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Dominant lethal phenotype of a mutation in the −35 recognition region of *Escherichia coli* σ^{70}

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ABSTRACT **A dominant lethal mutation in the *Escherichia coli rpoD* gene, which encodes σ^{70}, the promoter recognition subunit of RNA polymerase, was isolated after random mutagenesis. The lethal gene was maintained under control of the *lac* repressor on a low copy plasmid. An amount of lethal σ^{70} that was nearly equimolar with the chromosomally encoded σ^{70} was sufficient to cause cessation of growth. RNA synthesis per unit cell mass was unaffected, but protein synthesis was inhibited by the mutant σ^{70}. The amino acid change (Glu-585 to Gln) was in a region of σ^{70} thought to bind the −35 hexamer of the promoter, and the mutant σ^{70} caused increased expression from promoters with nonconsensus bases in the third position of the −35 hexamer. A null mutation of the *fis* gene could partially suppress the mutant phenotype. These properties are consistent with those expected of a σ^{70} insensitive to growth rate control of rRNA and tRNA promoters.**

In *Escherichia coli*, the intracellular concentration of ribosomes is proportional to the growth rate of the culture (1), except in very slowly growing or stationary phase cells, in which not all ribosomes are actively translating (2). The synthesis of ribosomes is regulated by controlling the initiation frequency from the P1 promoter at each of seven virtually identical rRNA operons (1, 3, 4). This regulation is termed "growth rate control." The other components of ribosomes, ribosomal proteins, are synthesized in response to this regulation of rRNA; if certain ribosomal proteins are produced in excess of the rRNA available for assembly of ribosomes, those unassembled ribosomal proteins inhibit translation of their gene and other ribosomal protein genes on polycistronic mRNAs (1). Most of the tRNA operons appear to be regulated in the same way as the rRNA operons (1). If additional rRNA operons are introduced on a multicopy plasmid, overall rRNA synthesis is unchanged, but expression of each chromosomal rRNA operon is decreased, compensating for expression of the plasmid-borne operons (5). This feedback phenomenon is presumed to be the same as growth rate control; however, the molecular details are unknown.

The P1 promoter region itself—including the −35 and −10 hexamers, the start site of transcription, but little flanking DNA—is sufficient for growth rate control (6). Extensive mutational analysis failed to reveal a possible binding site for a regulatory protein (7, 8). However, mutations that bring the promoter closer to the consensus, either a single base pair change at position 4 of the −35 hexamer or addition of a base pair to increase spacing between the −10 and the −35 hexamers to the optimal spacing (17 bp), eliminate growth rate control by permitting a high level of expression even at relatively slow growth rates (8). As a potential tool for study of growth rate control, we sought mutations in the promoter recognition subunit of *E. coli* RNA polymerase, σ^{70}, that would be analogous to these promoter mutations that eliminate regulation. That is, we sought altered forms of σ^{70} that would see the wild-type rRNA P1 promoter as if it were more nearly consensus. The effect of P1 promoter mutations had been evaluated using β-galactosidase fusions in single-copy λ lysogens (8), and expression of the seven chromosomal rRNA operons was unaffected. However, an unregulatable form of σ^{70} would affect expression of all rRNA operons and most tRNA operons. Such a mutant might be lethal. Since the desired mutant σ^{70} should express rRNA under conditions when the wild-type σ^{70} does not, the mutant we wished to isolate should be dominant. We therefore looked for dominant lethal mutations in *rpoD*, the gene encoding σ^{70}. Here, the isolation and properties of one such mutant are reported.

MATERIALS AND METHODS

Media and Growth Conditions. Minimal medium was Mops (9), and when appropriate, medium was supplemented with ampicillin (20 μg/ml), kanamycin (20 μg/ml), chloramphenicol (20 μg/ml), streptomycin (100 μg/ml), 5-bromo-4-chloro-3-indolyl β-D-galactoside (40 μg/ml), or isopropyl β-D-thiogalactoside (IPTG; 0.5 mM unless otherwise stated). Incubation temperature was 30°C.

