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Genetic and Physical Analyses of the Growth Rate-Dependent Regulation of *Escherichia coli zwf* Expression

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Growth rate-dependent regulation of the level of *Escherichia coli* glucose 6-phosphate dehydrogenase, encoded by *zwf*, and 6-phosphogluconate dehydrogenase, encoded by *gnd*, is similar during steady-state growth and after nutritional upshifts. To determine whether the mechanism regulating *zwf* expression is like that of *gnd*, which involves a site of posttranscriptional control located within the structural gene, we prepared and analyzed a set of *zwf-lacZ* protein fusions in which the fusion joints are distributed across the glucose 6-phosphate dehydrogenase coding sequence. Expression of β-galactosidase from the protein fusions was as growth rate dependent as that of glucose 6-phosphate dehydrogenase itself, indicating that regulation does not involve an internal regulatory region. The level of β-galactosidase in *zwf-lac* operon fusion strains and the level of *zwf* mRNA from a wild-type strain increased with increasing growth rate, which suggests that growth rate control is exerted on the mRNA level. The half-life of the *zwf* mRNA mass was 3.0 min during growth on glucose and 3.4 min during growth on acetate. Thus, *zwf* transcription appears to be the target for growth rate control of the glucose 6-phosphate dehydrogenase level.

A model system for studying growth rate-dependent regulation of nonribosomal genes is the *Escherichia coli gnd* gene, which encodes 6-phosphogluconate dehydrogenase (6PGD; EC 1.1.1.44), an enzyme of the pentose phosphate pathway. An interesting feature of *gnd* regulation is that it occurs at the posttranscriptional level and requires a negative control site that lies deep within the structural gene (3, 4). The site, called the internal complementary sequence (ICS), appears to function by forming a long-range mRNA secondary structure that sequesters the ribosome-binding region of *gnd* mRNA (9). The proposed regulatory role for the secondary structure is to reduce the translation initiation frequency and/or the stability of the mRNA. The effector of the regulation is unknown.

Although the formation of long-range mRNA secondary structures that sequester a translation initiation region on mRNA has been shown in other systems to be involved in mediating translational coupling (6, 21, 25), no other example of a role for such a structure in growth rate-dependent regulation has yet been reported. Since we were interested in determining whether the mechanism is used for growth rate control of other genes, particularly central metabolism genes, we decided to study the *zwf* gene, which codes for glucose 6-phosphate dehydrogenase (G6PD; EC 1.1.1.49), the enzyme catalyzing the first step in the pentose phosphate pathway. The physiological response of *zwf* to changes in growth rate is similar to that of *gnd*. The specific activity of G6PD increases with increasing growth rate during steady-state growth in minimal media, although the extent of the increase is less than that of 6PGD (33). Also, the accumulation of G6PD after a nutritional upshift follows the same kinetics as that of 6PGD (11).

In a previous publication, we reported the nucleotide sequence of *zwf* and the map of the 5′ ends of *zwf* mRNA (26). Transcription initiates from a promoter whose sequence resembles that of typical *E. coli* promoters recognized by the σ^{70} form of RNA polymerase, the start of the coding sequence is preceded by a consensus Shine-Dalgarno sequence, and the mRNA leader appears to be processed. Computer analysis did not reveal a thermodynamically stable mRNA secondary structure like the one in *gnd*, which is capable of sequestering the translation initiation region (26). However, a sequence within the structural gene was observed that is complementary to the region around the Shine-Dalgarno sequence.

In the work reported here, we prepared a set of *zwf-lacZ* protein fusions to probe genetically for whether growth rate-dependent regulation of the G6PD level depends on an ICS-like structure. Finding no evidence for a regulatory site within the structural gene, we used *zwf-lacZ* operon fusions and direct assays of *zwf* mRNA to show that the mechanism for regulation of *zwf* expression is at the level of transcription.

MATERIALS AND METHODS

Chemicals and enzymes. Glucose 6-phosphate, *ortho*-nitrophenyl-β-D-galactoside, and isopropyl-β-D-thiogalactoside were purchased from Sigma, St. Louis, Mo. NADP and 5-bromo-4-chloro-3-indolyl-β-D-galactoside were from Boehringer Mannheim Biochemicals, Indianapolis, Ind. Restriction enzymes were purchased from Bethesda Research Laboratories, Gaithersburg, Md.; International Biotechnologies Inc., New Haven, Conn.; and New England BioLabs, Beverly, Mass. Phage T4 DNA ligase was from New England BioLabs. Deoxynucleoside triphosphates were from Pharmacia P-L Biochemicals, Milwaukee, Wis., and [α-^{35}S]dATP, [α-^{32}P]dATP, and [α-^{32}P]dCTP were from New England Nuclear, Boston, Mass. Sequencing kits were from New England BioLabs or Promega Biotec, Madison, Wis.

Media, growth conditions, and measurements of enzyme specific activity. For genetic experiments we used standard rich media or appropriately supplemented minimal medium 63 (20), except that media for growth of strain MCL19 contained 1.25% potassium chloride. Antibiotics were used

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TABLE 1. Bacterial strains and plasmids

