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UDP-Glucose Is a Potential Intracellular Signal Molecule in the Control of Expression of σ^S and σ^S-Dependent Genes in *Escherichia coli*

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The σ^S subunit of RNA polymerase is the master regulator of a regulatory network that controls stationary-phase induction as well as osmotic regulation of many genes in *Escherichia coli*. In an attempt to identify additional regulatory components in this network, we have isolated Tn*10* insertion mutations that in *trans* alter the expression of *osmY* and other σ^S-dependent genes. One of these mutations confered glucose sensitivity and was localized in *pgi* (encoding phosphoglucose isomerase). *pgi*::Tn*10* strains exhibit increased basal levels of expression of *osmY* and *otsBA* in exponentially growing cells and reduced osmotic inducibility of these genes. A similar phenotype was also observed for *pgm* and *galU* mutants, which are deficient in phosphoglucomutase and UDP-glucose pyrophosphorylase, respectively. This indicates that the observed effects on gene expression are related to the lack of UDP-glucose (or a derivative thereof), which is common to all three mutants. Mutants deficient in UDP-galactose epimerase (*galE* mutants) and trehalose-6-phosphate synthase (*otsA* mutants) do not exhibit such an effect on gene expression, and an *mdoA* mutant that is deficient in the first step of the synthesis of membrane-derived oligosaccharides, shows only a partial increase in the expression of *osmY*. We therefore propose that the cellular content of UDP-glucose serves as an internal signal that controls expression of *osmY* and other σ^S-dependent genes. In addition, we demonstrate that *pgi*, *pgm*, and *galU* mutants contain increased levels of σ^S during steady-state growth, indicating that UDP-glucose interferes with the expression of σ^S itself.

The σ^S subunit of RNA polymerase in *Escherichia coli* is involved in the expression of a large number of genes and operons, of which more than 30 have been identified. Nearly all of these genes are induced during entry into stationary phase, and a similar regulation has also been observed for *rpoS*, the structural gene encoding σ^S (recently reviewed in references 22 and 23). This control of the intracellular level of σ^S operates at the transcriptional as well as posttranscriptional levels (34–37, 40, 42, 46) and also includes a mechanism that differentially influences the stability of σ^S, which is a very unstable protein (with a half-life of 1.4 min) in exponentially growing cells (35).

Within the large σ^S regulon, differential regulation has been observed for subsets of genes. For instance, *appCBA*, *hyaAB-CDEF*, and *appY* are also anaerobically induced, and σ^S is required for this induction (5, 6, 14, 16). The expression of a rather large subfamiliy of σ^S-dependent genes (e.g., *otsBA*, *treA*, *osmB*, *osmY*, and *bolA*) responds to changes in medium osmolarity (9, 19, 24, 25, 27, 59). These regulatory responses can be observed in growing cells that contain a low level of σ^S (18, 35, 54), indicating that the view of σ^S as a stationary-phase-specific sigma factor may be too narrow. In fact, we have recently presented evidence for a posttranscriptional induction of σ^S expression in response to high osmolarity in growing cells (35). Furthermore, during entry into stationary phase, the induction of various σ^S-dependent genes follows different kinetics. There are early-induced genes, such as the *otsBA* operon (24); genes like *osmY* (33, 56) or *osmB* (24, 27), whose expression is stimulated somewhat later; and genes with continuing expression until late stationary phase, such as *dps* (3). Taken together, all of these studies indicate that additional factors besides σ^S are involved in the fine regulation of these genes.

For a detailed analysis, we have chosen the *osmY* (*csi-5*) gene as a prototype of a σ^S-controlled gene. *osmY* was discovered independently as a growth phase-regulated and osmotically controlled *lacZ* fusion (25, 34, 56) and as the structural gene of a hyperosmotically inducible periplasmic protein of unknown function (59). Under all conditions, its expression is strongly reduced by mutations in *rpoS* (25, 34, 56, 58). *osmY* is expressed from a single promoter, which is probably directly recognized by σ^S (33, 58). In recent studies we have shown that in addition to σ^S, the cyclic AMP (cAMP)-cAMP receptor protein (cAMP-CRP) complex, Lrp, and integration host factor (IHF) are involved in modulating *osmY* transcription (33, 56). These factors act as negative transition state regulators whose physiological role may be to maintain repression of *osmY* during the early phase of transition into stationary phase, in which σ^S levels already are increasing but expression of *osmY* might not yet be appropriate. However, CRP, Lrp, and IHF are not essential for osmotic induction of *osmY* (33).

In a second approach to identify additional factors that influence the expression of *osmY*, we have isolated transposon insertion mutations that in *trans* alter the expression of a transcriptional *lacZ* fusion to *osmY* (*csi-5*::*lacZ*). In the present paper, we concentrate on the analysis of one of these mutations, which strongly increased the basal level of expression of *osmY* and other σ^S-controlled genes and reduced the factor of osmotic induction of these genes. The mutation was mapped in *pgi*, which encodes phosphoglucose isomerase (PGI). The finding that *pgm* and *galU* mutations have similar effects on *osmY* expression implicates UDP-glucose as a potential intracellular

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TABLE 1. Bacterial strains

Strain	Relevant genotype	Reference or source
MC4100	F$^-$ Δ(*arg-lac*)*U169 araD139 rpsL150 ptsF25 flbB5301 rpsR deoC relA1*	48
RH90	MC4100 *rpoS359*::Tn*10*	34
RO151	MC4100 Φ(*csi-5*::*lacZ*)(λp*lac*Mu55)	56
FF2032	MC4100 Φ(*otsA*::*lacZ*)7(λp*lac*Mu55)	19
JU25	MC4100 *pgi*::Tn*10*-23	This study
JU23	RO151 *pgi*::Tn*10*-23	This study
JU24	FF2032 *pgi*::Tn*10*-23	This study
PGM1	Hfr3000 *pgm-1*	2
RH117	MC4100 *pgm-1 zbf-3057*::Tn*10*	This study
JU1	RO151 *pgm-1 zbf-3057*::Tn*10*	This study
FF4001	MC4100 *galU95*	19
MG4	MC4100 *galU95 zch*::Tn*10*[a]	This study
JU2	RO151 *galU95 zch*::Tn*10*	This study
UE14	MC4100 *treA*::Tn*10*	10
MG5	RO151 *galU95 treA*::Tn*10*[b]	This study
FF4169	MC4100 *otsA1*::Tn*10*	19
JU3	RO151 *otsA1*::Tn*10*	This study
REI201	MC4100 *mdoA200*::Tn*10*[c] Φ(*glpD*::*lacZ*)λp*lac*Mu55 Φ(*malK*::*lacZ*) *malI*	J. Reidl
RH151	RO151 *mdoA200*::Tn*10*	This study
RH153	MC4100 *mdoA200*::Tn*10*	This study
DHB4	F′ *lacI*^{q} *pro araD139* Δ(*ara-leu*)*7697* Δ*lacX74* Δ*phoA*^{PvuII} *phoR* Δ*malF3 galE galK thi rpsL*	11
RH152	RO151 *galE galK zbh-29*::Tn*10*	This study
GM301	MC4100 Mu*cts62* Ap^r(pEG5005)	39
JU26	JU25 Mu*cts62* Ap^r	This study
13527	*malF3180*::Tn*10*Kan (cotransducible with *pgi*)	49
18560	*metF159*::Tn*10*Kan (cotransducible with *pfkA*)	49
18518	*zdi-3123*::Tn*10*Kan (cotransducible with *pfkB*)	49
18559	*nupG3157*::Tn*10*Kan (cotransducible with *fda* and *pgk*)	49
12122	*zea-3125*::Tn*10*Kan (cotransducible with *gapA*)	49
12182	*cysC3152*::Tn*10*Kan (cotransducible with *eno*)	49
18493	*zbh-29*::Tn*10* (cotransducible with *galEK*)	49

