

5S rRNA gene deletions cause an unexpectedly high fitness loss in *Escherichia coli*

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Received August 16, 1998; Revised and Accepted November 13, 1998

ABSTRACT

In *Escherichia coli*, ribosomal RNAs (16S, 23S and 5S) are co-transcribed in a highly regulated manner from seven genomically dispersed operons. Previous studies on the cellular effects of altered levels of two of these rRNAs (16S and 23S) have been useful in better understanding the regulation of rRNA expression. Furthering these studies, we have investigated the effect of 5S rRNA deficiencies on cell fitness through the sequential deletion of 5S rRNA genes. Our findings indicate that the loss of 5S rDNA from multiple genes decreases cell fitness more rapidly than loss of a similar number of 16S and 23S rRNA genes. These results suggest that the cell's innate ability to up-regulate rRNA operons does not compensate for 5S rRNA deficiencies, as was previously shown for 16S and 23S rRNAs. A plasmid-borne 5S rRNA gene is able to compensate for the deleted 5S rRNA genes.

INTRODUCTION

Escherichia coli 5S rRNA is a 120 nt rRNA found in the 50S ribosomal subunit which plays an uncertain role in proper ribosome function. 5S rRNA is transcribed along with 16S and 23S rRNA to produce a polycistronic precursor RNA in a highly regulated manner (1). *Escherichia coli* has seven rRNA operons: *rna*, B, C, D, E, G and H. The rRNAs in each operon have the order 16S–23S–5S with, depending on the operon, various tRNA genes interspersed as described in detail elsewhere (2). Operon *rrnD* is unusual in that it contains a second tandem copy of 5S rRNA. Thus, there are seven genes for 16S rRNA, seven genes for 23S rRNA and eight genes for 5S rRNA. Ribosomal RNA expression is intimately linked with the cells' translational potential (3–5), i.e. if the cell has less functional ribosomes than it needs, rRNA synthesis is increased by a generally equal up-regulation of all seven rRNA operons. Consistent with these views, it has been demonstrated that if 16S and 23S rRNA levels in the cell are artificially reduced, the cell will up-regulate rRNA operons to compensate for the loss (1). *Escherichia coli* has also been shown to degrade excess 16S and 23S rRNAs when artificially overexpressed in the cell, with relatively little increase in ribosome number (7,8). *Escherichia coli* therefore has some capacity to compensate for both imbalances and quantitative

deficiencies of at least some rRNAs. In contrast to 16S and 23S rRNA, 5S rRNA is not known to have additional regulatory functions in the cell. Thus, although not previously explored, the simpler nature of 5S rRNA would suggest that 5S rRNA deficiencies could be similarly, if not more easily, addressed by the cell. Herein, we report the creation of *E. coli* deletion mutants in which 5S rRNA genes have been deleted from the genome in sequentially increasing numbers. Surprisingly, the loss of 5S rRNA genes has a greater detrimental effect on cell fitness than was seen in previous studies involving deletion of equivalent numbers of 16S and 23S rRNA genes.

MATERIALS AND METHODS

Plasmids and bacterial strains

All PCR amplification and blunt-ending was performed with *Taq* polymerase and mung bean exonuclease (Promega), respectively. Deletion of chromosomal 5S rRNA genes was performed with recombination plasmids pKO3-(B, D, H and E), where the last letter represents the target operon that the recombination plasmid is specific for. The recombination cassettes in the four different recombination plasmids share a similar design (Fig. 1). The FRT-flanked kanamycin cassette was obtained from a *HindIII/SmaI* double digest of pCP15 (9). 5S rRNA flanking sequences on either side of the kanamycin cassette were obtained through PCR amplification of EMG2 chromosomal DNA between primer sets 1 (CCCAAGAATTCATATCGACGGC) and 2 (CCCAAGCTTCGCTACTGCCGCCAGGCA) for the 5S rRNA 5' flanking region and 3 (TCCCCCGGGAGTAGGGAAGTCCAGGCAT) and either primer B (ATTGCGCGTTCGCTGTGTAT), D (ATTGCGCGTTCGCTGTGTAT), H (GGCTCTCTTTCAGACTTGGG) or E (CTCATCCCCGGTGGGCTGAAC) for the 3' flanking regions. Primers B, D, H and E were specific for the operon represented by their name. Primers 1, 2 and 3 are non-specific and will hybridize to orthologous regions in all seven rRNA operons. Primers 2 and 3 contain restriction sites for *HindIII* and *SmaI*, respectively, to facilitate their ligation to the *HindIII-SmaI* fragment from pCP15. Primers 1 and B contained *EcoRI* and *KpnI* restriction sites, respectively, to facilitate ligation of the recombination cassette specific for operon B into pBluescript II KS(+/-). Recombination plasmid pKO3-B was created by ligating the *HindIII-SmaI* kanamycin cassette, the *HindIII*-cut PCR fragment generated with primers 1 and 2 along with the

