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Role of *uhp* Genes in Expression of the *Escherichia coli* Sugar-Phosphate Transport System

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The *uhpABCT* locus of *Escherichia coli* is responsible for expression of the sugar-phosphate transport system and its induction by external glucose 6-phosphate. Expression of *uhpT-lacZ* fusions depended on the function of *uhpA*, *uhpB*, and *uhpC* but not of *uhpT*. A plasmid carrying only *uhpT* conferred transport activity in a host strain deleted for the *uhp* region. Thus, *uhpT* encodes the polypeptide required for transport function, and the other three *uhp* genes regulate *uhpT* transcription. The presence of *uhpA* at elevated copy number resulted in a substantial increase in *uhpT* expression. This elevated expression was only about 50% of the level seen in induced haploid cells, and no further increase occurred after addition of inducer. Activation by multicopy *uhpA* was not affected by the status of *uhpC* but was decreased in the absence of *uhpB*, suggesting a role for UhpB in directly activating UhpA. Transcription of *uhpA*, monitored by expression of a *uhpA-lacZ* fusion, was not affected by either inducer or the presence of the wild-type *uhpA* allele. The presence of multiple copies of the *uhpT* promoter region reduced *uhpT* expression in strains with *uhpA* in single copy number but not in those with multiple copies, consistent with competition for the activator. Amino acid sequence comparisons showed that UhpA was homologous to a family of bacterial regulatory proteins, some of which act as transcriptional activators (OmpR, PhoB, NtrC, and DctD). The C-terminal portion of UhpB displayed matches to the corresponding portions of another family of proteins (EnvZ, PhoMR, NtrB, and DctB) that participate in regulation of gene expression in response to environmental factors.

Cells of *Escherichia coli* can accumulate various phosphorylated carbohydrates by means of the *uhpT*-encoded sugar-phosphate transport system. Its synthesis is controlled by a transmembrane signaling process termed exogenous induction. Induction occurs when glucose 6-phosphate (G6P) (or 2-deoxyglucose 6-phosphate) is present in the growth medium but not when G6P is formed inside the cell by transport and phosphorylation of glucose (3, 28). Studies with *uhpT-lac* fusions demonstrated that production of the transport system was regulated at the transcriptional level and that UhpT transport activity was not needed for *uhpT* induction (23). Regulation by external G6P avoids the constant expression that would otherwise occur in response to the presence of substantial levels of internal G6P under most growth conditions. The Uhp transporter has been shown to operate as a sugar phosphate/phosphate antiporter (1). Its unregulated synthesis could be detrimental to the cell by providing a route for loss of many phosphorylated metabolites as well as inorganic phosphate.

Genetic studies have provided information about the mechanism of exogenous induction. All mutations that specifically affect the Uhp transport system lie in the *uhp* locus at min 82.1 on the genetic map (2, 10). The nucleotide sequence of this region has been determined, and transposon insertions have been isolated throughout the cloned segment (7, 26). Comparison of the reading frames deduced from the sequence and the growth phenotypes caused by the transposon insertions revealed the existence of four *uhp* genes. They are transcribed in the same direction and arranged in the order *uhpABCT*. The polypeptide products of the four genes have been detected in maxicells and in a phage T7 polymerase-promoter expression system (26).

Insertion mutations in any of the four *uhp* genes on the chromosome resulted in a Uhp$^-$ phenotype. However, neither *uhpB* nor *uhpC* was needed for growth on G6P when *uhpA*$^+$ was present on a multicopy plasmid. Based on these results and the properties of the predicted Uhp polypeptides, we proposed that UhpA is a positive activator of *uhpT* transcription and that UhpB and UhpC are membrane proteins that detect the presence of external G6P and regulate the ability of UhpA to stimulate *uhpT* transcription. This model of the role of the *uhp* genes was based on complementation tests measuring the growth response of strains carrying chromosomal *uhp* mutations and plasmids with various segments of the *uhp* region. This paper presents quantitative measurements of *uhpT* transcription and function in these strains. Transcriptional activity was estimated by using *uhpT-lacZ* fusions; UhpT function was assayed by measuring G6P transport activity. These studies provide new information about the function of the *uhp* regulatory genes and about the aberrant expression of *uhpT* that occurs in response to overproduction of UhpA.

Additional information on the possible role of the products of the *uhp* regulatory genes came from detection of amino acid sequence homology to other bacterial proteins. Regulatory proteins that contain conserved sequence motifs are being found in increasing numbers. Often, it is seen that transcriptional gene control in response to external signals is mediated by members of these related families. For example, transcriptional regulation of the outer membrane porin proteins and the modulation of their levels in response to the medium's osmolarity depends on the *ompR* and *envZ* products (9). The *phoB*, *phoM*, and *phoR* products allow derepression of the genes of the *pho* regulon in response to phosphate limitation (25). The *ntrC* and *ntrB* products control numerous genes subject to regulation by the nitrogen supply (4, 18). Other pairs of transcription regulatory proteins include *sfrA* and *cpxA* (F pilin synthesis [5]), *virG* and

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TABLE 1. Bacteria and plasmids used