[a] Uncharacterized Tn*10* insertion >90% cotransducible with *galU*.
[b] *treA*::*lacZ* is approximately 5% cotransducible with *galU*.
[c] *mdoA200*::Tn*10*, originally described by Lacroix et al. (32), was mapped in *mdoH*, which together with *mdoG* constitutes the *mdoA* operon (31).

signal molecule in the control of expression of *rpoS*-controlled genes and of σ^S itself.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The strains used in this study are listed in Table 1. Cultures were grown aerobically at 37°C with vigorous shaking. Growth was monitored by measuring the optical density at 578 nm (OD_{578}). Luria-Bertani medium (LB) and M9 minimal medium were prepared as described previously (41). Minimal medium was supplemented with glucose, glycerol, maltose, and sorbitol in the concentrations indicated in Results and in the figure legends. Antibiotics were used in the concentrations recommended previously (41). For osmotic induction experiments, cells were grown in M9 medium with 0.4% glycerol as a carbon source to which 0.3 M NaCl was added at an OD_{578} of between 0.1 and 0.3, after the culture had been growing exponentially for more than five generations. Lactose MacConkey plates were prepared from premixed ingredients purchased from Difco. For the glucose sensitivity plate assay, 100 μl of an overnight culture of the strain to be tested was plated with top agar (0.9% NaCl, 0.9% agar) on M9–0.4% glycerol plates. Sterile filter platelets (5-mm diameter, prepared from Whatman glass microfiber filters [GF/B]) were placed on the plates and dotted with 10 μl of glucose solution (4 or 0.8%). Glucose-sensitive strains formed clear zones of growth inhibition visible after overnight growth at 37°C.

Genetic procedures. Standard techniques were used for generalized transduction with phage P1 and for growth and titer determination of bacteriophages (41, 48).

For the isolation of Tn*10* insertion mutations, a two-step procedure was followed. First, random Tn*10* insertions were obtained in strain MC4100 with λNK55, an abortive λ phage carrying Tn*10*, as described by Kleckner et al. (29). Approximately 30,000 tetracycline-resistant colonies were pooled and used to grow a P1 lysate (this P1 pool lysate was kindly provided by R. Lange). In a second step, strain RO151 was transduced with this pool lysate, and approximately 16,000 tetracycline-resistant transductants were replica plated on ordinary MacConkey plates as well as on MacConkey plates that were supplemented with 0.2 M NaCl in order to screen for strains with altered expression of *csi-5*::*lacZ*.

For cloning of chromosomal DNA fragments that complemented the Tn*10*-23 phenotype, the mini-Mu system was used and the preparation of lysates and infections were carried out as described previously (20). A thermoinduced lysate was prepared from strain GM301 (39), which is an MC4100 derivative carrying both Mu*cts62* as a helper phage in the chromosome and the mini-Mu plasmid pEG5005 (which carries *cts62* A^+ B^+ Kan^r rep_{pMB1} [20]). Strain JU26 (an MC4100 derivative carrying Tn*10*-23 as well as the Mu*cts62* helper phage) was infected with this lysate. The infected cultures were plated on LB-kanamycin plates (for obtaining the total number of kanamycin-resistant colonies) as well as on M9 plates containing 0.4% glycerol, 0.1% glucose, and kanamycin, on which should grow only strains that carry fragments of chromosomal DNA on pEG5005 that can complement or suppress the glucose-sensitive phenotype of strain JU26. More than 100 glucose-resistant colonies were obtained. The majority of these colonies could also grow on glucose as the sole carbon source (i.e., fully complemented the Tn*10*-23 mutation), whereas a few strains were glucose resistant but still unable to grow on glucose (multicopy second-site suppressors). Mini-Mu plasmids that fully complemented the Tn*10*-23 mutation were isolated from 10 strains by the standard alkaline-lysis plasmid preparation (48) and were found to carry overlapping chromosomal DNA fragments of various sizes.

DNA manipulations. For DNA manipulations such as plasmid and chromosomal DNA preparations, restriction digestions, ligation, transformation, and agarose gelelectrophoresis, standard procedures were followed (45, 48).

Southern transfer of DNA onto nylon membranes (Schleicher & Schuell) was performed as described previously (45). Digoxigenin (DIG) labeling of DNA probes, hybridization, and detection of DNA hybrids were performed by using a kit for nonradioactive DNA labeling, hybridization, and detection (Boehringer Mannheim) according to the instructions given by the manufacturer. Lumigen PPD (Boehringer Mannheim) was used as a chemiluminescent substrate for the detection reaction.

SDS-PAGE and immunoblot analysis. For the determination of the cellular σ^S protein levels, samples corresponding to 30 μg of total celluar protein were taken from exponentially growing cultures at an OD_{578} of 0.5 and were precipitated with 10% trichloroacetic acid. The samples were prepared for and separated by sodium dodecyl sulfate-12% polyacrylamide gel electrophoresis (SDS–12% PAGE) (with 15 μg of total cellular protein applied per lane) and subjected to immunoblot analysis with a previously characterized polyclonal serum against σ^S as described previously (35). 5-Bromo-4-chloro-2-indolylphosphate and 4-ni-

troblue tetrazolium chloride were used as chromogenic substrates for the detection reaction.

Enzyme assays. β-Galactosidase activity was assayed in SDS-chloroform-permeabilized cells by use of *o*-nitrophenyl-β-D-galactopyranoside as a substrate and is reported as micromoles of *o*-nitrophenol per minute per milligram of cellular protein (41). For qualitative estimation of β-galactosidase activity on plates, 5-bromo-4-chloro-3-indolylgalactopyranoside (XG) was used as an indicator substance.

For the determination of PGI and phosphoglucomutase (PGM) activities, LB-grown cultures were washed in M9 medium, resuspended in 50 mM Tris-Cl (pH 7.6), and disrupted by two passages through a French pressure cell at 103,500 kPa. The extracts obtained were dialyzed overnight in 50 mM Tris-Cl (pH 7.6) containing 5% glycerol and 1 mM dithiothreitol. For assay of PGI and PGM activities in these extracts, the respective enzymatic reactions were coupled to the oxidation of glucose-6-phosphate catalyzed by glucose-6-phosphate dehydrogenase, and the formation of NADPH was measured as an increase in extinction at 340 nm. The assay samples contained 50 mM Tris-Cl (pH 7.6), 10 mM $MgSO_4$, 0.3 mM $NADP^+$, 2 μg of glucose-6-phosphate dehydrogenase per ml, and an appropriate amount of the cellular extracts. The reactions were started by adding 1 mM fructose-6-phosphate or 1 mM glucose-1-phosphate for assay of the activity of PGI or PGM, respectively.