Strain[a] or plasmid	Genotype or description	Source or reference
E. coli		
DR702	W3110 Δ(*argF-lac*)*U169* Δ(*attλ-bio-uvrB*)	This study
GB1815	W3110 *supF* Δ(*argF-lac*)*U169* Δ(*edd-zwf*)*22* Δ(*sbcB-his-gnd-rfb*) *trpR trpA9605 kdgR*	5
HB301	W3110 Δ(*argF-lac*)*U169*	9
HB351	W3110 Δ(*argF-lac*)*U169 zeb-1*::Tn*10* Δ(*edd-zwf*)*22*	3
HB582	W3110 Δ(*argF-lac*)*U169 gnd-217*::ΔMu cts dII (Ap^r Lac)::λp1(209) (Lac$^+$) (Hyb)	4
JM109	*recA endA gyrA96 thi hsdR17 supE44 relA1* Δ(*lac-proAB*)/F′ *traD36 proAB*$^+$ *lacI*^{q} *lacZ*Δ*M15*	Laboratory stock
KK2186	Δ(*lac-proAB*) *supE thi endA sbcB15 strA hsdR4*/F′ *proAB*$^+$ *lacI*^{q} *lacZ*Δ*M15*	Laboratory stock
MCL19	Δ(*attλ-bio-uvrB*) *trkA405 trkD1 thi rha* Δ(*kdp-phr*)*214*	J. Hays
N5230	HfrC *gal*::Tn*5 proC*	M. Gottesman
NF3079	*dam-3 araD139* Δ(*araABOIC-leu*)*7679 rpsL galU galK* Δ(*lac*)*X74*	Laboratory stock
MB1485	W1485/F′::Tn*10*	M. Berman
Plasmids		
pDR17	1.9-kb *Pvu*II-*Sst*I fragment carrying *zwf*$^+$ in pBR322	26
pDR20	0.7-kb *Bam*HI-*Eco*RI fragment from mDR15b in pMLB1034	This study
pDR52	0.7-kb *Bcl*I fragment from pDR17 in pMLB1022	This study
pMLB1022	Transcription fusion cloning vector carrying (′*trpA-′lacO-lacZ-lacY*′) in pBR322	M. Berman
pMLB1034	Translation fusion cloning vector carrying ′*lacZ-lacY*′ in pBR322	29

[a] All strains are *E. coli* K-12 derivatives. Strain designations are according to Bachmann (2).

at the following concentrations: ampicillin, 50 mg/liter; kanamycin, 50 mg/liter; tetracycline, 25 mg/liter. Strains were grown at 37°C.

Physiological experiments were carried out as described previously (23, 33). Briefly, cultures were initially grown in MOPS (morpholinepropanesulfonic acid) minimal medium containing 1.9 mM glucose, which arrests exponential growth abruptly at a density of about 3×10^8 cells per ml (33). Bacteria were then inoculated into MOPS medium containing 20 mM glucose or 20 mM potassium acetate at densities of 10^5 to 10^6 cells per ml. After 8 to 10 generations of balanced growth in a given medium, samples were removed for assay of enzyme activity or preparation of RNA. The activity of G6PD in sonic extracts was assayed spectrophotometrically as described previously (33); 1 U of enzyme was equivalent to 1 nmol of NADPH formed per min at 25°C per mg of protein. The protein concentration was determined by the method of Bradford (7) with immunoglobulin G as the standard. β-Galactosidase activity was assayed in cells permeabilized by treatment with chloroform and sodium dodecyl sulfate and is expressed in Miller units (20). All specific activity values represent the averages of duplicate assays of at least two samples from each of two or more independent cultures. On a given day, the standard deviation of the assays for a particular strain was usually less than 10% of the mean value. The growth rate induction ratio is the specific activity of the enzyme during growth on glucose divided by the specific activity on acetate. Although mean specific activity values for a particular strain sometimes varied between days by more than 10%, the induction ratios were nearly constant.

Bacterial strains and plasmids. Table 1 shows the strains and plasmids used in this study. Standard genetic procedures were used (20).

Strain DR702, used as the host for integration of λ *zwf-lac* fusion phages at the *zwf* locus, was prepared as follows. A phage P1 lysate grown on the *gal*::Tn*5* strain N5230 was used to transduce the Δ(*attλ bio uvrB*) mutant MCL19 to Kan^r on galactose MacConkey agar medium supplemented with kanamycin and biotin. Kan^r Gal$^-$ transductants retaining the *attλ* deletion were identified by their Bio$^-$ phenotype on glucose minimal agar. A P1 lysate prepared on one such strain was used to transduce strain HB301 (Δ*lac*) to Kan^r on galactose MacConkey agar supplemented with kanamycin and biotin. After transductions were scored for the presence of the *attλ* deletion, Gal$^+$ revertants, which presumably arose by precise excision of the Tn*5* element, were isolated as Gal$^+$ papillae on the galactose indicator plates and scored for Kan^s and Bio$^-$. One clone was designated DR702.

To transfer the *zwf-lacZα* fusions from phage M13 to phage λ DR20 by homologous recombination in vivo, we needed an F$^+$ strain that was Δ*lac*, Δ*zwf*, and Rec$^+$. Accordingly, strain GB1815/F′::Tn*10* was prepared by mating strain W1485/F′::Tn*10* with GB1815; the desired transconjugants were identified by selecting for tetracycline resistance and scoring for Lac$^-$.

The *zwf-lac* operon fusion of plasmid pDR52 was prepared by cloning the *zwf* promoter-containing 0.7-kb *Bcl*I fragment (positions −200 to +483 with respect to the start of the G6PD coding sequence) from plasmid pDR17 into the *Bam*HI site of operon fusion cloning vector pMLB1022, a derivative of pBR322 that carries *bla*, a multiple cloning site, and the segment of the W205 *trpA-lac* fusion (22, 34) starting at codon 30 of *trpA* and extending to the *Ava*I site in *lacY*. The structure of pDR52 was verified by DNA sequencing across both *Bcl*I-*Bam*HI junctions.

Phage constructions and methods. Table 2 shows the phages used in the present study. The propagation and genetic manipulations of phages P1 *cm clr*100 (20), λ (29), and M13 (19) were by standard methods, except as modified previously (26). Hosts for growth of M13 phage were strains JM109, KK2186, and NF3079/F′::Tn*10*.