Strain or plasmid	Description	Reference or source
E. coli		
RK4353	*araD139* Δ(*argF-lac*)*U169 relA1 rpsL150 thi gyrA219 non*	23
RK4991	RK 4353 Δ*uhpT2050 recA56*	24
RK5000	RK5171 *pyrE*$^+$ Δ(*ilvB-uhpABCT'*)*2056 recA56*	24
RK5171	RK4353 *metE70 pyrE40*	26
RK5173	RK5171 *metE*$^+$	
RK5968	RK5173 *uhpT*::λp1(209)*2136 recA56*	
RK5969	RK5173 *uhpA*::λp1(209)*2134 recA56*	
RK7240	RK5171 *pyrE*$^+$ *uhpT2111*::miniKm	
RK7242	RK5171 *pyrE*$^+$ *uhpB2113*::miniKm	
RK7243	RK5171 *pyrE*$^+$ *uhpC2114*::miniKm	
RK7244	RK5171 *pyrE*$^+$ *uhpB2115*::Tn*1000*	
RK7245	RK5171 *pyrE*$^+$ *uhpC2116*::Tn*1000*	
RK7246	RK5171 *pyrE*$^+$ *uhpC2117*::Tn*1000*	
RK7247	RK5171 *pyrE*$^+$ *uhpB2118*::Tn*1000*	
RK7249	RK5171 *pyrE*$^+$ *uhpC2120*::Tn*1000*	
RK7251	RK5171 *pyrE*$^+$ *uhpT2122*::Tn*1000*	
RK7252	RK5171 *pyrE*$^+$ *uhpA2123*::miniKm	
RK7253	RK5171 *pyrE*$^+$ *uhpA2125*::miniKm	
RK7254	RK5171 *pyrE*$^+$ *uhpA2125*::miniKm	
RK7621	RK5171 *pyrE*$^+$ *uhpA2123*::miniKm *recA56*	
RK7622	RK5171 *pyrE*$^+$ *uhpB2113*::miniKm *recA56*	
RK7623	RK5171 *pyrE*$^+$ *uhpC2114*::miniKm *recA56*	
RK7624	RK5171 *pyrE*$^+$ *uhpT2111*::miniKm *recA56*	
Plasmids		
pAL2	pFR97 with 912-bp *Sau*3A fragment in *Bam*HI site; carries φ(*uhpT-lacZ*); Ap^r	
pDSE7	pMLB524 with *Eco*RI fragment from λ*uhp-lac* transducing phage 6j; carries *uhpT-lac* operon fusion and *uhpB*$^+$ *uhpC*$^+$; Ap^r	24
pGEM-4	Ap^r	
pRJK10	pBR322 with 6.5-kb *Hin*dIII-*Bam*HI fragment; Ap^r	
pLAW007	pGEM-4 with 2-kb *Cla*I fragment; Ap^r	26
pLAW014	pGEM-4 with *Hin*dIII-*Bam*HI deleted for 2-kb *Cla*I fragment; Ap^r	26
pLAW015	pGEM-4 with 2.8-kb *Eco*RV-4–*Eco*RV-5 fragment; Ap^r	26
pLAW024	pGEM-4 with 1.8-kb *Sph*I-*Bam*HI fragment; Ap^r	26
pLAW025	pGEM-4 with 1.6-kb *Cla*I-2–*Bgl*II fragment; Ap^r	26
pLAW027	pGEM-4 with 3.2-kb *Cla*I-2–*Bam*HI fragment; Ap^r	26
pLAW028	pGEM-4 with 5.3-kb *Hin*dIII-*Bgl*II fragment; Ap^r	26
pLAW030	pRJK10 deleted for 1.6-kb *Hpa*I fragment; Ap^r	26
p10-j1	pRJK10 *uhpA2123*::miniKm	26
p10-j2	pRJK10 *uhpB2113*::miniKm	26
p10-3b	pRJK10 *uhpC2114*::miniKm	26
p10-e1	pRJK10 *uhpT2111*::miniKm	26

virA (virulence traits in *Agrobacterium tumefaciens* [19, 27]), and *dctD* and *dctB* (dicarboxylic acid transport system in *Rhizobium leguminosarum* [20]).

Members of "two-component regulatory systems" share sequence motifs that might indicate a common origin and may be areas of interaction between the two protein components (18). The first members of each pair contain conserved sequences within the first 120 amino acids; subsets of these proteins display additional homologies within the carboxyl end. Many members of this set act as transcriptional activators. However, this family also includes the products of *cheY* and *cheB*, which participate in the chemotactic response (15). The second protein of each pair contains conserved sequences in its C-terminal 200 amino acids. Their amino-terminal ends are not conserved but often contain potential transmembrane regions. Members of this set may function as "sensors" of external signals. The existence of these related pairs of regulatory proteins raises the possibility of a common mode of regulation for these processes. In this paper, the relationship of UhpA and UhpB to these regulatory pairs is described.

MATERIALS AND METHODS

Bacterial strains and plasmids. Most of the strains of *Escherichia coli* used in this study were described previously (26) and are listed in Table 1. The construction of plasmids carrying portions of the *uhp* region or *uhp* transposon insertions was described previously (26). Plasmid pRJK10 carries the entire *uhp* region as a 6.5-kilobase (kb) *Hin*dIII-*Bam*HI fragment in the vector pBR322. The transposon insertions were isolated in the same fragment in the vector pACYC184 or pBR322. The *uhp* subclones were constructed in pGEM vectors. Figure 1 presents the restriction map of the *uhp* region and shows the locations of the *uhp* genes, the positions of the transposon insertions, and the extent of bacterial DNA carried on the plasmids used in this study. The isolation of chromosomal *uhp* transposon insertions and the transfer of insertions from plasmids onto the chromosome were described previously (24, 26). The transposon designated miniKm is Tn*10*Δ*16*Δ*17*Km.