RESULTS

Isolation of Tn*10* insertion mutants with altered expression of σ^S-dependent genes. As a reporter gene fusion for assaying osmotic and stationary-phase induction of a σ^S-dependent gene, the transcriptional *csi-5*::*lacZ* fusion located in the chromosomal copy of *osmY* was chosen. Growth phase-dependent and osmotic inductions of this fusion are between 20- and 50-fold (25, 56). As a plate assay for osmotic induction, lactose MacConkey plates were used. Strain RO151 (an MC4100 derivative carrying *csi-5*::*lacZ*) grows as white colonies on salt-free MacConkey plates and as dark red colonies on similar plates to which 0.2 M NaCl was added (data not shown). Starvation induction was qualitatively tested on M9 minimal medium plates containing 0.2 or 0.04% glucose and XG as an indicator for β-galactosidase activity (34).

Strain RO151 was mutagenized by P1 transduction with a P1 pool lysate (kindly supplied by R. Lange) obtained from approximately 30,000 tetracycline-resistant colonies of strain MC4100 mutagenized with Tn*10* (see Materials and Methods for details). Approximately 16,000 tetracycline-resistant transductants of strain RO151 were replica plated onto salt-free and 0.2 M NaCl-containing lactose MacConkey plates.

Three classes of transductants that phenotypically differed from the original strain could be distinguished. Strains of class I were dark red in either the absence or presence of NaCl (20 strains were isolated). When grown in LB medium, three representatives of this class exhibited a sharp transition into stationary phase already at an OD_{578} of 1, and supernatant from their overnight cultures in LB had a lower pH than that of the parent strain. These strains seemed to be impaired in respiratory metabolism and were not further analyzed. Class II consisted of more than 100 transductants that remained white on MacConkey plates containing NaCl. These strains were mostly kanamycin sensitive and thus had lost the *csi-5*::*lacZ* fusion (which together with a kanamycin resistance marker is located on λp*lac*Mu55 [13]) by cotransduction of the respective Tn*10* transposons with the *csi-5* (*osmY*) wild-type region. Among class II strains that were still kanamycin resistant, one mutant that exhibited an *rpoS* mutant phenotype (reduced glycogen synthesis and catalase activity) was observed. In the remaining class II strains, the phenotype on MacConkey plates was unstable, and therefore these mutants were not further analyzed.

Finally, class III consisted of mutants that seemed less red on NaCl-containing MacConkey plates than the parental strain. Among 40 mutants of this class, only three strains exhibited significantly altered expression and osmotic induction of β-galactosidase when assayed quantitatively in liquid medium. As expected from the phenotypes on MacConkey indicator plates, in two of these strains the basal level of *csi-5*::*lacZ* expression was not altered but its osmotic induction was reduced to some degree (data not shown). In contrast, the remaining strain (JU23) exhibited higher-level expression of *csi-5*::*lacZ* already during steady-state growth in the absence of NaCl (Fig. 1), indicating that its white phenotype on MacConkey plates is due to a failure to ferment lactose rather than to a decrease in expression of the fusion. Among the Tn*10* insertion mutations isolated here, the mutation in strain JU23 (termed Tn*10*-23) had the most significant influence on the expression of *csi-5*::*lacZ*. Therefore, this mutation was studied in more detail.

Effect of Tn*10*-23 on the expression of the σ^S-dependent genes *osmY* and *otsBA*. By P1 transduction, Tn*10*-23 was also transferred into FF2032, an MC4100 derivative carrying a transcriptional *lacZ* fusion in the chromosomal copy of *otsA* (encoding trehalose-6-phosphate synthase). Like for *osmY*, osmotic and stationary-phase inductions of the *otsBA* operon are dependent on the presence of σ^S (24, 28). Figure 1 shows the influence of Tn*10*-23 on basal expression and osmotic induction of *csi-5*::*lacZ* and *otsA*::*lacZ* in the MC4100 background. In both cases, the basal levels of expression of the fusions during steady-state growth were strongly elevated. The addition of 0.3 M NaCl still elicited osmotic induction, but induction was less than 2-fold for both fusions, whereas induction in the parental strains was 15- and 4.5-fold for *csi-5*::*lacZ* and *otsA*::*lacZ*, respectively (Fig. 1A and C). These data indicate that the effect of Tn*10*-23 on gene expression is not restricted to *osmY* but is of a more general nature. In addition, it appeared that Tn*10*-23-carrying strains were less able to cope with hyperosmotic stress, since the reduction of the growth rate after the addition of NaCl was stronger than that for the parental strains (Fig. 1B and D; Table 2).

Tn*10*-23 also affected growth phase-related expression of *csi-5*::*lacZ* in LB. Whereas the basal level of expression in rapidly growing cells was not altered, induction during entry into stationary phase was somewhat larger, and a 25% higher level of β-galactosidase activity in JU23 than in the parental strain RO151 was finally reached. The growth rates during the whole experiment were unaffected by Tn*10*-23 (data not shown).

Tn*10*-23 is located in *pgi*, the structural gene for PGI. During our analysis of the phenotypes of Tn*10*-23-carrying strains, we noted that these strains were unable to grow on glucose minimal plates. Since the inability to grow on certain carbon sources might indicate the function and identity of the gene disrupted by Tn*10*-23, growth properties were studied in more detail for a Tn*10*-23 derivative of MC4100 (JU25). The following results can be derived from Table 2. (i) When strain JU25 was transferred from minimal glycerol medium to minimal glucose medium, lysis occurred, and a similar result was obtained after several hours of incubation in minimal medium containing maltose, a sugar that is metabolized via glucose-6-phosphate. (ii) No lysis was observed in minimal medium containing sorbitol, which is metabolized via fructose-6-phosphate. (iii) When the strains were grown in minimal glycerol medium, the addition of NaCl resulted in a greater increase of doubling times for the Tn*10*-23 mutant than for the parental strain, yet the addition of NaCl prevented lysis of the Tn*10*-23 strain when it was growing in glucose minimal medium and even resulted in more rapid growth than in glycerol medium containing the same concentration of NaCl.