The vector for transfer of *zwf-lacZα* fusions from M13 to the bacterial chromosome, λ DR20, was prepared by cloning the *Eco*RI-*Bam*HI fragment of M13 phage mDR15b, which carries the segment of the *zwf* locus extending from positions −705 to −18 with respect to the start of the G6PD coding sequence, into the corresponding sites of pMLB1034, a plasmid containing *bla* and a multiple cloning site at codon 8 of *lacZ* (29). Ap^r transformants of strain GB1815 were

TABLE 2. M13 and λ bacteriophages

Phage	Genotype[a] or description	Source or reference
M13 phages		
mDR14b	1.0-kb *Hin*cII-*Sal*I fragment of *zwf* in *Hin*cII site of M13mp19 (antisense)	26
mDR15b	0.7-kb *Sal*I-*Hin*cII fragment upstream of *zwf* coding sequence in *Sma*I site of M13mp18 (sense)	26
mDR26b	Φ(*zwf*-'*lacZα*$^+$)*161*(Hyb) codon 161 of *zwf* fused in frame to *lacZα* of M13mp18	26
mDR101	Φ(*zwf*-'*lacZα*$^+$)*19*(Hyb) codon 19 of *zwf* fused in frame to *lacZα* of M13mp18	26
mDR102	Φ(*zwf*-'*lacZα*+)*302*(Hyb) codon 302 of *zwf* fused in frame to *lacZα* of M13mp18	26
mDR104	Φ(*zwf*-'*lacZα*$^+$)*215*(Hyb) codon 215 of *zwf* fused in frame to *lacZα* of M13mp18	26
mDR106	Φ(*zwf*-'*lacZα*$^+$)*136*(Hyb) codon 136 of *zwf* fused in frame to *lacZα* of M13mp18	26
mDR111	Φ(*zwf*-'*lacZα*$^+$)*115*(Hyb) codon 115 of *zwf* fused in frame to *lacZα* of M13mp18	26
mDR114	Φ(*zwf*-'*lacZα*$^+$)*263*(Hyb) codon 263 of *zwf* fused in frame to *lacZα* of M13mp18	26
mDR161	Φ(*zwf*-'*lacZα*$^+$)*76*(Hyb) codon 76 of *zwf* fused in frame to *lacZα* of M13mp18	26
mDR165	Φ(*zwf*-'*lacZα*$^+$)*125*(Hyb) codon 125 of *zwf* fused in frame to *lacZα* of M13mp18	26
λ phages		
λ *c*I		Laboratory stock
λ *c*I c17		Laboratory stock (28)
λ RZ5	*'bla 'lacZYA'*	R. Zagursky
λ DR20	Φ(*zwf*-'*lacZYA'*) (Lac$^-$) *bla*$^+$	This study
λ DR52	Φ(*zwf*-*trpA*-'*lacOZYA'*) (Lac$^+$) *bla*$^+$	pDR20 × λ RZ5
λ DR101	Φ(*zwf*-'*lacZYA'*)*19*(Hyb) *bla*$^+$	mDR101 × λ DR20
λ DR102	Φ(*zwf*-'*lacZYA'*)*302*(Hyb) *bla*$^+$	mDR102 × λ DR20
λ DR104	Φ(*zwf*-'*lacZYA'*)*215*(Hyb) *bla*$^+$	mDR104 × λ DR20
λ DR106	Φ(*zwf*-'*lacZYA'*)*136*(Hyb) *bla*$^+$	mDR106 × λ DR20
λ DR111	Φ(*zwf*-'*lacZYA'*)*115*(Hyb) *bla*$^+$	mDR111 × λ DR20
λ DR114	Φ(*zwf*-'*lacZYA'*)*263*(Hyb) *bla*$^+$	mDR114 × λ DR20
λ DR127	Φ(*zwf*-'*lacZYA'*)*161*(Hyb) *bla*$^+$	mDR26b × λ DR20
λ DR161	Φ(*zwf*-'*lacZYA'*)*76*(Hyb) *bla*$^+$	mDR161 × λ DR20
λ DR165	Φ(*zwf*-'*lacZYA'*)*125*(Hyb) *bla*$^+$	mDR165 × λ DR20
λ DR1022	*'trpA-'lacOZYA'* (Lac$^-$) *bla*$^+$	pMLB1022 × λ RZ5

[a] The allele designation for each protein fusion corresponds to the codon number of the G6PD coding sequence that is fused in frame to *lacZ*.

selected on lactose MacConkey plates containing ampicillin. The recombinant plasmid pDR20 is LacZ$^-$, because, although it carries the *zwf* promoter oriented for transcription of *lacZ*, the mRNA lacks a ribosome binding site and hence is not translated. Strain GB1815(pDR20), which does not revert to Lac$^+$, was then infected with phage λ RZ5, a *lac* fusion rescue vector that contains the carboxy-terminal coding sequence of *bla* adjacent to a *lac* operon segment extending from the *Eco*RI site in *lacZ* into the N-terminal coding sequence of *lacA*. As described previously (9), recombination may take place between the homologous *bla* and *lac* segments of pDR20 and λ RZ5, thereby transferring the intervening plasmid DNA, including the *zwf* sequences, to the phage. The resulting lysate was used to transduce strain GB1815 to Ap^r on lactose MacConkey agar containing ampicillin. A lysate of the recombinant phage λ DR20 was prepared by UV induction of a Lac$^-$ Ap^r transductant.

The in-frame *zwf-lacZα* fusions of recombinant M13 phages were transferred to phage λ DR20 by in vivo recombination between the *zwf* and *lacZ* regions of homology on the two phages. A 0.1-ml aliquot of an overnight culture of strain GB1815/F'::Tn*10* was infected with about 10^9 PFU of a hybrid M13 phage and incubated at 37°C for 30 min. About 200 PFU of λ DR20 (Ap^r Lac$^-$) were added; the mixture was incubated for 20 min at room temperature and then plated with top agar onto lactose MacConkey agar. After incubation at 37°C for 24 h, about 95% of the λ plaques contained Lac$^+$ lysogens. Lac$^+$ Ap^r lysogens were cloned from the turbid centers of the plaques by streaking onto lactose MacConkey plates containing ampicillin. Recombinant λ phages were recovered from cultures of the respective lysogens, and high-titer lysates were prepared. To verify that the λ phages carried *zwf-lac* protein fusions corresponding to the fusions on the respective M13 phages, λ DNA was isolated, digested with *Pvu*II, and subjected to Southern analysis with the 1.7-kb *Sal*I fragment of pDR9 radiolabeled by the random priming method (13) as a probe. The hybridization signals comigrated with those obtained with DNA from the parental M13 phages. The λ phages carrying the *zwf-lacZ* protein fusions were named after the M13 phages from which they were derived.

