Determination of the Growth Rate-Regulated Steps in Expression of the *Escherichia coli* K-12 *gnd* Gene

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In *Escherichia coli* K-12 strain W3110, the amount of 6-phosphogluconate dehydrogenase relative to that of total protein, i.e., the specific enzyme activity, increases about threefold during growth in minimal media over the range of growth rates with acetate and glucose as sole carbon sources. Previous work with *gnd-lac* operon and protein fusion strains indicated that two steps in the expression of the *gnd* gene are subject to growth rate-dependent control, with at least one step being posttranscriptional. With both Northern (RNA) and slot blot analyses, we found that the amount of *gnd* mRNA relative to that of total RNA was 2.5-fold higher in cells growing in glucose minimal medium than in cells grown on acetate. Therefore, since the total mRNA fraction of total RNA is essentially independent of the growth rate, the amount of *gnd* mRNA relative to that of total mRNA increases about 2.5-fold with increasing growth rate. This indicates that most of the growth rate-dependent increase in 6-phosphogluconate dehydrogenase can be accounted for by the growth rate-dependent increase in *gnd* mRNA level. We measured the decay of *gnd* mRNA mass in the two growth conditions after blocking transcription initiation with rifampin and found that the stability of *gnd* mRNA does not change with growth rate. We also used a *gnd-lacZ* protein fusion to measure the functional mRNA half-life and found that it too is growth rate independent. Thus, the growth rate-dependent increase in the level of *gnd* mRNA is due to an increase in *gnd* transcription, and this increase is sufficient to account for the growth rate regulation of the 6-phosphogluconate dehydrogenase level. The dilemma posed by interpretation of the properties of *gnd-lac* fusion strains and by direct measurement of *gnd* mRNA level is discussed.

Growth rate-dependent regulation refers to the process that coordinates gene expression with the cellular growth rate. In *Escherichia coli* K-12, the level of the pentose phosphate pathway enzyme 6-phosphogluconate dehydrogenase (6PGD) is about threefold higher during growth in minimal medium with glucose as the carbon source, where the doubling time is about 1 h, than during growth on acetate, where the doubling time is about 4 h (4, 30). Since the 6PGD level also decreases when the growth rate is decreased by limiting the rate of glucose uptake, *gnd* gene expression is regulated by growth rate per se, not by the specific carbon source (30).

Inferences concerning the mechanisms responsible for the growth rate regulation of *gnd*, which encodes 6PGD, have been drawn primarily from the properties of *gnd-lac* operon and protein fusions. Thus, for strains carrying operon fusions prepared with phage MudI and located within the *gnd* gene at its normal chromosomal location, the level of β-galactosidase is constant over the fourfold range of growth rates (2). This implies that two steps of *gnd* gene expression are subject to growth rate control. For example, both the rate of *gnd* transcription and the translational efficiency of the mRNA may be proportional to growth rate; alternatively, regulation could be exerted on *gnd* mRNA stability instead of on one of the other two steps. The properties of *gnd-lacZ* protein fusions prepared with phage MudII and located within the normal *gnd* gene were also illuminating (3). In all but one protein fusion strain, the level of β-galactosidase increased threefold with increasing growth rate, just like the growth rate dependence of the 6PGD level in a wild-type strain (3). With the remaining fusion, which had the smallest amount of the *gnd* structural gene fused to *lacZ*, the level of β-galactosidase during growth on acetate was elevated threefold, and it increased only slightly during growth on glucose (3). This suggests that growth rate regulation requires a negative control site that lies within the 6PGD coding sequence (3). Subsequent 3′ deletion analysis of a protein fusion carried on a λ prophage integrated at attL, together with the use of synthetic deoxyoligonucleotides, identified the internal regulatory site as the segment of *gnd* mRNA from codon 71 to 74 (6). Since this region is complementary to the Shine-Dalgarno sequence and surrounding nucleotides in the *gnd* mRNA leader, we proposed that its negative control function is to sequester the *gnd* ribosome binding site (RBS) into a long-range mRNA secondary structure, thereby reducing the frequency of translation initiation (6). From this we argued that if the rate of formation or the stability of the secondary structure formed between the internal complementary sequence (ICS) and the RBS was proportional to growth rate, then the translational efficiency of *gnd* mRNA would be growth rate dependent (6). We also recognized the possibility that the RBS-ICS secondary structure might be a target for the growth rate-dependent cleavage of the mRNA (6).

