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Notes:

A single base change in the Shine–Dalgarno region of 16S rRNA of *Escherichia coli* affects translation of many proteins

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ABSTRACT A single base mutation was constructed at position 1538 of *Escherichia coli* 16S rRNA, changing a cytidine to a uridine. This position is in the Shine–Dalgarno region, thought to be involved in base-pairing to mRNA during initiation of protein synthesis. The mutation was constructed by using a synthetic oligodeoxynucleotide that differs in sequence by one base from the wild-type sequence of 16S rRNA. This oligonucleotide was used as a primer on single-stranded DNA of phage M13, into which was cloned a specific region of DNA encoding 16S rRNA. The mutation is lethal when expressed from the normal promoters of rRNA operons, *P1* and *P2*, in a high-copy-number plasmid. Expression can be repressed by a temperature-sensitive repressor, *cI857*, in combination with the bacteriophage λ *P_L* promoter. Induction of transcription by temperature shift yields mutant 16S rRNA that is processed and assembled into functional ribosomal subunits. The presence of mutant ribosomes retards cell growth and dramatically alters incorporation of [³⁵S]methionine into a large proportion of the cellular proteins. The change in level of synthesis of individual proteins correlates with the change in base-pairing between mutant rRNA and the Shine–Dalgarno region of the mRNA.

One of the goals of studies on ribosomes is to understand the role of rRNA in translation. One of the best-characterized functional regions of *Escherichia coli* rRNA is the 3' minor domain of 16S rRNA that extends from position 1400 to the 3' end at position 1542. This domain is associated with tRNA decoding (1), subunit association (2–4), control of translational fidelity (5), and initiation of translation (6).

The process of initiation requires recognition of the appropriate sequences on the mRNA by the ribosome. A major factor in this recognition is an mRNA–rRNA interaction involving base-pairing between a purine-rich sequence in the mRNA 5' to the initiation codon and a pyrimidine-rich sequence near the 3' end of 16S rRNA. This base-pairing interaction, first proposed by Shine and Dalgarno (7), was confirmed by the work of Steitz and Jakes (8). Subsequently, considerable genetic and biochemical evidence has been accumulated in support of this mechanism (9, 10). Synthetic oligonucleotides complementary to the 3' end of 16S rRNA block initiation by preventing mRNA–ribosome binding (11, 12). Mutations isolated in a number of mRNAs also indicate that the Shine–Dalgarno (SD) interaction plays a pivotal role in the initiation of translation of prokaryotic mRNAs (13–19). There can be little doubt that complementary sequences on the ribosome and mRNA do base-pair during initiation. The relative importance of this interaction has been difficult to evaluate because of the multiplicity of other sequence effects on both sides of the SD sequence, including sequences in the coding region (15, 16), which appear to modulate the efficiency of translation (17, 20–22). Our interest in rRNA has led us to approach this question from another point of view. By

altering a specific base in the SD sequence of the 16S rRNA, we have obtained direct evidence for the importance of the SD interaction in regulating protein levels in *E. coli*. We show that a single base alteration in rRNA has catastrophic consequences for the cell, which are manifested as gross alterations in the synthesis of many proteins.

MATERIALS AND METHODS

Strains and Plasmids. *E. coli* strain K5637 is W3110 (F^- , *hsdR^-*, *hsdM^+*) *cI857* Δ Bam *cro*²⁷ *Oam*²⁹ and was the gift of H. Miller (Genentech, South San Francisco). The gene *cI857* in K5637 codes for the temperature-sensitive repressor *cI* of bacteriophage λ . *E. coli* strains HB101 and JM101 have been described (23, 24). The plasmid pKK3535 contains the entire *rrnB* operon cloned into pBR322 (25). pNO2680, a derivative of pKK3535 in which the *rrn* promoters *P1* and *P2* are replaced by the λ *P_L* promoter and operator (26), was a gift from R. Gourse (University of Georgia) and M. Nomura (University of California, Irvine). pWJ1538T is pNO2680 with a C→T transition in rRNA-encoding DNA (rDNA), at the position corresponding to position 1538 in 16S rRNA. *P_L*-*Sma*6 is pNO2680 with a 770-nucleotide deletion between the two *Sma* I sites in 16S rDNA. Cells were grown in M9 medium supplemented with 0.4% Casamino acids and 0.4% glycerol (27). Cells containing plasmids were grown in media containing ampicillin (200 μ g/ml) as appropriate.