To transfer the *zwf-lac* operon fusion of plasmid pDR52 to phage λ, strain GB1815(pDR52) was infected with λ RZ5 and the resulting lysate was used to transduce GB1815 to Lac$^+$ Ap^r. Phage λ DR52 was isolated from one such transductant by the method described above. A control phage carrying the *amp-lac* segment of the parental pMLB1022 vector was also prepared.

Lysogens carrying λ *zwf-lac* protein and operon fusion phages integrated at the *zwf* locus were prepared by infecting strain DR702 with the respective λ phages and selecting Ap^r Lac$^+$ specialized transductants on lactose-MacConkey plates containing ampicillin. The transductants were screened for monolysogens with λ *c*Ic17 (28). Monolysogens with their prophage at *zwf* were identified by generalized transduction with a lysate prepared on strain HB351, whose *zeb*::Tn*10* marker is 50% linked to *zwf*; monolysogens that gave rise to Tc^r Lac$^-$ Ap^s transductants were considered to have their prophage at *zwf*. One such monolysogen of each fusion phage was chosen for further study.

Recombinant DNA methods, DNA sequencing, and Southern analysis. Recombinant DNA methods were standard

procedures (1, 18), except as modified previously (26). DNA sequencing was by the chain termination method (27). The oligonucleotide primer for sequencing across *zwf-lacZ* fusion joints has been described (26). The oligonucleotide for sequencing across the *zwf-trpA* fusion joint of plasmid pDR52 had the sequence CGGCTTCAATTAGCGTATCG and is complementary to codons 37 to 44 of *trpA*. The oligonucleotide for sequencing the *zwf-bla* junction of pDR52 was described previously and is complementary to codons 1 to 7 of *zwf* (26). The structure of λ *zwf-lac* phages was verified by Southern analysis as previously described (30), and λ DNA was isolated by the method of Silhavy et al. (29).

RNA isolation and Northern RNA analysis. For determining the *zwf* mRNA fraction of total RNA, cultures were grown in glucose and acetate MOPS minimal media under standard physiological conditions (33), and RNA was isolated as previously described (26). RNA concentration was determined by monitoring the A_{260} of the solutions (18). The RNA preparations were subjected to electrophoresis in agarose (1.5%)–formaldehyde (6%) gels and transferred to GeneScreen membrane (New England Nuclear) according to the manufacturer's instructions. The efficiency of RNA transfer to the membrane was monitored by the method of Denis et al. (10). Hybridizations were carried out at 42°C for 16 to 24 h in a hybridization chamber purchased from Hoeffer Scientific Instruments, San Francisco, Calif., under the conditions described in the GeneScreen manual. The probe for the hybridizations was prepared by the method of Hu and Messing (16), with M13 phage mDR14b as the template and [α-^{32}P]dCTP as the radioactive nucleotide. After hybridization, blots were scrubbed twice at room temperature in a solution containing 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and 0.1% sodium dodecyl sulfate and then washed three times by incubation for 20 min at 37°C in a solution of 0.1× SSC and 0.1% sodium dodecyl sulfate, as described by the manufacturer of the hybridization apparatus. Autoradiograms, made with XRP film purchased from Eastman Kodak Co., Rochester, N.Y., were scanned with a video densitometer (Bio-Rad Laboratories, Richmond, Calif.), and the relative intensity of the hybridization signals was determined with computer software provided with the instrument. The intensity of the hybridization signals was directly proportional to the amount of input RNA.

For determining the half-life of the *zwf* mRNA mass, exponential-phase cultures grown under standard physiological conditions were treated with rifampin at a concentration of 500 μg/ml, and samples were removed at various times thereafter. RNA was isolated and analyzed by Northern analysis as described above. The half-life was calculated from the linear regression line of a semilogarithmic plot of hybridization signal intensity, quantified by densitometry as described above, as a function of time.

RESULTS

Preparation of *zwf-lacZ* protein fusions. The approach for determining whether the metabolic control of *zwf* expression requires an internal regulatory region was similar to the one that revealed the ICS of the *gnd* gene (4, 9). In the earlier work, we prepared a set of *gnd-lacZ* protein fusions in which increasing amounts of the 6PGD-coding sequence were fused in frame to *lacZ*. Expression of β-galactosidase from fusions with fusion joints downstream of the ICS was induced by the growth rate to the same extent as 6PGD was from *gnd* itself, whereas expression from fusions lacking the element was growth rate derepressed; i.e., the level of β-galactosidase produced from these latter ICS$^-$ fusions under slow growth conditions (on acetate) was as high as the level from ICS$^+$ fusions at the faster growth rate (on glucose) (4). In anticipation of applying this strategy to *zwf*, we previously prepared a set of 3′ deletions that gave rise to in-frame fusions of the *zwf* structural gene to the *lacZα* gene segment of a recombinant M13 phage (26). The junctions between *zwf* and *lacZα* in the fusions were dispersed at fairly regular intervals across the promoter-proximal 60% of the G6PD coding sequence and thus were useful in sequencing the *zwf* gene (26).

