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Altered Growth-Rate-Dependent Regulation of 6-Phosphogluconate Dehydrogenase Level in *hisT* Mutants of *Salmonella typhimurium* and *Escherichia coli*

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In *Escherichia coli*, the level of 6-phosphogluconate dehydrogenase is directly proportional to the cellular growth rate during growth in minimal media. This contrasts with the report by Winkler et al. (M. E. Winkler, J. R. Roth, and P. E. Hartman, J. Bacteriol. 133:830–843, 1978) that the level of the enzyme in *Salmonella typhimurium* LT-2 strain SB3436 is invariant. The basis for the difference in the growth-rate-dependent regulation between the two genera was investigated. Expression of *gnd*, which encodes 6-phosphogluconate dehydrogenase, was growth rate uninducible in strain SB3436, as reported previously, but it was 1.4-fold growth rate inducible in other *S. typhimurium* LT-2 strains, e.g., SA535. Both the SB3436 and SA535 *gnd* genes were growth rate inducible in *E. coli* K-12. Moreover, the nucleotide sequences of the regulatory regions of the two *S. typhimurium* genes were identical. We concluded that a mutation unlinked to *gnd* is responsible for the altered growth rate inducibility of 6-phosphogluconate dehydrogenase in strain SB3436. Transductional analysis showed that the altered regulation is due to the presence of a mutation in *hisT*, the gene for the tRNA modification enzyme pseudouridine synthetase I. A complementation test showed that the regulatory defect conferred by the *hisT* mutation was recessive. In *E. coli*, *hisT* mutations reduced the extent of growth rate induction by the same factor as in *S. typhimurium*. The altered regulation conferred by *hisT* mutations was not simply due to their general effect of reducing the polypeptide chain elongation rate, because *miaA* mutants, which lack another tRNA modification and have a similarly reduced chain growth rate, had higher rather than lower 6-phosphogluconate dehydrogenase levels. Studies with genetic fusions suggested that *hisT* mutations lower the *gnd* mRNA level. The data also indicated that *hisT* is involved in translational control of *gnd* expression, but not the aspect mediated by the internal complementary sequence.

Growth-rate-dependent regulation alters the synthesis rate and/or the relative amount of a given protein in response to changes in growth rate (39; reviewed in reference 53). The accumulation rate of some proteins is proportional to growth rate, such that the amount of the protein relative to total protein is constant. For other proteins, the accumulation rate increases in proportion to the square of the growth rate, and thus the relative amount of these proteins increases in proportion to the growth rate. The accumulation rate for a third class of proteins is constant, with the result that the level of these proteins is inversely proportional to the growth rate. The mechanism for this regulation is not yet completely understood for any gene (for reviews, see references 17, 24, and 26).

The *gnd* genes of *Escherichia coli* K-12 and *Salmonella typhimurium* LT-2 are among the few nonribosomal genes whose growth-rate-dependent regulation is currently under investigation. The *gnd* gene encodes 6-phosphogluconate dehydrogenase (6PGD; EC 1.1.1.44), an enzyme of the hexose monophosphate shunt. The relative amount of 6PGD increases approximately threefold over the fivefold range of growth rates obtained with cells growing in minimal medium on acetate and on glucose, and the level does not increase further when cells are growing faster (55). Because the relative amount of β-galactosidase is constant in strains carrying operon fusions of the *E. coli* K-12 *gnd* and *lacZ* genes (6), the accumulation rate of *gnd* mRNA varies in proportion to growth rate, and so does translational efficiency. In other words, growth-rate-dependent regulation of 6PGD expression in *E. coli* K-12 is subject to translational control as well as to control of either *gnd* transcription or mRNA stability. Regulation of translational efficiency requires a negative control site that lies in the 6PGD-coding sequence (7; P. Carter-Muenchau and R. E. Wolf, Jr., manuscript in preparation). Recently, the internal regulatory region has been shown to be the segment at codons 69 to 74 of the *gnd* structural gene (16). The region is complementary to the ribosome-binding site of *gnd* and includes the complement of the Shine-Dalgarno sequence. Evidence has been presented that suggests that this internal complementary sequence (ICS) mediates the translational control by sequestering the ribosome-binding site into a secondary structure and therefore functions as a *cis*-acting antisense RNA (16). The ICS and its ability to sequester the ribosome-binding site into a secondary structure are conserved in the *gnd* genes of *E. coli* B/r and four other *E. coli* strains from natural populations, all of which are growth rate inducible (8, 9). Although a mechanism has been proposed for the growth-rate-dependent regulation of translational efficiency (16), the effector of this regulation is unknown. Moreover, essentially nothing is known of the mechanism that regulates the growth rate dependence of the accumulation rate of *gnd* mRNA.

In studies of the growth-rate-dependent regulation of the histidine operon in *S. typhimurium* LT-2 strain SB3436, Winkler et al. used *gnd* as a control and found that the level of 6PGD does not vary with growth rate (51). This was in

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accord with the preconceptions of the day about expression of "constitutive" enzymes. However, Wolf et al. subsequently showed that the 6PGD level in *E. coli* K-12 increases in proportion to growth rate during growth in minimal media (55). The work described here followed up on this discrepancy, because it offered the possibility of opening a new route toward characterizing the mechanisms of growth-rate-dependent regulation of 6PGD level. For example, the *S. typhimurium* and *E. coli gnd* genes might differ in the nucleotide sequence of a regulatory site, in which case determining the responsible nucleotide differences might identify a site for either type of regulation. Alternatively, *S. typhimurium* SB3436 might be defective in a component of a system involved with the capacity to carry out growth-rate-dependent regulation of 6PGD level; in this case, understanding the basis for the defect might shed light on the mechanism of either type of regulation or on the nature of an effector of regulation.

In this paper we confirm the observation of Winkler et al. (51) that the level of 6PGD is invariant in strain SB3436, but we show that the level increases with increasing growth rate in other *S. typhimurium* LT-2 strains. By cloning the *gnd* gene of strain SB3436 into phage λ we show that expression of this gene in *E. coli* K-12 is as growth rate inducible as the *E. coli* K-12 gene itself. Moreover, the nucleotide sequence of the regulatory region of the SB3436 gene is identical to that of the gene from a growth-rate-inducible *S. typhimurium* strain. Thus, a mutation unlinked to *gnd* is responsible for the altered regulation.

