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Gratuitous Overexpression of Genes in *Escherichia coli* Leads to Growth Inhibition and Ribosome Destruction

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We attempted to test the idea that the relative abundance of each individual tRNA isoacceptor in *Escherichia coli* can be altered by varying its cognate codon concentration. In order to change the overall codon composition of the messenger pool, we have expressed in *E. coli lacZ* with the aid of T7 RNA polymerase and, separately, we have expressed a truncated *tufB* gene (designated Δ*tufB*) with the aid of *E. coli* RNA polymerase so that their respective gene products individually accounted for 30% of the total bacterial protein. Unexpectedly, the maximum expression of either test gene has no specific effect on the relative rates of synthesis of the tRNA species that we studied. Instead, we find that there is a cumulative breakdown of rRNAs, which results in a loss of ribosomes and protein synthetic capacity. After either of the test genes is maximally induced, there is a growing fraction of protein synthesis invested in β-galactosidase or Δ*tufB* that is matched by a comparable decrease of the fraction of normal protein synthesis. We have also observed enhanced accumulation of two heat shock proteins during overexpression. Finally, after several hours of overexpression of either test protein, the bacteria are no longer viable. These results are relevant to the practical problems of obtaining high expression levels for cloned proteins.

There is a good correlation between the frequencies of codon usage in the mRNA pools and the abundance of the corresponding tRNA species for bacteria growing in rich laboratory media (10). Furthermore, when the quality of the culture media is systematically varied, the tRNA abundance changes in expected ways. In particular, the relative fraction of major tRNA species that translate major codons increases as the growth rates of the bacteria increase (4, 6). This variation is thought to be parallel to an increased usage of major codons in the mRNA pool at the higher growth rates (3, 5, 14). The present experiments were initiated to study the influence of the codon frequencies in the mRNA pool on the synthesis of individual tRNA species by bacteria.

We have followed the consequences for *Escherichia coli* of overexpressing to very high levels genes whose protein products have no function for the cells, so-called gratuitous proteins. In this way, we have manipulated the codon composition of the mRNA pool, and we have measured the influence of this manipulation on the abundance of a number of tRNA species. The two gratuitous proteins that we have used were chosen because they are encoded by mRNA species with very different codon frequencies. One such protein is β-galactosidase, which is metabolically useless under the growth conditions we studied and which has an mRNA with a predominantly minor codon composition (21). The other is a truncated version of elongation factor Tu, called ΔEF-Tu, which is functionally inactive and is encoded primarily by major codons (21). The results of overexpressing these two proteins were similar and not at all the expected ones.

Expression of either β-galactosidase or ΔEF-Tu to as much as 30% of the total bacterial protein has no specific effect on the relative rates of synthesis of the tRNA species that we studied. Instead, we find a cumulative breakdown of rRNA associated with a progressive loss of protein synthesis capacity. We have also observed enhanced accumulation of two heat shock proteins during overexpression. After several hours of overexpression of either test gene, the bacteria are no longer viable.

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MATERIALS AND METHODS

Bacterial strains and plasmids. *E. coli* HDS106 Δ(*lac pro*) *ara gyrA argE recA* (λDE3)/F′ *lacI*^{q} *pro*$^+$ is a bacteriophage λ lysogen, containing a single chromosomal copy of the T7 RNA polymerase gene under the control of the *lacUV5* promoter (23). HDS117 is a *recA* derivative of *E. coli* MG1655. It was constructed through conjugation of MG1655 with US567 (HfrK16 *sbl*::Tn*10 recA56 ile thi his thr300 rpsE30*) using tetracycline as a selection marker.

pHD14 was constructed by cloning a *Bam*HI-*Bam*HI DNA fragment harboring the *lacZ* gene from pFJU175 (12) into the *Bam*HI site of pET3-b (23). pTuB12.1 contains the *E. coli tufB* gene with an in-frame deletion between nucleotides 248 and 491 of the *tufB* gene (25). pHD67 was constructed by ligating the *Bam*HI-*Pst*I fragment containing the Δ*tufB* gene from pTuB12.1 with a pTrc99c (1) cleaved with *Bam*HI and *Pst*I (see Fig. 2). The cloning was carried out in HB101 (19).

Media and growth of the cells. The media used were Luria broth (15) and M9-glycerol (0.4%, wt/vol) supplemented with thiamine and all amino acids except methionine and proline (12). In addition, adenine, cytosine, thymine, and xanthine were added to the M9-glycerol medium at a final concentration of 0.2 mM. Ampicillin and tetracycline were added at concentrations of 200 and 20 μg/ml, respectively.

For growth rate assays, overnight cultures of HDS106(pHD14) or HDS117 (pHD67) were diluted 1:40 in M9-glycerol media and the cells were grown at 37°C to an optical density at 540 nm (A_{540}) close to 0.5. The cells were rediluted (1:20) in prewarmed M9-glycerol medium with or without isopropyl-β-D-thiogalactopyranoside (IPTG). The cultures were grown at 37°C with vigorous shaking, and the growth rates of the cultures were monitored spectrophotometrically.