Oligonucleotide-Directed Mutagenesis. Mutagenesis was performed by the two-primer modification of the basic method (28, 29). The mutagenic oligonucleotide, designated 1538C/T, had the sequence 5' TTGGATCACCT(T)CTTACCTT 3'. The position of the mutation is indicated by parentheses.

Preparation of Plasmid-Encoded rRNA. The rRNA transcripts from wild-type and mutant plasmids were specifically labeled in maxicells as described previously (30), with one modification. Cells were grown at 30°C, irradiated with UV light for 75 sec, and allowed to recover for 7 hr in the dark at 30°C. D-Cycloserine was added (100 μ g/ml final concentration) after 1 hr. Immediately before addition of (³²P)orthophosphate (carrier-free, 75 μ Ci/ml; 1 Ci = 37 GBq), the cells were transferred to 42°C. Labeling was continued for 10 hr at 42°C. Cell lysis and electrophoretic analysis of plasmid-encoded rRNA were performed as described (30).

RNA Fingerprinting. Cells containing plasmids pNO2680 or pWJ1538T were grown for several generations in supplemented M9 medium at 30°C and then transferred to 42°C. After 4 hr at 42°C, the cells were treated for 10 min with rifampicin (100 μ g/ml) to remove mRNA and lysed (30). Ribosomes were isolated and 200 *A*₂₆₀ units were treated with 400 μ g of colicin E3 for 30 min at 37°C (31); the colicin

Abbreviations: SD, Shine–Dalgarno; rDNA, rRNA-encoding DNA.

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fragment comprising the 49 3'-terminal nucleotides of 16S rRNA was isolated as described (32). The colicin fragment was 3'-end-labeled with ^{32}P by use of cytidine 5'-[^{32}P]phosphate 3'-phosphate (specific activity 3000 Ci/mmol) and RNA ligase and then was digested with RNase T1. The resulting RNA fragments were separated on a two-dimensional chromatography system as described by Barrell (33).

Two-Dimensional Gel Electrophoresis of Proteins. Samples were prepared and analyzed by two-dimensional gel electrophoresis as described (34–36).

RESULTS

Construction of a C→T Mutation at Position 1538 of 16S rDNA. The presence of multiple copies of rRNA operons in *E. coli* has greatly hampered genetic studies of rRNA. The availability of a cloned copy of the *rrnB* operon in the plasmid pKK3535 (25) permits mutations to be constructed *in vitro* and then introduced into *E. coli* (37). Transcription of the operon is initiated from two tandem promoters, *P1* and *P2*, which are regulated in the same way as the chromosomal operons (26, 38). It has been estimated that pKK3535, present in about 15 copies per cell, can supply at least 50% of the ribosomes in the cell (30).

To test the SD hypothesis, we chose to alter one of the bases most frequently used in the complementary interaction with mRNA, but in a way that would be least disruptive of the rRNA secondary structure at the 3' end (32). Thus, we changed the base at position 1538 from cytosine to thymine (cytosine to uracil in RNA) (Fig. 1).

Oligonucleotide-directed mutagenesis using recombinant M13 phage containing rDNA coding for the 3' end of 16S rRNA was carried out as described in *Materials and Methods*. A 320-base-pair *Xma*I–*Xba*I fragment of pKK3535, containing the coding region for the 3' end of 16S rRNA, was cloned into M13mp11 replicative-form DNA. Plaques containing mutant phage were identified by a combination of plaque hybridization, restriction analysis, and dideoxy sequencing (see refs. 28 and 29 for methods).

Introduction of the Mutant into Plasmids pKK3535 and pNO2680. A 320-base-pair *Xma*I–*Xba*I fragment was removed from pKK3535 and replaced with its mutant counterpart isolated from replicative-form M13. Transformation of HB101 with the mutant construction yielded very few colonies. The plasmids had the 1538T mutation but also contained other mutations in the upstream portion of 16S rDNA, which blocked processing (and expression) of 16S rRNA in maxicells. [Similar results have been obtained by Hui and de Boer (39).]