Isolation of *uhp*::Mu dII mutants. The *uhp* gene fusions used in this study were obtained by in vivo transposition of

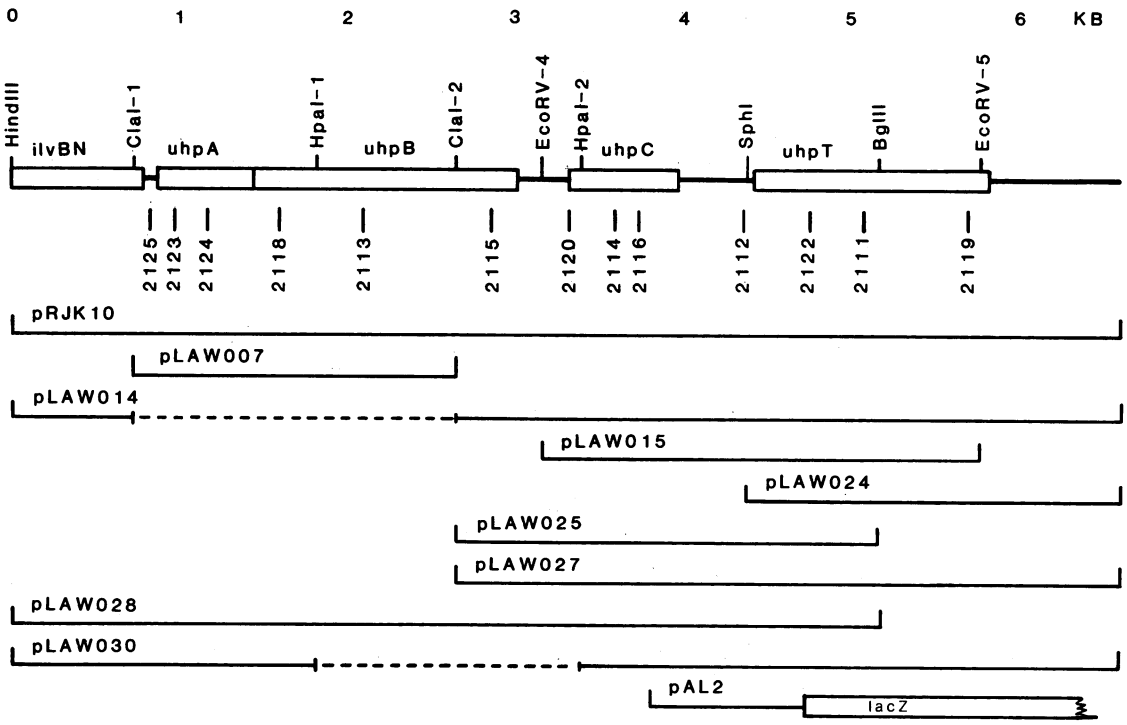

FIG. 1. Restriction map of the *uhp* region, location of transposon insertions, and material carried on *uhp* plasmids used in this study. The transposon insertions are indicated by their allele numbers below the restriction map. Sequences deleted from plasmid pRJK10 to form plasmids pLAW014 and pLAW030 are indicated by dashed lines. All genes shown here are transcribed from left to right.

the Mu dII*301*(Ap *lac*) fusion-generating phage (kindly provided by P. J. Bassford), with selection for resistance to fosfomycin. The fusions arising from single insertions of the phage were stabilized by replacement of Mu sequences with λp1(*209*) by the method of Komeda and Iino (11). The target gene disrupted by the insertion was identified by complementation tests with various *uhp* plasmids. Only fusions to *uhpT* displayed induction of β-galactosidase by G6P. Different *uhpT-lacZ* gene fusions exhibited a fourfold range in induced β-galactosidase levels. Presumably, the activity or stability of the membrane-bound UhpT-LacZ hybrid proteins varies depending on the location of the fusion joint.

Plasmids bearing *uhpT-lacZ* fusions. Plasmid pAL2 was constructed by ligating *Sau*3A-digested DNA from plasmid pRJK10 into the *Bam*HI site of plasmid pFR97 (22). Selection was for ampicillin-resistant transformants in strain RK4991 that displayed G6P-inducible β-galactosidase activity. The resulting plasmid, pAL2, was shown to contain the 912-base-pair (bp) fragment that extends from the distal portion of *uhpC* to codon 103 of *uhpT*, so that the *uhpT* sequence is in phase with the *lacZ* gene of the vector. Plasmid pDSE7 has been described previously (24).

Media and growth conditions. Minimal medium was medium A (16) supplemented with 0.5% glycerol, thiamine (1 μg/ml), and required amino acids (100 μg/ml). For enzyme or transport assays, this medium was supplemented with 0.5% casein hydrolysate. Plasmid-bearing strains were routinely grown in the presence of ampicillin (25 μg/ml) or chloramphenicol (25 μg/ml), as appropriate. Cells were exposed to 0.35 mM G6P for at least three doublings before harvest and assay. All transport and enzyme assays were performed in duplicate and with several isolates of each strain.

Enzyme and transport assays. β-Galactosidase was assayed as described previously (24) by measuring the continuous increase in absorbance at 420 nm of 2 mM *o*-nitrophenyl-β-D-galactopyranoside. Whole cells were permeabilized with $CHCl_3$–sodium dodecyl sulfate (16). Enzyme activity is expressed as nanomoles of *o*-nitrophenol formed per minute per microliter of cell water (equivalent to 0.27 mg of protein).

Cells were washed once and suspended to a density of 4×10^8/ml in growth medium containing chloramphenicol (100 μg/ml). A portion of the cell suspension was incubated at 37°C for 2 min before the assay was initiated by the addition of [^{14}C]G6P (200 μM and 0.1 μCi/ml, final values). Samples were collected at 0.3 and 1.3 min on membrane filters (Millipore Co., 0.45-μm pore size) and washed with 5 ml of medium A. Transport activity is expressed as nanomoles of G6P retained by the cells per minute per microliter of cell water, with correction for radioactivity retained on the filter in the absence of cells.

Homology search and sequence alignment. Amino acid sequences were compared by using the computer program FASTP of Lipman and Pearson (13). The statistical significance of sequence matches was evaluated by comparison with random sequences of the same amino acid composition with the program RDF (13). Multiple sequences were aligned visually by adjusting the pairs of computer alignments to achieve the best overall arrangement.