Protein quantification. Exponentially growing bacteria were uniformly labelled with 15 μCi of L-[^{35}S]methionine per ml (specific activity, 1,000 Ci/mmol; Amersham) and 20 μM nonradioactive methionine for two generations (100 min) before they were induced with 1 mM IPTG, and the cells were continuously labelled in the presence of the inducer. Prior to and at different time points after induction, 1-ml aliquots of cells were removed and chilled on ice. The cells were pelleted, resuspended in 100 μl of protein lysis buffer (12), and boiled for 3 min. Fifteen-microliter aliquots from the cell lysates were applied onto sodium dodecyl sulfate (SDS)-polyacrylamide gels (8% polyacrylamide for the β-galactosidase experiments and 10% polyacrylamide for the ΔEF-Tu experiments). The proteins were separated on two gels. One gel was electrophoresed at 100 V for 12 h for fractionation of the proteins. The other gel was run for only 1.5 h, allowing all proteins to be excised as one band, and used to estimate total cellular proteins. Subsequent treatments of the two gels were identical. After fixation in 7% acetic acid for 20 min, the gels were washed with water, dried, and autora-

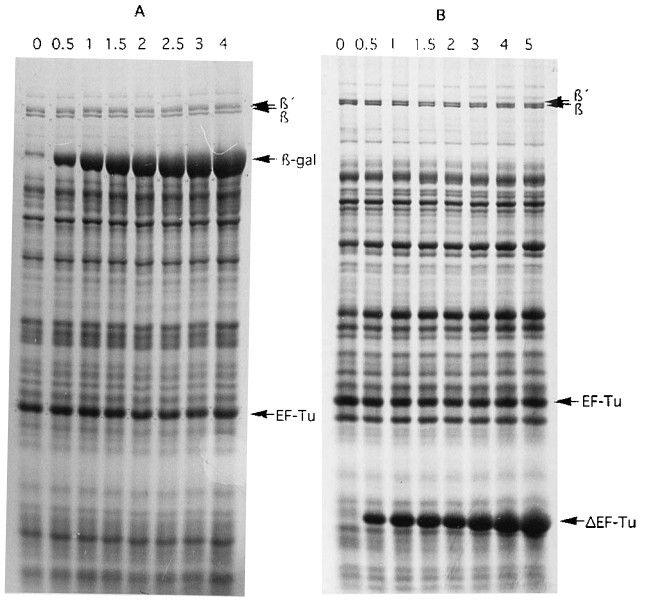

FIG. 1. Autoradiograms of total cell proteins. (A) Position of β-galactosidase (β-gal) on an SDS–8% polyacrylamide gel; (B) position of ΔEF-Tu on an SDS–10% polyacrylamide gel. The electrophoretic components corresponding to EF-Tu and the ββ′ subunits of RNA polymerase are also indicated. The time (in hours) is indicated over each lane.

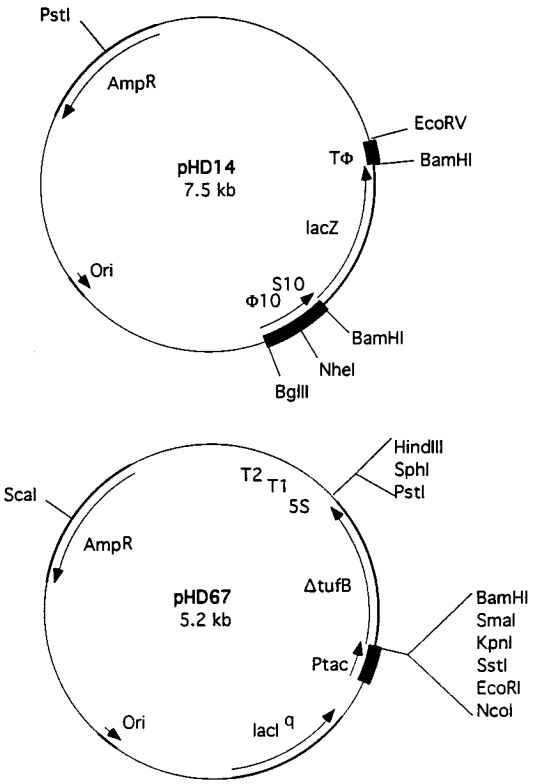

FIG. 2. Plasmids pHD14 and pHD67. Here, ϕ10, S10, and Tϕ represent the promoter, the leader sequence, and the terminator of T7 gene *10*, respectively. 5S, 5S rRNA; T1 and T2, transcriptional terminators of the *rrnB* operon. Δ*tufB* is the *E. coli tufB* gene containing a *Sma*I-*Sma*I fragment deletion that corresponds to nucleotides 248 to 491. The orientations of the *lacZ* and Δ*tufB* genes in each plasmid are indicated.

diographed (Fig. 1). The protein bands corresponding to β-galactosidase or ΔEF-Tu as well as EF-Tu and the ββ′ subunits of RNA polymerase were excised from the gels. The gel slices were incubated in 37% H_2O_2 for 48 h at 37°C and neutralized with catalase (12). The samples were dried at 110°C and incubated in a scintillation cocktail containing a tissue solubilizer (Zisser Analytic) for 20 h at room temperature before the radioactive contents of the samples were counted.

The responses of major cellular proteins to the overexpression of *lacZ* and Δ*tufB* were studied with a two-dimensional polyacrylamide gel. Exponentially growing bacteria were uniformly labelled with 15 μCi of L-[^{35}S]methionine per ml as described above. In addition, an exponentially growing culture was labelled for 2 min with 20 μCi of L-[4,5-^{3}H]-leucine per ml (specific activity, 164 Ci/mmol; Amersham) and chased with 200 μM nonradioactive leucine for 10 min. These cells were used as reference cells. The samples obtained prior to and at different time points after IPTG induction were mixed with the reference cells. The cell lysates were prepared from the mixed samples for resolution of the cellular proteins on two-dimensional polyacrylamide gels (16, 24). The protein spots corresponding to EF-Tu, EF-G, β subunit of RNA polymerase, ribosomal proteins S1 and L7/L12, NusA, and heat shock proteins GroEL and DnaK were excised and their isotope contents were determined as described above. The radioactivities of total cell proteins were determined by 5% trichloroacetic acid precipitation of the cell lysates. The relative level of each protein was defined as the ^{35}S/^{3}H ratio divided by the same isotope ratio of unfractionated mixed extracts.

tRNA quantification. Total RNA was prepared from the cells before and after induction, and the levels of the individual tRNA species were measured by Northern (RNA) blotting as described elsewhere (4).