Tn*10*-23 not only interferes with growth on glucose as a carbon source but also confers glucose sensitivity. When small filter discs with glucose were placed onto a lawn of Tn*10*-23

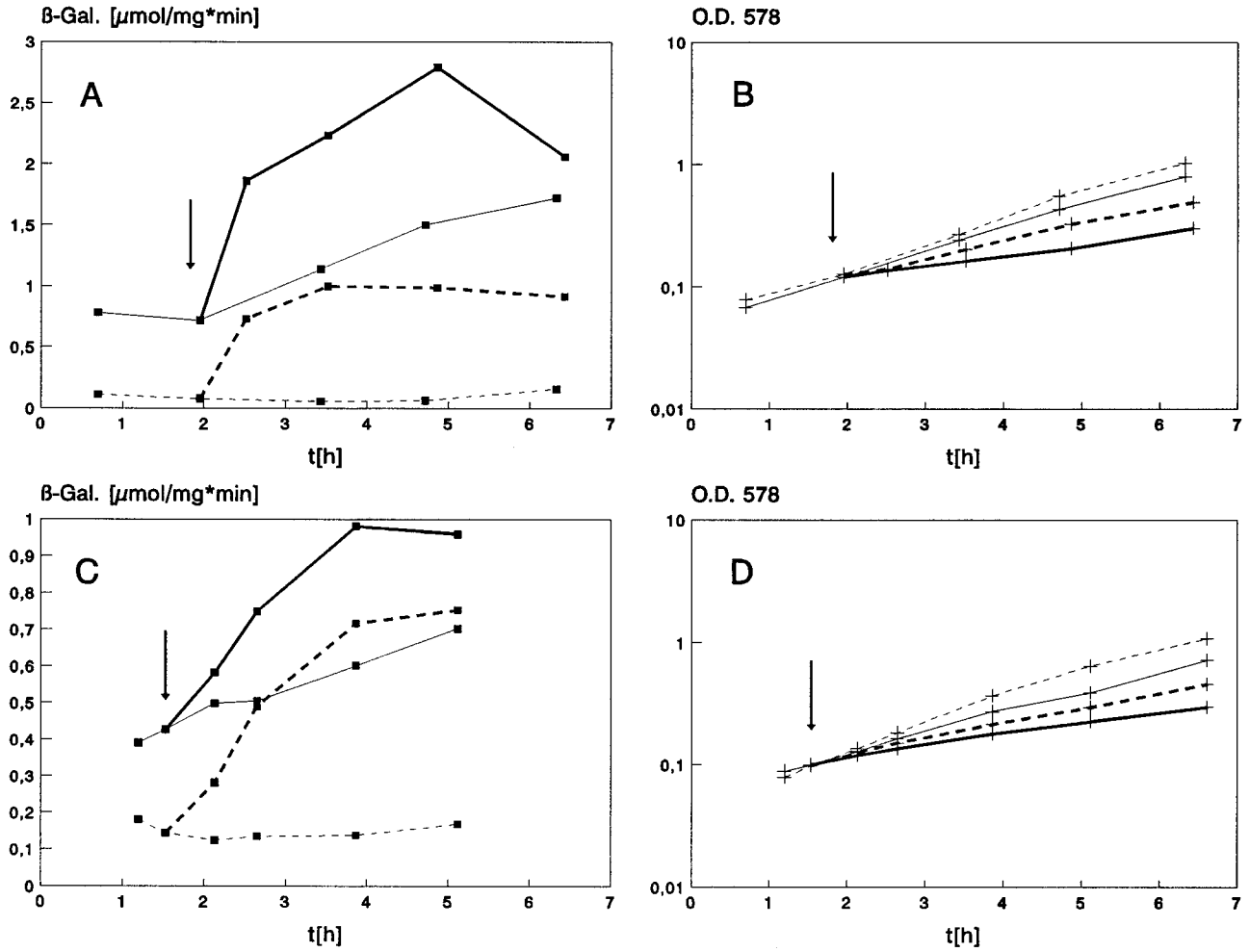

FIG. 1. Effect of Tn*10*-23 on the basal level of expression and osmotic induction of *csi-5*::*lacZ* and *otsA*::*lacZ*. Cultures of the *csi-5*::*lacZ*-carrying strains RO151 (parental) and JU23 (containing Tn*10*-23) (A and B) and the *otsA*::*lacZ*-carrying strains FF2032 (parental) and JU24 (containing Tn*10*-23) (C and D) were grown in M9 medium containing 0.4% glycerol for more than five generations (not completely shown) before the cultures were divided into two aliquots and 0.3 M NaCl was added to one aliquot in each case (indicated by the arrows). Specific β-galactosidase (β-Gal.) activities (A and C) and OD_{578}s (B and D) were determined for Tn*10*-23-carrying strains (solid lines) and the corresponding parental strains (dashed lines) in the presence (thick lines) and absence (thin lines) of NaCl.

mutant cells on minimal glycerol plates, distinct zones of growth inhibition around the filter discs could be observed after overnight growth (data not shown). Glucose sensitivity has been described previously for strains with defects in several glycolytic enzymes. These strains include those carrying mutations in *fda*, encoding fructose-1,6-diphosphate aldolase (7), and in *gapA*, *pgk*, and *eno*, encoding glyceraldehyde-3-phosphate dehydrogenase, glycerate-3-phosphate kinase, and enolase, respectively (26). In the case of the latter three mutants, it was also observed that increased osmolarity rescued the cells from glucose sensitivity (26). We therefore tested whether Tn*10*-23 could be located in any of these genes or in other genes that code for glycolytic enzymes. The Tn*10*-23 mutant JU23 was transduced with P1 lysates obtained with strains carrying Tn*10*Kan insertions (49) cotransducible with *pgi* (encoding PGI), *pfkA* and *pfkB* (encoding two phosphofructokinase isoenzymes), *fda*, *gapA*, *pgk*, and *eno*. Only the transduction with a *malF*::Tn*10*Kan marker resulted in tetracycline-sensitive colonies, with a frequency of 69%. *malF* is located at 91.44 centisomes on the physical map of the *E. coli* chromosome (44). The cotranduction frequency indicated a distance of 0.23 min between *malF* and Tn*10*-23, which is equivalent to the distance between *malF* and *pgi*.

In accordance with a putative location of Tn*10*-23 in *pgi*, we found a more-than-100-fold reduction in PGI activity in strain JU23 (0.025 µmol min^{-1} mg^{-1}) in comparison to that in the parental strain RO151 (2.80 µmol min^{-1} mg^{-1}). As a control, PGM activity was determined and was found to be identical in the two strains (0.161 µmol min^{-1} mg^{-1}).