In the work reported here, we tested some of the conclusions described above by determining directly the effect of growth rate on *gnd* mRNA level and also by determining the effect of growth rate on the chemical and functional decay of *gnd* mRNA. Finding that the *gnd* mRNA fraction of total mRNA increases with increasing growth rate by approximately the same threefold factor as the increase in the 6PGD fraction of total protein and also that *gnd* mRNA decay is independent of growth rate, we conclude that most or all of the growth rate regulation of 6PGD level may be accomplished by a growth rate-dependent increase in *gnd* transcription. These new data raise the question of the role of the ICS in *gnd* expression.
MATERIALS AND METHODS

Chemicals and enzymes. 6-Phosphogluconate was purchased from Sigma, St. Louis, Mo., and NADP was purchased from Boehringer Mannheim Biochemicals, Indianapolis, Ind. Deoxynucleoside triphosphates were from Pharmacia P-L Biochemicals, Milwaukee, Wis., and $\alpha^{-35}$S-dATP, $[\alpha^{32}P]$dATP, $[\alpha^{32}P]$dCTP, and $[\alpha^{-35}]$methionine were from New England Nuclear, Boston, Mass. Sequencing kits were from New England Biolabs, Beverly, Mass., or Promega Biotec, Madison, Wis. Reagent grade phenol was from International Biotechnologies, Inc., New Haven, Conn. An RNA molecular weight ladder was from Bethesda Research Laboratories, and protein molecular weight standards were from Bio-Rad Laboratories, Richmond, Calif. Reinforced nitrocellulose membranes (BA-NC) were from Schleicher & Schuell, Inc., Keene, N.H., and GeneScreen nylon membranes were from New England Nuclear. The M13 hybridization primer was from New England Biolabs. Anti-β-galactosidase serum was from Promega Biotec, and protein A-Sepharose beads were from Pharmacia.

Strains, media, growth conditions, and measurement of enzyme specific activity. All strains are derivatives of E. coli K-12 strain W3110. Strain HB301 is $\Delta$(arg-fuc)-U169 (6), and strain RW231 is trpR lacZ(Am) trpa9605 kdgR(Con) (24-26). The pMN6 plasmid of strain RW231(pMN6) is a derivative of pBR322 and carries the entire gnd gene (18). Strain HB518 is $\Delta$(arg-fuc)-U169 gnd(-278):Amu cts dII(Ap' Lac::Ap1209)(Lac')(Hyb); its gnd-lacZ protein fusion is equal to codon 122 of gnd (3). LB broth (15) was the standard broth used for routine bacterial subculture. Morpholinopropane sulfonic acid (MOPS) minimal medium supplemented with glucose or acetate (4 g/liter) as the carbon source was used in physiological experiments as previously described (19, 30). Growth rates (μ) are expressed as doublings per hour.

6PGD specific activity was measured spectrophotometrically as previously described (30).

Isolation of total RNA and quantitation of gnd mRNA by Northern (RNA) and slot blot analyses. Total cellular RNA was isolated from cultures of strain HB301 growing exponentially in acetate and glucose minimal media by the method of von Gabain et al. (29), with some previously described modifications (24, 25). The quality of the RNA preparations was assessed by electrophoresis through a 1.5% agarose gel and subsequent staining with ethidium bromide; only preparations yielding unsmeared RNA bands were used for the Northern and slot blot analyses. The RNA concentration was determined spectrophotometrically at 260 nm, using the relationship that one $A_{260}$ unit corresponds to 40 μg/ml (1).

Appropriate dilutions of the total RNA were subjected to Northern analysis as described previously (24), using either nylon or reinforced nitrocellulose membranes. The probe for detecting gnd-specific mRNA was prepared by the method of Hu and Messing (11) as described in the directions accompanying the M13 hybridization probe-primer (New England Biolabs), using DNA from M13 phage HB562-9 (17) as the template and $[\alpha^{32}P]$dCTP as the radioactive nucleotide. The gnd DNA in phage HB562-9 is complementary to the 5' end of the message and covers 1,260 of the 1,500 bases of gnd mRNA (17). Hybridizations were carried out in a chamber purchased from Hoefer Scientific, San Francisco, Calif., as described previously (24). The filters were subjected to autoradiography, and hybridization signals were quantified by a Bio-Rad video densitometer. Multiple exposures of X-ray film were always done on each filter to ensure that the signal was in the linear range of the film.