RESULTS

Three *uhp* regulatory genes are required for *uhpT* transcription. *E. coli* mutants with transposon insertions in any of the four *uhp* genes were unable to grow on G6P or fructose 6-phosphate (26). As will be shown later, these strains lost G6P transport activity. The expression of *uhpT-lac* fusions offered a way to determine which of the *uhp* products were

TABLE 2. Expression of plasmid-borne *uhpT-lacZ* fusions in *uhp* host strains

Host strain	Relevant genotype	β-Galactosidase activity[a] (U)			
		Plasmid pAL2		Plasmid pDSE7	
		No G6P	With G6P	No G6P	With G6P
RK5173	*uhp*$^+$	ND[b]	ND	3	249
RK4991	Δ*uhpT2050*	2	322	ND	ND
RK5000	Δ*uhpABCT'2056*	1	2	3	7
RK7254	*uhpA2125*::miniKm	3	24	2	157
RK7252	*uhpA2123*::miniKm	2	1	3	2
RK7253	*uhpA2124*::miniKm	5	5	1	1
RK7247	*uhpB2118*::Tn*1000*	3	3	3	16
RK7242	*uhpB2113*::miniKm			1	7
RK7244	*uhpB2115*::Tn*1000*	7	6	3	43
RK7249	*uhpC2120*::Tn*1000*	5	3		
RK7243	*uhpC2114*::miniKm	2	3	2	50
RK7245	*uhpC2116*::Tn*1000*	2	3	2	41
RK7246	*uhpC2117*::Tn*1000*	2	1	1	52
RK7251	*uhpT2122*::Tn*1000*	1	56	ND	ND
RK7240	*uhpT2111*::miniKm	3	78	3	450

[a] Units of enzyme activity are defined in Materials and Methods. Cells were grown for at least three doublings in medium A with 1% glycerol, 0.5% casein hydrolysate, and ampicillin (25 μg/ml), with or without 0.35 mM G6P as indicated.
[b] ND, Not determined.

involved in regulation of *uhpT* transcription and which were necessary for formation of the functional transport system. To test this, *uhpT-lacZ* fusions were introduced on plasmids into host strains with transposon insertions in the individual chromosomal *uhp* genes. The levels of β-galactosidase were measured after growth in the absence or presence of 0.35 mM G6P.

The *uhpT-lacZ* fusion plasmid pAL2 carries a 912-bp *Sau*3A fragment that spans the distal portion of *uhpC*, the upstream regulatory region of *uhpT*, and the first 309 bp of *uhpT* in frame with *lacZ*. Plasmid pAL2 did not restore utilization of G6P to any *uhp* mutant and thus carries no intact *uhp* gene. Synthesis of β-galactosidase was induced about 100-fold by G6P in a *uhp*$^+$ host and in *uhpABC*$^+$ hosts with either deletions or transposon insertions in *uhpT* (Table 2). Production of β-galactosidase was eliminated by transposon insertions in any of the other three *uhp* genes. Substantial activity was expressed by the *uhpA2125* mutant. This insertion lay just outside the *uhpA* coding region and appeared to reduce production of the normal *uhpA* product. Thus, the transport function encoded by *uhpT* is not necessary for regulation of its own synthesis, but the other three *uhp* genes are required for *uhpT* transcription.

Plasmid pDSE7 carries a *uhpT-lac* operon fusion and about 3 kb of DNA upstream of *uhpT*, containing *uhpB* and *uhpC* but only the distal end of *uhpA* (24). The exact endpoint within the *uhp* region has not been determined, but the *Eco*RV site in *uhpA* is absent. This plasmid complemented *uhpB* and *uhpC* but not *uhpA* mutations for growth. Synthesis of β-galactosidase was induced about 100-fold by G6P in wild-type and *uhpT* strains carrying this plasmid. No expression was seen in *uhpA* hosts, but in contrast to pAL2, inducible expression occurred in *uhpB* and *uhpC* hosts. The G6P-induced enzyme levels were lower in these strains than in the wild-type strain, perhaps because the absence of the *uhpA* promoter region results in decreased expression of the *uhpBC* genes. These results showed that *uhpB* and *uhpC* function could be provided in *trans*.

Effect of *uhp* plasmids on *uhpT* transcription. The presence of multicopy *uhp* plasmids results in altered regulation of *uhpT* transcription. We showed previously that elevated gene dosage of *uhpA*$^+$ conferred high-level constitutive expression of *uhpT* (24, 26). To examine the effect of increased copy number of the *uhp* genes, various plasmids (Fig. 1) were introduced into a *recA56* strain harboring a chromosomal *uhpT-lacZ* fusion. The production of β-galactosidase provided an assay for *uhpT* transcription, and G6P transport activity gave a measure of the expression of *uhpT*$^+$ genes present on some of the plasmids.

The Φ(*uhpT-lacZ*)*2136* fusion strain RK5968 exhibited G6P-inducible β-galactosidase synthesis but possessed no significant G6P transport activity (Table 3) as a result of the disruption of the chromosomal *uhpT* allele by fusion formation. The other chromosomal *uhp* genes were intact. The degree of induction (25- to 50-fold) was somewhat lower than that for the plasmid-borne fusions. This difference is probably not significant owing to the uncertainty in the measurement of the low levels of activity in uninduced cells. The induced level of β-galactosidase in the chromosomal fusion strain was about 10% of that in the strains carrying a *uhpT-lacZ* plasmid (Table 2), reflecting the lower copy number of the chromosomal fusion.

The presence of any plasmid that carries *uhpA* [pRJK10, pLAW007, pLAW028, pLAW030, pRJK10-j2 (*uhpB2113*::miniKm), pRJK10-3b (*uhpC2114*::miniKm), or pRJK10-el (*uhpT2111*::miniKm)] conferred greatly elevated uninduced levels of β-galactosidase (Table 3). Plasmids that did not carry *uhpA* did not engender this elevated basal expression. The presence of *uhpA* on the plasmid was the sole feature that correlated with this aberrant regulatory behavior. The levels of β-galactosidase in strains with multicopy *uhpA* were usually ca. 50% of that found in induced cells of the same strain with the vector alone. Surprisingly, no further increase in β-galactosidase activity occurred when the *uhpA*$^+$-plasmid-bearing cells were grown with G6P. A moderate decrease occurred upon addition of inducer to most strains. This lack of inducibility was not solely the result of catabolite repression, since it occurred even in strain RK5968(pLAW007) with no detectable transport or metabolism of G6P. The only strain in which G6P elicited a substantial increase in β-galactosidase activity over the uninduced level was that harboring plasmid p10-e1 with a transposon in *uhpT*.