Determination of the genomic 16S rDNA and 16S rRNA content. The amount of 16S rRNA relative to that of 16S rDNA was measured as follows: 5-ml aliquots of bacterial cultures were centrifuged. The pellets were resuspended in 200 μl of lysis buffer (23), and the cells were lysed for 2 min in boiling water. The lysates were extracted once with phenol, pH 7.5, and then with chloroform.

The samples were treated with RNase-free DNase I (Pharmacia) for rRNA quantifications and DNase-free RNase (Sigma) for rDNA measurements. The samples were slot blotted onto GeneScreen Plus membranes (NEN). The membranes were hybridized with an α-^{32}P-labelled probe made from a DNA fragment complementary to nucleotides 79 to 647 of 16S rRNA (2).

In order to follow the radioactive decay of the prelabelled 16S rRNAs, the exponentially growing cells were labelled with [5-^{3}H]uridine (20 μCi/ml; specific activity, 25 Ci/mmol; Amersham) for 100 min. Then, 1 mM IPTG and 200 μg of uridine per ml were added. One-milliliter aliquots of culture were centrifuged, and the cells were lysed after resuspension in 60 μl of lysis buffer (23) by boiling the samples for 2 min in a 100°C water bath. Then, 10 μl of the RNA lysates was mixed with 10 μl of formamide and loaded onto a 1.2% agarose formaldehyde gel (19). After electrophoresis and ethidium bromide staining, the RNA bands corresponding to 16S rRNAs and total tRNAs were excised from the gel. The gel slices were treated with 0.4 M NaOH for 20 h at 37°C and dried before their radioactive contents were measured.

Ribosome preparation. Ribosomes were quantitatively prepared as follows. The samples containing equal amounts of cells from induced and unperturbed cultures were chilled on ice. The cells were pelleted, washed in Poly-Mix buffer (11), and resuspended in the same buffer. The cells were lysed with a French press. After addition of RNase free DNase I (1 U/ml; Pharmacia) and a 15-min incubation at 4°C, the cell debris was pelleted by centrifugation for 10 min at 4,300 × g_{av} at 4°C. One milliliter of the supernatant was applied onto a 40-ml 10 to 40% sucrose gradient in Poly-Mix buffer. After centrifugation for 14 h at 55,000 × g_{av} in an SW28 rotor at 4°C, fractions of 1 ml were collected from the bottom to the top of each gradient and measured at 260 nm.

RESULTS

Growth rate response. In this work, we have studied the consequences of expressing at high levels two gene products that do not contribute to the metabolism of the bacterium under the growth conditions used in these experiments. One of these is the product of a *lacZ* gene that is under the control of a T7 promoter and that is transcribed by T7 RNA polymerase after induction by IPTG. The other is a truncated version of EF-Tu that is produced by a Δ*tufB* gene which is under the control of a *tac* promoter; Δ*tufB* is transcribed by *E. coli* RNA polymerase. Each of the two test genes is carried on a high-copy-number plasmid, and each is induced by the addition of IPTG to the growth medium (Fig. 1). The *lacZ* gene used here is preceded by a Shine-Dalgarno sequence from T7 gene *10* that is thought to be much stronger than the normal *E. coli* Shine-Dalgarno element (17, 23). In contrast, the Δ*tufB* gene contains an *E. coli* consensus Shine-Dalgarno sequence (20).

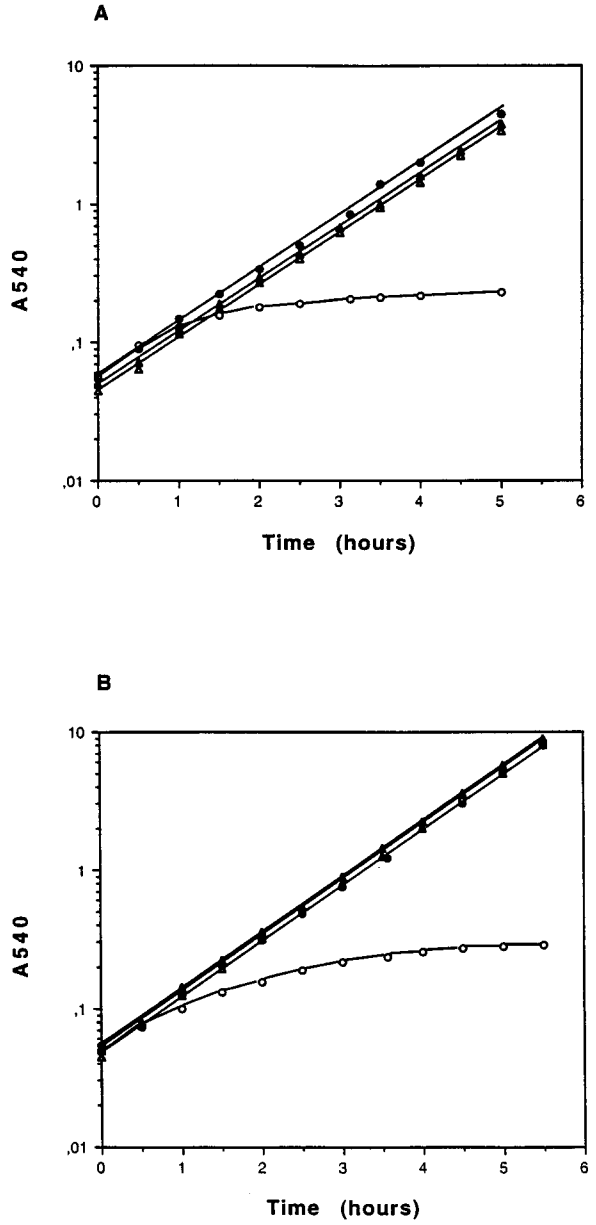

FIG. 3. Bacterial growth curves. (A) Response of a culture to overexpression of *lacZ*; (B) response of a culture to overexpression of Δ*tufB*. The turbidities of growing cultures in the presence (○) and in the absence (●) of 1 mM IPTG were assayed as described in Materials and Methods and plotted as a function of time. As a control, growth of strains HDS106(pET-3b) (A) and HDS117(pTrc99c) (B) with (△) and without (▲) 1 mM IPTG was assayed.