Strains. NO3940 was constructed from NCM533, which is K12 F⁻ λ⁺ *lacI*^{q} *lacZ*::Tn*5* (10), by first replacing λ⁺ with *cI857 lacZ*⁺ using P1 transduction and then curing λ*cI857* by heat pulse. CAG339 is *str his arg rpoD800*. RLG851 (CAG4000) and RLG1863 are *fis*⁺ and *fis*⁻ (*fis 767*::*kan*), respectively, and are otherwise isogenic *lac*⁻ derivatives of MG1655 (11). The NO3940 derivatives carrying fusions to *lacZ* of P22 *ant* promoter variants were constructed as follows. P22 tail protein was added to P22 lysates of each variant (12) and spotted onto a plate spread with MS3034, which carries a chloramphenicol-resistance F′ vector bearing a P22 *att* site, pOX38BC (13). Colonies were streaked from the spots and scored for the Lac⁺ and kanamycin-resistance phenotypes of the P22 phages. pOX38BC derivatives, now lysogenized with the P22 *ant* promoter variant fusions, were transferred to the restriction-negative *E. coli* strain MC1061 (*leu*⁻, *lac*⁻, *str*^{R}) by conjugation and selection for chloramphenicol and streptomycin resistance. Conjugation of the MC1061 derivatives with NO3940 and selection for Leu⁺ and chloramphenicol resistance yielded the host strains, which were then transformed with pNO3099 and used to generate the data in Table 2. β-Galactosidase assays and standard genetic methods were as described (14, 15).

Plasmids and Mutagenesis. For conditional expression of the *E. coli rpoD* gene, a 7.9-kb ampicillin-resistance low copy plasmid, pNO3097, was constructed, which is a derivative of pJEL126 (16). It also carried the wild-type *lac* promoter, a polylinker downstream of it, and the gene for the *lac* repressor. The sequence and a complete description of pNO3097 have been deposited in the GenBank data base (accession no. L05669). The 2.2-kb *Hpa* I–*Apa*LI fragment carrying the *E.*

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Abbreviation: IPTG, isopropyl β-D-thiogalactoside.

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coli rpoD gene (17) was obtained from phage 19F2 of the Kohara library (18), and it was cloned downstream of the *lac* promoter in pNO3097 such that it was flanked by unique *Eco*RI and *Xba* I sites, resulting in pNO3098. Primers for PCR that annealed outside these sites were chosen. Mutagenic PCR reactions contained 20 mM Tris·HCl (pH 8.3), 50 mM KCl, 0.001% gelatin, 0.5 mM $MnCl_2$, one dNTP at 0.2 mM, the other three dNTPs at 1 mM, 0.4 nM linearized template, each primer at 0.3 μM, and, as optimized for this primer set, 3.2 mM $MgCl_2$ (19). Amplification was for 25 cycles. The product was cut with *Eco*RI and *Xba* I and ligated to unmutagenized vector that had been cut with the same enzymes. After identification of the mutant, the fragment of *rpoD* responsible for the mutant phenotype was cloned into unmutagenized pNO3098 to give pNO3099 (see text). The R588H mutation (20) was cloned on the *Cla* I–*Apa*LI fragment into pNO3098, resulting in pNO3100.

RESULTS

Rationale and Mutant Isolation. A dominant lethal mutation in σ^{70} was sought; therefore, it was necessary to be able to conditionally express a mutant allele. This was achieved by cloning the gene for σ^{70}, *rpoD*, downstream of the wild-type *lac* promoter on a low copy plasmid, so that its expression was under control of the *lac* repressor. In the absence of IPTG (or other inducers of the *lac* operon), expression of σ^{70} from the plasmid is undetectable, but in its presence, plasmid-encoded σ^{70} can accumulate to an amount nearly equimolar with chromosomally encoded σ^{70} (see below).

The *E. coli rpoD* gene was mutagenized by PCR (19) and introduced into strain NO3940. Transformants were selected on glucose minimal medium (plus antibiotic). On these plates, the *lac* promoter is repressed due to the lack of inducer and also by glucose catabolite repression. Transformants were screened by replica plating onto glycerol minimal medium plus IPTG, with proline instead of ammonia as a nitrogen source (inducing medium) and then onto glucose minimal medium as a control for transfer. Candidate dominant lethal mutants were recovered from the master plates. The one that had the most severe growth defect, as judged by colony size on inducing plates, was chosen for further study. After preparation of plasmid DNA from the mutant strain, the wild-type host strain (NO3940) was retransformed, and an identical phenotype resulted, demonstrating that the mutation responsible for the defect resided on the plasmid.