The most obvious difference between strain SB3436 and the strains in which the 6PGD level is growth rate inducible is that strain SB3436 has a *hisT* mutation. *hisT* mutations were originally identified among mutants selected for having derepressed expression of the histidine operon (18). For their studies of the metabolic regulation of *his* operon expression, Winkler et al. used a *hisT* mutant because the mutation eliminated attenuation-specific control (51). *hisT* maps near 47 min on the chromosome of *S. typhimurium* (41, 42), about 5 min from *his*, and encodes pseudouridine synthetase I (PSUI), a tRNA modification enzyme that introduces pseudouridine into the anticodon loop of many tRNA species (19, 48). Although PSUI is not essential for growth, mutations in *hisT* are pleiotropic, with effects on growth rate (14, 25), polypeptide chain elongation rate (37), amino acid analog resistance (48), and derepression of amino acid biosynthetic operons (20, 25). *hisT* has been cloned, sequenced, and found to be part of a complex multigene operon (3, 4, 29, 36).

Here we demonstrate that the altered growth-rate-dependent regulation of 6PGD level in *S. typhimurium* SB3436 is, indeed, due to its *hisT* mutation and that *E. coli hisT* mutations have a similar effect on regulation. By physiological and genetic experiments we show that the altered regulation is not a trivial consequence of several of the known pleiotropic effects of *hisT* mutations. With *gnd-lac* fusions we investigated the step in *gnd* expression that is affected by *hisT* mutations. Finally, we report that the level of 6PGD is elevated in *S. typhimurium* and *E. coli miaA* mutants, which lack tRNA Δ^2-isopentenylpyrophosphate transferase, the enzyme that catalyzes the first step in the modification of the adenosine residue adjacent to the anticodon of several tRNA species (10, 13).

MATERIALS AND METHODS

Media and growth conditions. Physiological experiments were carried out by using MOPS (morpholinepropanesulfonic acid)-minimal medium supplemented with acetate and glucose (55). Auxotrophic requirements of various strains were met by appropriate concentrations of the required nutrients (21, 33). Where indicated, the media for the growth of *hisT* mutants were supplemented with 0.4 mM adenine, 0.4 mM isoleucine, and 80 μg of uracil per ml (14). Growth rates are expressed as the specific growth rate constant ($k = \ln 2/\mu$). The following nutrient media were used: LB broth (31) for routine subculture; TB broth and top agar (47) for the propagation of strains during manipulations with bacteriophage λ; YT agar plates and broth (30) for the isolation, cloning, and propagation of M13 phage and M13 DNA; TBYCM broth and MC buffer (31) for the preparation of phage P1 lysates from *E. coli*; P22 broth (21) for the preparation of phage P22 lysates from *S. typhimurium*; and F-top and H-top agars (31) for generalized transductions. Antibiotics included ampicillin (50 μg/ml), chloramphenicol (15 μg/ml for selection of P1 *cml clr-100* lysogens), and tetracycline (25 μg/ml). Minimal medium 63 (31) with appropriate supplements was used for genetic selections. Strains lysogenic for bacteriophage P1 *cml clr-100* or for λ NF1955 and its derivatives were cultured at 30°C. All other strains were grown at 37°C.

Bacterial strains. Table 1 shows the *S. typhimurium* LT-2 and the *E. coli* K-12 strains used in this study.

Lysogens carying the λ *gnd* phages integrated at *att*λ were prepared with strain GB1815, which contained deletions of the lactose operon and *gnd* to prevent recombination between regions of sequence homology on the phage and the bacterial chromosome. The strain also carried *supF* to permit the propagation of the λ phages, which were derived from phage λ NF1955 (47). Strain GB1815 was prepared as follows. Strain RW181, which contains a large deletion of the *gnd* locus and amber mutations in *lacZ* and *trpA*, was transduced to Lac^+ Trp^+ on lactose-minimal medium with a phage P1 generalized transducing lysate prepared on the amber-suppressor-containing strain LE392. Transductants were scored for SupF by their ability to support the formation of plaques of λ NF1955. The lactose operon of one such transductant, GB1811, was deleted by cotransduction of the mutation Δ(*argF-lac*)*U169* with the *proC*::Tn*10* mutation of strain SG47. Tetracycline-resistant transductants were selected and scored for Lac^- Pro^-. The *proC*::Tn*10* mutation of one of these transductants, strain GB1814, was repaired by transduction to growth on glucose minimal medium with a P1 lysate prepared on strain RW181. Strain GB1815 was a Pro^+, tetracycline-sensitive transductant that remained Lac^-.

Strain EF2 was prepared by specialized transduction of strain EF1 to His^+ Gnd^+ with φ80 d*his gnd* phage isolated and purified from strain XX30 as described previously (54).

S. typhimurium hisT alleles were transduced into other *S. typhimurium* strain backgrounds with phage P22. $HisT^+$ and $HisT^-$ cells were distinguished by the morphology of colonies growing on minimal agar plates with 2% glucose, where wild-type colonies are smooth and *hisT* mutants are rough (18). The phenotypes were verified by assaying the activity of histidinol dehydrogenase (27), which is derepressed in *hisT* mutants (18, 41). In every case, cells that produced rough colonies had histidinol dehydrogenase levels about fivefold higher than those of cells that produced smooth colonies.