Finally, Tn*10*-23 was physically mapped by Southern hybridization. For obtaining an appropriate DNA probe, DNA fragments that complemented the glucose-sensitive phenotype of JU23 were cloned by using the mini-Mu system developed by Groisman and Casadaban (20). Two classes of mini-Mu pEG5005 derivatives carrying different inserts of chromosomal DNA were isolated. Whereas most of the clones fully complemented the Tn*10*-23 phenotype and allowed normal growth on glucose, a few mini-Mu plasmids complemented glucose sensitivity but did not allow growth on glucose as a sole carbon source. From a representative clone of the first class that carried approximately 22 kb of chromosomal DNA, random fragments obtained with various restriction enzymes were sub-

TABLE 2. Effect of Tn*10*-23 on growth in various media

Medium[a]	Doubling time (min)	
	MC4100	JU25
LB	24	24
M9–0.4% glycerol	85	100
M9–0.4% glycerol–0.2 M NaCl	96	180
M9–0.4% glycerol–0.3 M NaCl	123	218
M9–0.4% glycerol–0.4 M NaCl	152	231
M9–0.2% glucose	75	—[b]
M9–0.2% glucose–0.3 M NaCl	102	171
M9–0.2% sorbitol	68	95
M9–0.2% maltose	69	330[c]

[a] Cultures not growing with glycerol as a carbon source were started by resuspension in the medium indicated, of an exponential culture that had been growing in M9–0.4% glycerol.
[b] Lysis.
[c] Lysis after several hours.

cloned into pBR322. A pBR322 derivative containing a 5.9-kb *Sph*I insert (pJU1) that fully complemented the Tn*10*-23 mutant phenotype was chosen for further analysis. The restriction map of the *Sph*I insert corresponded to the restriction map in the *lysC-pgi* region of the *E. coli* chromosome (30, 44), which allowed us to place the insert of pJU1 flanked by the two *Sph*I restriction sites on the physical map (Fig. 2A).

For physical mapping of Tn*10*-23 by Southern hybridization, chromosomal DNAs of MC4100 and JU25 as well as pJU1 plasmid DNA were digested with *Sph*I, separated by agarose gel electrophoresis, blotted onto a nylon membrane, and probed with the DIG-labeled plasmid pJU1 (Fig. 2B). Whereas a single hybridizing band of the same size as the pJU1 insert was observed for MC4100, the Tn*10*-23-carrying strain JU25 produced two bands of approximately 4.7 and 1.9 kb. Tn*10* contains *Sph*I restriction sites at distances of 347 bp from the ends of IS*10*$_L$ and IS*10*$_R$ (55). Therefore, the point of insertion of Tn*10*-23 can be calculated to be at a distance of 1.55 kb from one of the two *Sph*I restriction sites (the two theoretically possible insertions points on the chromosomal map are indicated by arrows in Fig. 2A). The right-hand one of these two physically determined potential insertion points is in the first third of the *pgi* gene, which is consistent with the genetic mapping by P1 transduction as well as with the loss of PGI activity (see above). We conclude that Tn*10*-23 is located in *pgi.*

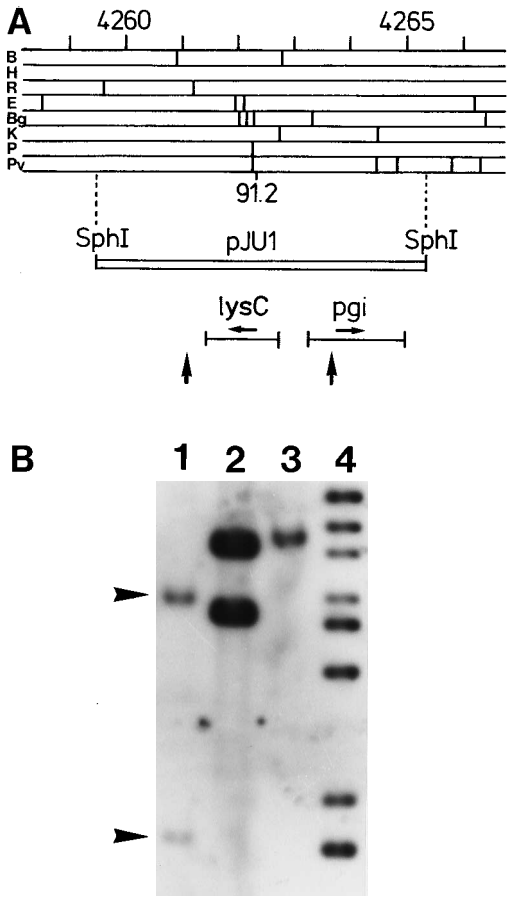

FIG. 2. Localization of Tn*10* in *pgi*. (A) Physical map in the 91-min region of the *E. coli* chromosome (30, 44). The location of the chromosomal insert present on pJU1 and the locations and directions of transcription of *lysC* and *pgi* are indicated. Vertical arrows point to the two possible positions of Tn*10*-23 as derived from Southern hybridization (B). B, *Bam*HI; Bg, *Bgl*I; E, *Eco*RV; H, *Hin*dIII; K, *Kpn*I; P, *Pst*I; Pv, *Pvu*II; R, *Eco*RI. (B) DIG-labeled pJU1 was used as a probe for hybridization to a Southern blot of *Sph*I-digested chromosomal DNA of the Tn*10*-23-carrying strain JU25 (lane 1), pJU1 (lane 2), chromosomal DNA of MC4100 (lane 3), and a DIG-labeled DNA standard (lane 4; 7.4, 6.9, 5.7, 4.8, 4.3, 3.7, 2.3, and 1.9 kb from top to bottom). Arrowheads indicate two hybridizing bands of approximately 4.7 and 1.9 kb in the chromosomal DNA of JU25.

Identification of UDP-glucose as a putative signal molecule for the expression of *rpoS*-dependent genes. From the localization of Tn*10*-23 in *pgi*, the question arose of why a disruption of the glycolytic pathway at the level of PGI resulted in increased expression and a reduced factor of osmotic induction of *osmY* and *otsBA*. When grown in glycerol minimal medium, a *pgi* mutant does not synthesize glucose-6-phosphate or any of its derivatives. We therefore hypothesized that such a component might have some negative signal function for the expression of *osmY* and other σ^S-dependent genes. If so, the addition to the *pgi* mutant of sublethal amounts of glucose that replenish the intracellular pools of glucose-6-phosphate and its derivatives should result in a decrease in the expression of σ^S-dependent genes. As shown in Fig. 3A, this was the case for *csi-5*::*lacZ* when strain RO151 was grown on minimal glycerol medium to which 0.002% glucose was added.

In wild-type cells a derivative of glucose-6-phosphate is glucose-1-phosphate, which is produced by PGM (encoded by *pgm*). We found that in a *pgm* mutant (strain JU1), which should have wild-type levels of glucose-6-phosphate but lacks glucose-1-phosphate and its derivatives, the expression of *csi-5*::*lacZ* was strongly elevated (Fig. 3B). Moreover, the addition of 0.008% galactose resulted in a reduction of *csi-5*::*lacZ* expression (Fig. 3B) very similar to that observed for the addition of glucose to the *pgi* mutant (Fig. 3A). After uptake, galactose is phosphorylated and converted into glucose-1-phosphate by the action of the enzymes encoded by the *galETK* operon (1) and thus supplies a *pgm* mutant with the missing glucose-1-phosphate (see Fig. 4 for the relevant metabolic reactions). In the *pgi* mutant (Fig. 3A), as well as in the *pgm* mutant (Fig. 3B), the rate of decrease in specific β-galactosidase activity compared with the rate of cell growth (data not shown) is consistent with dilution of preexisting β-galactosidase, indicating that for a certain period after the addition of glucose and galactose, respectively, the expression of *csi-5*::*lacZ* is nearly completely inhibited.