Growth Properties and Viability of the Mutant. The effect of the dominant σ^{70} mutation on growth in liquid medium is shown in Fig. 1. The uninduced culture showed no growth defect; its generation time in glycerol minimal medium at 30°C was 85–90 min, the same as a control strain harboring the vector alone. After induction of synthesis of the mutant

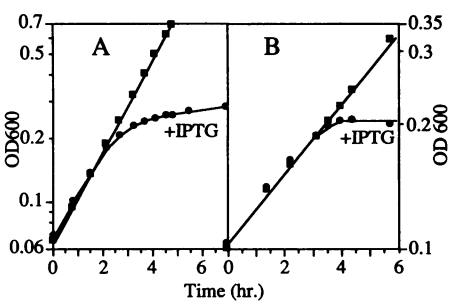

FIG. 1. Growth inhibition after induction of mutant σ^{70} synthesis by IPTG. NO3940 containing pNO3099 was grown in minimal medium with glycerol (*A*) or with D-alanine (*B*) as the carbon source. ●, IPTG was added at time 0 to cultures; ■, control cultures without IPTG.

σ^{70} by addition of IPTG, the growth rate of the induced culture gradually decreased, beginning about 2 hr after induction at 30°C (Fig. 1*A*). By about 3.5–4 hr after induction, growth had almost completely ceased. In glycerol minimal medium, an induced mutant culture grew about two generations before cessation of growth, whereas in minimal medium with D-alanine as a carbon source, which supports a growth rate about 2-fold less than does glycerol, an induced mutant culture grew only about one generation before cessation of growth (Fig. 1*B*). In this particular K12 genetic background (NO3940), an induced mutant culture grown in rich medium like Luria–Bertani (LB) broth exhibits no growth defect during logarithmic phase; however, we believe that this is because the mutant σ^{70} is poorly expressed (see below). In other genetic backgrounds such as MG1655, MC1000, or CSH50, cessation of growth was observed in LB as well as in glycerol minimal medium.

To determine whether cessation of growth was simply arrested growth or was irreversible (lethal), samples of induced cultures were diluted and plated under permissive, noninducing conditions on LB plates with no IPTG. After cultures induced in glycerol minimal medium ceased growth (4 hr after induction), the colony-forming units per OD_{600} ranged from 0.5% to 5% of the value for logarithmic phase uninduced control cultures. Although no loss of viability was seen in logarithmic phase-induced cultures in LB (in the NO3940 strain), upon entry into stationary phase they exhibited a dramatic loss of viability. Over a period of 2–3 hr after entry into stationary phase, the colony-forming units per OD_{600} of the induced mutant culture decreased to <0.1% of the value for the uninduced control culture entering stationary phase. Thus, the mutant phenotype appears to be lethal. The survivors, when retested, exhibited the full mutant phenotype.

The Amount of Mutant σ^{70} Expressed in Induced Cells. To estimate the amount of the dominant lethal σ^{70} present in growth-inhibited cells, the plasmid bearing the mutant *rpoD* gene was introduced into strain CAG339, whose chromosomally encoded σ^{70} migrates anomalously fast in SDS/PAGE because of a 14-aa internal deletion (21). A culture of this strain was divided, and several concentrations of IPTG were added to induce synthesis of the dominant lethal σ^{70} to different extents (Fig. 2*A*). This resulted in varying degrees of growth inhibition, from complete inhibition (0.12 mM IPTG) to only slight inhibition (0.06 mM IPTG). Samples taken at times designated "#1" and "#2" were subjected to Western blot analysis (Fig. 2*B*). In the sample from uninduced cells (0 IPTG, sample #2), the upper band is undetectable, indicating that the plasmid-encoded σ^{70} is not expressed. In a sample taken after growth had been completely inhibited, the dominant lethal, plasmid-encoded σ^{70}, which is the same size as wild-type σ^{70} (Fig. 2*B*, 0.12 mM IPTG, sample #2, upper band), was less than or equal in concentration to the chromosomally encoded σ^{70} (lower band). Just prior to complete cessation of growth, the concentration of the dominant mutant σ^{70} is about half that of the reference σ^{70} species (Fig. 2*B*, 0.12 mM IPTG, sample #1 and 0.09 mM IPTG, sample #2). Even a relatively small amount of mutant σ^{70}, about one-fifth of the chromosomally encoded σ^{70}, decreases growth rate to some extent (0.06 mM IPTG, sample #2). We conclude that expression of the mutant phenotype does not require a large excess of the mutant σ^{70} and that the degree of growth inhibition roughly correlates with the relative amount of lethal σ^{70}.