A P22 lysate of strain TT317 was used to introduce the *hisT*-linked selectable marker *purF*::Tn*10* into strain SB3436 by infecting it with the lysate and selecting for tetracycline-resistant (Tc^r) transductants. A lysate of a *hisT* Tc^r trans-

TABLE 1. Bacterial strains

Strain	Genotype or phenotype[a]	Source or reference
S. typhimurium LT-2		
ara-9	Prototroph	P. E. Hartman (51)
GT522	Prototrophic isogen to GT523	G. R. Björk (22)
GT523	*miaA1*	G. R. Björk (22)
hisT1504	*hisT1504*	P. E. Hartman (18)
LB5010	r_{LT}^- m_{LT}^+; r_{SA}^- m_{SA}^+; r_{SB}^- m_{SB}^+	L. B. Bullas (15)
SA535	HfrK5 *serA13*	M. J. Voll (50)
SB3436	*hisT1504*	P. E. Hartman (51)
SB3436(pψ300)	*hisT1504* (*hisT*$^+$)	SB3436 with pψ300
TR36	*hisT1529*	J. R. Roth (42)
TT317	*purF1741*::Tn*10*	J. R. Roth (42)
TT5866	*hisT290*::Tn*5*	J. R. Roth (42)
WJ167	SB3436 [*purF1741*::Tn*10*][b]	P22 (TT317) × SB3436
E. coli K-12[c]		
EF1	F$^-$ Δ(*sbcB-his-gnd-rfb*)	This laboratory
EF2	EF1(ϕ80 d*his gnd*)	ϕ80 d*his gnd* × EF1
GB1811	RW181 *supF58*	This study
GB1814	GB1811 Δ(*argF-lac*)*U169 proC*::Tn*10*	This study
GB1815	GB1814*proC*$^+$	This study
GB1816	GB1815(λ *gndK1*)	This study
GB1817	GB1815(λ *gndK6*)	This study
GB1818	GB1815(λ *gndS3*)	This study
GB1819	GB1815(λ *gndS4*)	This study
GB23152	RW231 Ca^r *hsdR*	8
GB23152(pGB3436E)	GB23152(pGB3436E)	8
GB23152(pGB535E)	GB23152(pGB535E)	This study
HB354	W3110 Δ(*argF-lac*)*U169 gnd-128*::ΔMu cts d1(Ap^r Lac)::λp1(209) (Lac$^+$)	6
HB545	W3110 Δ(*argF-lac*)*U169 gnd-48*::ΔMu cts dII(Ap^r Lac)::λp1(209) (Lac$^+$) (Hyb)	7
HB552	W3110 Δ(*argF-lac*)*U169 gnd-118*::ΔMu cts dII(Ap^r Lac)::λp1(209) (Lac$^+$) (Hyb)	7
JK334	*fadL*::Tn*10 hisT76*	J. Parker (38)
JM109	*recA1 endA1 gyrA96 thi hsdR17 supE44 relA1* Δ(*lac-proAB*) (F′ *traD36 proA*$^+$*B*$^+$ *lacI*^{q} Δ(*lacZ*)M15)	S. Bingham
KK2186	Δ(*lac-pro*) *supE thi endA sbcB15 rpsL20 hsdR4* (F′ *traD36 proA*$^+$*B*$^+$ *lacI*^{q} Δ(*lacZ*)M15)	O. Karlström
LE392	F$^-$ Δ*hsdR14 supE44 supF58 lacY1* or Δ(*lacIYZ*)*6 galK2 galT22 metB1 trpR55*	L. W. Bergman
MMS345	λ *cos2* Δ*SR1-2 red3* γam210 cIts857 *nin5 S*am7	M. Stahl (40)
NU57	*hisT76*(pψ300)	M. E. Winkler (4)
NU426	W3110 prototroph	M. E. Winkler (4)
NU611	NU426 [*hisT*::Km^r]	M. E. Winkler (4)
RW181	F$^-$ *trpR lacZ*(Am) *trpA9605*(Am) *kdgR*^{c} Δ(*edd-zwf*)*22* Δ(*sbcB-his-gnd-rfb*)	52
RW181(pMN3)	RW181(pMN3)	32
RW231Ca	RW181 *recA rpsL20* Ca^r	8
SG47	F$^-$ *araD139* Δ(*argF-lac*)U169 *flbB ptsF relA rpsL thi deoC proC*::Tn*10*	M. L. Berman
W3110	F$^-$ prototroph	This laboratory
W3110-16	Isogenic with W3110-18 but *miaA*	C. Yanofsky
W3110-18	*trpR tna* [Tn*10*]	C. Yanofsky
WJ300	W3110 [*fadL*::Tn*10*]	P1 (JK334) × W3110
WJ301	W3110 [*fadL*::Tn*10 hisT*]	P1 (JK334) × W3110
WJ320	HB545 [*fadL*::Tn*10*]	P1 (JK334) × HB545
WJ321	HB545 [*fadL*::Tn*10 hisT*]	P1 (JK334) × HB545
WJ324	HB354 [*fadL*::Tn*10*]	P1 (JK334) × HB354
WJ325	HB354 [*fadL*::Tn*10 hisT*]	P1 (JK334) × HB354
WJ328	HB552 [*fadL*::Tn*10*]	P1 (JK334) × HB552
WJ329	HB552 [*fadL*::Tn*10 hisT*]	P1 (JK334) × HB552
XX30	Δ(*his-gnd*) *thi* Δ(*lac-pro*)	50

[a] The notations used are those of Bachmann (5). Ca^r, Calcium resistant.
[b] Brackets indicate that a gene was introduced by transduction.
[c] All strains are *E. coli* K-12 except MMS345, which is *E. coli* C.

ductant, strain WJ167, was subsequently used to introduce the *hisT* mutant allele into the *S. typhimurium* LT-2 prototroph ara-9 by selecting for Tc^r transductants and scoring for HisT. In a reciprocal transduction, a phage stock prepared on strain ara-9 was used to replace the *hisT* mutant allele in strain WJ167 with a *hisT*$^+$ allele by selecting for adenine independence and scoring for HisT. For the complementation test, plasmid DNA carrying the *E. coli hisT* operon was isolated by the alkaline lysis method (12), transformed into the restriction-deficient and modification-proficient *S. typhimurium* LT-2 strain LB5010 (15) in the presence of 100 mM calcium chloride (21), and then reisolated and transformed into *S. typhimurium* LT-2 strain SB3436 by the same methods.

A *hisT* mutation was introduced into the *E. coli* W3110 background by using a phage P1 lysate of strain JK334, which contains the *hisT*-linked selectable marker *fadL*::Tn*10*.