Major compounds produced from glucose-1-phosphate are ADP-glucose and UDP-glucose, which are synthesized by ADP-glucose pyrophosphorylase (encoded by *glgC*) (43) and by UDP-glucose pyrophosphorylase (encoded by *galU*) (57),

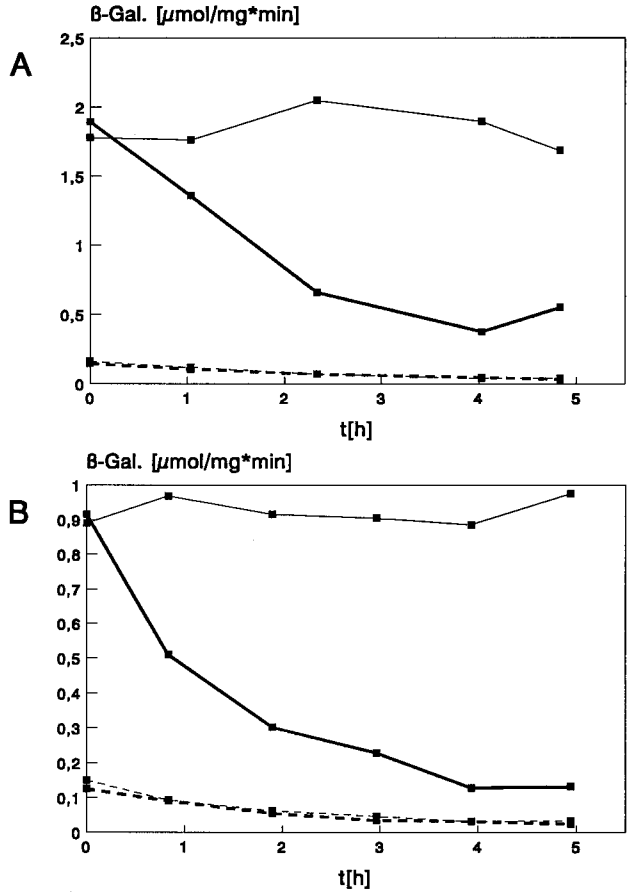

FIG. 3. Effects on the expression of *csi-5*::*lacZ* of glucose added to the *pgi* mutant (A) and of galactose added to the *pgm* mutant (B). Cultures of strain RO151 and its Tn*10*-23-carrying derivative JU23 (A) and of RO151 and its *pgm-1*-carrying derivative JU1 (B) were grown in M9 medium containing 0.4% glycerol for more than five generations (not shown). Two cultures of each strain were grown, and to one of the cultures 0.002% glucose (A) or 0.008% galactose (B) was added ($t = 0$). Specific β-galactosidase (β-Gal.) activities were determined for the parental strain RO151 (dashed lines) and the *pgi* and *pgm* mutants (solid lines) growing in the presence of glucose or galactose (thick lines) or in the absence of sugar additions (thin lines). During the experiment shown, the cultures grew exponentially between ODs of 0.05 and 1, and the addition of glucose and galactose in these low concentrations did not interfere with growth (data not shown).

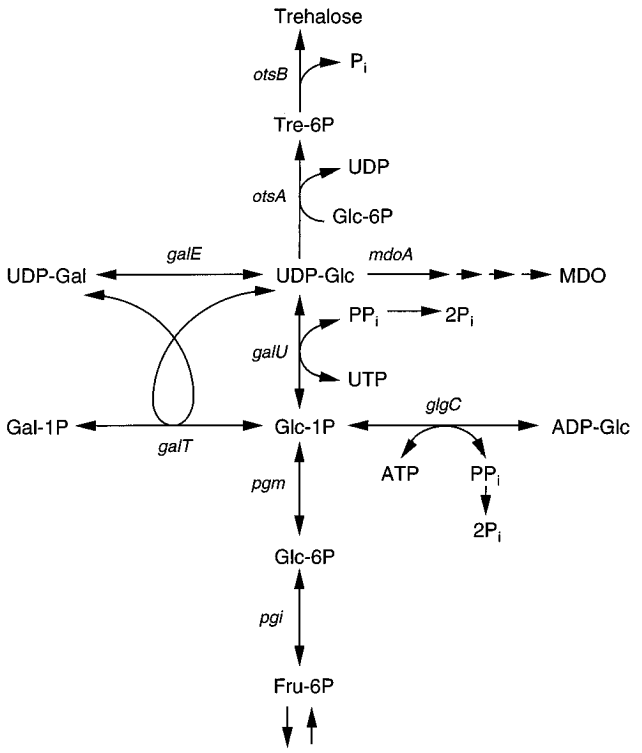

FIG. 4. Metabolic reactions involved in the synthesis and consumption of UDP-glucose. Tre, trehalose; P, phosphate; Glc, glucose; Gal, galactose; Fru, fructose.

respectively (Fig. 4). Whereas ADP-glucose is a precursor for glycogen synthesis (43), UDP-glucose is required for the biosyntheses of lipopolysaccharide (17, 53), membrane-derived oligosaccharides (MDO) (47), capsular polysaccharide (38), and trehalose (19). We observed that a *glgC* mutant exhibits normal expression of *csi-5*::*lacZ* (data not shown). However, a *galU* mutant expresses higher levels of *csi-5*::*lacZ* than the isogenic *galU*$^+$ strain (Fig. 5A), indicating that UDP-glucose somehow plays a role in repressing expression of *osmY* and perhaps other σ^S-controlled genes.

In order to test whether UDP-glucose itself or one of the derivatives mentioned above is the crucial substance involved in this regulatory effect, we also determined expression of *csi-5*::*lacZ* in mutants with lesions in *galEK*, *otsA*, and *mdoA* (Fig. 4). We observed no increase in the basal level of *csi-5*::*lacZ* expression in the *galEK* and *otsA* mutants, but the *otsA* mutant exhibited reduced osmotic induction of *csi-5*::*lacZ* (data not shown). The *mdoA* mutant showed an increased basal level of *csi-5*::*lacZ* expression, but this effect was not as pronounced as it was in the *galU* mutant (Fig. 5B).

***pgi*, *pgm*, *galU*, and *mdoA* mutants contain increased levels of σ^S.** We have previously shown that the expression of *osmY* is controlled by several additional global regulatory factors (cAMP-CRP, Lrp, and IHF) whose action is not dependent on the presence of σ^S (33). Therefore, it seemed possible that the mutations found to have an effect on *osmY* expression influence one of these components. On the other hand, UDP-glucose might influence the expression of σ^S itself and thereby have a pleiotropic effect on the expression of other σ^S-dependent genes (e.g., *otsBA*) as well.

In order to distinguish between these possibilities, the intracellular σ^S content in the mutants deficient in *pgi*, *pgm*, *galU*, and *mdoA* was determined by immunoblot analysis (Fig. 6). In comparison to the isogenic wild-type strain, *pgi*, *pgm*, and *galU* mutants exhibited approximately fourfold-increased levels of σ^S during steady-state growth. The *mdoA* mutant showed an intermediate level of σ^S (1.8-fold increase) and was thus less affected than the mutants with defects in *pgi*, *pgm*, and *galU*. This indicates that these mutations influence the synthesis of σ^S itself and that increased σ^S levels in the mutants are the basis of their increased expression of *osmY* and probably also other σ^S-dependent genes.