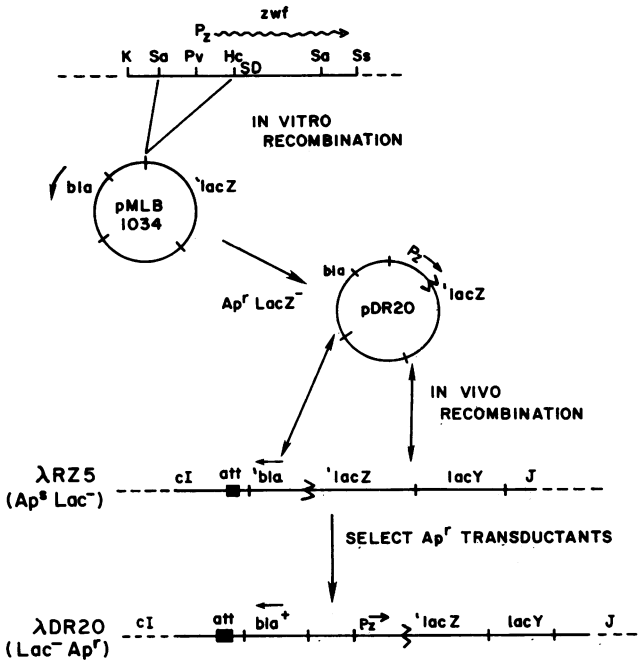

FIG. 1. Construction of λ DR20, a vector for transferring *zwf-lacZ* fusions from recombinant M13 phages to the bacterial chromosome. The top line is a restriction map of the *zwf* locus (26). The *Sal*I-to-*Hin*cII region containing the *zwf* promoter was cloned as a *Hin*cII fragment into the *Sma*I site of M13, forming mDR15b (26). The *Eco*RI-*Bam*HI fragment containing the *zwf* promoter was then cloned into the corresponding sites of pMLB1034, forming pDR20. The cross yielding λ DR20 was done as described in the text. K, *Kpn*I; Sa, *Sal*I; Pv, *Pvu*II; Hc, *Hin*cII; Ss, *Sst*I; SD, Shine-Dalgarno sequence; P_Z, *zwf* promoter; cI and J, genes of λ.

To facilitate using the protein fusions carried on M13 phages as a means of searching for an ICS-like element in the G6PD coding sequence, we prepared a fusion rescue phage, λ DR20 (Fig. 1); we then transferred the fusions from M13 phages mDR26b, mDR101, mDR102, mDR104, mDR106, mDR111, mDR114, mDR161, and mDR165 onto λ DR20 by in vivo homologous recombination (Fig. 2) and integrated the resulting phages at the *zwf* locus of strain DR702 (Δ*lac* Δ*att*λ). This scheme had several advantages compared with using the original M13 phages, plasmids derived from them, or λ fusion phages integrated at *att*λ. First, the fusions carried on the λ DR20 derivatives are between *zwf* and a full-length *lacZ* gene and therefore encode an enzymatically active β-galactosidase, whereas the fusions on the M13 phages express a truncated β-galactosidase whose activity depends on complementation with the α-receptor polypep-

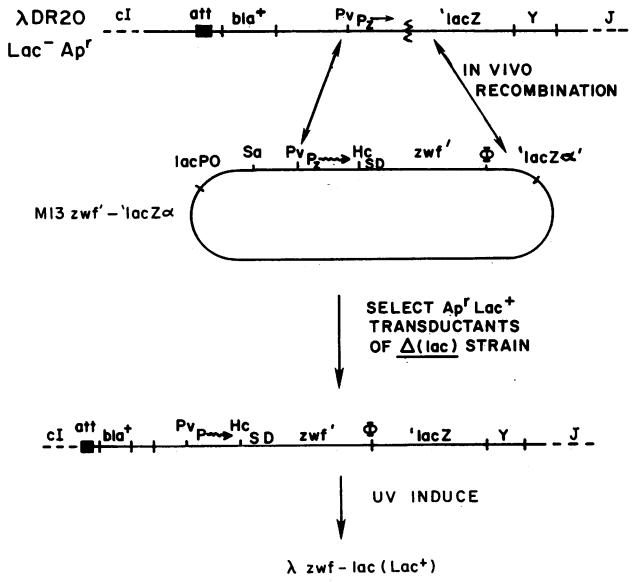

FIG. 2. Preparation of λ phages carrying *zwf-lacZ* protein fusions by in vivo homologous recombination between λ DR20 and recombinant M13 phages. The cross was done as described in the text. Sa, *Sal*I site at position −708; Pv, *Pvu*II site at position −370; Hc, *Hin*cII; P_Z, *zwf* promoter; SD, Shine-Dalgarno sequence of *zwf*; cI and J, genes of λ; Φ, in-frame fusion joint between *zwf* and *lacZ*.

tide. In addition, the λ fusion phages encode lactose permease. Second, placing the fusions at the normal region of the bacterial chromosome avoided potential complications of gene dosage, location, and orientation. Accordingly, strain DR702 was infected with the λ phages carrying the respective protein fusions (λ DR101, λ DR102, λ DR104, λ DR106, λ DR111, λ DR114, λ DR127, λ DR 161, λ DR165), and monolysogens with their prophage at *zwf* were isolated as described in Materials and Methods.

Growth rate dependence of β-galactosidase level in lysogens carrying λ *zwf-lac* protein fusion phages. The parental strain DR702 was grown in acetate and glucose minimal media and assayed for G6PD activity. With acetate as the carbon source, the doubling time was about 4 h and the enzyme specific activity was 54 U/mg, whereas with glucose as the carbon source the doubling time was about 1 h and the specific activity of G6PD was 103 U/mg. Thus, the growth rate induction ratio for G6PD, defined as the ratio of enzyme activity during growth on glucose to the value for cells grown on acetate, was 1.9. For unknown reasons, the extent of the growth rate induction of G6PD in strain DR702 is about half of the value reported previously for strain W3110 (33).