Since the 1538T mutation appeared to be lethal when expressed in pKK3535, it was cloned into the conditional expression vector pNO2680 and transformed into strain K5637. The new plasmid, pWJ1538T, is plasmid pNO2680 with a C→T substitution at the position corresponding to nucleotide 1538 of 16S rRNA. The genes encoding 5S, 16S,

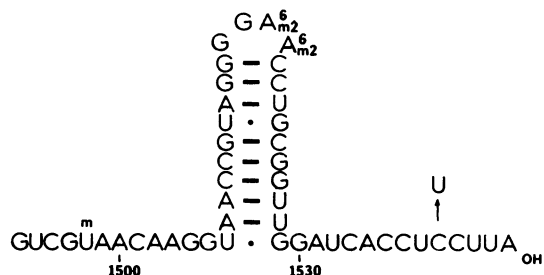


FIG. 1. Secondary structure of the 49 3'-terminal nucleotides of 16S rRNA. The C→U mutation of position 1538 is indicated. m, Methyl; A_m^2 , N^6 -dimethyladenosine.

and 23S rRNAs are under the control of the λP_L promoter. Transcription from P_L is repressed at 30°C but not at 42°C in strain K5637.

Growth Rate at 30°C and 42°C of *E. coli* Strain K5637 Containing Mutant Plasmid. K5637 cells with repressed plasmids (at 30°C) grew with slightly longer doubling times than K5637 with no plasmid (110 min vs. 95 min). In this strain, cells carrying pNO2680, pWJ1538T, and P_L -Sma6 all grew with a generation time of 110 min. The plasmid P_L -Sma6 (37) yields a shorter 16S rRNA transcript that is not assembled into 30S particles. In the absence of repressor, cells with plasmid P_L -Sma6 grew much more slowly than cells carrying pNO2680. These results are consistent with complete repression in K5637 at 30°C and indicate that mutants can be maintained stably under these conditions.

To study the effects of mutant rRNA, cells growing at 30°C were shifted to 42°C, inducing transcription of plasmid-encoded rRNA. Growth rates were measured after 2 hr at 42°C, following the marked change in rate reported for cells containing P_L plasmids (26). In cells with wild-type pNO2680, the doubling time was 110 min, whereas plasmid pWJ1538T increased the doubling time to 145 min. The effect of P_L -Sma6 was intermediate between the doubling times of pNO2680 and pWJ1538T (data not shown).

Synthesis of Mutant RNA. The modified maxicell technique (30) was used to examine the rRNA transcripts synthesized from plasmid pWJ1538T. Cells were irradiated with UV light and, after 8 hr, were transferred from 30°C to 42°C to induce transcription from the plasmid. The plasmid transcript was specifically labeled with [^{32}P]orthophosphate. Aliquots of lysate were extracted with phenol and electrophoresed in an agarose/acrylamide composite gel (40). Fig. 2 shows a one-dimensional separation of 23S and 16S rRNA. In addition, precursor 17S RNA was also separated. Lanes 1 and 5 show rRNAs derived from cells with pNO2680. Nearly all of the small- and large-subunit RNAs were present in their mature 16S and 23S forms. Lane 4 shows RNA isolated from cells with pWJ1538T. The RNA species were identical with wild type, showing that processing is not affected by the 1538T mutation. In contrast, lane 3 shows RNA extracted from maxicells carrying a plasmid with both the 1538T mutation and a mutation that partially blocks processing of the 17S precursor RNA to 16S rRNA. Many of the plasmids isolated in the initial cloning experiments were of this type. Lane 2 shows the P_L -Sma6 transcripts. The mutant 16S rRNA with a 770-nucleotide deletion was easily separable from the wild-type transcript. The absence of a band in the