Similarly, G6P transport activities in strains with both

TABLE 3. Expression of chromosomal *uhpT-lac* and *uhpA-lac* gene fusions and G6P transport activity in strains carrying *uhp* plasmids

Strain and plasmid	Multicopy[a]			Activity (U)			
				β-Galactosidase[b]		G6P transport[c]	
	A	P_T	T	No G6P	With G6P	No G6P	With G6P
RK5968 [ϕ(*uhpT-lacZ*)][d]							
None	−	−	−	0.5	27.2	ND[e]	ND
pGEM3	−	−	−	0.9	26.0	0.2	0.3
pRJK10	+	+	+	15.2	11.8	19.7	22.1
pLAW007	+	−	−	18.5	13.9	1.2	0.2
pLAW014	−	+	+	1.1	0.8	1.3	36.9
pLAW015	−	+	−	0.7	1.8	0.2	0.1
pLAW024	−	−	+	1.0	25.4	2.7	2.4
pLAW025	−	+	−	0.1	2.1	1.3	0.2
pLAW027	−	+	+	0.1	1.4	2.8	17.9
pLAW028	+	+	−	13.8	15.9	0.1	0.1
pLAW030	+	+	+	15.1	10.3	25.6	21.5
p10-j1 (*uhpA*)	−	+	+	0.1	2.1	0.3	13.6
p10-j2 (*uhpB*)	+	+	+	9.7	7.2	19.4	19.7
p10-3b (*uhpC*)	+	+	+	11.5	8.7	18.9	18.5
p10-e1 (*uhpT*)	+	+	−	10.5	35.4	1.1	0.1
RK5969 [Φ(*uhpA-lacZ*)][d]							
None				3.7	3.2	0.2	0.2
pRJK10	+	+	+	2.6	2.7	15.9	19.0
pLAW007	+	−	−	3.7	3.2	10.8	10.5
RK5173 (*uhp*$^+$)						0.6	11.1

[a] A, P_T, and T, Presence on plasmid of *uhpA*$^+$, the *uhpT* upstream regulatory region, and *uhpT*$^+$, respectively.
[b] Units of β-galactosidase are defined in Materials and Methods. Cells were grown as described in Table 2, footnote *a*.
[c] Cells were harvested and assayed for G6P transport activity as described in Materials and Methods.
[d] Fusion-bearing strains were RecA$^-$.
[e] ND, Not determined.

uhpT and *uhpA* on the plasmid were elevated and not increased further upon growth with inducer. The levels of transport activity were only two to three times higher than that of a haploid strain.

Plasmids that did not carry intact *uhpA* conferred a different regulatory behavior in RK5968. Transport activities conferred by plasmids pLAW014, pLAW027, and p10-j1 were subject to induction by G6P. The induced transport levels were comparable to those elicited by plasmids that carried both *uhpA* and *uhpT*, which indicates that the chromosomal *uhpA* product was fully functional. β-Galactosidase synthesis was also inducible by G6P, but the induced levels were very low, less than 10% of that seen in the strain with the pGEM vector. The only plasmid that did not cause this marked reduction in G6P-induced β-galactosidase activity was pLAW024, which lacks both *uhpA* and the *uhpT* regulatory region. It carries the complete *uhpT* coding region but not its promoter, which lies upstream of the *Sph*I site. Strains carrying this plasmid exhibited low levels of transport activity that were not affected by inducer or, as shown later, any other *uhp* gene. Transcription of *uhpT* on this plasmid appears to be driven from weak vector promoters.

It is thus likely that the decreased *uhpT-lacZ* expression seen in the presence of the other plasmids that did not carry *uhpA* was the result of competition for the limiting amounts of the UhpA activator protein between the *uhpT* regulatory region on the multicopy plasmids and that of the chromosomal fusion. Plasmid pAL2 engendered an equivalent decrease in transport activity when it was present in a *uhp*$^+$ host (data not shown). The maximal level of expression when these plasmids were present was roughly 1/20 of that seen with plasmids carrying *uhpA*, consistent with a stoichiometric titration of the activator. Thus, elevated levels of *uhpA* and of the region upstream of *uhpT* had profound effects on *uhpT* transcription.

Effect of *uhp* plasmids on G6P transport activity. The effect of the presence of the other *uhp* genes on *uhpT* expression and its response to excess *uhpA* could not be determined from the previous experiments owing to the presence on the chromosome of the wild-type allele of each gene. Hence, strains were constructed that carried portions of the *uhp* region on plasmids and transposon insertions in individual chromosomal *uhp* genes. Expression of *uhpT* was determined from measurements of G6P transport activity. All strains were *recA* to prevent recombination of the plasmid with the chromosomal *uhp* region. Uhp regulatory behavior in *recA*$^+$ strains was inducible as a result of reduced plasmid copy number (data not shown).

Plasmid pRJK10, carrying the entire *uhp* region, conferred high and constitutive levels of transport activity in all hosts (Table 4). As was seen with the strains described in Table 3, transport activities were only about twice that of the induced wild-type strain.

As expected, strains with an insertion in any *uhp* gene and carrying the vector pGEM4 lacked transport activity. As suggested from growth experiments (26) and consistent with the expression of *uhpT-lac* fusions, at least a single copy of both *uhpA* and *uhpT* had to be present for expression of transport activity (Table 4). Neither *uhpB* nor *uhpC* was required if *uhpA* was present in multiple copies. However, their absence did affect transport activity in the presence of either excess or haploid copies of *uhpA*.