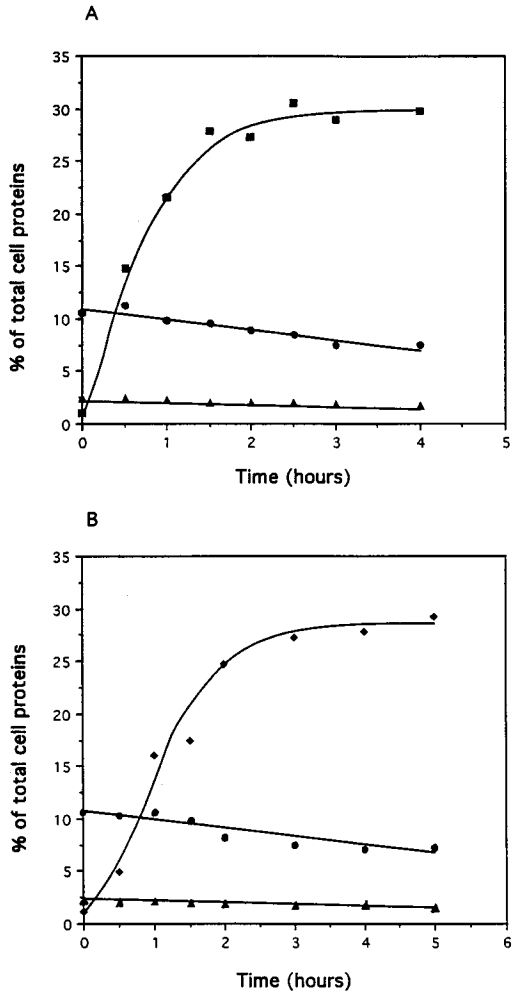

FIG. 4. Accumulation of selected proteins as a function of time after induction. Data were obtained from the experiments of Fig. 1 as detailed in Materials and Methods. (A) *lacZ* overexpression; (B) Δ*tufB* overexpression. ■, β-galactosidase; ◆, ΔEF-Tu; ▲, ββ′ subunits of RNA polymerase; ●, EF-Tu.

The growth of the cells after addition of IPTG was monitored by measuring the optical densities (A_{540}) of the growing cultures (Fig. 3). The growth rate of the cells decreases after induction of either test protein with IPTG, and it ceases after roughly 5 h in both cases. Control cultures in which cells carrying the homologous plasmids lacking both *lacZ* and Δ*tufB* genes were treated with the same concentrations of IPTG behaved as though they were uninduced (Fig. 3).

Gene expression. The expression levels of selected gene products were estimated in long-term labelling experiments. A logarithmically growing culture was uniformly labelled with [^{35}S]methionine for two generations prior to IPTG addition. Continuous labelling was maintained throughout the time course of the experiment. The amounts of accumulated proteins were quantified as described in Materials and Methods. Data for the two test proteins as well as intact EF-Tu and the ββ′ subunits of RNA polymerase are summarized in Fig. 4. Both β-galactosidase and ΔEF-Tu are maximally expressed to a level of about 30% of the total cell proteins. In parallel, there is a roughly proportional reduction in the levels of intact EF-Tu and the ββ′ subunits of RNA polymerase.

Table 1 summarizes the data from a similar experiment in which the accumulation of a number of other proteins was monitored after induction of either ΔEF-Tu or β-galactosidase. Here, we observe that for six proteins—EF-Tu, EF-G, L7/12, S1, the β subunit of RNA polymerase, and NusA—there is a progressive decrease in accumulation that is associated with and proportional to the increased accumulation of the gratuitous proteins, which corresponds to roughly 30%. In contrast, the heat shock proteins DnaK and GroEL increase by roughly 90 and 27%, respectively. Accordingly, there is both a graded decrease in the accumulation of uninduced proteins and a specific enhancement of heat shock proteins in response to the overexpression of either β-galactosidase or ΔEF-Tu.

These changes in expression levels are associated with a net

TABLE 1. Responses of major cellular proteins to overexpression of the test genes

Protein	Relative amt of protein[a] after induction of:					
	Δ*tufB*			*lacZ*		
	1 h	3 h	5 h	1 h	2 h	4 h
EF-Tu	0.80	0.74	0.62	0.95	0.68	0.65
EF-G	0.85	0.82	0.62	0.73	0.63	0.62
β[b]	0.96	0.71	0.65	0.92	0.69	0.68
S1	0.86	0.73	0.60	0.82	0.71	0.64
L7/L12	0.85	0.78	0.75	0.82	0.75	0.71
NusA	0.61	0.57	0.50	0.60	0.60	0.52
DnaK	1.09	2.09	1.95	1.14	1.86	1.81
GroEL	1.11	1.32	1.27	1.01	1.30	1.26

[a] Measured from two-dimensional polyacrylamide gels as described in Materials and Methods. The change in the relative amount of the sampled protein was calculated by normalizing the amount of protein after IPTG induction to that (assigned a value of 1.0) present in the uninduced cells.
[b] Beta subunit of RNA polymerase.

loss of protein synthesis capacity after overexpression of either gratuitous protein. For example, a plot of the protein synthesis rate normalized to mass as a function of time after induction (Fig. 5) shows that the translation rate normalized to bacterial mass, measured as radioactive amino acid incorporation per optical density of culture, decreases dramatically in response to the induction of the overexpressed proteins. This disproportionate decrease of protein synthesis efficiency is correlated with the loss of growth rate (Fig. 3).