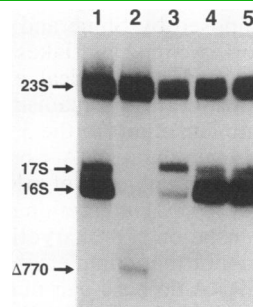


FIG. 2. Labeling of plasmid-encoded rRNA in maxicells. Cells carrying plasmids with wild-type or mutant forms of the *rrnB* operon were labeled with [^{32}P]orthophosphate by using the maxicell technique described in *Materials and Methods*. Labeled rRNAs were visualized by autoradiography after separation by gel electrophoresis. The positions of mature 23S rRNA, precursor 16S rRNA (17S), mature 16S rRNA, and deleted 16S rRNA ($\Delta 770$) are indicated. Lanes: 1 and 5, pNO2680; 2, P_L -Sma6; 4, pWJ1538T; 3, plasmid with undefined second site mutation in 16S rDNA (in addition to 1538T) that retards processing of 17S rRNA.

16S position indicates that there was no labeling of chromosomal RNA and that the RNAs visible in this and the other lanes were authentic plasmid transcripts. The mutant 16S band from P_L-Sma6 is fainter because it is smaller and less stable than wild-type 16S rRNA. The 16S band from pWJ1538T is the same intensity as the wild-type rRNA, indicating that its stability is similar to that of wild-type rRNA.

Assembly of Ribosomes Containing a C→U Change in 16S rRNA. The biogenesis of ribosomal subunits with plasmid-encoded mutant rRNA was studied in the maxicell system. After [³²P]orthophosphate incorporation, ribosomes in cell lysates were separated by a two-dimensional gel electrophoresis technique. Electrophoresis in the first dimension was carried out in the presence of 10 mM Mg²⁺ to separate 70S, 50S, and 30S particles. The RNA species in each particle were then separated in the second dimension after deprotection by soaking the gel slice in EDTA and NaDodSO₄ (30). The results (Fig. 3) show that wild-type and mutant particles are identical. The 70S particles contain plasmid-encoded ³²P-labeled 23S and 16S rRNA; 50S and 30S particles contain 23S and 16S rRNAs, respectively. Some precursor 50S particles were noted in the 50S region and contained precursor 23S RNA. The mutant 16S rRNA (U¹⁵³⁸) is found both in 30S subunits and in 70S ribosomes.

Percentage of Ribosomes in Strain K5637 that Contain U¹⁵³⁸ in 16S rRNA. To determine the fraction of the total ribosome pool composed of mutant ribosomes, 70S ribosomes were isolated from cells grown at 42°C for 4 hr and treated with colicin E3 as described in *Materials and Methods*. The colicin fragment was isolated and labeled at the 3' end, and following RNase T1 digestion, the 3'-terminal dodecamer fragments containing either uridine or cytidine at position 1538 were separated. Approximately 65% of the terminal dodecamers of colicin fragments isolated from cells containing mutant plasmid had the mobility of the mutant oligonucleotide, indicating that 65% of the ribosomes in these cells contained the plasmid-encoded mutant 16S rRNA (data not shown). During the 4-hr incubation at 42°C, the cell mass approximately doubled, and the value of 65% for mutant ribosomes reflects this fact.

Functional Analysis of Mutant Ribosomes. To assay the functional activity of the mutant ribosomes, cells with

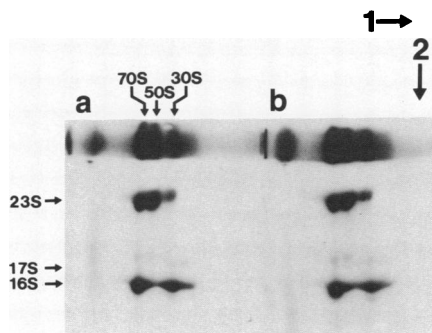


FIG. 3. Two-dimensional gel electrophoresis of ribosomal particles. Cells carrying pNO2680 or pWJ1538T were grown and labeled with [³²P]orthophosphate as described in *Material and Methods*. After labeling, aliquots of lysates were electrophoresed in the first-dimension gel to separate 70S ribosomes and subunits. To analyze the rRNA content of the ribosomal particles, the gel was incubated at room temperature for 1 hr in Tris/borate/EDTA buffer and then for an additional hour in the same buffer containing 0.2% (wt/vol) NaDodSO₄. The gel strip was then polymerized into a second gel (3% acrylamide/0.5% agarose), and rRNAs were separated by electrophoresis for 12 hr at 100 V and 3°C (40). Ribosomal particles (70S, 50S, and 30S) are identified by the presence of mature 23S and 16S rRNA. (a) pNO2680. (b) pWJ1538T.