To measure the sum of hybridizable gnd mRNA, rather than the single, full-length mRNA detected by Northern analysis, slot blot analysis was done. The Hybri-Slot filtration manifold from Bethesda Research Laboratories was used for the slot blots. RNA was diluted into 15 × SSC (1 × SSC is 0.15 M sodium chloride plus 0.015 M sodium citrate), denatured by incubation at 65°C for 15 min, and blotted onto nitrocellulose or nylon filters according to the manufacturers' directions. The filters were probed with $^{32}P$-labelled DNA as described above.

Determination of mass decay. To measure decay of gnd mRNA mass, transcription initiation was inhibited by adding rifampin (dissolved in dimethyl sulfoxide at 50 μg/ml) to 500 μg/ml to cells growing exponentially in either glucose or acetate minimal medium as described previously (24). Control experiments determining the kinetics of inhibition by rifampin of the incorporation of $[3H]$uridine into trichloroacetic acid-precipitable material and modeled after experiments of Pato and von Meyenberg (21) showed that this concentration of the antibiotic rapidly blocked transcription initiation (22). At various times after the addition of the rifampin, samples of the cultures were taken and total RNA was isolated by the method of Erickson et al. (9). In this method, 3 × lysis medium (3% sodium dodecyl sulfate, 24 mM EDTA, 300 mM sodium chloride) and cell culture were mixed at a volume ratio of 2:1 and incubated at 100°C for 5 min; the RNA was then extracted by the hot-phenol method as described previously (25). Although we used this method when isolating RNA for half-life determinations because it was faster than the method described above and hence might reduce possible mRNA degradation by endogenous RNases, we found that the two methods did not differ with respect to either the quantity or the quality of the RNA recovered.

The half-life of gnd mRNA mass was determined by carrying out Northern analysis on equal amounts of total RNA isolated at appropriate times after rifampin addition. The amount of the gnd-specific mRNA hybridization signal in each sample was quantified by microdensitometry and expressed relative to the value at zero time, which was set at 100%. The half-life of the mRNA was calculated from the slope of a least-squares regression line of a semilogarithmic plot of percent gnd mRNA remaining as a function of time. Since the level of gnd mRNA after rifampin addition rises above the amount at zero time, the half-life was calculated by using time points during which decay was exponential.

Determination of functional mRNA half-life. The decay of the capacity to synthesize a given protein after transcription initiation is blocked with rifampin is a measure of the functional decay of the mRNA encoding the protein. Accordingly, we used strain HB518, which carries an ICS + gnd-lacZ protein fusion, and determined the rate of synthesis of the 6PGD-β-galactosidase fusion protein at appropriate time intervals after drug addition. The strain was grown in glucose and acetate minimal media, and transcription initiation was blocked with rifampin as described above. One-milliliter samples were taken at various times, pulsed with 10 μCi of $[35]$methionine for 1 min, and chased for 2 min with excess nonradioactive methionine (100 μCi of a 10-mg/ml solution of methionine). The chase was terminated by adding 4 μl of a 50-mg/ml solution of chloramphenicol, and the samples were chilled on ice.

Two methods were used for quantitating the amount of $[35]$methionine-labelled fusion protein in each sample. In one method, the fusion protein was immunoprecipitated by use of an anti-β-galactosidase monoclonal antibody (1), while in the other method the fusion protein was cut directly from the gel after electrophoresis in a 7.5% polyacrylamide gel prepared as described by Silhavy et al. (28) and staining with Coomassie
blue (27). In the gel slice method, the region containing the fusion protein was excised from the gel, and the gel slices were digested with 200 μl of hydrogen peroxide for 2 h at 70°C; the hydrogen peroxide was inactivated by two rounds of incubation with 75 μl of catalase (0.5 mg/ml). The digested gel slices were then added to Aquasol scintillation fluid (New England Nuclear) and incubated overnight to ensure that the gel slices were completely dissolved, and the amount of radioactivity was determined by scintillation counting. We identified the band containing the fusion protein by the absence of a corresponding band in an extract of the Δlac strain HB301. In addition, we carried out gel electrophoresis on extracts prepared from several Δlac strains harboring multicopy plasmids carrying gnd-lacZ protein fusions with different fusion joints, since the relative mobilities of these fusion proteins had been determined previously (3).