Bacteriophage. Lysates of phage λ NF1955 (47) were prepared by lytic infection of strain LE392 (28). Lysates of the λ *gnd* phages were prepared by heat induction and specialized transductions performed as described previously (31). Lysates of λ *cI90 c17* were prepared by the plate method (31). Monolysogens carrying the λ *gnd* phages were identified by a plaque test with λ *cI90 c17* (45). All manipulations of bacteriophage M13 were performed as described by Messing (30), with KK2186 and JM109 as the host strains. Phage P22 HT105/1 (P22; 44) lysates were prepared, and generalized transduction was performed as described by Davis et al. (21). Phage P1 *cml clr-100* (P1) lysates were prepared by heat induction of lysogens, and P1 generalized transductions were performed as described by Miller (31).

Recombinant DNA techniques. Plasmid DNA was isolated by the alkaline lysis method (12) and further purified as described previously (8). Restriction enzymes (Bethesda Research Laboratories, Inc., Gaithersburg, Md., or New England BioLabs, Inc., Beverly, Mass.), were used according to the recommendations of the suppliers. Other standard recombinant DNA techniques were used (28).

Plasmids, DNA cloning, and DNA sequencing. To clone the *gnd* genes of *S. typhimurium* and *E. coli* K-12 into λ, DNA of λ NF1955 was isolated by the rapid method of Silhavy et al. (47), and the cohesive ends were ligated for 6 h at 16°C under conditions favoring intramolecular ligation. The DNA was cleaved at the unique *Eco*RI restriction site. The *gnd* gene of *E. coli* K-12 was obtained from plasmid pMN3 (32) as a 3.8-kilobase *Eco*RI fragment, and the *gnd* gene of *S. typhimurium* SB3436 was obtained from plasmid pGB3436 (8) as an 8.8-kilobase *Eco*RI fragment. The fragments were purified from agarose gels by the method of Benson (11) and ligated to the *Eco*RI-cleaved λ NF1955 DNA. The recombinant DNAs were packaged in vitro by the method of Rosenberg et al. (40) by using cell extracts prepared from strain MMS345. Strain GB1811 was infected with the resulting phage particles, and phage lysates were prepared by the plate method (31). Strain GB1811 was infected with the low-frequency transducing lysates, and Gnd$^+$ specialized transductants were selected on gluconate minimal medium containing histidine at 30°C. High-frequency transducing lysates were prepared by heat induction of the transductants. To verify the presence of the correct *gnd* gene and to determine the orientation of the gene in the respective λ *gnd* phages, DNA was isolated from the lysates and subjected to restriction analysis. Phages λ *gnd*K1 and λ *gnd*K6 carry the *E. coli* K-12 gene in the two possible orientations, and phages λ *gnd*S3 and λ *gnd*S4 carry the gene from *S. typhimurium* SB3436.

The 8.8-kilobase *Eco*RI fragment containing the *gnd* gene of *S. typhimurium* SA535 was cloned from chromosomal DNA into plasmid pBR322 to form plasmid pGB535 as described previously for the cloning of the gene from strain SB3436 (8). For DNA sequencing of the control region of the SA535 gene, the 760-base-pair *Hin*dIII fragment of pGB535 was cloned in both orientations into phage M13mp18 to form phages mGB535H182 and mGB535H184. The *Hin*dIII fragment extends from position −165 with respect to the start of the coding sequence to codon 200. DNA sequencing was by the chain termination method (43).

Measurements of enzyme specific activity. The activities of 6PGD and glucose 6-phosphate dehydrogenase in sonic extracts were assayed spectrophotometrically as previously described (55); specific activity is expressed as nanomoles of NADPH formed per minute per milligram of protein. Cultures in exponential growth were collected at ~10^8 cells per ml, concentrated fivefold by centrifugation, and sonically disrupted. When HisT$^+$ and HisT$^-$ transductants of *S. typhimurium* were being scored for the growth rate inducibility or uninducibility of 6PGD level, individual transductants were grown in 8 ml of MOPS-minimal medium containing a limiting concentration of glucose (12.5 mM), such that bacteria were arrested in exponential growth at ~10^9 cells per ml. Samples (1 ml) of these cultures were sonically disrupted without prior concentration and assayed for 6PGD activity by the standard method (55). The activity of histidinol dehydrogenase in sonic extracts was assayed spectrophotometrically as described by Loper and Adams (27). β-Galactosidase activity was assayed in cells permeabilized by treatment with chloroform and sodium dodecyl sulfate and is expressed in Miller units (31). All values represent the average of duplicate assays of at least two samples from each of two independent cultures and are followed by their standard deviations, expressed as the percentage of the mean value. Most of the experiments were repeated, and day to day variations were less than 10%. The growth rate induction ratio for 6PGD, glucose 6-phosphate dehydrogenase, or β-galactosidase is the specific activity of the enzyme during growth on glucose divided by the specific activity on acetate. Propagated standard deviations for the induction ratios are given as the square root of the sum of the square of the percent standard deviation of the respective specific activities.

RESULTS

Growth rate dependence of 6PGD level in *S. typhimurium*. To determine whether the growth-rate-invariant level of 6PGD in strain SB3436 was a general property of *S. typhimurium* LT-2 strains, we measured the effect of growth rate on the level of the enzyme in the parental ara-9 prototroph and in strain SA535. Strain SA535 was used because it is the strain from which a φ80 transducing phage carrying *S. typhimurium gnd*, used later in this study, was isolated (50). Table 2 shows the specific activity of 6PGD during steady-state growth for the three strains growing in acetate- and glucose-MOPS-minimal media. As reported by Winkler et al. (51), 6PGD level was growth rate invariant in strain SB3436, but it was about 1.4-fold growth rate inducible in strains ara-9 and SA535. Although the extent of the growth rate induction with strains ara-9 and SA535 was small, only about half as large as that in *E. coli* K-12 (55), it was reproducible. Moreover, whereas the level of 6PGD under slow growth conditions, with acetate as the sole carbon source, was about the same in the three *S. typhimurium* LT-2 strains, the enzyme level in cells grown on glucose was always about

TABLE 2. Growth-rate-dependent regulation of 6PGD levels in *hisT* mutants of *S. typhimurium*

Strain	*hisT* allele	Acetate		Glucose		Induction ratio ± %
		k	Sp act ± %	*k*	Sp act ± %	
ara-9	+	0.30	66 ± 8	0.89	87 ± 1	1.3 ± 8
SA535	+	0.28	63 ± 2	0.81	91 ± 7	1.4 ± 7
SB3436	−	0.20	55 ± 7	0.81	51 ± 6	0.9 ± 9
SB3436(pψ300)	−/+	0.29	41 ± 5	0.93	60 ± 7	1.5 ± 9

1.4-fold higher in strains ara-9 and SA535 than in strain SB3436. Thus, *S. typhimurium* LT-2 and *E. coli* K-12 do not differ in their capacity for growth rate induction of *gnd* expression. Rather, strain SB3436 appears to be defective in growth rate induction of 6PGD.

