes are termed C, R, and A. The *uhpA* product is a positive activator of *uhpT* transcription. In the uninduced state, the A product may be complexed to the C regulatory product and be unavailable for transcription activation. A third regulatory component is located in the cytoplasmic membrane. Binding of G6P to an external site of that transmembrane protein could result in an altered conformation of an internal domain. Exposure of this internal domain could trigger release of the UhpA product from its inactive complex by interaction with the C component. Predictions of this model are that mutational loss of either *uhpA* or the gene for the membrane component results in the Uhp$^-$ phenotype. Reversion of *uhpA* mutants should be a rare event, requiring either restoration of UhpA function or an alteration of the *uhpT* promoter to a sequence no longer requiring activation by the A product. On the other hand, loss of the membrane component could be compensated by a second-site mutation that eliminates the C product. Mutants lacking the C protein should exhibit constitutive *uhp* expression, whether the membrane component is active or not. On the basis of the observed reversion frequencies and patterns (13), it is tempting to predict that the *uhpR* product is the membrane component. Consistent with this view is the finding that *uhpR*::Tn*10* mutants reverted readily to constitutive expression by second mutations in the *uhp* region, some of which appeared to lie to the left of *uhpR*, opposite from *uhpA*. It is possible that these second mutations leading to constitutivity define the C component, but fine-structure mapping and complementation analysis are necessary before these conclusions can be drawn.

The high uninduced level of *uhp* expression in cells carrying cloned *uhpA* is not fully explicable. The possibility that *uhpA* represents an operator site whose elevated amount titrates repressor is not tenable, because *uhpA* function can be supplied in a *trans* situation. Other possibilities include the absence of the gene for the C product, which inactivates the A product, from the plasmid, so that the levels of C product are not elevated. Also, perhaps the overproduction of both interacting proteins results in the presence of sufficient uncomplexed *uhpA* product to allow a high uninduced level of transcription. Transcription of *uhpT* from the plasmid with the cloned operon fusion was not fully constitutive, but subject to two- to threefold further induction, which could indicate that not all of the *uhpA* product is able to activate transcription without inducer.

This tentative model is rather complex and requires considerable further testing. The complexity and involvement of both positive and negative elements might be expected from the need to transmit a regulatory signal from the exterior of the cell to the genome. Possibly relevant is the finding of multiple positive regulatory genes controlling the *pho* operon (23); however, some of these elements may be involved in control of the synthesis of other regulatory components (22).

ACKNOWLEDGMENTS

We gratefully acknowledge the technical contributions of S. Wood and Y. Stacey and the generous provision of materials and methods from M. Smith. We thank B. Bachmann and T. Silhavy for providing bacterial strains.

This work was supported by research grant PCM 78-26711 from the National Science Foundation. D.M.S.-E. was supported by Public Health Service predoctoral training grant GM07082 and R.J.K. was the recipient of Public Health Service Research Career Development Award GM00019, both from the National Institute of General Medical Sciences.

LITERATURE CITED

1. **Bachmann, B. J., and K. B. Low.** 1980. Linkage map of *Escherichia coli* K-12, edition 6. Microbiol. Rev. **44**:1–56.
2. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1524.

3. **Clarke, L., and J. Carbon.** 1975. Biochemical construction and selection of hybrid plasmids containing specific segments of the *Escherichia coli* genome. Proc. Natl. Acad. Sci. U.S.A. **72**:4361–4365.
4. **Clarke, L., and J. Carbon.** 1976. A colony bank containing synthetic Col E1 hybrid plasmids representative of the entire *E. coli* genome. Cell **9**:91–99.
5. **Csonka, L. N., and A. J. Clark.** 1980. Construction of an Hfr strain useful for transferring recA mutations between *Escherichia coli* strains. J. Bacteriol. **143**:529–530.
6. **Dagert, M., and S. D. Ehrlich.** 1979. Prolonged incubation in calcium chloride improves the competence of *Escherichia coli* cells. Gene **6**:23–28.
7. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
8. **Dills, S. S., A. Apperson, M. R. Schmidt, and M. H. Saier, Jr.** 1980. Carbohydrate transport in bacteria. Microbiol. Rev. **44**:385–418.
9. **Essenberg, R. C., and H. L. Kornberg.** 1977. Location of the gene specifying hexose phosphate transport (uhp) on the chromosome of *Escherichia coli*. J. Gen. Microbiol. **99**:157–169.
10. **Goldenbaum, P. E., and K. S. Farmer.** 1980. *uhp*-directed, glucose-6-phosphate membrane receptor in *Escherichia coli*. J. Bacteriol. **142**:347–349.
11. **Greener, A., and C. W. Hill.** 1980. Identification of a novel genetic element in *Escherichia coli* K-12. J. Bacteriol. **144**:312–321.
12. **Kadner, R. J.** 1973. Genetic control of the transport of hexose phosphates in *Escherichia coli*: mapping of the *uhp* locus. J. Bacteriol. **116**:764–770.
13. **Kadner, R. J., and D. M. Shattuck-Eidens.** 1983. Genetic control of the hexose phosphate transport system of *Escherichia coli*: mapping of deletion and insertion mutations in the *uhp* region. J. Bacteriol. **155**:1052–1061.
14. **Kadner, R. J., and H. H. Winkler.** 1973. Isolation and characterization of mutations affecting the transport of hexose phosphates in *Escherichia coli*. J. Bacteriol. **113**:895–900.
15. **Leathers, T. D., J. Noti, and H. E. Umbarger.** 1979. Physical characterization of *ilv-lac* fusions. J. Bacteriol. **140**:251–260.
16. **Lee, C. A., G. R. Jacobson, and M. H. Saier.** 1981. Plasmid directed synthesis of enzymes required for D-mannitol transport and utilization in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **78**:7336–7340.
17. **Maloy, S. R., and W. D. Nunn.** 1981. Selection for loss of tetracycline resistance by *Escherichia coli*. J. Bacteriol. **145**:1110–1111.
18. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **Parker, R. C.** 1980. Conversion of circular DNA to linear strands for mapping. Methods Enzymol. **65**:415–426.
20. **Shattuck-Eidens, D. M., and R. J. Kadner.** 1981. Exogenous induction of the *Escherichia coli* hexose phosphate transport system defined by *uhp-lac* operon fusions. J. Bacteriol. **148**:203–209.
21. **Teather, R. M., B. Muller-Hill, U. Abrutsch, G. Aichele, and P. Overath.** 1978. Amplification of the lactose carrier protein in *Escherichia coli* using a plasmid vector. Mol. Gen. Genet. **159**:239–248.
22. **Tommassen, J., P. de Geus, B. Lugtenberg, J. Hackett, and P. Reeves.** 1982. Regulation of the *pho* regulon of *Escherichia coli* K-12. Cloning of the regulatory genes *phoB* and *phoR* and identification of their gene products. J. Mol. Biol. **157**:265–274.
23. **Wanner, B. L., and P. Latterell.** 1980. Mutants affected in alkaline phosphatase expression: evidence for multiple positive regulators of the phosphate regulon in *Escherichia coli*. Genetics **96**:353–366.
24. **Yamada, M., Y. Ebina, T. Miyata, T. Nakazawa, and A. Nakazawa.** 1982. Nucleotide sequence of the structural gene for colicin E1 and predicted structure of the protein. Proc. Natl. Acad. Sci. U.S.A. **79**:2827–2831.