In the immunoprecipitation method, radiolabelled cells were lysed and incubated with anti-β-galactosidase antibody, and immune complexes were precipitated with protein A coupled to Sepharose beads, as described previously (1). After the beads were washed, the fusion protein-antibody complexes were released from the beads by incubation in gel loading buffer (28) for 5 min at 95°C. The beads were removed by centrifugation, and the volume of the supernatants was reduced by about one-half by lyophilization. The samples were then subjected to acrylamide gel electrophoresis and staining as described above. The fusion proteins were recovered from the gel, and radioactivity in the fusion proteins was determined by scintillation counting as described above.

Regardless of the purification method, the rate of fusion protein synthesis at a given time was expressed as the amount of radioactivity in the fusion protein per milliliter of culture. The rate immediately prior to addition of rifampin was defined as the 100% rate of fusion protein synthesis, and rates at various times after drug addition were expressed relative to the value at zero time. The rates of fusion protein synthesis were plotted semilogarithmically as a function of time after drug addition. The half-life for decay of the protein synthesis capacity was calculated by least-squares regression analysis of the plots, as described above for the mass decay analysis. Correlation coefficients of the regression lines were ~0.95 or better.

In some early experiments, we controlled for variation in loading and recovery by adding reference cells to the [35S]methionine-labelled samples prior to electrophoresis. The reference cells were prepared by labelling a culture of the same strain for three generations with [3H]leucine at 20 μCi/ml (23). We then determined the 35S/3H ratio of the fusion protein in the different samples. However, the same half-lives were obtained by measuring only the 35S radioactivity. Thus, the sample-to-sample errors in loading and recovery were small enough that we did not use the reference cells in subsequent experiments.

RESULTS

The growth rate independence of β-galactosidase level in gnd-lac operon fusion strains suggested that growth rate-dependent regulation is exerted on a posttranscriptional step in gnd expression; this regulation was presumed to be of translational efficiency, but regulation of mRNA stability was also considered possible (2). Moreover, the properties of gnd-lacZ protein fusions lacking the ICS (2, 3) were consistent with either possibility. For example, the ICS itself or the RBS-ICS secondary structure could be the target for an endoribonuclease whose activity is inversely related to growth rate. Alternatively, since the ICS is complementary to the RBS of gnd mRNA (6), the translation initiation frequency of gnd mRNA might be directly proportional to growth rate. These two hypotheses make different predictions. If growth rate regulation is achieved by differential mRNA stability, then both the level and the stability of gnd mRNA should increase about threefold with increasing growth rate. On the other hand, if differential translation efficiency is the posttranscriptional mechanism for growth rate control, then both gnd mRNA level and half-life should be independent of growth rate.

Effect of growth rate on the level of gnd mRNA. The gnd mRNA fraction of total RNA was determined for strain HB301 growing in glucose and acetate minimal media. We did this by Northern analysis, which evaluates putatively full-length mRNA molecules, and by slot blot analysis, which measures the sum of all species of the target mRNA, both full-length molecules and mRNAs in the process of being degraded. In each case, the probe was DNA from M13 phase HB562-9, which carries an internal portion of the template strand of the gnd structural gene (17).

Figure 1A shows a representative Northern blot of total RNA isolated from glucose- and acetate-grown cells. The
observed hybridization signal represents gnd mRNA because it is absent in the RNA obtained from Δgnd strain RW231; moreover, the detected RNA was approximately 1,500 nucleotides in length, the size predicted for gnd mRNA from the DNA sequence of the gene and mapping of the 5' end of gnd mRNA (17). In addition, the probe was in excess to its target, as evidenced by the direct proportionality between the hybridization signal, measured by microdensitometry, and the amount of input total RNA. In six independent experiments, there was 2.6 (±0.6) times more gnd mRNA relative to total RNA in glucose-grown cells than in cells grown on acetate. Smaller species of gnd mRNA, i.e., decay intermediates, were not observed.