In addition, it is possible to study the relationship between the amounts of gratuitous protein accumulated and the growth rates of the bacteria by measuring both parameters at different concentrations of IPTG. The results of such experiments are shown in Fig. 6, which shows that the progressive decrease of growth rate is a steep linear function of the amount of either test protein accumulated. In particular, growth virtually ceases when only 30% of the normal proteins are replaced by gratuitous protein. This result suggests that the cessation of both growth and translation is not simply a result of a proportionate dilution of normal proteins. This conclusion is reinforced by the observation that heat shock proteins are accumulated at enhanced rates in response to overexpression from either test gene.

Abundance of tRNA species. This study was initiated to test the effect of altering the codon composition of the mRNA pool on the distribution of different tRNA species. We chose to overexpress *lacZ* and Δ*tufB* genes because they represent genes with either a low codon usage bias or a strong bias towards major codons, respectively. In these experiments aliquots of bacterial cultures prior to and at different times after induction with IPTG were processed for analysis with the aid of a Northern blot technique as described previously (4). The data in Table 2 show that there is apparently a uniform change in the composition of the tRNA populations in response to the induction of either gene coding for one of the gratuitous proteins: individual tRNA/16S rRNA ratios increase by a factor of 2. However, there are no proportional responses of any of the tested tRNA species to changes in the frequencies of their cognate codons in the mRNA pool.

Degradation of ribosomes. There are at least two extreme interpretations of the response of the tRNA/16S rRNA ratios to overexpression of genes coding gratuitous proteins: either the rates of synthesis of all the tRNA species tested had increased equally while ribosome accumulation was constant or the amounts of 16S rRNA had decreased while the rates of

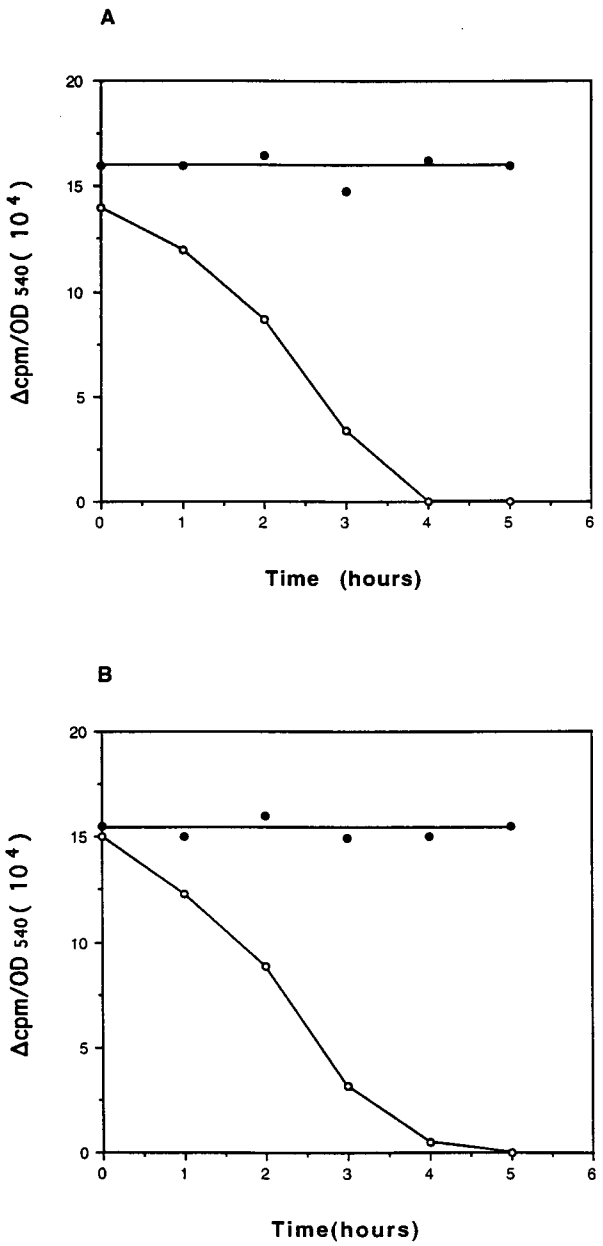

FIG. 5. Synthesis rates for total cell protein. A logarithmically growing culture in M9-glycerol medium was labelled with L-[^{35}S]methionine as detailed in Materials and Methods. Then, the culture was split; one subculture was subsequently induced with 1 mM IPTG at zero time, and the other served as a control without IPTG. Duplicated 50-μl samples of cells were periodically taken from both the induced (○) and the control culture (●). In parallel, the cell densities of the cultures were monitored. The radioactive content of [^{35}S]methionine precipitated with trichloroacetic acid was determined. The amount of radioactivity accumulated per time unit (expressed as change in counts per minute) was normalized to optical density at 540 nm and plotted as a function of the induction time. (A) β-Galactosidase; (B) ΔEF-Tu.

accumulation of tRNA were unaffected. In order to distinguish these alternatives, we measured the amounts of 16S rRNA in the bacteria prior to and after IPTG induction of the genes coding for the gratuitous proteins. Here, hybridization experiments were carried out, and the chromosomal rDNA content, measured as 16S rDNA-hybridizable DNA, was used as an internal standard to which the amounts of both 16S rRNA and individual tRNA species were normalized. We find that the