pNO2680, pWJ1538T, or P_L-Sma6 were labeled for 20 min with [³⁵S]methionine at 4 hr after transfer to 42°C. Aliquots of lysates were analyzed by two-dimensional (isoelectric focusing/NaDodSO₄) gel electrophoresis. The intensities of the different protein spots on the autoradiogram are related to their abundance in the cell lysate, as well as to the number of methionine and cysteine residues in the protein. Two-dimensional electrophoresis was done according to the standard conditions of Neidhardt *et al.* (34), and proteins were identified by their mobilities in the gel (T. A. Phillips, F. C. Neidhardt, and S. Pedersen, personal communication). Representative proteins (A–E) are indicated by arrows on the autoradiogram in Fig. 4a. The relative amount of each protein present in a given cell extract can be estimated by relating the intensity of the spot of interest with the intensities of neighboring spots. In addition, the levels of a protein present in different cell extracts can be compared, taking into account slight variations in spot pattern and gel exposure.

At 30°C, when plasmid-encoded rRNA is not expressed, relative intensities of all of the spots are the same on all three gels (data not shown). In contrast, after 4 hr at 42°C, major differences in spot intensities are apparent. The protein patterns for cells carrying plasmids pNO2680 and P_L-Sma6 are virtually identical, suggesting that the presence of mutant rRNA itself, even as a defective particle, does not lead to any gross perturbations in the levels of different proteins. How-

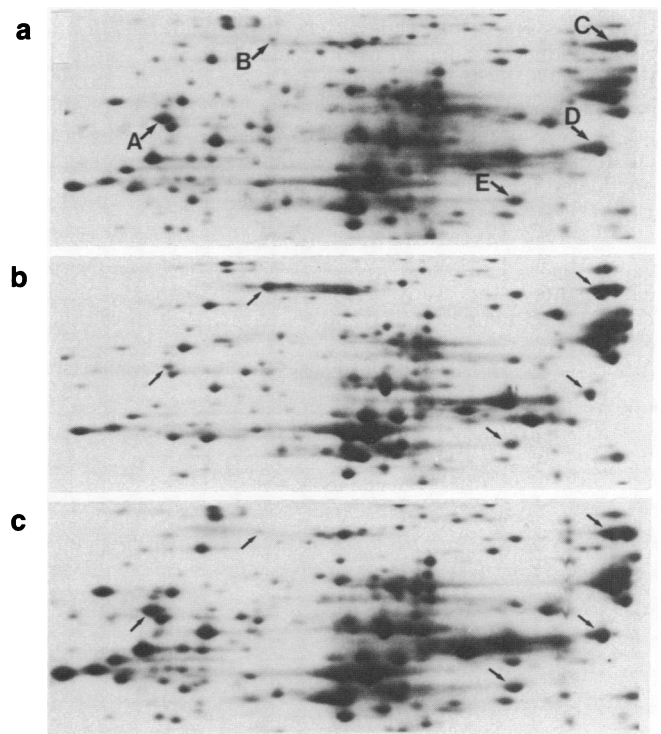


FIG. 4. Two-dimensional gel analysis of *E. coli* K5637 proteins grown at 42°C. Cells containing pNO2680 (a), pWJ1538T (b), or P_L-Sma6 (c) were grown at 30°C in M9 medium supplemented with a mixture of all 20 common amino acids except cysteine and methionine (final concentration 100 μg/ml). Cells were transferred to 42°C for 4 hr and then labeled for 20 min with [³⁵S]methionine (50 μCi/ml). Samples were prepared and analyzed by two-dimensional gel electrophoresis as described (34). Aliquots of lysate were subjected to equilibrium isoelectric focusing (pH 5–7) in the first dimension (8200 V·hr) and then electrophoresed in the second dimension in an 11.5% polyacrylamide/NaDodSO₄ gel (34, 35). Proteins indicated by arrows are as follows: A, α subunit of Ca²⁺, Mg²⁺-ATPase; B, methionyl-tRNA synthetase; C, ribosomal protein S1; D, β subunit of Ca²⁺, Mg²⁺-ATPase; E, S-adenosylmethionine synthetase (*metK* gene product).