Figure 1B shows a slot blot analysis of gnd mRNA from the same strain. In these experiments, the gnd mRNA/total RNA ratio increased 2.4-fold with increasing growth rate. Thus, approximately the same value was obtained for the growth rate-dependent increase in the level of gnd mRNA when either full-length mRNA or the total hybridizable molecules were measured. This congruence suggests that the ratio of partially degraded to full-length gnd mRNA molecules is constant in cells growing under the two conditions. More importantly, since the total mRNA fraction of total RNA is essentially independent of growth rate (5), the gnd mRNA/total mRNA ratio increases about 2.5-fold with increasing growth rate, approximately the same factor by which the 6PGD/total protein ratio increases (200 U/mg in glucose-grown cells, 70 U/mg in acetate-grown cells). According to the argument presented above, this result predicts that the stability of gnd mRNA mass should increase with increasing growth rate.

**Effect of growth rate on decay of gnd mRNA mass.** We used Northern blot analysis to quantitate the decay of gnd mRNA mass in cells of strain HB301 growing in acetate and glucose minimal media after inhibition of transcription initiation with rifampin. Figure 2 shows a representative experiment measuring the decay of gnd mRNA in strain HB301. The autoradiogram shown in Fig. 2A and others resulting from exposure of the gel to X-ray film for different lengths of time were scanned with a video densitometer, and the areas under the peak representing the hybridization signal were plotted semilogarithmically against time after rifampin addition (Fig. 2B). Interestingly, in both the acetate and glucose cultures, exponential decay of gnd mRNA did not begin for more than 10 min after drug addition. Before that, the level of the mRNA actually increased two- to threefold over the first 5 to 6 min after rifampin addition, even though control experiments had established that the concentration of rifampin used (500 μg/ml) blocks transcription initiation in less than 1 min (22).

Linear regression analysis of the hybridization signals in the 15- to 30-min interval after rifampin addition was done for each of four experiments with cells growing on glucose and for two experiments with cells growing on acetate. The correlation coefficients of the respective regression lines were 0.99 or better. The average half-life of gnd mRNA in glucose-grown cells was 7.3 ± 0.9 min, while the half-life in the acetate cultures was 5.8 ± 0.1 min. This difference in gnd mRNA half-life is not statistically significant, but even if true, it could account for only a small part of the growth rate-dependent increase in gnd mRNA level. Therefore, most or all of the 2.5-fold increase in the level of gnd mRNA over the range of growth rates is due to increased gnd transcription. Moreover, the growth rate-dependent increase in gnd transcription is of sufficient magnitude to account for the growth rate-dependent regulation of 6PGD level. This conclusion was not predicted by either interpretation of the operon and protein fusion data.

The observed increase in gnd mRNA level after blocking of transcription initiation is unusual and reflects some difference between gnd and other genes, since decay of zwf mRNA mass following rifampin addition begins immediately, in the same strain and under the same conditions as in the experiments described here (24).

**Growth rate independence of functional mRNA half-life.** The level of β-galactosidase in ICS gnd-lacZ protein fusion strains increases with increasing growth rate by approximately the same threefold factor as the increase in 6PGD produced from a wild-type strain (3). This allowed us to investigate the effect of growth rate on functional mRNA half-life by determining the decay of the capacity to synthesize the 6PGD-β-galactosidase fusion protein after blocking of transcription initiation with rifampin. Knockout strain HB518 growing exponentially in acetate and glucose minimal media was treated with rifampin, and samples of equal culture volume taken at various times thereafter were pulse-labelled with [35S]methionine and chased with excess nonradioactive methionine as described in Materials and Methods. The 6PGD-β-galactosidase fusion protein in the various samples was purified by either (i) polyacrylamide gel electrophore-
sis of total cell protein and extraction of the hybrid protein from a gel slice or (ii) immunoprecipitation with anti-β-galactosidase antiserum. We determined the amount of radioactivity in each sample and expressed the values relative to that in the sample taken immediately before drug addition.

Figure 3 shows an autoradiogram of total labelled protein separated by polyacrylamide gel electrophoresis in one such experiment, and Fig. 4 shows a plot of the rate of synthesis of the fusion protein as a function of time after drug addition. Just as the level of gnd mRNA mass increased by twofold or more during the first 5 to 6 min after rifampin treatment, so did the relative rate of fusion protein synthesis. Accordingly, the functional mRNA half-life was calculated by using values from the time interval during which synthesis capacity decayed exponentially.