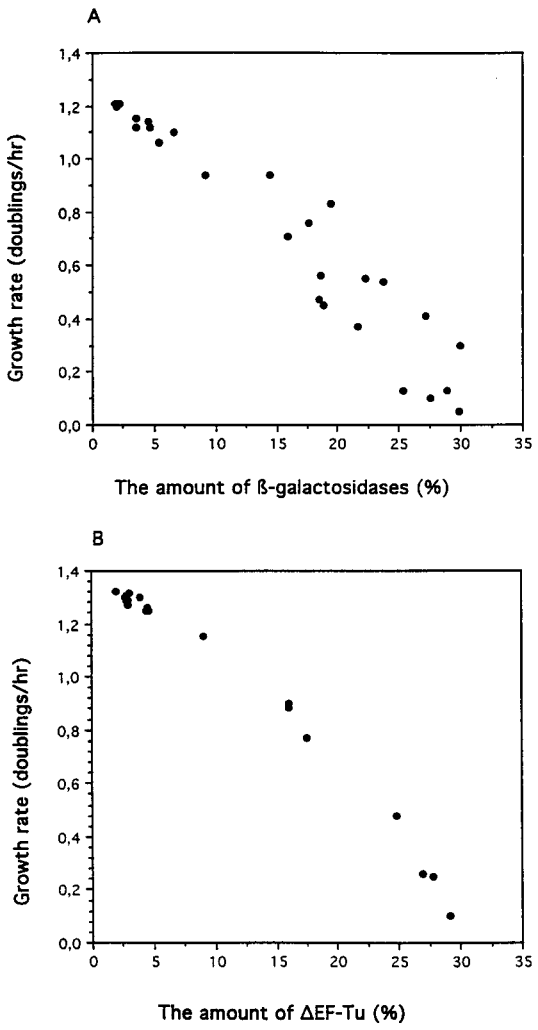

FIG. 6. Correlations between the bacterial growth rate and the amounts of overexpressed gene products. Exponentially growing cells in M9-glycerol media at 37°C were induced with IPTG at concentrations corresponding to 0, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} M, respectively. The growth rates and the amounts of induced gene products at different time points for both induced and control cultures were assayed (see Materials and Methods).

DNA-normalized amounts of 16S rRNAs decrease (Fig. 7) while the DNA-normalized amounts of individual tRNA isoacceptors remain constant (Table 2) in response to overexpression of the genes for the gratuitous proteins.

A pulse-chase experiment was conducted to determine the stability of 16S rRNA following the induction of gratuitous proteins by IPTG. Exponentially growing cells were uniformly labelled with [5-^{3}H]uridine for two generations (100 min). The labelled culture was divided into two portions. Both IPTG and nonradioactive uridine were simultaneously added to one subculture in order to follow the fate of the prelabelled 16S rRNA after induction of genes coding for gratuitous protein. Only nonradioactive uridine was added to the other subculture in order to monitor the stability of 16S rRNA during continued normal growth. Aliquots of cells were removed periodically, and total RNA preparations were prepared from these cells so that 16S rRNA as well as tRNA could be monitored. Since the tRNA/rDNA ratio is constant in such experiments (Table 2), the ratio of labelled uridine in the 16S rRNAs normalized to that in total tRNA is a measure of rRNA stability. The results summarized in Fig. 8 show that the prelabelled 16S rRNA is quite unstable and is progressively lost from the cells in response to IPTG-induced overexpression of genes coding for gratuitous proteins. In contrast, the 16S rRNA from the uninduced culture is stable.

In order to confirm these results, cell extracts from uninduced cultures as well as IPTG-induced cultures were fractionated on sucrose density gradients (Fig. 9). This experiment was carried out by adding to each sucrose gradient an extract prepared from a fixed amount of bacterial material, as described in Materials and Methods. Therefore, the relative amount of UV-absorbing material in the ribosomal peaks resolved in the sucrose gradients is an index of the relative amounts of ribosomes in the cells for each sample. It is evident from the profiles in Fig. 9 that there is a substantial and progressive decrease of the ribosome content of the bacteria as they accumulate increasing amounts of the gratuitous proteins.

DISCUSSION

The experiments described here have identified novel responses of bacteria to the overexpression of genes whose protein products do not contribute to their growth under the assayed conditions. The two gratuitous gene products that we studied are a truncated, inactive version of elongation factor Tu (25, 26), referred to as ΔEF-Tu, and β-galactosidase, which is superfluous to the growth of the cells in the presence of glycerol (13). In these experiments the overexpression of β-galactosidase is dependent on transcription by T7 RNA poly-

TABLE 2. Distribution of tRNA species after overexpression of *lacZ* and Δ*tufB*

tRNA	Anticodon (5′-3′)	Recognized codon(s) (5′-3′)	No. of codons used in:		tRNA concn in unperturbed cells[a] (μM)	Relative ratio of tRNA/16S rRNA[b,c] following overexpression of:								Relative ratio of tRNA/rDNA[c] following overexpression of:							
			lacZ	Δ*tufB*		*lacZ*				Δ*tufB*				*lacZ*				Δ*tufB*			
						1 h	2 h	3 h	4 h	1 h	2 h	3 h	4 h	1 h	2 h	3 h	4 h	1 h	2 h	3 h	4 h
Leu-1	CAG	CUG	54	19	14.6	1.54	1.84	1.83	1.91	1.20	1.85	1.85	1.92	1.1	1.0	1.0	1.0	1.1	1.1	1.0	1.0
Leu-2	GAG	CUU, CUC	9/9	0/0	3.0	1.55	1.77	1.88	1.88	1.13	1.80	1.86	1.92	1.1	0.9	1.1	0.9	1.0	1.0	1.0	1.0
Leu-3	UAG	CUA, CUG	6/54	0/19	1.3	1.50	1.70	1.80	1.90	1.20	1.70	1.80	1.90	1.0	0.9	0.9	1.0	1.1	0.9	0.9	1.0
Leu-4	CAA	UUG	11	0	3.1	1.47	1.84	1.84	1.89	1.14	1.78	1.85	1.92	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Leu-5	UAA	UUA, UUG	7/11	0/0	3.5	1.45	1.80	1.85	1.85	1.20	1.80	1.86	1.90	0.9	0.9	1.0	0.9	1.1	1.0	1.0	0.9