Table 3 shows the effect of growth rate on the level of β-galactosidase in monolysogens of DR702 carrying the protein fusion phages. For all nine strains, the activity was higher during exponential growth on glucose compared with that during growth on acetate, and the mean induction ratio for the set of fusions was 1.8. Since the growth rate induction ratio for the expression of β-galactosidase from the *zwf-lac* protein fusions was about the same as that for G6PD from *zwf* itself, we conclude that metabolic control of *zwf* is different from that of *gnd* in that it does not depend on a negative control site that lies deep within the structural gene. In particular, the previously noted sequence TCTCC at codons 249 to 250 (26), which is complementary to a segment of the *zwf* mRNA translation initiation region, is not such a regulatory element, since fusions without this sequence do not confer growth rate-derepressed expression. Moreover, the data indicate that all of the sequences necessary for growth rate regulation lie upstream of codon 19, the fusion joint of fusion DR101.

TABLE 3. Growth rate dependence of β-galactosidase level in monolysogens carrying λ *zwf-lac* protein or operon fusions[a]

Strain	Fusion joint[b]	β-Galactosidase activity (U) on:		Induction ratio
		Acetate	Glucose	
Protein fusions				
DR702 (λ DR101)	19	222	366	1.7
DR702 (λ DR161)	76	19	43	2.3
DR702 (λ DR111)	115	83	154	1.9
DR702 (λ DR165)	125	103	172	1.7
DR702 (λ DR106)	136	110	188	1.7
DR702 (λ DR127)	161	198	333	1.7
DR702 (λ DR104)	215	196	310	1.6
DR702 (λ DR114)	263	137	292	2.1
DR702 (λ DR102)	302	171	333	1.9
Operon fusions				
DR702 (λ DR52)	NA	616	1,006	1.6
HB301 (λ DR1022)[c]	NA	28	29	NA

[a] Strains were grown in acetate and glucose minimal media and assayed for enzyme activity as described in Materials and Methods. NA, not applicable.
[b] The number of *zwf* codons fused to *lacZ*.
[c] In this strain, the λ prophage is located at *att*λ.

Within the set of fusions, β-galactosidase activity during growth under a given condition varied over a 10-fold range. The basis for the difference in enzyme activities between the various fusions was not studied further, but it could be due to differences in the stability or activity of the respective hybrid polypeptides.

Growth rate-dependent expression of β-galactosidase from a *zwf-lac* operon fusion. Plasmid pDR52, which carries a *zwf-lac* operon fusion, was prepared by cloning a restriction fragment containing the *zwf* promoter into plasmid pMLB1022 as described in Materials and Methods. The operon fusion was transferred to phage λ by in vivo homologous recombination with λ RZ5, and the resulting phage, λ DR52, was integrated into the *zwf* locus of strain DR702 as described above for the λ phages carrying the *zwf-lacZ* protein fusions. In the λ DR52 lysogen, *lacZ* transcription is under control of all of the sequences normally upstream of the *zwf* structural gene. Translation initiating at the *zwf* ribosome binding site of the operon fusion terminates at an out-of-frame nonsense codon early in *'trpA*, and translation of β-galactosidase is initiated at the native *lacZ* ribosome binding site.

Strain DR702 (λ DR52) was grown in acetate and glucose minimal media and assayed for β-galactosidase activity. The acetate-grown cells contained 616 Miller units of β-galactosidase, and the glucose grown cells contained 1006 Miller units, which yields a growth rate induction ratio of 1.6 (Table 3). The similarity between the growth rate induction ratio for β-galactosidase in the operon fusion strain and the value of 1.9 obtained for G6PD in strain DR702 and the values obtained for the protein fusion strains suggest that it is the level of *zwf* mRNA that is controlled by the cell growth rate.

Effect of growth rate on the level of *zwf* mRNA. Total RNA was isolated from strain HB582 growing exponentially on acetate and glucose and subjected to Northern analysis. A single mRNA species of about 1.7 kb was detected in both

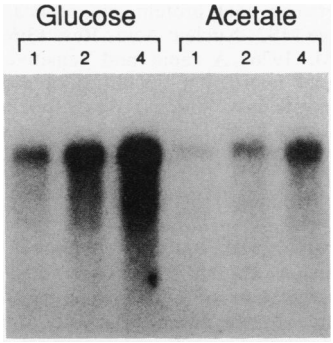

FIG. 3. Growth rate dependence of *zwf* mRNA level. Northern blots were prepared for 1, 2, and 4 μg of total RNA isolated from strain HB582 growing in acetate and glucose minimal media. The blots were probed and analyzed by densitometry as described in the text. The slope of the least-squares fit of a plot of hybridization signal as a function of total RNA for glucose-grown cells was 3.2-fold higher than that for acetate-grown cells.

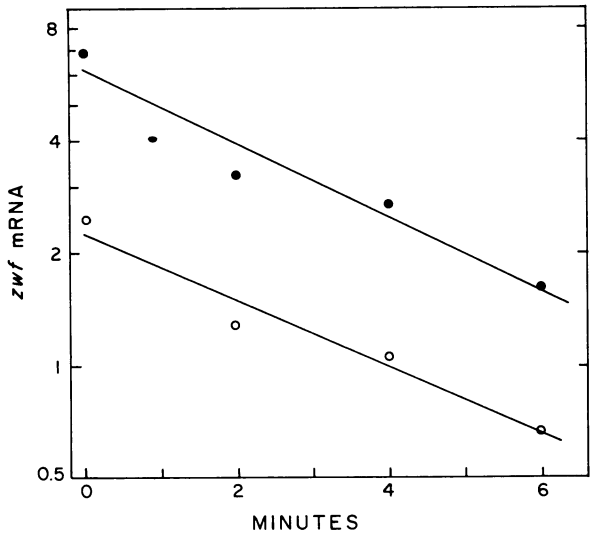

FIG. 4. Stability of *zwf* mRNA as a function of growth rate. Northern blots were analyzed by densitometry as described in the text. The amount of *zwf* mRNA is the hybridization signal intensity in arbitrary units. ●, RNA from glucose-grown cells; ○, RNA from acetate-grown cells.