In three independent experiments with cells growing on acetate, the functional half-life was 4.0 ± 0.3 min, and it was 4.0 ± 0.2 min in two independent experiments with cells growing on glucose. Thus, like the decay of gnd mRNA mass, the functional decay of gnd-lacZ fusion mRNA does not change with increasing growth rate. Moreover, as concluded above, the threefold increase in 6PGD level over the range of growth rates between acetate and glucose as carbon sources can be accounted for by increased gnd transcription without invoking a requirement for differential mRNA stability.

In addition to the control experiments described in Materials and Methods, we conducted two other control experiments to ensure that the elevated rate of synthesis of the gnd-lacZ protein fusion was not an artifact of the experimental protocol. First, we used the gel slice method to determine the effect of rifampin on the capacity to synthesize four unknown proteins, labeled I, H, A, and X in Fig. 3. With proteins I, A, and X, the exponential decay of synthesis capacity began in less than 2 min without a significant increase above the value at zero time, and the respective functional half-lives were 2 to 4 min (22). Also, although the functional half-life for protein H was 9 min, synthesis capacity rose by about 50% and exponential decay began by 2 min (22). Thus, while the synthesis capacity for the gnd-lacZ fusion increased severalfold in the 5- to 8-min interval after rifampin addition, the rate of synthesis of other proteins declined normally. Moreover, the observed rifampin effect is not simply due to the long length of the fusion mRNA, because decay of the rate of synthesis of the β’ subunit of RNA polymerase, labeled D in Fig. 3, began within 2 min, even though, in agreement with previous reports (reviewed in reference 16), the rate initially increased about twofold.

In a second control experiment, we determined the effect of rifampin on the rate of synthesis of another fusion protein, the zwf-lacZ protein fusion of strain DR702(ADR104) (24). Similar to the previously reported decay of zwf mRNA mass (24), exponential decay of the capacity to synthesize the zwf-lacZ...
fusion protein began within 2 min, without an initial increase, and the functional half-life was 2 min in both glucose- and acetate-grown cells (22).

**DISCUSSION**

The primary objective of the work presented here was to determine the steps in gnd expression that are subject to growth rate-dependent regulation. The properties of gnd-lac operon and protein fusions had suggested previously that regulation was exerted at the posttranscriptional level, i.e., at the level of either mRNA stability or translation initiation, and that this posttranscriptional regulation involved the ICS, a negative control site located within the 6PGD coding sequence that is complementary to the translation initiation region of gnd mRNA (2, 3). Implicit in the arguments for posttranscriptional control was the expectation that the gnd fraction of total mRNA transcription would remain constant as growth rate increases. To our surprise, the data presented here indicate that nearly all of the growth rate-dependent regulation of 6PGD synthesis can be accounted for by a growth rate-dependent increase in the level of gnd mRNA, which is achieved by the gnd fraction of total mRNA transcription increasing in direct proportion to growth rate.

The following formal description of the relationships among the amount of 6PGD relative to total protein (P), the amount of gnd mRNA relative to the amount of total mRNA, and the translational efficiency of gnd mRNA (e\_g) relative to the translational efficiency of bulk mRNA (e\_m) is presented as a means of facilitating an understanding of the apparent paradox between conclusions pertaining to gnd regulation that are drawn from the data presented in this paper and those derived from the properties of gnd-lac operon and protein fusions. The relationships presented here are based in part on the derivations and equations described in more detail by Bremer and Dennis (5) and Hernandez and Bremer (10).

We start by asserting that under a given growth condition the relative amount of 6PGD produced (6PGD/P; i.e., enzyme specific activity) equals the product of the relative amount of gnd mRNA (gnd mRNA/total mRNA) times the relative translational efficiency of gnd mRNA (e\_g/e\_m). Defining the translation efficiency of gnd mRNA as the rate of 6PGD synthesis (r\_6PGD) per amount of gnd mRNA, the translation efficiency of bulk mRNA as the rate of total protein synthesis (r\_p) per amount of total mRNA, the rate of gnd transcription as e\_g, the rate of total mRNA synthesis as e\_m, and the half-lives of gnd mRNA, bulk mRNA, 6PGD, and total protein as t\_1/2\_g, t\_1/2\_em, t\_1/2\_6PGD, and t\_1/2\_m respectively, we proceed as follows.