[a] From reference 4.
[b] Determined by the method described in reference 4.
[c] Ratios for uninduced cells were assigned a value of 1.0.

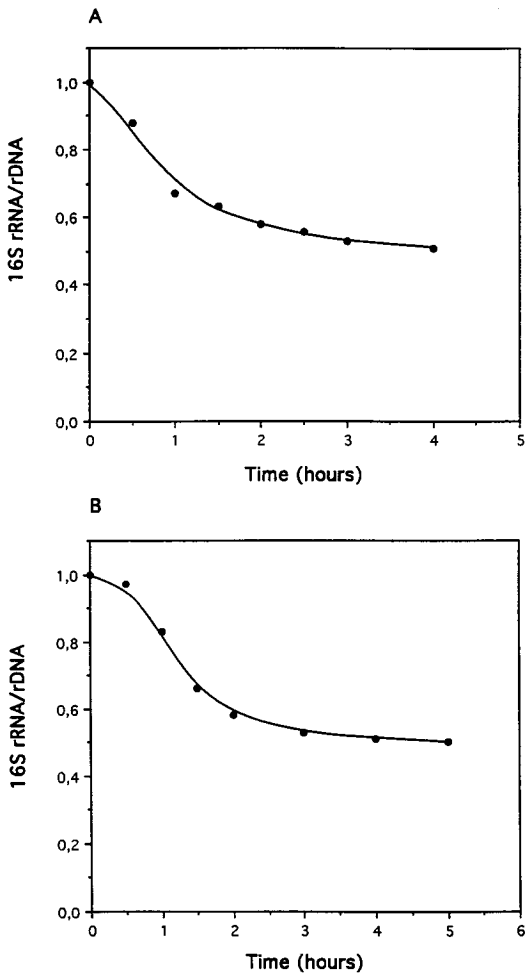

FIG. 7. Response of cellular 16S rRNA to overexpression. The amounts of cellular 16S rRNA normalized to the corresponding chromosomal rDNA content were obtained by blotting (see Materials and Methods). The data are plotted against time after induction of β-galactosidases (A) and ΔEF-Tu (B).

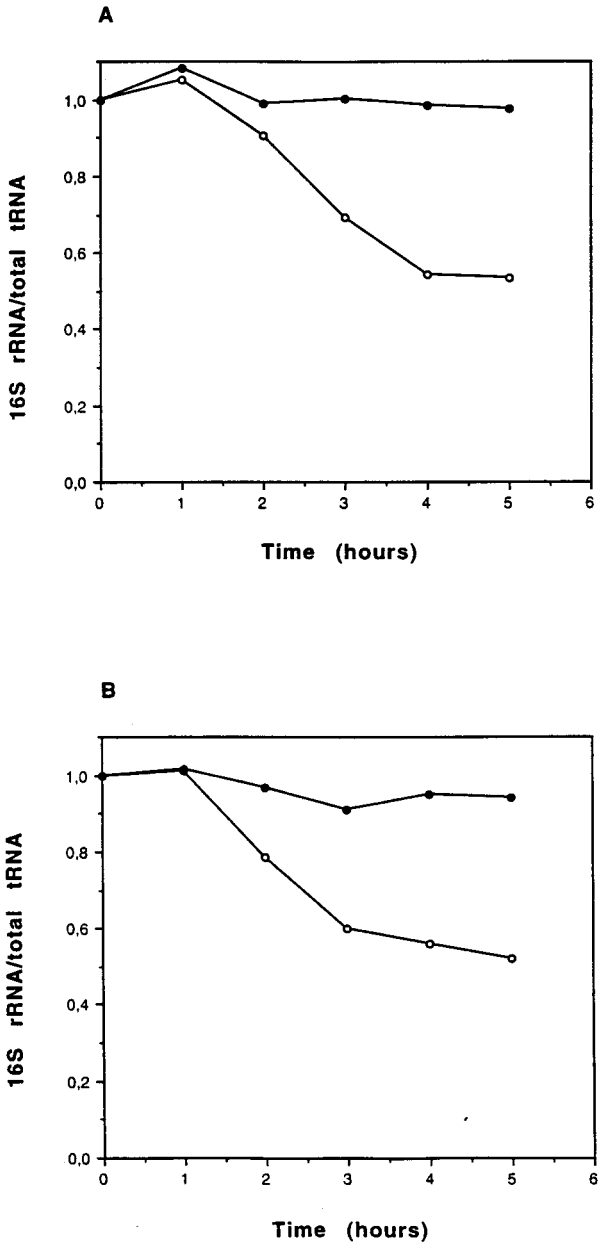

FIG. 8. Decay of 16S rRNAs. Exponentially growing cultures of HDS106 (pHD14) and HDS117(pHD67) were labelled for two generations with [5-^{3}H] uridine and then chased with nonradioactive uridine following induction with (○) or without IPTG (●). The amount of radioactive 16S rRNA was measured and normalized to total tRNA (see Materials and Methods). (A) Overexpression of *lacZ*; (B) overexpression of Δ*tufB*.

merase and that for ΔEF-Tu is dependent on transcription by *E. coli* RNA polymerase. Accordingly, the parallel effects observed by overexpression of either of the corresponding genes cannot be ascribed to the idiosyncrasies of either the T7 or the *E. coli* transcription process.