ever, the pattern observed for cells carrying pWJ1538T is markedly different from that of cells carrying either pNO2680 or P_L-Sma6. For example, the α subunit of Ca²⁺,Mg²⁺-ATPase (see A in Fig. 4a) is markedly lessened in intensity in pWJ1538T extracts (Fig. 4b), both relative to neighboring proteins within the gel and compared to its abundance in either pNO2680 or P_L-Sma6 extracts. The β subunit of Ca²⁺,Mg²⁺-ATPase (D) is similarly reduced in abundance in cells with pWJ1538T. Two proteins that are increased in intensity in cells carrying pWJ1538T are methionyl-tRNA synthetase (B) and ribosomal protein S1 (C). We conclude, therefore, that the single base mutation in pWJ1538T may result in a distinct reduction or increase in the level of protein in *E. coli* when the mutation is expressed.

Not all of the spots change, and not all spots that change do so in such a readily observable way. Only about 200 of the more than 1000 proteins resolved in two-dimensional gels have been identified (34), and only a fraction of the genes for those proteins have been sequenced. Of the spots that were observed to change in these experiments, only eight were identified whose genes have been sequenced (Table 1). The changes in all eight are compatible with the changes in SD complementarity. For example, in the ATPase α -subunit mRNA, the 1538T mutation replaces a G-C base pair with a G-U base pair and reduces its translation (GGAG to GGAG, where underlined bases are complementary to 16S rRNA). Conversely, methionyl-tRNA synthetase mRNA gains a base pair and its translation is increased (UAAGAAG to UAAGAAG). An important observation is made with the *metK* gene product, *S*-adenosylmethionine synthetase (protein E, Fig. 4). The 1538T mutation in rRNA is outside the proposed base-pairing of the SD region and would therefore be predicted not to affect translation (AGGUGA to AGGUGA). As indicated, the levels of *S*-adenosylmethionine synthetase remained unaffected in all of the strains examined.

DISCUSSION

The results of this study provide direct evidence for the role of the SD sequence at the 3' end of 16S rRNA during initiation

of protein synthesis (7). We have shown that base-pairing between rRNA and mRNA is, indeed, a very important step in this process and that an alteration in this interaction by a single base change in the rRNA has a dramatic effect on the level of synthesis of most cellular proteins. Although many regions of mRNA are important for determining translational efficiency (16), the SD sequence is of major importance.

In this study, we constructed a mutation in a plasmid-encoded *rrnB* operon at position 1538 of 16S rRNA of *E. coli*. We have shown that rRNA containing this mutation can be maintained stably in cells only when its transcription is repressed. When induced, the mutant rRNA is transcribed, processed, and incorporated into functional ribosomes. Four hours after induction, mutant ribosomes comprise $\approx 65\%$ of the total ribosomes in the cell. The presence of these mutant ribosomes has a catastrophic effect on the cell, altering the relative levels of production of many proteins.

Interpretation of the data obtained from the two-dimensional gel autoradiograms is difficult but, in this instance, crucial. Two criteria must be met: the identity of a spot must be unequivocal, and the DNA sequence of the gene for that protein has to be available. Thus, testing the consequences of the mutation has only been possible for a limited number of proteins (Table 1). Within these limits, we conclude that where mRNA:rRNA base-pairing has been disrupted by the expression of the 1538T mutation, protein production is reduced; where base-pairing is strengthened, protein production is increased; and in one case, where base-pairing is unaffected, so is the level of protein production. This conclusion is strengthened even further when the levels of these same proteins are examined at 30°C (data not shown). At this temperature, the 1538T mutation is silent and, importantly, the relative levels of all these proteins remain unaffected. Thus, we conclude that the amount of protein produced (as indicated by the relative spot intensity on a two-dimensional gel) reflects the effect of the change in translational efficiency at initiation due to a change in complementarity between mRNA and rRNA at the SD sequence. We predict that similar conclusions will apply to other proteins for which