\[
\text{specific activity} = \frac{6 \text{PGD}}{P} = \frac{\text{gnd mRNA}}{\text{total mRNA}} \times \frac{e_g}{e_m}
\]

Thus, if

\[
\text{amount of mRNA} = \frac{r_{\text{synthesis}}}{r_{\text{decay}}} = \frac{\ln 2}{r_{\text{decay}}}
\]

then,

\[
\text{amount of mRNA} = \frac{r_{\text{synthesis}}}{(\frac{\ln 2}{r_{\text{decay}}})} = \frac{r_{\text{synthesis}} \times t_{1/2}}{\ln 2}
\]

and thus

\[
gnd \text{ mRNA} = \frac{e_g \times t_{1/2}}{\ln 2} \Rightarrow \frac{r \times t_{1/2}}{r_m \times t_{1/2_m}}
\]

\[
\text{total mRNA} = \frac{r_m \times t_{1/2_m}}{(\ln 2)} \Rightarrow \frac{t_m}{t_{1/2_m}}
\]

Now since

\[
e_g = \frac{6 \text{PGD}}{P} \times \frac{t_{1/2_p}}{t_{1/2_g}} \times \frac{\text{total mRNA}}{\text{gnd mRNA}}
\]

then, when \(t_{1/2_p} = 1\)

\[
e_g = \frac{6 \text{PGD}}{P} \times \frac{\text{total mRNA}}{\text{gnd mRNA}}
\]

Finally

\[
\text{specific activity} = \frac{6 \text{PGD}}{P} = \frac{\text{gnd mRNA}}{\text{total mRNA}} \times \frac{\text{6 PGD}}{\text{P}} \times \frac{\text{total mRNA}}{\text{gnd mRNA}}
\]

\[
\text{specific activity} = \frac{6 \text{PGD}}{P} = \frac{r_g}{r_m} \times \frac{t_{1/2}}{t_{1/2_m}} \times \frac{e_g}{e_m}
\]

According to the relationships above, the threefold increase in 6PGD/P with increasing growth rate is accounted for nearly completely by the 2.5-fold increase in gnd mRNA/total mRNA; thus, the translation efficiency of gnd mRNA relative to that of bulk mRNA increases little if at all with increasing growth rate. This does not mean that the translation efficiency of gnd mRNA is independent of growth rate; rather, it means only that the translation efficiency of gnd mRNA is about the same as that of the mRNA for an average gene. Indeed, the translation efficiencies of both gnd mRNA and bulk mRNA increase in direct proportion to growth rate. [For example, the rate of 6PGD accumulation per cell mass unit (M), d(6PGD/M)/dt, is 6PGD/M × k\_p, where k = ln 2/60 and k \_p = doublings hour \^-1\;]; thus, since 6PGD/M increases about 3-fold over the 3.6-fold range of growth rates, the rate of 6PGD accumulation per mass unit increases about 10.8-fold. Finally, since the amount of gnd mRNA per mass unit increases about 3.3-fold with increasing growth rate, the translation efficiency of gnd mRNA increases about 3-fold with growth rate (22).]