Growth-rate-inducible expression in *E. coli* K-12 of the *gnd* gene of strain SB3436. There are two general ways in which growth-rate-dependent regulation of 6PGD level could be defective in strain SB3436. One is that the *gnd* gene of strain SB3436 carries a mutation that prevents growth rate induction. Alternatively, strain SB3436 might carry a mutation in a gene for a *trans*-acting factor involved in growth-rate-dependent regulation of *gnd* expression or in a gene whose product is involved in sensing differences in growth rate.

To address the first possibility we cloned (as described in Materials and Methods) the *gnd* gene of strain SB3436 in both possible orientations into λ NF1955, an integration-proficient cloning vector. As a control we also prepared λ phages carrying the *E. coli* K-12 gene in both possible orientations. The effect of growth rate on the specific activity of 6PGD was determined in strains monolysogenic for the four λ *gnd* phages. In all four strains the level of the enzyme was about 1.9-fold higher in glucose-grown cells than in acetate-grown cells (data not shown). Moreover, 6PGD was also growth rate inducible in strain EF2, which is lysogenic for a ϕ80 specialized transducing phage that carries the *gnd* gene of strain SA535 (data not shown). Thus, the two *S. typhimurium gnd* genes that are regulated differently in their native backgrounds are similarly regulated in *E. coli*. Moreover, the *gnd* gene of strain SB3436 has all sequences necessary and sufficient for growth-rate-dependent regulation in *E. coli* K-12.

The growth rate induction ratio was less for the lysogens carrying the *E. coli* K-12 *gnd* gene on a λ prophage than the threefold induction obtained when *gnd* is at its normal chromosomal position (55). A similar effect of chromosomal position has also been observed recently with *gnd-lacZ* fusions (16).

Identical DNA sequence of the regulatory regions of the *gnd* genes of strains SA535 and SB3436. The fact that the *gnd* gene of strain SB3436 is as growth rate inducible in *E. coli* K-12 as the native *E. coli* gene suggested that the growth rate uninducibility of the gene in *S. typhimurium* SB3436 was due to a mutation located elsewhere in the genome and not to a mutation in a *gnd* regulatory site. To confirm this conclusion, we cloned the *gnd* gene of strain SA535 as described in Materials and Methods, determined the nucleotide sequence of the regulatory regions, and compared the sequence to that of the gene from strain SB3436. The DNA sequence was identical to the sequence previously determined for the *gnd* gene of strain SB3436 (data not shown) (9). Therefore, the altered growth rate inducibility of 6PGD in strain SB3436 is not due to a regulatory mutation in *gnd* but rather to a mutation located elsewhere. The most apparent candidate was the *hisT* mutation.

Growth rate invariance of 6PGD level in *hisT* mutants of *S. typhimurium*. To determine whether the apparent growth rate uninducibility of strain SB3436 is due to its *hisT* mutation, the levels of 6PGD during growth on glucose and acetate were determined in strain hisT1504, which is the parent of strain SB3436, and in strains TR36 and TT5866, which contain different *hisT* mutations. The level of 6PGD was growth rate invariant in these *hisT* mutants, just as it was in strain SB3436 (data not shown). Thus, the growth-rate-invariant 6PGD level is a general property of *S. typhimurium hisT* mutants.

***trans* complementation of altered regulation in strain SB3436 by a *hisT*$^+$ plasmid.** A plasmid carrying the entire *E. coli hisT* operon, pψ300, was transformed into strain LB5010 and subsequently into strain SB3436 by selection for ampicillin resistance (Ap^r). The plasmid complemented the *his* operon regulatory defect of the strain, in that strain SB3436(pψ300) formed smooth colonies on 2% glucose plates and had repressed levels of histidinol dehydrogenase. The level of 6PGD was growth rate inducible in this transformant (Table 2) but not in a transformant carrying a control plasmid derived from pψ300 that contained only part of the *hisT* gene (data not shown). These results show that the growth-rate-uninducible expression of 6PGD in strain SB3436 is recessive to the wild-type *hisT* allele. Moreover, they also rule out the possibility that a mutation unlinked to *hisT*, putatively present as a compensatory mutation in all *hisT* mutants, is solely responsible for the regulatory defect.

Cotransduction of *hisT* mutation and growth rate invariance of 6PGD level. Because *hisT* mutations are highly pleiotropic, we next considered the possibility (similar to the one above) that the altered regulation of 6PGD level is due to two mutations, one in *hisT* and the other a compensatory mutation unlinked to *hisT* that is present in all *hisT* mutants. We tested this possibility by examining the growth rate dependence of 6PGD level in two pairs of isogenic strains made by reciprocal transduction crosses. The strain constructions were facilitated by the availability of the closely linked marker *purF*::Tn*10*. In one cross, the *hisT* mutation of strain WJ167 was replaced with the *hisT*$^+$ allele of strain ara-9. This was accomplished by using a phage P22 lysate prepared on strain ara-9 to transduce strain WJ167, a *purF*::Tn*10* derivative of strain SB3436, to adenine independence and scoring for HisT$^+$. In the other cross, the *hisT* mutation of strain WJ167 was transduced into the wild-type genetic background of strain ara-9 by selecting Tc^r transductants and scoring for HisT$^-$. A HisT$^+$ and a HisT$^-$ transductant were chosen from each cross, and the growth rate dependence of the 6PGD level was determined for each pair.