RNA preparations (Fig. 3); the size of the transcript is sufficient to encode the 490-amino-acid residue G6PD polypeptide. The relative amount of the 1.7-kb transcript was 10- to 15-fold higher in RNA isolated from a strain carrying the zwf^+ plasmid pDR17, and no signal was detected with RNA prepared from strain GB1815, which carries a *zwf-edd* deletion (26; data not shown). Thus, the 1.7-kb transcript is *zwf* mRNA. Densitometric analysis of the autoradiogram showed that the *zwf* mRNA fraction of total RNA is 3.2-fold higher in the glucose-grown cells than in cells grown on acetate; similar results were obtained in other experiments (data not shown). As discussed below, these data are consistent with the conclusion drawn from the growth rate dependence of β-galactosidase level in the operon fusion strain that the regulation of *zwf* expression is on the level of the mRNA.

Effect of growth rate on the stability of *zwf* mRNA. The growth rate-dependent changes in *zwf* mRNA level could be due to regulation of transcription or of mRNA stability. To distinguish between these possibilities, we measured *zwf* mRNA levels after treatment of cells growing exponentially in acetate and glucose minimal media with rifampin, an inhibitor of transcription initiation. RNA was extracted from cultures of strain HB301 at various times after the addition of the inhibitor, and equal amounts were subjected to Northern hybridization analysis. The resulting autoradiograms were scanned with a densitometer. The half-lives of *zwf* mRNA mass, calculated by a least-squares fit of a semilogarithmic plot of the relative amount of *zwf* mRNA as a function of time, were 3.0 min in the glucose-grown cells and 3.4 min for the cells grown on acetate (Fig. 4). Since the half-life of *zwf* mRNA does not change significantly with growth rate, growth rate-dependent regulation appears to be exerted on transcription.

We also made the following observations. First, the relative amount of *zwf* mRNA at zero time, i.e., before rifampin addition, was 2.8-fold higher for the cells growing on glucose compared with the value for the cells growing on acetate; this induction ratio agrees with the ratio obtained as described above for strain HB582. Second, unlike the decay of several other mRNAs (31), the decay of *zwf* mRNA did not give rise to stable decay intermediates. Third, the decay of *zwf* mRNA began almost immediately after rifampin addition, whereas the decay of *gnd* mRNA does not begin until several minutes after the addition of the antibiotic (24).

DISCUSSION

The work described here demonstrates that, although the growth rate affects the G6PD and 6PGD levels in similar ways during both steady-state growth and after nutritional shifts (11, 33), the underlying mechanisms regulating expression of the respective *zwf* and *gnd* genes are different. With *gnd*, a set of protein fusions with fusion joints dispersed across the coding sequence for 6PGD revealed the presence of a negative control site in the codon 70 region of the structural gene (4). However, since no *zwf-lac* protein fusions conferred unregulated expression, *zwf* appears to lack an internal regulatory region. Thus, the properties of the *zwf-lac* protein fusions agree with the previous computer analysis of *zwf* mRNA, which showed that the most stable secondary structure of the mRNA does not involve long-range base pairing of the ribosome binding site to a segment of the coding sequence (26).

Expression of β-galactosidase from a *zwf-lac* operon fusion strain was about 1.6-fold higher in glucose-grown cells than in cells grown on acetate, whereas the growth rate induction ratios for G6PD itself and for β-galactosidase from *zwf-lac* protein fusion strains were about 2.0. The close agreement of these values suggests that the growth rate-dependent regulation of G6PD expression is due to regulation of the *zwf* mRNA level.

Direct measurement of the *zwf* mRNA fraction of total RNA also showed that growth rate control of G6PD expression derives from regulation of *zwf* mRNA level. In the Northern analyses reported here, the amount of *zwf* mRNA relative to total RNA was about 3.0-fold higher in glucose-grown cells than in cells grown on acetate; in addition, we previously observed that the relative amount of the 5′ ends of *zwf* mRNA, detected by S1 nuclease protection and primer extension techniques, is about 3.0-fold higher in the faster-growing cells (26). Thus, since the amount of total

RNA per genome increases in direct proportion to the growth rate (8), we calculate that the amount of *zwf* mRNA per genome is 12-fold higher in glucose-grown cells, for which the doubling time is 1 h, than in cells grown on acetate, for which the doubling time is 4 h. Moreover, by taking into account the 1.4-fold increase in total protein per genome over the fourfold range of growth rates (8), we calculate that the 2.0-fold higher level of G6PD in glucose-grown cells compared with that in cells grown on acetate is due to an 11.2-fold increase in the accumulation rate of G6PD per genome. Thus, the growth rate-dependent increase in the amount of *zwf* mRNA per genome matches almost exactly the growth rate-dependent increase in the accumulation rate per genome of G6PD.

The half-life of *zwf* mRNA was about 3 min in both glucose- and acetate-grown cells. Accordingly, the growth rate-dependent increase in *zwf* mRNA level is due to increased *zwf* transcription. This growth rate-dependent regulation of *zwf* transcription is most likely due to control of *zwf* promoter activity, not transcription elongation, because (i) the only recognizable transcription termination sequence is the one at the end of the gene; and (ii) the 5′ ends of *zwf* mRNA are the same during growth in glucose and acetate minimal media (26). In contrast, the growth rate control of 6PGD level appears to involve posttranscriptional regulation (3).

Expression of G6PD is also induced by agents that endogenously generate superoxide free radicals (17). Using the *zwf-lac* operon fusion described here (λ DR52 was previously called λ B11), Greenberg et al. showed that this induction, like that of several other members of the superoxide radical regulon, increases the mRNA level and is dependent on *soxR* (14, 15). In work to be reported elsewhere, we have found that growth rate-dependent regulation of G6PD level is independent of the *soxR* control (12). Thus, *zwf* is an example of a gene whose specific regulation, i.e., induction by oxidative stress, is superimposed upon the more basic growth rate-dependent regulation (32). Accordingly, it will be interesting to determine not only the site(s) and mechanism for growth rate control of *zwf* expression but also the orientation of this regulatory site with respect to the target of *soxR* action.