Macromolecular Synthesis Rates. We monitored RNA and protein synthesis rates as growth slowed after induction of expression of the dominant lethal σ^{70}. Table 1 presents results of pulse labeling at various times after induction. The strain and growth conditions were the same as for Fig. 1*A*, and the growth curve was also similar. As the growth rate slowed

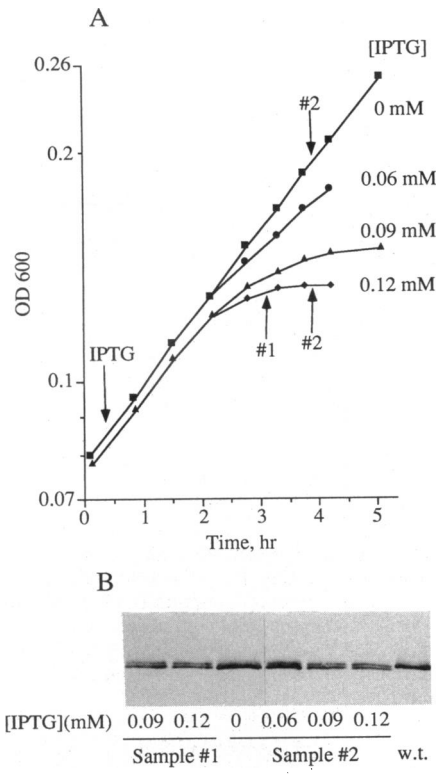

FIG. 2. Correlation of the relative amount of lethal σ^{70} with the degree of growth inhibition. (*A*) Strain CAG339 containing pNO3099 was grown in LB at 30°C. It was in steady-state growth when IPTG was added at 20 min to the final concentrations indicated to induce synthesis of the lethal σ^{70}. (*B*) At the times indicated as "#1" and "#2", samples were withdrawn from each of the cultures; cells were pelleted, resuspended in SDS loading buffer, and frozen. Later, the samples, from equal volumes of culture and uncorrected for culture density, were electrophoresed in a 10% SDS gel at 25 mA until the dye front had reached the bottom of the gel and then for another 3 hr longer. The Western blot was probed with monoclonal antibody 2F8 directed against σ^{70} (22) and visualized by colorimetric detection of alkaline phosphatase-conjugated secondary antibody. The wild-type sample (w.t.) is from NO3940.

after induction, the rate of [^{14}C]lysine incorporation per unit cell mass decreased, almost proportionally. The rate of [^{3}H]uridine incorporation per unit cell mass was unchanged (within experimental error) as the growth rate decreased. Thus, the bulk RNA synthesis rate appears to be unaffected by expression of the dominant lethal σ^{70}, whereas the protein synthesis rate is inhibited.

In a separate experiment using chemical assays for RNA and protein, we demonstrated accumulation of RNA in cells whose growth was inhibited by the mutant σ^{70} (data not shown), consistent with the undiminished RNA synthesis rates but decreased growth rates in Table 1. RNA·DNA hybridization analysis of pulse-labeled RNA showed that the proportion of total RNA that is rRNA was constant during growth inhibition (data not shown). The level of ppGpp, the nucleotide that mediates the stringent response, was the same, within experimental error, in induced mutant cells as in uninduced cells, indicating that an elevated level of this nucleotide was not responsible for the growth inhibition by the mutant σ^{70} and suggesting that translation was not substrate limited (data not shown).

Localization of the Mutation. The mutation was localized to the carboxyl terminus of the plasmid-borne *rpoD* gene by restriction fragment exchange. A fragment of the mutant gene extending downstream from the *Cla* I site to the *Xba* I site outside the gene, which included the carboxyl terminal 8% of

Table 1. Pulse-labeling of RNA and protein in the absence and presence of the dominant lethal σ^{70}

Time after induction, min	Growth rate,* hr^{-1}	dpm × 10^{-3}/OD_{600}†	
		[^{3}H]Uridine	[^{14}C]Lysine
0‡	0.48	435	53
67	0.48	556	62
116	0.38	546	53
153	0.27	440	26
184	0.17	475	21
215	0.12	508	17
245	0.10	489	14

At various times after addition of IPTG to a glycerol minimal culture of NO3940 bearing the dominant lethal *rpoD* mutant, 190-μl samples of culture were pulse-labeled with a mixture of [^{3}H]uridine (0.3 μCi/μmol, 6 μCi/ml final concentration) and [^{14}C]lysine (2.7 μCi/μmol, 20 μCi/ml final concentration) for 2 min, then chased with a 350-fold excess of unlabeled uridine and lysine for 5 min. After trichloroacetic acid precipitation in the presence of RNA and protein carriers, the radioactivities of the trichloroacetic acid precipitates were determined, corrected for counting efficiency, and then divided by the OD_{600} of the culture at the time of sampling.