DISCUSSION

For the present study we have isolated Tn*10* insertion mutations that in *trans* altered the expression of the growth phase-regulated and osmotically inducible *osmY* (*csi-5*) gene, which belongs to a large family of σ^S-dependent genes in *E. coli*. Characteristically, all mutations that we have been able to obtain only partially affected the expression of *csi-5*::*lacZ*.

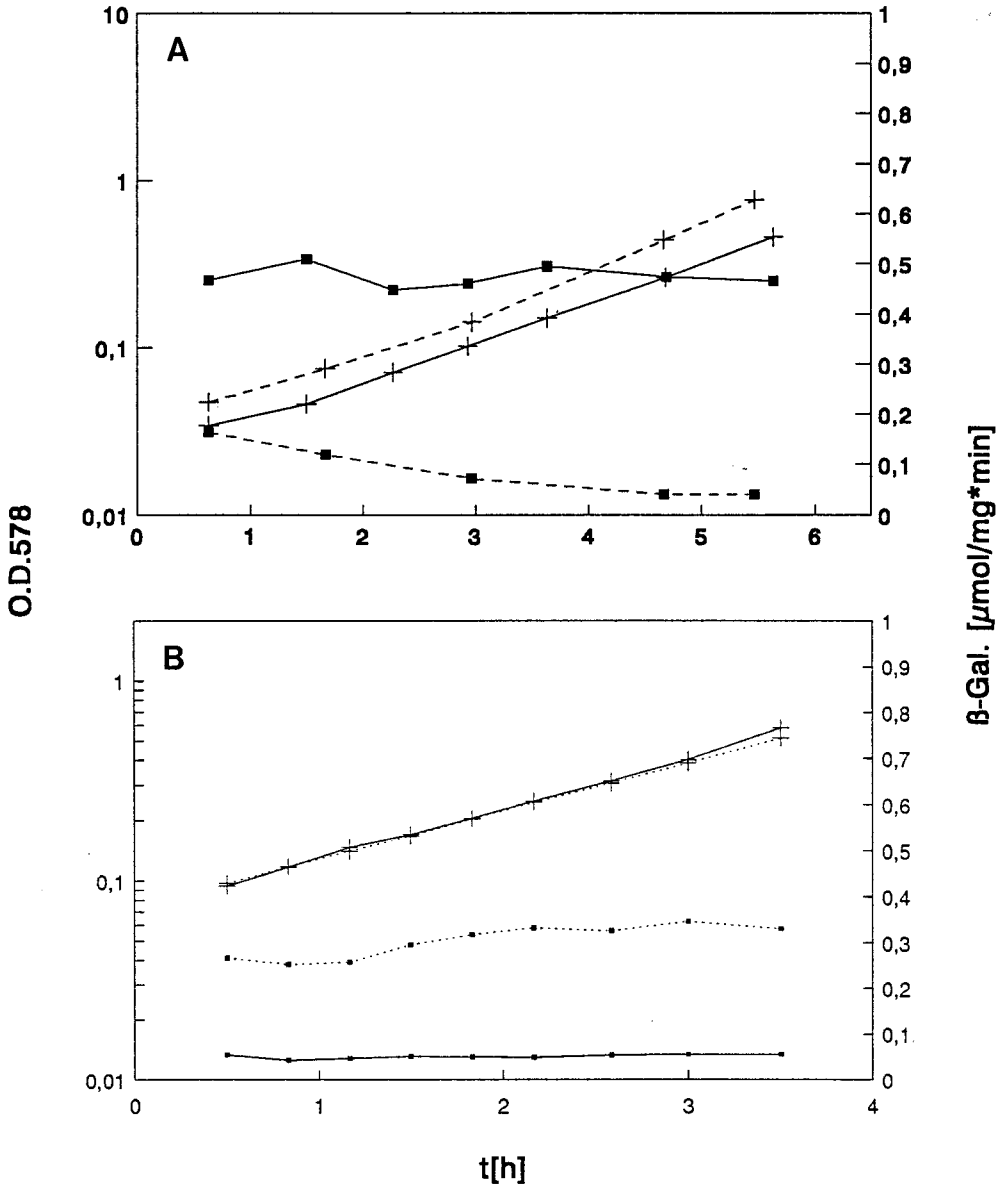

FIG. 5. Effect of mutations in *galU* (A) and *mdoA* (B) on the expression of *csi-5*::*lacZ*. (A) Cultures of strain RO151 (dashed lines) and its *galU* derivative JU2 (solid lines) were grown in M9 medium containing 0.4% glycerol, and specific β-galactosidase (β-Gal.) activities (squares) and OD_{578}s (crosses) were determined. (B) Cultures of strain RO151 (solid lines) and its *mdoA* derivative RH151 (dotted lines) were grown and specific β-galactosidase activities and OD_{578}s were determined, as described for the experiment shown in panel A. The cultures were grown exponentially for at least five generations before the beginning of the experiment (not shown).

From other recent studies performed in our laboratory it is now clear that *osmY* not only is under the control of σ^S (25, 34, 56) but also is regulated by multiple additional factors such as the cAMP-CRP complex, Lrp, and IHF (33). Moreover, the regulation of these factors themselves may be complex, as recently demonstrated for σ^S, whose cellular concentration is controlled at the levels of transcription, translation, and protein stability (35). A single mutation in any one of these components or levels of control can have only a partial effect on the expression of *osmY*, and we therefore did not obtain mutations with a strong all-or-none phenotype (with the exception of *rpoS* mutations, but even these do not totally abolish the expression of *osmY*, and they even allow for some osmotic induction at a low absolute level of expression [33, 56]).

Among the mutations isolated, Tn*10*-23 had the strongest effect on *osmY* expression and therefore has been characterized in detail. Tn*10*-23 is located in *pgi*, the structural gene for PGI. This was demonstrated by genetic and physical mapping as well as by the finding that a Tn*10*-23-carrying strain had a more than 100-fold-reduced PGI activity. We have shown here that this *pgi* null mutation, like other mutations affecting glycolytic enzymes (7, 26), confers a glucose-sensitive phenotype that is probably due to the accumulation of toxic phosphorylated metabolic intermediates. A mutation in *pgi* also interferes with fermentation of lactose. This explains the white phenotype of the Tn*10*-23 mutant on lactose MacConkey plates despite increased expression of the *lacZ* fusion in *osmY*. Although they are finally converted to glucose-6-phosphate, lactose and maltose are less toxic for the mutant, probably because they enter the cell as nonphosphorylated compounds

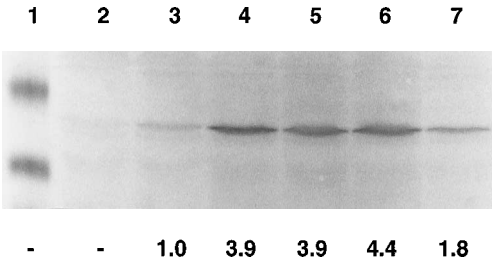

FIG. 6. Cellular σ^S content in *pgi*, *pgm*, *galU*, and *mdoA* mutants. Strains RH90 (*rpoS*::Tn*10*) (lane 2), MC4100 (lane 3), JU25 (*pgi*::Tn*10*-23) (lane 4), RH117 (*pgm-1*) (lane 5), MG4 (*galU*) (lane 6), and RH153 (*mdoA*::Tn*10*) (lane 7) were grown in M9 medium containing 0.4% glycerol. At an OD_{578} of 0.5, samples were taken for immunoblot analysis with a serum against σ^S (see Materials and Methods for details). Size standards (lane 1) have molecular masses of 49.5 and 32.5 kDa. Numbers indicate densitometric quantitation of the σ^S bands (relative to the value obtained for MC4100).