Consider first the behavior of strains bearing plasmids which did not carry *uhpA* (pLAW014, pLAW024, and p10-j1). With plasmids pLAW014 and p10-j1, transport function was dependent on the presence of chromosomal *uhpA*. In

TABLE 4. G6P transport activity in strains carrying *uhp* plasmids and chromosomal *uhp* mutations

Plasmid	G6P transport activity[a] (U)							
	RK7621 (*uhpA*)		RK7622 (*uhpB*)		RK7623 (*uhpC*)		RK7624 (*uhpT*)	
	No G6P	With G6P	No G6P	With G6P	No G6P	with G6P	No G6P	With G6P
pGEM4	0.1	0.1	0.1	0.1	0.1	0.1	0.3	0.1
pRJK10	21.4	21.6	21.8	21.6	20.2	22.9	19.2	22.4
pLAW007	11.4	14.7	9.1	8.9	4.4	5.9	0.7	0.3
pLAW014	0.6	0.8	2.8	2.2	1.3	9.0	0.9	21.1
pLAW024	1.4	1.7	3.0	2.3	3.1	2.2	2.8	3.1
pLAW028	4.4	4.9	4.2	4.2	2.8	3.4	0.2	0.1
pLAW030	9.2	14.0	5.7	10.7	21.4	26.0	24.7	24.1
p10-j1	0.1	0.3	0.6	14.2	0.7	14.0	0.8	16.8
p10-j2	ND[b]	ND	5.2	7.9	18.4	19.7	17.2	19.0
p10-3b	ND	ND	12.2	10.9	17.9	18.0	20.9	20.1
p10-e1	8.1	11.9	6.8	11.3	6.4	8.8	0.2	0.5

[a] The *uhp* genotypes of the host strains were: RK7621, *uhpA2123*::miniKm; RK7622, *uhpB2113*::miniKm; RK7623, *uhpC2114*::miniKm; *uhpT2111*::miniKm. All strains were *recA*. When used, G6P was present at 0.35 mM.

[b] ND, Not determined.

most *uhpA*$^+$ hosts carrying either of these plasmids, transport activity was inducible by G6P, but the induced level was substantially lower (by 30 to 50%) than when *uhpA* was carried on the plasmid. This result suggests that the level of chromosomally encoded activator is limiting when there are several copies of the *uhpT* regulatory region. Plasmid pLAW014, which is deleted for *uhpA* and part of *uhpB*, complemented the *uhpC* host almost completely, showing that UhpC is expressed from this plasmid. The low level of activity seen in the *uhpB* host RK7622 was not regulated, and its origin is unclear. Plasmid p10-j1, with a transposon insertion in *uhpA*, conferred inducible transport activity in all *uhpA*$^+$ hosts. This result indicates that the transposon does not have a strongly polar effect on expression of the distal *uhp* regulatory genes on the plasmid.

Expression from plasmid pLAW024 was low level and constitutive in all hosts, confirming that this expression is driven by weak vector promoters and is independent of inducer and *uhp* control. This result indicates that *uhpT* is the only gene whose product is required for transport activity.

Plasmids that carried *uhpA* conferred constitutive transport activity, as expected, although the levels of activity were affected by the presence of the other *uhp* genes. The constitutive expression of transport function in the *uhpA* host RK7621 in response to plasmid pLAW007, which carries only *uhpA*, was substantially lower than that seen with pRJK10, which carries the entire *uhp* region. The absence of *uhpB* and *uhpC* depressed activity by an additional 25 and 50%, respectively. Similarly, plasmid pLAW030, which is deleted for part of *uhpB*, conferred high constitutive levels of transport in the *uhpC* and *uhpT* hosts. Transport was partially inducible and somewhat lower in the *uhpA* strain RK7621 and the *uhpB* strain RK7622, consistent with a role for *uhpB* in stimulating activation by UhpA, even in the presence of multicopy *uhpA*. An identical response (substantial decrease in transport activity in the absence of *uhpB*) occurred in strains carrying plasmid p10-j2, with a transposon insertion in *uhpB*.

The absence of *uhpC* did not affect the high-level expression of *uhpT* in response to excess *uhpA*. Strain RK7623(p10-3b), with transposon insertions in both the plasmid and chromosomal copies of *uhpC*, displayed high-level constitutive transport activities.

Plasmid pLAW028, which carries *uhpABC* intact but only the proximal portion of *uhpT*, conferred low-level constitutive transport activity in all *uhpT*$^+$ hosts. Its reduced level of expression is explained by competition for the activator between the *uhpT* promoter on the chromosome and the multiple copies of the promoter preceding the truncated *uhpT* gene on the plasmid.

The results from transport and fusion expression in different strains were generally consistent with each other and led to the following conclusions. Increased *uhpA* copy number was associated with elevated transcription of *uhpT* that was usually not increased further by addition of inducer. This elevated expression was increased by a factor of 2 to 3 by the presence of *uhpB*, whereas the presence of *uhpC* did not seem to affect this response. Strains carrying multiple copies of the *uhpT*-regulatory region exhibited decreased transport or β-galactosidase activity, consistent with the titration of the activator protein.

Regulation of *uhpA* expression. Derepression of the proteins of the *pho* regulon during phosphate limitation is associated with increased synthesis of the PhoB activator protein (8). To test whether a similar situation might occur in the control of the Uhp system, the expression of *uhpA* was examined by means of a *uhpA-lacZ* gene fusion.

The *recA* strain RK5969 carries a stabilized Mu(*lac*) gene fusion to *uhpA* and exhibits a Uhp$^-$ phenotype that is complemented only by *uhpA*$^+$ plasmids. Production of β-galactosidase by this strain was 10 to 15% of that engendered by *uhpT-lac* fusions. Enzyme levels were not substantially affected by addition of G6P or by the presence of the *uhpA*$^+$ plasmid pRJK10 or pLAW007 (Table 3). Both of these plasmids conferred constitutive G6P transport activity. Thus, expression of *uhpA* is constant during induction of *uhpT* transcription, and there is no indication of autogenous regulation of *uhpA* transcription.