Table 1. Changes in autoradiographic intensity of proteins identified in two-dimensional gels after induction of mutant rRNA

Gene product	SD sequence	Change in no. of base pairs	Change in intensity
	(U)		
3' end of 16S rRNA	3' AUUCCUCCACUAG 5'		
Ca ²⁺ , Mg ²⁺ -ATPase α subunit	5' UAAGGGGACUGG*GAGCAUG 3'	-1	Down
β subunit	5' CGUAGAGGATTTAAGAUG 3'	-1	Down
Methionyl-tRNA synthetase	5' AUUAAGA*AGUAAUGCCUACUAUG 3'	+1	Up
Elongation factor EF-G	5' UAAACG*AGGAAACAAUG 3'	-1	Down
Ribosomal proteins L7/L12	5' UAUUCAGGA*ACAAUUUAAAUG 3'	-1	Down
S1	5' CCTGAAGA*UUUAAAUG 3'	+1	Up
RNA polymerase β subunit	5' AGCGAGCUGAG*GAACCCUAUG 3'	-1	Down
<i>S</i> -Adenosylmethionine synthetase	5' UUUAGGUGAUUUUAAAUG 3'	0	None

Sequences were taken from the indicated references: 16S rRNA (7); Ca²⁺,Mg²⁺-ATPase (*uncA*, *uncB*) (41); methionyl-tRNA synthetase (42); elongation factor EF-G (*fus*) (43); L7/L12 (*rplL*) (44); S1 (*rpsA*) (45); *S*-adenosylmethionine synthetase (*metK*) (46); RNA polymerase β subunit (*rpoB*) (44). Proteins were identified by T. A. Phillips, F. C. Neidhardt, and S. Pedersen (personal communication). Asterisks indicate positions affected by the 1538T mutation, which is indicated above the sequence for the 3' end of 16S rRNA.

identification from the gels has been possible but for which the sequence has not yet been established.

The remarkable effect of the SD mutant ribosomes on the cellular proteins can account quite adequately for the lethality of this mutant in plasmid pKK3535. It was necessary to keep the mutation repressed when in a high-copy-number plasmid. Interestingly, there was no detectable effect on growth rate when the mutant was cloned into a low-copy-number plasmid (D. K. Jemiolo, personal communication), indicating that the phenotype is gene-dosage-dependent and related to the amount of mutant rRNA in the cell.

It was necessary to rule out the possibility that factors other than the presence of mutant ribosomes might account for the changes in relative levels of different proteins. The results obtained with the P_L-Sma6 plasmid showed that the presence of mutant rRNA *per se* does not lead to perturbations in protein levels. Transcription of an *rrnB* operon with the Sma6 mutation led to an increase in generation time when this mutant was present in either pKK3535 or pNO2680. The Sma6 transcript was neither fully assembled into functional particles nor stable. However, while the cell was stressed by the presence of this mutation, it produced the same protein pattern as that observed in cells with the wild-type *rrnB* (pNO2680). (Note, however, that ribosomal proteins other than S1 are not separated in this gel.) Another factor to consider was the heat shock response as a result of transferring the cells from 30°C to 42°C during induction of the P_L-*rrnB* operon. The heat shock response in *E. coli* starts within seconds of temperature shift and lasts for 20–25 min, after which time a new equilibrium is established (47, 48). In our experiments, none of the proteins that were identified as being changed in cells carrying pWJ1538T has been identified as a heat shock protein, and the labeling of the cells occurred at 4 hr, long after a new equilibrium would have been established.

We cannot rule out the possibility that some of the protein changes that we observed were the result of secondary effects, rather than due primarily to alterations in SD complementarity. An alteration in one protein might affect levels of proteins encoded in its operon or regulon. The limited set of protein identifications that have been made do not permit further discussion on this point, but this must be considered if proteins are identified later that change in ways not explained in terms of SD complementarity.

This paper and ref. 39 report rRNA mutants with a functional effect defined at the molecular level. These data strongly support the hypothesis (7) that interaction between bases at the 3' end of the 16S rRNA complementary to a purine-rich region upstream of the AUG initiation codon in mRNA is crucial for the correct initiation of protein synthesis.

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