(21) that can be excreted as acetylated derivatives when present at a high intracellular concentration (4, 12). The location of Tn*10*-23 in *pgi* also explains the fact that the mutant grows almost normally on sorbitol, which is converted to fructose-6-phosphate and thus enters the glycolytic pathway below the block imposed by Tn*10*-23. In addition, our finding that Tn*10*-23 interfered with the ability to cope with increased medium osmolarity but that, on the other hand, increased osmolarity rescued the mutant from glucose sensitivity now has an explanation. Upon osmotic upshift in minimal medium, *E. coli* synthesizes trehalose as a compatible solute and an osmoprotectant (15). In a *pgi* mutant growing on glycerol, this osmotic stress reaction is not possible, since trehalose is synthesized from glucose-6-phosphate and UDP-glucose (19). However, if otherwise toxic glucose is added to the mutant growing in high-osmolarity medium, not only can trehalose accumulate but trehalose synthesis also provides a pathway to remove excess glucose-6-phosphate, since trehalose can be excreted into the medium (52).

Rather unexpectedly, our *pgi* mutant exhibited increased basal expression and reduced levels of osmotic induction of *osmY*, *otsBA*, and perhaps other σ^S-dependent genes. There is, however, a precedent for a link between a mutation in a glycolytic gene and gene expression. A temperature-sensitive mutation which at nonpermissive temperatures interferes with stable RNA synthesis and the expression of the *rrnB* gene was localized in *fda*, encoding fructose-1,6-diphosphate aldolase (50, 51). It is intriguing that this block in glycolysis reduces the expression of *rrnB*, a gene that exhibits positive growth rate control and whose expression is strongly reduced in stationary phase, whereas a second block in glycolysis (caused by the *pgi* mutation) results in increased expression of stationary phase-induced genes such as *osmY* and *otsBA*. It seems likely that the two effects are related, although the molecular details have yet to be elucidated.

A *pgi* mutant does not synthesize glucose-6-phosphate or any of its derivatives, and we therefore assumed that it might be the absence of any such compound in the cell that by an unknown mechanism derepressed the expression of *osmY* and *otsBA*. Mutations in *pgm* and *galU* (which interfere with the synthesis of glucose-1-phosphate and UDP-glucose, respectively) were found to have the same phenotype with regard to expression of *osmY* as the *pgi* mutation did. This indicated that this phenotype has something to do with the lack of UDP-glucose that is common to all three mutants under the growth conditions used in this study (see Fig. 4 for relevant metabolic pathways). In *pgi* and *pgm* mutants, the internal UDP-glucose pools can be replenished by adding sublethal amounts of glucose and galactose, respectively. Such a treatment indeed resulted in a decrease of *osmY* expression, which is consistent with our hypothesis that the lack of UDP-glucose (or perhaps a derivative thereof [see below]) is involved in the observed effects on gene expression. Alternatively, different growth rates for the mutants and the parental strain in minimal glycerol medium might account for increased expression of σ^S and therefore of *osmY* and other σ^S-dependent genes. However, such differences in growth rate are minimal for the *pgi* mutant (Table 2) and are not found at all for the *galU* mutant (Fig. 5A).

From these results the question arose of whether UDP-glucose itself is the crucial component involved in the signaling process that controls the expression of *osmY* and other σ^S-dependent genes. UDP-glucose is a precursor for the synthesis of trehalose, and it is involved in the biosyntheses of MDO (which accumulate under conditions of low osmolarity) (47), of lipopolysaccharide (directly and via UDP-galactose) (17, 53), and of the capsular constituent colanic acid (38). In principle, it could have been the lack (or incompleteness) of any of these components rather than that of UDP-glucose itself that in *pgi*, *pgm*, and *galU* mutants, was responsible for the observed effects. However, *otsA* and *galEK* mutants, which are unable to synthesize trehalose and UDP-galactose, respectively, did not exhibit elevated expression of *osmY* during steady-state growth. This excludes trehalose and UDP-galactose as signal compounds. However, we also observed that the *otsA* mutant exhibited less osmotic induction of *osmY*, which implicates the process of conversion of UDP-glucose into trehalose in an osmotic signaling mechanism. In minimal medium an increase in osmolarity results in a massive synthesis of the osmoprotectant trehalose. This is due to an activation of trehalose-6-phosphate synthase (encoded by *otsA*) that may be mediated by the increased cellular concentration of K^+ (19). One may speculate that as a consequence of trehalose synthesis, the intracellular pool of UDP-glucose may be lowered, which could serve as an internal signal for high medium osmolarity. This hypothesis is consistent with preliminary data showing that an *otsA* mutant exhibits an approximately twofold-higher intracellular concentration of UDP-glucose than an isogenic wild-type strain when grown in the presence of 0.3 M NaCl (41a).

Interestingly, the *mdoA* mutant also exhibits partially increased expression of *osmY*. However, it seems unlikely that the regulatory effects described above are simply mediated by the absence of MDO, because (i) the phenotype of the *mdoA* mutant with respect to the expression of *osmY* and σ^S itself is less pronounced than that observed for the *pgi*, *pgm*, and *galU* mutants, and (ii) the pathway from UDP-glucose to trehalose seems to be involved in signaling (see above). The rate of metabolic flux through the MDO biosynthetic pathway may be dependent on the concentration of UDP-glucose as a probably limiting substrate. In addition, it is dependent on medium osmolarity (enzymatic activity in the MDO pathway is inhibited at high external osmolarities [8]). It is therefore possible that some component of this pathway has a sensory function in the signal transduction pathway that controls the expression of *osmY* and other σ^S-dependent genes.

We have also shown here that the mutants that exhibit increased expression of *osmY* also have increased cellular levels of σ^S during steady-state growth. This provides a direct explanation for the finding that the expression of *otsBA* also is stimulated in a *pgi* mutant and suggests that these mutations may pleiotropically affect the expression of other σ^S-controlled genes as well. The regulation of σ^S expression is complex and

involves transcriptional and posttranscriptional control mechanisms (35, 37, 40). In addition, the stability of σ^S is differentially controlled (35). Preliminary results indicate that in UDP-glucose-free strains, *rpoS* expression is stimulated at the posttranscriptional level (41a). Further studies to clarify the details of this novel signal transduction and regulatory pathway are in progress.

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