*Specific growth rate, equal to the product of ln 2 and the mass doublings per hour.

†Trichloroacetic acid precipitable dpm.

‡Average of three pulse-labelings at cell densities comparable to those of the induced culture.

rpoD (amino acids 566–613), was sufficient to confer the complete mutant phenotype when ligated to wild-type *rpoD*. This plasmid, pNO3099, was used for all of the experiments described in this paper. Only a single-base-pair change from the wild-type sequence was found in the *Cla* I fragment, a G to C substitution resulting in a change of Glu-585 (GAA) to Gln (CAA), designated E585Q. The limiting nucleotide in the mutagenic PCR reaction that yielded this mutation was dCTP, consistent with the initial substitution occurring on the noncoding strand. This amino acid change lies between two previously described σ^{70} mutations at positions 584 and 588 (20, 23). Each of these mutations alters the promoter specificity of RNA polymerase such that it recognizes nonconsensus bases at a certain position in the −35 hexamer better than does polymerase with wild-type σ^{70}. These mutations, together with supporting biochemical evidence (24), define the −35 recognition region of σ^{70}. Accordingly, we tested our dominant lethal mutation, E585Q, for altered specificity.

Promoter Specificity of the Mutant σ^{70}. The plasmid bearing the dominant lethal σ^{70} mutation was transformed into a series of strains that carried *lacZ* fused to variants of the phage P22 *ant* promoter (12). The variants included the wild-type *ant* promoter, which has a consensus −35 hexamer, TTGACA, and promoters with substitutions at the second, third, fourth, or fifth positions of the −35 hexamer. Table 2 presents β-galactosidase activities of control, uninduced cultures and of cultures in which synthesis of the E585Q mutant σ^{70} has been induced. In uninduced cultures, only chromosomally encoded wild-type σ^{70} is present, whereas in induced cultures, both mutant and wild-type σ^{70} are present. The E585Q mutation causes about 6-fold or 3-fold increased expression from the −33A or −33C promoters, respectively, in induced versus uninduced cells.

A previously described *rpoD* mutation at amino acid 588 (R588H) exhibits specificity similar to the dominant lethal E585Q mutation (20). We cloned the R588H mutation into the same conditional expression vector used for E585Q. After synthesis of the R588H mutant σ^{70} was induced with IPTG in glycerol minimal medium, the generation time increased only slightly, from 84 min to 96 min. For the R588H mutant, the differential rate of β-galactosidase expressed from the −33A promoter variant in induced cells was 7100 Miller units, a 2.5-fold greater value than was obtained for the lethal E585Q

Table 2. β-Galactosidase activity expressed from variants of the P22 *ant* promoter

Promoter	β-Galactosidase activity, Miller units		Ratio of induced to uninduced
	Uninduced	Induced	
	Experiment 1		
Wild-type	20,300	16,400	0.81
−33A	420	2,170	5.2
−32T	4,080	3,380	0.83
−32C	4,670	3,860	0.83
−32G	858	600	0.70
−31T	1,510	1,030	0.68
−31G	1,030	720	0.70
−31A	6,130	5,180	0.84
	Experiment 2		
−34A	3,000	2,200	0.73
−34C	2,200	1,400	0.64
−33A	330	2,160	6.5
−33C	530	1,520	2.9
−33T	2,670	3,900	1.5

Parallel steady-state cultures in glycerol minimal medium were left uninduced or were induced for synthesis of the dominant lethal σ^{70} (E585Q) by addition of IPTG at culture densities between 0.05 and 0.09 OD_{600} units. For experiment 1, the activities are averages of samples taken 1.8 hr and 3.2 hr after induction. For experiment 2, the activities were determined from differential rate plots of five samples taken between 1.2 and 3.2 hr after induction. The −35 hexamer for the wild-type promoter from −35 to −30 is TTGACA.

mutant in the same experiment, 2900 Miller units. (β-Galactosidase activities in uninduced control cells were 410 and 350 Miller units for the R588H and E585Q mutants, respectively.) The viability of induced R588H cells in this experiment, which was tested 6.5 hr after induction, was 67% of uninduced cells, whereas the viability of induced E585Q cells was 0.5% of uninduced cells. Thus, although the R588H σ^{70} transcribes from the −33A promoter variant about twice as efficiently as the E585Q σ^{70}, it is not lethal.