Sequence homologies of the Uhp proteins. Sequence homologies can provide insights into the evolution and function of particular proteins. UhpT displays about 30% amino acid identity (about 60% if conservative replacements are allowed) with GlpT, the glycerol phosphate transporter that also acts as a phosphate antiport (6, 12). Furthermore, UhpC displays the same degree of relatedness to the middle portion of UhpT, suggesting that UhpC might also possess a sugar-phosphate-binding site (7). At the time the nucleotide sequence of the *uhp* region was determined, no significant matches of UhpA or UhpB to proteins in the data banks were found. Recent comparisons have revealed some interesting relationships.

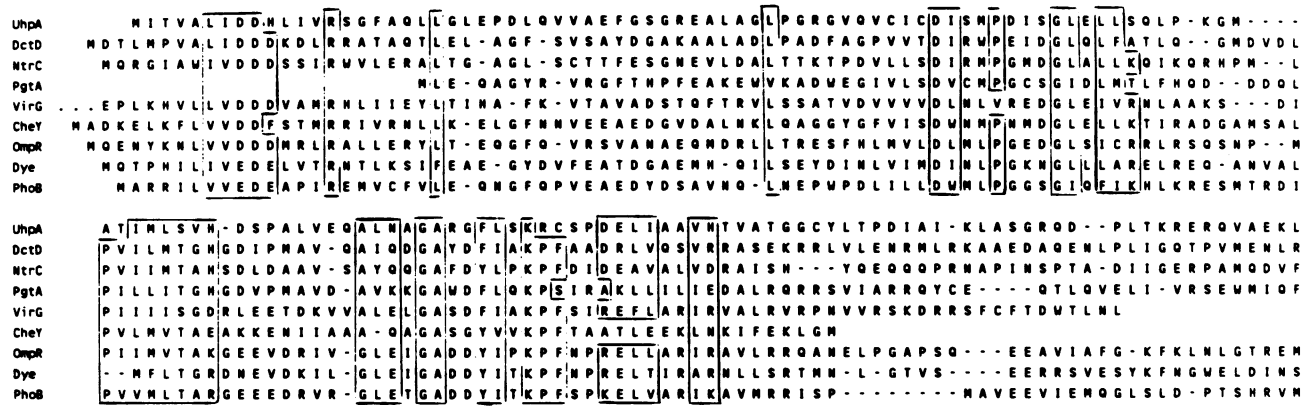

FIG. 2. Alignment of UhpA with homologous proteins. Amino acids enclosed by boxes indicate amino acids that are identical or similar between UhpA and its homologs. Only the first 120 amino acids are shown. References for the sequences are given in the text.

There are two families of bacterial regulatory proteins, some of whose members interact to control gene expression in response to external signals (4, 5, 14, 15, 18, 20, 25, 27). One family includes OmpR, PhoB, SfrA, VirG, CheB, CheY, Spo0A, Spo0F, NtrC, and DctD. Many of these proteins function as transcription activators. All members of this family have related sequences that are located within the first 120 amino acids; homologies located further along the polypeptide chain are displayed by subsets of these proteins. The second family includes EnvZ, PhoR, PhoM, CpxA, VirA, NtrB, and DctB. The conserved sequences that define this family are located within the carboxyl half of the chain.

The sequence of the DctD protein, which controls expression of the dicarboxylic acid transport system in *Rhizobium leguminosarum* (20), provided the key to the relationship of UhpA to the first family of regulatory proteins. UhpA showed the closest match to DctD, with 30% identity. The match to the other proteins, although obvious, was weaker, ranging from about 25% identity with NtrC and CheY to 19% for OmpR, SfrA, and PhoB. An alignment of the first 120 amino acids of several of these proteins is shown in Fig. 2, with the most highly conserved positions enclosed in boxes. There was strong statistical evidence for relatedness within this portion of the sequence. The matches of UhpA with DctD, NtrC, PhoB, and CheY were at least 12 standard deviations (SDs) above random. Surprisingly, the matches of UhpA to OmpR and VirG were not statistically significant (<0.5 SD above random), despite the obvious alignments possible. Within the aligned regions of these proteins, 60% of the amino acid residues in UhpA were found in an identical position in at least one of the other proteins.

There is a sharp divergence after the first 120 amino acids. The *ompR*, *sfrA*, *phoB*, and *virG* products share sequences over their entire lengths of about 240 amino acids. The distal regions of the nitrogen-regulatory *ntrC* and *dctD* proteins are also highly homologous to each other and to the corresponding portion of *nifA* products (18). Alignment of the remainder of UhpA (195 amino acids) to either of these groups of proteins would require extensive insertion of gaps and was not detected by the FASTP search program. Hence, UhpA only has an N-terminal motif (about 120 amino acids) in common with the OmpR group and the NtrC group of regulatory proteins. Within the C-terminal region specific to UhpA is a potential helix-turn-helix motif that could constitute a DNA-binding domain (7).

The PgtA protein is a transcription activator for the externally induced phosphoglycerate transport system, PgtP, of *Salmonella typhimurium* (21, 29). UhpA exhibits homology to PgtP, although the match is relatively weak. PgtA shows a closer match to the nitrogen-regulatory proteins than to the OmpR family.