Growth rate induction was regained when the *hisT* mutant was transduced to *hisT*$^+$, and growth rate uninducibility was obtained when a *hisT* mutation was transduced into the wild-type genetic background (data not shown). From these results we conclude that the altered regulation is due solely to a mutation in the *hisT* region of the chromosome, presumably in *hisT* itself. Moreover, the altered regulation does not depend on the presence of a compensatory mutation unlinked to *hisT*. These conclusions were confirmed by construction of additional strains, where the *hisT1529* and *hisT290*::Tn*5* mutations were introduced by transduction into strain ara-9, and by transduction of strain TR36 to *hisT*$^+$. Again, the 6PGD level was growth rate independent in the *hisT* mutants and growth rate dependent in the *hisT*$^+$ strain (data not shown).

Despite these results, it was still possible that the altered growth rate inducibility of 6PGD was not due solely to a mutation in *hisT* but rather to a second mutation closely linked to *hisT*, either alone or in combination with it. Testing this possibility required a rapid method of screening *hisT*$^+$ and *hisT* mutant transductants for growth-rate-inducible and -uninducible phenotypes of *gnd*. This was accomplished by assaying 6PGD activity in cultures of transductants growing on glucose, since under these conditions the enzyme level of a *hisT* mutant is always lower than that of a *hisT*$^+$ strain (Table 2). Cells were grown in minimal medium containing a limiting concentration of glucose, which arrests cells in exponential growth at a density high enough for assay of

TABLE 3. Growth-rate-dependent regulation of 6PGD levels in *hisT* mutants of *E. coli*

Strain	*hisT* allele	Acetate		Glucose		Induction ratio ± %
		k	Sp act ± %	*k*	Sp act ± %	
WJ300	+	0.26	42 ± 2	0.79	114 ± 4	2.7 ± 5
WJ301	−	0.18	50 ± 4	0.68	98 ± 2	2.0 ± 4
NU426	+	0.14	59 ± 7	0.69	167 ± 1	2.8 ± 7
NU611	−	0.09	60 ± 3	0.46	134 ± 2	2.2 ± 4

enzyme activity without prior concentration of the cultures. Control experiments with strains ara-9 and SB3436 showed that the difference in enzyme level obtained with the two strains in exponential growth was also evident for glucose-limited cultures.

Thirty-two isolated transductants from each of the reciprocal crosses between strains ara-9 and WJ167 were scored for colony morphology on 2% glucose plates, grown in small volumes, and assayed for 6PGD level. The results demonstrated 100% linkage of $hisT^+$ and inducibility and of $hisT^-$ and uninducibility (data not shown). The conclusion drawn from these experiments is that altered growth-rate-dependent regulation of *gnd* expression in strain SB3436 is due to the *hisT* mutation, not to some other mutation in the strain background. Moreover, the *hisT* mutation alone is sufficient for the growth rate uninducibility.

Growth rate dependence of 6PGD level in *E. coli hisT* mutants. Because of the availability of operon and protein fusions of *gnd* to *lacZ* (6, 7), further analysis of *hisT* effects on growth-rate-dependent regulation was done in *E. coli*. Moreover, the magnitude of growth rate induction of 6PGD level is greater in *E. coli* K-12 than it is in *S. typhimurium* LT-2 (2.8-fold versus 1.4-fold). Accordingly, the growth rate dependence of the 6PGD level was determined in two isogenic pairs of strains. One *hisT* mutant, WJ301, carried the *hisT76* allele, whereas the other, NU611, carried an insertion mutation. The induction ratio was about 2.0 for the *hisT* mutants and about 2.8 for the respective $hisT^+$ strains (Table 3). Thus, the induction ratio of the *E. coli* wild-type strains is about 1.4-fold higher than that of the *hisT* mutant, a difference of the same magnitude as that seen when comparing *S. typhimurium* $hisT^+$ and *hisT* strains (Table 2). This indicates that *E. coli hisT* mutations reduce the extent of growth rate induction by one-third, just as *S. typhimurium* mutations. In other words, reduced expression of 6PGD during growth on glucose is common to *S. typhimurium* and *E. coli hisT* mutants.

Pleiotropic effects of *hisT* mutations. Bruni et al. reported that the *E. coli hisT* mutant FB105 grows at half the rate of the wild type on glucose (14). Under our growth conditions, the mutant grew on glucose at about the same rate as the wild type and on acetate at about 75% of the wild-type rate. They also reported that the growth rate in glucose-minimal medium is enhanced to the wild-type rate by supplementation with adenine, isoleucine, and uracil. The same effect was observed when strains WJ300 and WJ301 were grown in acetate- and glucose-minimal media containing these supplements. However, when $hisT^+$ and *hisT* strains were growing at the same rate on acetate and almost the same rate on glucose, the induction ratio of the *hisT* mutant was significantly lower than that of the wild type (data not shown). To minimize the difference in growth rates on acetate between $hisT^+$ and *hisT* strains, all subsequent experiments involving *E. coli hisT* mutants were performed in the presence of these supplements. In any event, the growth rate differences we observed between $hisT^+$ and *hisT* strains were not large enough to account for the differences in 6PGD levels.

TABLE 4. Growth-rate-dependent regulation of 6PGD levels in *miaA* mutants of *S. typhimurium* and *E. coli*

Strain	*miaA* allele	Acetate		Glucose		Induction ratio ± %
		k	Sp act ± %	*k*	Sp act ± %	
S. typhimurium						
GT522	+	0.34	83 ± 5	0.92	110 ± 3	1.3 ± 6
GT523	−	0.25	162 ± 7	0.71	201 ± 7	1.2 ± 10
E. coli						
W3110-18	+	0.14	65 ± 5	0.57	158 ± 4	2.4 ± 6
W3110-16	−	0.10	133 ± 8	0.55	259 ± 1	1.9 ± 8

To test the possibility that the reduced growth rate induction is due to the 20 to 25% reduction in polypeptide chain elongation rate caused by *hisT* mutations (37), the growth rate dependence of 6PGD level was examined in *miaA* mutants, which are defective in another tRNA modification enzyme and have a polypeptide chain elongation rate that is approximately 30% lower than that of the wild type (22). Table 4 shows the effect of *miaA* mutations in *S. typhimurium* and *E. coli* on growth rate induction of 6PGD level. Unlike *hisT* mutations, the *miaA* mutations did not reduce 6PGD levels; rather they increased them. Thus, the reduced extent of growth rate induction in *hisT* mutants is not an indirect effect of decreased polypeptide chain growth rate, nor is it due to a general deficiency in tRNA modification.