Partial Suppression of the Mutant Phenotype. We searched for conditions or genetic backgrounds that could suppress the growth defect of the E585Q σ^{70} mutant. To control for the possibility that a simple decrease in the intracellular concentration of the mutant σ^{70} could mimic suppression, the −33A promoter variant fused to β-galactosidase was employed as an indicator of E585Q σ^{70} expression. By this criterion, in the NO3940 strain, the E585Q σ^{70} was not sufficiently expressed after addition of IPTG during logarithmic-phase growth in LB, consistent with the absence of a growth defect under these conditions. However, for strain RLG851 (whose genetic background is MG1655) growing in logarithmic phase in LB medium, synthesis of the E585Q σ^{70} was induced as judged by the growth defect (Fig. 3) and by 9-fold increased expression of the −33A *lac* fusion. A *fis* null mutation in the isogenic strain, RLG1863, afforded partial suppression of the growth defect in LB medium (Fig. 3). The differential rate of expression from the −33A promoter in the *fis*$^-$ strain after induction of the E585Q σ^{70} in LB was 10-fold increased relative to the uninduced control culture, indicating that comparable intracellular concentrations of the mutant σ^{70} were present in induced cultures of both *fis*$^+$ and *fis*$^-$ strains. Thus, absence of the Fis protein prevents some of the growth inhibition caused by the dominant σ^{70}, E585Q. Suppression of the growth defect in glycerol minimal medium by the *fis*$^-$ mutation was not evident, perhaps because more slowly growing *E. coli* contain little Fis protein (25), and hence the Fis content of the *fis*$^+$ cells might be similar to that of the *fis*$^-$ cells in this medium.

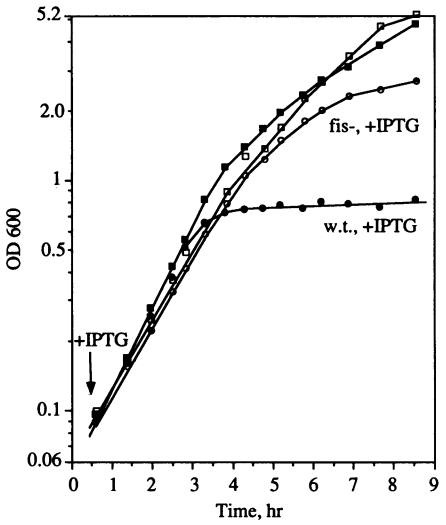

FIG. 3. Suppression of the growth defect caused by E585Q σ^{70} by a *fis* null mutation. Strains RLG851 (*fis*$^+$; filled symbols) and RLG1863 (*fis*$^-$; open symbols) containing pNO3099, which carries the *rpoD* E585Q dominant lethal mutation, were grown in LB. Synthesis of the E585Q σ^{70} was induced with IPTG as indicated by the arrow (circles), or uninduced control cultures were grown in parallel (squares).

DISCUSSION

By screening for a severe growth defect, we have isolated a dominant lethal mutation in the *rpoD* gene, which encodes the major promoter recognition subunit, σ^{70}, of *E. coli* RNA polymerase. The mutation is conditional in that expression of the mutant σ^{70} (called E585Q) is from the *lac* promoter and therefore expression requires inducer (IPTG).

Several lines of evidence indicate that the mutant σ^{70}, E585Q, is not dominant simply because of loss of function and sequestering of core RNA polymerase into an inactive holoenzyme. First, not enough of the mutant σ^{70} is expressed to sequester core polymerase: by the time cell growth has halted, mutant σ^{70} and chromosomally encoded wild-type σ^{70} were equally abundant. Since the ratio of σ^{70} to core is about 0.3 (26), only about one-third of core polymerase could be sequestered. Also, the overall rate of RNA synthesis per unit cell mass is unaffected by accumulation of the mutant σ^{70}. Rather, it appears that the E585Q σ^{70} is functional but has altered activity, because it causes more frequent transcription from promoters with a nonconsensus base in the highly conserved third position of the −35 hexamer, −33A or −33C, than does wild-type σ^{70}.