When we induce the overexpression of either of the test genes, the bacteria accumulate after several hours as much as 30% of their total protein as one or the other of the corresponding gene products. This accumulation is accompanied by a progressive decrease in the growth rates of the cells until growth ceases at about the same time that the accumulation of gratuitous protein levels off. It has been observed by Vind et al. (27) that induction of β-galactosidase production from a high-copy-number plasmid leads to an inhibition of the accumulation of normal cellular proteins. The present data confirm these results and suggest that at least two separate effects are responsible for the reduced production of normal cellular proteins when gratuitous proteins are accumulated.

One component of the progressive decrease in biosynthetic capacity is the degradation of rRNA that we have observed. The other is the competition between the mRNA species corresponding to the normal and gratuitous proteins for a dwindling supply of ribosomes. For example, we find that, aside from two heat shock proteins, the fraction of total protein synthesis devoted to normal proteins decreases roughly to the same extent that the fraction of gratuitous protein increases over the time course of our experiments. In contrast, the degradation of rRNA is cumulative and associated with a progressive loss of protein synthesis capacity. We are at present selecting mutants that escape the lethal effects of overexpression to see if we can separate experimentally these two effects on the growth rates.

The overexpression of the gratuitous genes in our experiments results in a dramatic increase of both transcription and translation of the corresponding gene products. We do not

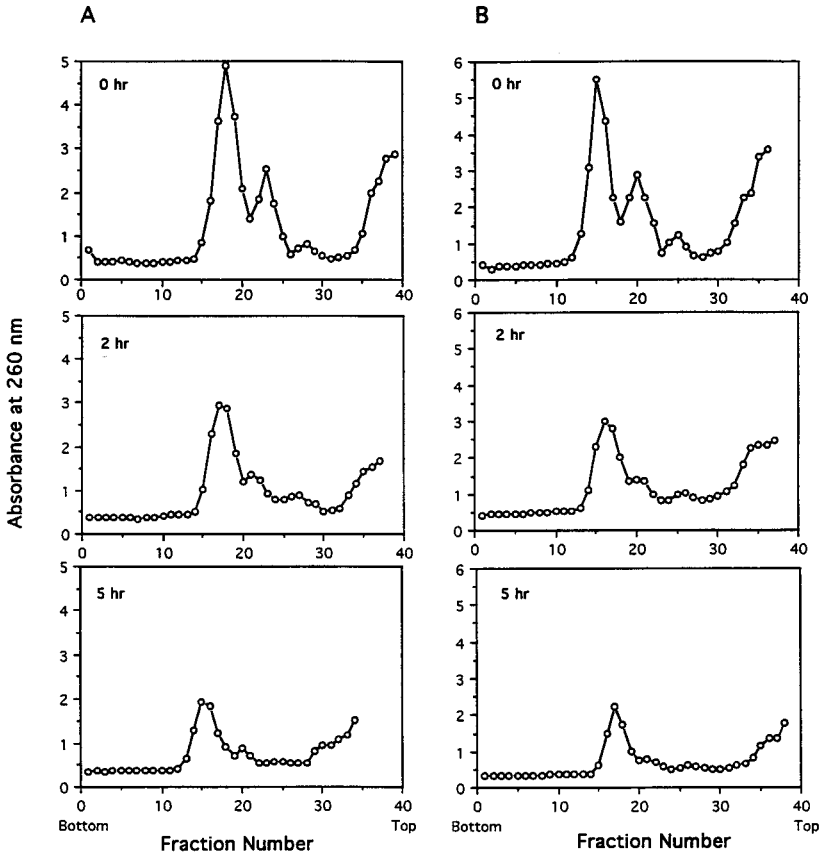

FIG. 9. Recovery of ribosomes. Ribosomes were fractionated by sucrose gradient centrifugation as described in Materials and Methods. Extracts were prepared from bacteria carrying plasmid pHD14 (A) or pHD67 (B) prior to (0 h) or 2 or 5 h after IPTG induction.

know which of these provides the signal for the destruction of ribosomes. It has been observed previously that under conditions of amino acid starvation there is a preferential breakdown of rRNA and a reduction in the number of ribosomes in the bacteria (7, 18). The replacement of normal proteins by gratuitous ones under conditions in which the capacity to synthesize proteins is fixed or decreased may be likened to an internal starvation state. In addition, it is known that failure to match the accumulation of rRNA with that of ribosomal proteins leads to the degradation of the newly synthesized rRNA (8, 9, 22). In the present case, however, preexisting ribosomes are being destroyed. Therefore, while there are precedents that identify pathways for degradation of rRNA, the mechanism through which these might be activated in response to overexpression of genes coding for gratuitous proteins is unknown.

There is a significant discrepancy between the roughly twofold reduction of 16S rRNA content and the complete loss of protein synthetic capacity for the fully induced bacteria. It remains to be determined whether the remainder of ribosomes in the inactivated cells are partially degraded. Likewise, we have observed enhanced production of heat shock proteins in response to the overexpression of either test gene, but we do not understand the relationship of this response to the death of the cells. In general, the identification of mutants that escape the lethal effects of overexpression should help in addressing some of these unanswered questions. Such mutants might also provide attractive vehicles for high-level expression of gratuitous proteins in biotechnical applications.

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