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REFERENCES

1. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl.** 1987. Current protocols in molecular biology. John Wiley & Sons, Inc., New York.
2. **Bachmann, B. J.** 1990. Linkage map of *Escherichia coli* K-12, edition 8. Microbiol. Rev. **54**:130–197.
3. **Baker, H. V., II, and R. E. Wolf, Jr.** 1983. Growth rate-dependent regulation of 6-phosphogluconate dehydrogenase level in *Escherichia coli* K-12: β-galactosidase expression in *gnd-lac* operon fusion strains. J. Bacteriol. **153**:771–781.
4. **Baker, H. V., II, and R. E. Wolf, Jr.** 1984. Essential site for growth rate-dependent-regulation within the *Escherichia coli gnd* structural gene. Proc. Natl. Acad. Sci. USA **81**:7669–7673.
5. **Barcak, G. J., and R. E. Wolf, Jr.** 1988. Growth-rate-dependent expression and cloning of *gnd* alleles from natural isolates of *Escherichia coli*. J. Bacteriol. **170**:365–371.
6. **Berkhout, B., and J. van Duin.** 1985. Mechanism of translational coupling between coat protein and replicase genes of RNA bacteriophage MS2. Nucleic Acids Res. **13**:6955–6967.
7. **Bradford, M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
8. **Bremer, H., and P. P. Dennis.** 1987. Modulation of chemical composition and other parameters of the cell by growth rate, p. 1527–1542. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
9. **Carter-Muenchau, P., and R. E. Wolf, Jr.** 1989. Growth-rate-dependent regulation of 6-phosphogluconate dehydrogenase level mediated by an anti-Shine-Dalgarno sequence located within the *Escherichia coli gnd* structural gene. Proc. Natl. Acad. Sci. USA **86**:1138–1142.
10. **Denis, N., D. Corcos, J. Kruh, and A. Kitzis.** 1988. A rapid and accurate method for quantitating total RNA transferred during Northern blot analysis. Nucleic Acids Res. **16**:2354.
11. **Farrish, E. E., H. V. Baker II, and R. E. Wolf, Jr.** 1982. Different control circuits for growth rate-dependent regulation of 6-phosphogluconate dehydrogenase and protein components of the translational machinery in *Escherichia coli*. J. Bacteriol. **152**:584–594.
12. **Fawcett, W. P., and R. E. Wolf, Jr.** Unpublished observations.
13. **Feinberg, A. P., and B. Vogelstein.** 1984. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **137**:266–267.
14. **Greenberg, J. T., and B. Demple.** 1989. A global response induced in *Escherichia coli* by redox-cycling agents overlaps with that induced by peroxide stress. J. Bacteriol. **171**:3933–3939.
15. **Greenberg, J. T., P. Monach, J. H. Chou, P. D. Josephy, and B. Demple.** 1990. Positive control of a global antioxidant defense regulon activated by superoxide-generating agents in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **87**:6181–6185.
16. **Hu, N., and J. Messing.** 1982. The making of strand-specific M13 probes. Gene **17**:271–277.
17. **Kao, S. M., and H. M. Hassan.** 1985. Biochemical characterization of a paraquat-tolerant mutant of *Escherichia coli*. J. Biol. Chem. **260**:10478–10481.
18. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **Messing, J.** 1983. New M13 cloning vectors for cloning. Methods Enzymol. **101**:20–78.
20. **Miller, J.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Min Jou, W., G. Haegeman, M. Ysebaert, and W. Fiers.** 1972. Nucleotide sequence of the gene coding for the bacteriophage MS2 coat protein. Nature (London) **237**:82–88.
22. **Mitchell, D., W. Reznikoff, and J. Beckwith.** 1975. Genetic fusions defining *trp* and *lac* operon regulatory elements. J. Mol. Biol. **93**:331–350.
23. **Neidhardt, F. C., P. L. Block, and D. F. Smith.** 1974. Culture medium for enterobacteria. J. Bacteriol. **109**:736–747.
24. **Pease, A. J., and R. E. Wolf, Jr.** Unpublished observations.
25. **Petersen, C.** 1989. Long-range translational coupling in the *rplJL-rpoBC* operon of *Escherichia coli*. J. Mol. Biol. **206**:323–332.
26. **Rowley, D. L., and R. E. Wolf, Jr.** 1991. Molecular characterization of the *Escherichia coli* K-12 *zwf* gene encoding glucose 6-phosphate dehydrogenase. J. Bacteriol. **173**:968–977.
27. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
28. **Shimada, K., R. A. Weisberg, and M. E. Gottesman.** 1972. Prophage lambda at unusual chromosomal locations. I. Location of the secondary attachment sites and the properties of the lysogens. J. Mol. Biol. **63**:483–503.
29. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

30. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
31. **von Gabain, J., J. G. Belasco, J. L. Schottel, A. C. Y. Chang, and S. N. Cohen.** 1983. Decay of mRNA in *Escherichia coli*: investigation of the fate of specific segments of transcripts. Proc. Natl. Acad. Sci. USA **80**:653–657.
32. **Wolf, R. E., Jr.** 1985. Growth-rate-dependent regulation of a central metabolism gene, p. 202–211. *In* M. Schaechter, F. C. Neidhardt, J. L. Ingraham, and N. O. Kjeldgaard (ed.), The molecular biology of bacterial growth. Jones and Bartlett, Boston.
33. **Wolf, R. E., Jr., D. M. Prather, and F. M. Shea.** 1979. Growth-rate-dependent alteration of 6-phosphogluconate dehydrogenase and glucose 6-phosphate dehydrogenase levels in *Escherichia coli* K-12. J. Bacteriol. **139**:1093–1096.
34. **Xian-Ming, Y., L. M. Munson, and W. S. Reznikoff.** 1984. Molecular cloning and sequence analysis of *trp-lac* fusion deletions. J. Mol. Biol. **172**:355–362.