Effects of *hisT* and *miaA* mutations on *zwf* expression. To determine whether *hisT* mutations exert a general effect on growth-rate-dependent regulation of central metabolism genes, we examined the expression of glucose 6-phosphate dehydrogenase in a *hisT* mutant. This enzyme, encoded by *zwf*, is also an NADP-dependent dehydrogenase of the hexose monophosphate shunt, and growth-rate-dependent regulation of its level is similar to that of 6PGD in steady-state growth and during nutritional upshifts (23, 55). However, growth rate induction of glucose 6-phosphate dehydrogenase in *E. coli* was unaffected by a *hisT* mutation (data not shown). Also, a *miaA* mutation had no effect on glucose 6-phosphate dehydrogenase expression (data not shown). Thus, the effects of *hisT* and *miaA* mutations on growth-rate-dependent regulation are not common to central metabolism genes.

Effect of *hisT* mutation on β-galactosidase levels in operon fusions. It is now well established that measurement of β-galactosidase activity in strains carrying operon fusions of a target gene to the *lac* operon can be used as an indirect assay of the effect of a variety of physiological and genetic conditions on the mRNA level of the target gene (46). Thus, to determine whether the effect of *hisT* mutations on growth rate inducibility of 6PGD is due to an effect on *gnd* mRNA level, we introduced a *hisT* mutation by transduction into an *E. coli gnd-lac* operon fusion strain. If *hisT* mutations affect the relative amount of *gnd* mRNA, the level of β-galactosidase during growth on glucose should be reduced by the same factor as 6PGD level is in a normal strain. On the other hand, if *hisT* mutations affect the translational efficiency of *gnd* mRNA, the β-galactosidase level should be unaffected. The level of β-galactosidase in the $hisT^+$ control strain, WJ324, did not increase with increasing growth rate (Table 5), as expected (6). The growth rate induction ratio for

TABLE 5. Growth-rate-dependent regulation of β-galactosidase levels in *hisT* mutants of *gnd-lac E. coli* fusion strains[a]

Strain	ICS[b]	*hisT* allele	Acetate		Glucose		Induction ratio ± %
			k	β-gal[c] ± %	*k*	β-gal ± %	
WJ324	NA	+	0.33	623 ± 3	0.92	460 ± 2	0.7 ± 4
WJ325	NA	−	0.15	414 ± 4	0.69	343 ± 2	0.8 ± 2
WJ328	+	+	0.16	554 ± 2	0.69	1,496 ± 3	2.7 ± 4
WJ329	+	−	0.16	618 ± 1	0.67	1,269 ± 1	2.0 ± 1
WJ320	−	+	0.17	1,859 ± 1	0.69	2,456 ± 3	1.3 ± 3
WJ321	−	−	0.18	2,067 ± 3	0.65	2,006 ± 4	1.0 ± 5

[a] Grown in the presence of adenine, isoleucine, and uracil.
[b] Presence (+) or absence (−) of the ICS regulatory element in the fusion. NA, Not applicable, because the strains carry operon fusions and the ICS has no effect on expression of *gnd-lac* operon fusions (6; Carter-Muenchau and Wolf, in preparation).
[c] β-Galactosidase (β-gal) activity is expressed in Miller units (MU; 31).

β-galactosidase in the *hisT* mutant operon fusion strain, WJ325, was the same as that of its isogenic partner, but the level of the enzyme was about one-third lower during growth on both glucose and acetate.

Interaction between *hisT* and the internal regulatory sequence of *gnd*. The ICS is a negative control element lying within the *gnd* structural gene that is necessary for growth-rate-dependent regulation of 6PGD level (7). It is highly complementary to the ribosome-binding site of *gnd* mRNA and is proposed to mediate regulation of *gnd* translational efficiency by forming an mRNA secondary structure that sequesters the Shine-Dalgarno sequence (16). In searching for a possible role of PSUI in *gnd* regulation we noticed that the core of the ICS, which contains the anti-Shine-Dalgarno sequence, matches six of seven bases at positions 40 to 46 in $tRNA^{His}$, the region modified by PSUI in many tRNAs (2). Moreover, the putative secondary structure of this segment of *gnd* mRNA (16) is similar overall to the PSUI-modified segment of tRNA, with a region of base pairing adjacent to unpaired bases. These observations suggested that PSUI might be directly involved in regulating *gnd* expression by binding to and unwinding the ICS structure. Indeed, Ames et al. have proposed that enzymes that bind tRNA, in particular modification enzymes, might play a regulatory role by binding to secondary structures in mRNA similar to those in tRNA (1). The recently demonstrated translational autoregulation of threonyl-tRNA synthetase expression lends support to this general idea (49).

To test the hypothesis, we determined the epistatic relationship between *hisT* and the ICS with strains carrying *gnd-lacZ* protein fusions (Table 5). A *hisT* mutation was introduced by transduction into strain HB552, which has an intact internal regulatory region, and into isogenic strain HB545, which contains an ICS^- protein fusion. In the control strain with an ICS^+ protein fusion, the *hisT* mutation reduced the extent of growth rate induction of β-galactosidase (Table 5, lines 3 and 4) by the same factor as it reduced induction of 6PGD expression from *gnd* itself (Table 3). The *hisT* mutation had the same effect on expression in the ICS^- fusion strain (Table 5, lines 5 and 6). Thus, the *hisT* mutation is epistatic to the absence of the ICS.

DISCUSSION

The growth rate invariance of 6PGD level observed by Winkler et al. (51) with *S. typhimurium* LT-2 strain SB3436 was the result expected at the time for a so-called constitutive gene. We had not yet reported that the level of 6PGD in *E. coli* K-12 is directly proportional to the cellular growth rate during growth in minimal media (55). In the present work we showed that the discrepancy was not due to a general difference between the two genera nor to a *gnd* regulatory mutation. We pursued the regulatory defect in strain SB3436 because few mutations unlinked to a growth rate regulated gene are known which affect the growth-rate-dependent regulation of the gene. In fact, aside from guanosine 3′-diphosphate, 5′-diphosphate (17), and as yet incompletely characterized mRNases (35), the effectors of growth-rate-dependent regulation of nonribosomal genes are unknown. Thus, identifying and characterizing the mutation in *S. typhimurium* SB3436 responsible for the altered growth rate regulation of 6PGD level was important because understanding the basis for the defect might provide insight into the mechanism of *gnd* regulation.