Common sequences in UhpB and members of the second family of regulatory proteins were present but were less striking. Sequence conservation within this family is usually less pronounced than within the first family. Figure 3 presents segments of some proteins in which common sequences were observed. These regions are all near the C termini of the polypeptides. In six of the seven regions shown, at least half of the amino acid residues in UhpB were present at the same position in one of the other proteins. As was the case with UhpA, UhpB had the most matches with DctB. Thus, sequence comparisons show that UhpA and

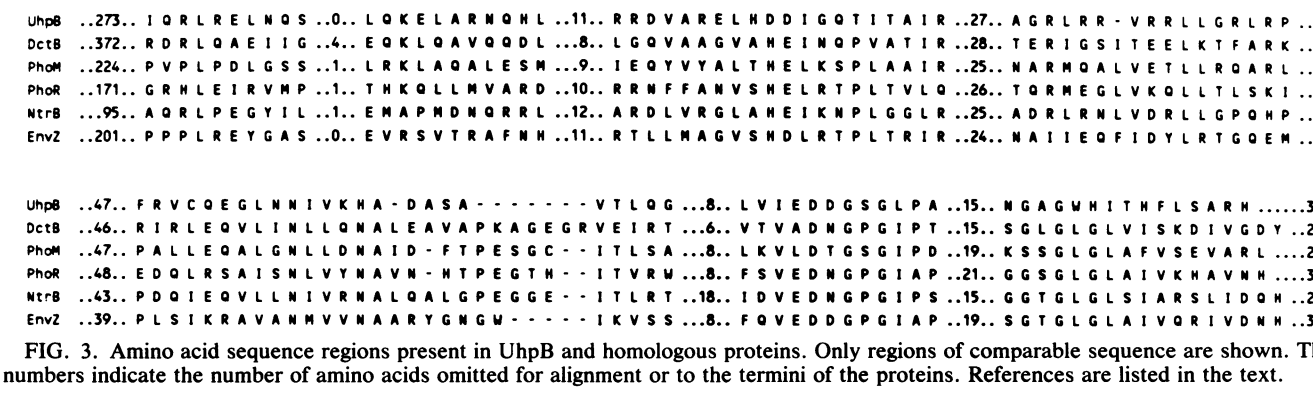

FIG. 3. Amino acid sequence regions present in UhpB and homologous proteins. Only regions of comparable sequence are shown. The numbers indicate the number of amino acids omitted for alignment or to the termini of the proteins. References are listed in the text.

UhpB are members of a large group of bacterial regulatory proteins.

DISCUSSION

Previous studies of the structure and expression of the genes controlling the sugar-phosphate (Uhp) transport system revealed the presence of four genes necessary for transport function (28). The observations described here show that *uhpT* transcription requires the action of three genes, *uhpABC*, whereas *uhpT* does not affect its own expression. *uhpT* appears to be the only gene necessary for formation of the transporter, although this conclusion must await reconstitution of purified UhpT protein.

Results with *lac* fusions agreed in general with the results of assays of G6P transport activity. The UhpA protein appears to function as a positive activator of transcription from the *uhpT* promoter. It is the only protein that is invariably required for *uhpT* transcription. Its overproduction by multicopy plasmids obviates the requirement for either of the other *uhp* regulatory genes or for inducer. The UhpA protein bears a substantial sequence relationship to other proteins that function as transcriptional activators. Uninduced expression is often elevated in strains that overproduce specific transcription activators.

The other two regulatory proteins are associated with the cytoplasmic membrane and appear to regulate the availability of the UhpA activator. The fact that UhpC was homologous to sequences in UhpT suggested that UhpC might be a transmembrane protein with a sugar-phosphate-binding site, consistent with a role for this protein as a receptor for the inducer.

The UhpB protein has a bipartite structure, with a very nonpolar amino-terminal half likely to be embedded in the cytoplasmic membrane and a highly polar carboxyl-terminal half. There are several possible mechanisms by which the UhpB protein could participate in regulation. It could bind UhpA and remove it from the vicinity of the genome. This possibility is unlikely, since it predicts that *uhpB* mutants would exhibit constitutive expression, whereas they are actually mute. It is more likely that UhpB protein serves to alter UhpA covalently to convert it to a form that stimulates *uhpT* transcription. For example, transcription from the *glnAp2* promoter is activated when the NtrC protein is phosphorylated by the action of the NtrB protein (17). This possibility could account for most of the results described here. Excess *uhpA* results in elevated expression of *uhpT* that cannot be further increased by inducer. UhpA, when overproduced, could be a substrate for modification by any of the other members of the second family described above. The activation of UhpA by these other proteins would not respond to the presence of UhpB or inducer. Although excess *uhpA* does cause greatly elevated expression by itself, the presence of UhpB appears to increase this expression further. The UhpA protein should interact more effectively with UhpB than with any other member of that family, and thus the loss of UhpB would have a substantial effect on UhpA's activity.

Constitutive expression of the *Agrobacterium tumefaciens vir* genes occurs when the *virG* activator gene is present at increased copy number (19). The presence of *virA* was necessary for further *vir* expression in response to the phenolic plant products that induce the virulence genes. The *uhp* system did not display further induction when *uhpA* was multicopy. The difference between these two regulatory systems could be related to the following feature. The *virA* product is likely to be the only protein needed to serve as sensor for the inducer's presence. In contrast, both *uhpB* and *uhpC* must interact for transmission of the signal indicating the presence of external G6P. To understand the basis for the lack of further induction when *uhpA* is multicopy will require information about the nature of the activation of UhpA and the effect of this putative modification on the DNA-binding properties of the protein. Perhaps an excess of the unmodified form can still bind to DNA, as is the case with NtrC (17), and interfere with activation by the correctly modified form. Future work must examine the possible modifications and interactions of the *uhp* proteins.

Another noteworthy finding was that transport activity did not increase in relation to the copy number of the *uhpT* gene. The increase in transport activity resulting from an increase in *uhpT* copy number was much less than the increase in β-galactosidase activity when chromosomal and plasmid-borne *uhpT-lac* fusions were compared. The lack of proportionality between transport activity and gene dosage does not seem to result from a limitation of transcription activity. There may be a barrier either to insertion of excess transporter proteins into the membrane in functional form or to their expression of full activity in standard transport assays. This result limits the conclusions that can be drawn from regulatory studies with plasmids, although the results were consistent with those obtained by using fusions.

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