This altered promoter specificity exhibited by the E585Q σ^{70} seems insufficient to account for its lethal phenotype because a previously characterized mutation located only 3 aa away, R588H (20), causes 2-fold more frequent transcription from the same nonconsensus promoters than does E585Q, but R588H is not lethal. Thus, the growth defect and lethality of E585Q σ^{70} are not due to excessive or inappropriate transcription from promoters similar to the nonconsensus −33A or −33C promoter variants. It is possible that a class of promoters exists that are recognized by E585Q σ^{70} but not by R588H or by wild-type σ^{70}, whose −35 hexamers differ either by context or by multiple nonconsensus bases from the set of promoters that was surveyed here. Another possible explanation is that, in addition to altering promoter specificity, E585Q also alters an interaction with a regulatory molecule or protein, perhaps with an effector whose interaction with σ^{70} depends on a particular conformation of σ^{70}, which would be assumed when it binds to a certain −35 sequence, and E585Q fails to assume that particular conformation, resulting in inappropriate transcription.

How might a mutation in the promoter recognition subunit of RNA polymerase cause inhibition of translation? It is unlikely that the inhibition is due to a failure to synthesize ribosomes, because in D-alanine medium, upon induction of E585Q σ^{70} synthesis, growth ceases after only one mass doubling, at which point preexisting ribosomes would have been diluted only 2-fold. Bulk RNA synthesis, as well as its distribution into stable RNA and mRNA, seems unaffected by the lethal σ^{70}, consistent with the fact that at least half of the σ^{70} in the cell is wild type, even after growth has ceased. Therefore, the decrease in protein synthesis cannot be explained by a general decrease in mRNA synthesis. The ppGpp levels are unchanged, indicating that aminoacyl-tRNA pools are not depleted, and since RNA synthesis continues unabated as growth slows, the cellular energy charge is probably maintained. It appears then that the substrates necessary for translation are present, and perhaps translation is being actively inhibited. One possible answer is that the inappropriate transcription caused by the altered specificity discussed above is of a gene encoding a general inhibitor of translation. Another explanation is that the translational inhibition is a normal cellular regulatory response that has been triggered by excessive or inappropriate transcription. These two possible explanations will be considered further below.

To discuss the implications of the partial suppression of the E585Q mutant growth defect by a *fis* null mutation, we summarize what is currently understood about the role for the Fis protein in *E. coli* physiology. The Fis protein binds upstream of rRNA and tRNA promoters and stimulates their rates of initiation (11, 27). The protein is most abundant in cells grown in rich medium, especially immediately after dilution of stationary phase cells into fresh medium. Fis protein is scarce in cells grown in poorer medium like glycerol minimal and is also scarce in stationary phase cells (25). For a *fis*$^-$ strain in rich medium, the loss of stimulation of stable RNA promoters is compensated for by the growth rate control system (or feedback control system), so that the rates of rRNA and tRNA synthesis are unchanged (11). This regulatory system is serving its normal purpose, that of controlling the proportion of cellular resources dedicated to protein synthesis in response to the overall nutritional quality of the medium by an unknown homeostatic feedback mechanism. In a *fis*$^-$ strain in rich medium, the growth rate control system is in a state that is 4- to 5-fold more derepressing than in a *fis*$^+$ strain (11).

One explanation for the *fis*$^-$ suppression effect is simply that expression of the hypothetical inappropriately transcribed gene, which encodes a translational inhibitor, depends on or is stimulated by Fis. However, Fis is present in high amounts at times when the genes for the protein synthetic machinery need to be highly expressed; therefore, Fis-mediated expression of a translational inhibitor would require additional regulation. Another scenario is as follows: if the E585Q mutant σ^{70} is insensitive to the growth rate control system, then as the mutant σ^{70} accumulates after induction, excessive stable RNA transcription would follow, except that the wild-type σ^{70} is still sensitive and the growth rate control system could compensate by repressing the activity of wild-type σ^{70} at stable RNA promoters. As mutant σ^{70} continues to accumulate, repression of wild-type σ^{70} activity at stable RNA promoters would become progressively more severe. The result would be an unchanged rate of stable RNA synthesis, but a state of the growth rate control system that becomes more and more repressing. To account for the inhibition of translation observed, we must postulate that initiation of translation can also be repressed by the growth rate control system, although it would be less sensitive than is initiation of stable RNA synthesis. There is precedent for regulation of translation, though the nature of the regulator is unknown: at very slow growth rates, a proportion of ribosomes is not engaged in protein synthesis (2). In summary, expression of a mutant σ^{70} that is insensitive to the homeostatic growth rate control system would cause that system to assume a state exerting much more repression. In a *fis*$^-$ strain the growth rate control system is in a state exerting 4- or 5-fold more derepression than wild type, which would compensate for the effect of the mutant σ^{70} and partially suppress its phenotype.