Transductional analyses and a complementation test showed conclusively that the altered regulation of strain SB3436 is due solely to its *hisT* mutation, which is recessive. Moreover, regulation was similarly affected by two other *S. typhimurium hisT* mutations, including an insertion mutation. Although *hisT* is the third gene of a complex four-gene operon (4, 29), it is unlikely that the altered regulation is due to deficiency of the product of the downstream gene, because neither the *hisT1504* nor the *hisT1529* mutation used in this study is suppressed by nonsense suppressors (18), and *hisT* does not appear to be translationally coupled to the downstream gene (4).

The availability of *gnd-lacZ* fusions prompted us to determine the growth rate dependence of 6PGD level in *E. coli hisT* mutants. Also, with the growth rate induction ratio of wild-type *E. coli* being about twice that of wild-type *S. typhimurium*, we anticipated a quantitatively larger effect of the *E. coli hisT* mutations. However, the *E. coli hisT* mutations decreased the induction ratio from about 3 in the wild-type strain to about 2 in the mutant, an effect of the same magnitude as the decrease in ratio from 1.4 to 1.0 brought about by the *S. typhimurium* mutations. Thus, rather than having an all-or-none effect, i.e., conferring total growth rate uninducibility, the *hisT* mutations of both *S. typhimurium* and *E. coli* may only reduce the extent of induction by about one-third.

The possibility that the altered regulation might be due to the decrease in polypeptide chain elongation rate brought about by *hisT* mutations (37) was considered, especially because uncoupling of transcription and translation has been proposed as an essential step in regulating the translational efficiency of *gnd* mRNA (16). However, this is not the case, since the level of 6PGD is higher in *miaA* mutants, whose chain growth rate is also about 25% lower than that of the wild type (22). Also, since growth-rate-dependent regulation of the level of another hexose monophosphate shunt enzyme, glucose 6-phosphate dehydrogenase, is normal in *hisT* and *miaA* mutants, deficiency in tRNA modification enzymes does not have a global effect on expression of central metabolism genes.

Operon and protein fusions were used as a way of determining whether *hisT* mutations affect regulation of *gnd* mRNA level or translational efficiency and in particular whether ICS function is altered. The *hisT* mutation had the same effect on the growth rate dependence of β-galactosidase level from both the ICS^- and the ICS^+ protein fusions, showing that the mutation does not act on the known regulatory element of translational efficiency. Thus, the similarity described above between the sequence of a por-

tion of the ICS and a segment of $tRNA^{His}$ that includes the site for PSUI modification has no functional significance.

Interpretation of the results obtained with the operon fusion strains is more complex (Table 5). The *hisT76* mutation did not change the growth rate induction ratio for β-galactosidase in the operon fusion strain, but the overall level of the enzyme was about one-third lower in both acetate- and glucose-grown cells. This indicates that *hisT* mutations reduce the level of *gnd* mRNA and hence its accumulation rate by the same one-third factor and that this reduction is growth rate independent. Thus the data argue that $hisT^+$ has a positive effect on *gnd* mRNA level. The effect could be on the rate of *gnd* transcription or on the decay rate of *gnd* mRNA.

The operon fusion data also indicate that $hisT^+$ has a negative effect on translational efficiency under slow growth conditions. During growth on acetate *hisT* mutations have no effect on the level of 6PGD (Tables 2 and 3), but *hisT* mutation reduces β-galactosidase level in an operon fusion strain by one-third. In other words, during growth on acetate the reduction in *gnd* mRNA level brought about by the *hisT* mutation is offset by increased translational efficiency, whereas during growth on glucose only the *gnd* mRNA level is affected. From the protein fusion data discussed above, it appears that an aspect of translational efficiency that does not involve the ICS is negatively regulated.

The interpretation presented above of the results obtained with the operon fusion strains rests on the assumptions that the effect of the *hisT76* mutation on the β-galactosidase level in the operon fusion strain is due to an effect on the level of the fusion mRNA and that the mutation has the same effect on the *gnd* mRNA level. When the properties of the operon fusion strains were initially reported, we pointed out that, because of translational coupling, polarity, or other unknown phenomena, the effect of growth rate on level of β-galactosidase might not accurately reflect the level of *gnd* mRNA (6). To test this possibility we subsequently determined the growth rate dependence of galactoside transacetylase in *gnd*::Mu dII strains, since their *gnd-lacZ* protein fusions were also *gnd-lacA* operon fusions (7). The level of the transacetylase was growth rate invariant, just like the level of β-galactosidase in the *gnd*::Mu dI strains. Thus, since the same conclusion concerning the effect of growth rate on *gnd* mRNA level could be drawn from data uncomplicated by the possibility of polarity, etc., as from measurement of β-galactosidase in the *gnd*::Mu dI strains, we believe that the above-stated assumptions and the interpretations based on them are probably valid.

Although speculation about possible mechanisms for the putative positive and negative regulatory roles of $hisT^+$ would be premature, it is worth considering the physiological significance of the effect. The altered *gnd* regulation in *hisT* mutants could be evidence of a global regulatory circuit that coordinates the roles of protein synthesis and central metabolism, both of which have established links to nucleotide modification (13). Although *zwf* expression was not affected, expression of other central metabolism genes might be. The fact that the effect of *hisT* mutations is relatively small might be because the primary role of the affected regulation is not for steady-state growth but rather for some other physiological condition, e.g., adaptation to a different nutrient environment. The rationale for coordination of macromolecular synthesis and metablism has been discussed (34).

It is also interesting that *gnd* expression was altered by *hisT* and *miaA* mutations, whereas expression of *zwf*, which responds like *gnd* to changes in growth rate, was unaffected. Perhaps there is a fundamental difference between the basic mechanisms regulating these two central metabolism genes.

In conclusion, the research reported here has uncovered a new layer of *gnd* regulation. It will be interesting to determine whether the opposing effects of *hisT* and *miaA* mutations are direct or indirect and whether they are mechanistically related.

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