The data presented in Fig. 2 show that the half-life of gnd mRNA mass, t\_1/2\_g, is growth rate independent, as is the half-life of bulk mRNA, t\_1/2\_m (21). This means that the ratio t\_1/2\_g/t\_1/2\_m does not change with growth rate. Accordingly, the observed 2.5-fold increase in gnd mRNA/total mRNA with increasing growth rate is due to an increase in the rate of gnd transcription, r\_g, relative to the rate of total mRNA transcription, r\_m.
We next use the equations as a framework for resolving the above-mentioned paradox in which direct measurement of the growth rate dependence of the relative amount and half-life of gnd mRNA seems to rule out a role for posttranscriptional control of the growth rate-dependent regulation of 6PGD. We first consider the ICS. In ICS+ gnd-lacZ protein fusions, β-galactosidase activity is about 600 Miller units during growth on acetate and is about 1,800 Miller units during growth on glucose, whereas in an ICS- gnd-lacZ protein fusion, β-galactosidase activity is about 1,800 Miller units during growth on acetate and about 2,400 Miller units during growth on glucose (3). In other words, in the ICS+ protein fusions, expression increases about threefold with increasing growth rate, but in the ICS- fusion, expression is nearly constant with growth rate. This means that if the ratio of ICS+ fusion mRNA to total mRNA increases with increasing growth rate by the same 2.5-fold factor as the ratio of gnd mRNA to total mRNA increases in a normal strain, then the translation efficiency of ICS- mRNA relative to that of bulk mRNA must decrease by this amount. This decrease in relative translation efficiency would occur (i) if the translation efficiency of ICS- mRNA during growth on acetate is elevated about 2.5-fold compared with those of normal gnd mRNA and ICS+ mRNA and (ii) if the translation efficiency during growth on glucose is unaffected by the absence of the ICS. Indeed, this argument is consistent with the aforementioned properties of the ICS- fusion. A second possibility, also consistent with the properties of the ICS- fusion, would be that the half-life of ICS- fusion mRNA relative to that of bulk mRNA decreases with increasing growth rate, while the relative transcription rate and relative translation efficiency are the same as in a normal strain. This would occur if the half-life of ICS- fusion mRNA during growth on acetate was about 2.5-fold longer than that of normal ICS+ mRNA, while the half-life of ICS- fusion mRNA during growth on glucose was the same as in the normal strain. In summary, regardless of whether the ICS affects translation efficiency or mRNA half-life, which could be determined by measurements of mRNA level and mass decay in ICS+ and ICS- fusion strains, it appears as if the presence of the ICS makes the translation efficiency or the half-life of gnd mRNA more like that of an average gene.

The arguments offered to explain the properties of the ICS- protein fusion can also be applied to the growth rate invariance of β-galactosidase level in gnd-lac operon fusion strains. If the transcription rate of the operon fusion is the same as that of the normal gnd gene, then either the relative translation efficiency or the relative half-life of operon fusion mRNA must decrease with increasing growth rate. In the case of the operon fusion, it is also possible, as mentioned previously (2), that the nature of the fusion joint leads to transcriptional polarity whose efficiency is inversely related to growth rate; if this was the case, then both the translation efficiency and half-life of the mRNA would be similar to those of the normal gnd gene. Again, we could distinguish among these possibilities by direct measurement of the growth rate dependence of the amount and half-life of operon fusion mRNA.

Other than for genes encoding components of the translational apparatus (7, 8, 12), the growth rate-regulated steps in expression have been determined for relatively few genes. For example, the growth rate dependence of the level of the outer membrane protein OmpA has been shown to be due to opposing changes in mRNA stability and translational efficiency (14, 20), while growth rate control of zwf, the gene for glucose 6-phosphate dehydrogenase, is exerted only on transcription (24).

In experiments aimed at determining the half-life of gnd mRNA, we observed that the level of gnd mRNA increased two- to threefold in the first 5 to 6 min after blocking of transcription initiation with rifampin. Similarly, the rate of synthesis of a gnd-lacZ fusion protein was severalfold higher 5 min after rifampin addition than it was in the steady-state condition prior to drug treatment. This phenomenon must reflect some relatively unusual property of the gnd gene, rather than being an artifact of the experimental regimens, since such an increase did not occur when we measured the decay of zwf mRNA mass after rifampin addition (24), nor was there an increase in the rate of synthesis of a zwf-lacZ fusion protein after drug treatment (22).

We do not know the basis for the above-described phenomenon, but most likely rifampin treatment, either directly or indirectly, stimulates gnd transcription or stabilizes gnd mRNA. For example, Morgan and Hayward (16) reported that rifampin stimulates transcription of the rpoBC genes by enhancing readthrough of an upstream transcription terminator. However, while we have not experimentally tested this hypothesis, the gnd leader does not contain an obvious transcription termination structure (17). Alternatively, as total mRNA decays after blocking of transcription initiation, the pool of free ribosomes should gradually increase. Thus, ribosome binding should increase on longer-lived mRNAs whose RBBS are relatively weak and undersaturated, leading to elevated translational yields on these mRNAs and perhaps indirectly to increased stability. Indeed, Pederson et al. (23) found that the protein synthesis capacity of some mRNAs increased after rifampin addition, although that of most mRNAs did not. Thus, since the half-life of gnd mRNA appears to be longer than average, this mechanism could apply, provided that the gnd RBBS is undersaturated.

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