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1. Jinks-Robertson, S. & Nomura, M. (1987) in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, ed. Neidhardt, F. C. (Am. Soc. Microbiol., Washington, DC), pp. 1358–1385.
2. Norris, T. E. & Koch, A. L. (1972) *J. Mol. Biol.* **64**, 633–649.
3. Kiss, A., Sain, B. & Venetianer, P. (1977) *FEBS Lett.* **79**, 77–79.
4. Sarmientos, P. & Cashel, M. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 7010–7013.
5. Jinks-Robertson, S., Gourse, R. L. & Nomura, M. (1983) *Cell* **33**, 865–876.
6. Gourse, R. L., deBoer, H. A. & Nomura, M. (1986) *Cell* **44**, 197–205.
7. Gaal, T., Barkei, J., Dickson, R. R., deBoer, H. A., deHaseth, P. L., Alavi, H. & Gourse, R. L. (1989) *J. Bacteriol.* **171**, 4852–4861.
8. Dickson, R. R., Gaal, T., deBoer, H. A., deHaseth, P. L. & Gourse, R. L. (1989) *J. Bacteriol.* **171**, 4862–4870.
9. Neidhardt, F. C., Bloch, P. L. & Smith, D. F. (1974) *J. Bacteriol.* **119**, 736–747.
10. Shand, R. F., Mierke, L. J. W., Mitra, A. K., Fong, S. K., Stroud, R. M. & Betlach, M. C. (1991) *Biochemistry* **30**, 3082–3088.
11. Ross, W., Thompson, J. F., Newlands, J. T. & Gourse, R. L. (1990) *EMBO J.* **9**, 3733–3742.
12. Moyle, H., Waldburger, C. & Susskind, M. M. (1991) *J. Bacteriol.* **173**, 1944–1950.
13. Waldburger, C., Gardella, T., Wong, R. & Susskind, M. M. (1990) *J. Mol. Biol.* **215**, 267–276.
14. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
15. Davis, R. W., Botstein, D. & Roth, J. R. (1980) *Advanced Bacterial Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
16. Valentin-Hansen, P., Albrechsten, B. & Larsen, J. E. L. (1986) *EMBO J.* **5**, 2015–2021.
17. Burton, Z., Burgess, R. R., Lin, J., Moore, D., Holder, S. & Gross, C. A. (1981) *Nucleic Acids Res.* **9**, 2889–2903.
18. Kohara, Y., Akiyama, K. & Isono, K. (1987) *Cell* **50**, 495–508.
19. Leung, D. W., Ellison, C. & Goeddel, D. V. (1989) *Technique* **1**, 11–15.
20. Gardella, T., Moyle, H. & Susskind, M. M. (1989) *J. Mol. Biol.* **206**, 579–590.
21. Hu, J. C. & Gross, C. A. (1983) *Mol. Gen. Genet.* **191**, 492–498.
22. Strickland, M. S., Thompson, N. E. & Burgess, R. R. (1988) *Biochemistry* **27**, 5755–5762.
23. Siegele, D. A., Hu, J. C., Walter, W. A. & Gross, C. A. (1989) *J. Mol. Biol.* **206**, 591–603.
24. Dombroski, A. J., Walter, W. A., Record, M. J., Siegele, D. A. & Gross, C. A. (1992) *Cell* **70**, 501–512.
25. Nilsson, L., Verbeek, H., Vijgenboom, E., van Drunen, C., Vanet, A. & Bosch, L. (1992) *J. Bacteriol.* **174**, 921–929.
26. Engbaek, F., Gross, C. & Burgess, R. R. (1976) *Mol. Gen. Genet.* **143**, 291–295.
27. Verbeek, H., Nilsson, L., Baliko, G. & Bosch, L. (1990) *Biochim. Biophys. Acta* **1050**, 302–306.