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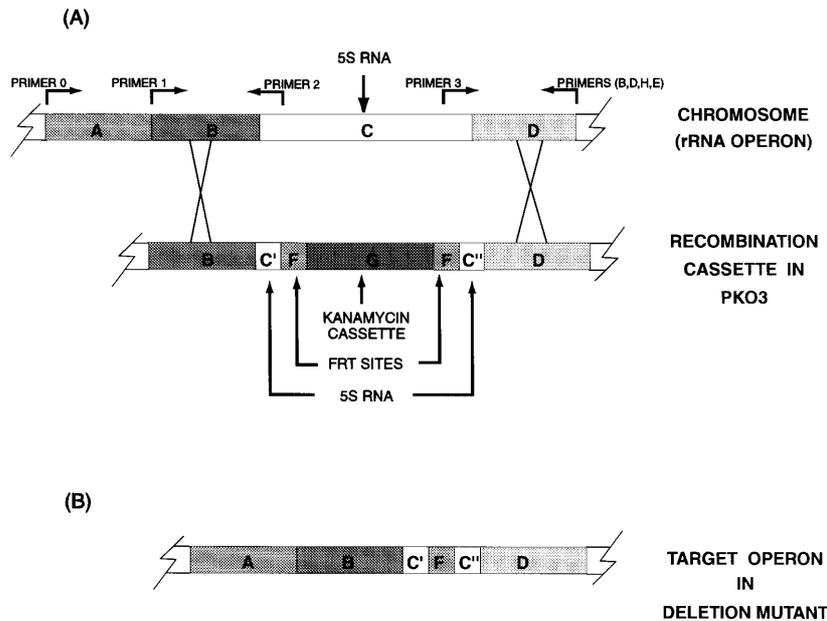


Figure 1. (A) The double crossover event between the recombination cassette in pKO3-(B, D, H or E) and the corresponding chromosomal operon in *E. coli* strain EMG2 is illustrated. (B) Composition of a 5S rRNA deleted rRNA operon after Flp-mediated excision of the kanamycin gene. Letters represent different DNA regions. A, B and D are regions of 468, 531 and 517 bp, respectively, which flank the 5S rRNA. Region C is 120 bp, region G is ~1500 bp and region F is ~75 bp. Regions C' and C'' are 18 and 20 bp in length, respectively, and represent the terminal 18 and 20 nt of the 5S rRNA gene that are complementary to primers 2 and 3. FRT is a DNA sequence recognized by Flp recombinase resulting in the loss of one FRT site and the entire DNA between the two FRT sites.

*Sma*I-cut PCR fragment generated with primers 3 and B. The ligation mix was then cut with *Kpn*I and *Eco*RI. The ligated fragment representing recombination cassette B was isolated from a 0.6% agarose gel and ligated to pBluescript II KS(+/-) cut with *Kpn*I and *Eco*RI, giving plasmid pBlu-B. The *Kpn*I-*Eco*RI fragment from pBlu-B was blunt-ended and ligated into the blunt-ended *Bam*HI site of pKO3 giving pKO3-B. Recombination plasmids pKO3-(D, H and E) were created by ligating the *Eco*RI-*Sma*I fragment from pBlu-B (comprising the 5' flanking region and the kanamycin cassette) with blunt-ended PCR fragments generated with primers 3 and D, H or E. The ligation mix was then further amplified with primers 1 and D, H or E and the resulting fragments blunt-ended and ligated into the *Sma*I site of pKO3, giving pKO3-(D, H and E). 5S rRNA deletions were performed in *E. coli* strain EMG2 (F', λ +). Plasmid pKO3 and *E. coli* strain EMG2 were obtained from George M. Church (Harvard Medical School, Boston, MA). A description of plasmid pKO3 was obtained from the Web page of Dr Church. The URL is <http://arep.med.harvard.edu/labgc/pKO3.html>. Plasmids pCP15 and pCP20 were obtained from Wilfried Wackernagel (Universität Oldenburg, Oldenburg, Germany).

Deletion of chromosomal 5S rRNA genes

The gene deletion protocol utilized was derived from Hamilton *et al.* (10). In brief, cells were transformed with recombination plasmid pKO3-(B, D, H or E). A single transformation colony was plated on YT/chloramphenicol plates (80 μ g/ml) at 42°C. The recombination plasmids are temperature sensitive and cannot replicate at 42°C. Thus, only cells in which the plasmid had integrated into the chromosome were able to replicate the plasmid-encoded gene for chloramphenicol resistance. A single colony from the 42°C plate was then plated onto YT plates

supplemented with kanamycin (50 μ g/ml) and sucrose (5% w/v) and grown at 30°C, a permissible temperature for plasmid replication. Cells carrying the chromosomally integrated plasmid cannot grow efficiently at 30°C due to the plasmid's functional origin of replication. Thus, colonies that do grow result from a second crossover event in which the plasmid (and host 5S rRNA gene) are lost from the chromosome, leaving behind the kanamycin cassette (Fig. 1). The recombination plasmids also contain the *Bacillus subtilis sacB* gene whose gene product is detrimental to *E. coli* cells in the presence of sucrose. Thus, on sucrose, only cells that have lost the chromosomally excised plasmid survive. Plasmid pCP20, which carries the gene for Flp recombinase, was then used to excise the kanamycin gene from between the FRT sites (9), resulting in mutant strains B, BD, BDH and (B)DHE. Deletion strains were created in the order B, D, H and then E. (B)DHE denotes a strain derived from strain BDH with 5S rDNA deletions in operons D, H and E but which has regained a 5S rRNA gene in operon B.

Sequence analysis of 5S rRNA deletion mutants

DNA template was generated by PCR amplification of chromosomal DNA between primer 0 (ACCGGAATTCCTCCGAGACT-CAGTGAAATTG) and the operon-specific primers B, D, H and E. Primer 0 is homologous to a region 467 nt 5' of primer 1 on the chromosome. In all cases chromosomal DNA amplified was from strain (B)DHE, except for operon B, which was obtained from strain BDH. PCR amplification was performed directly on bacterial cells, for 30 cycles of 95°C for 30 s, 60°C for 30 s, 72°C for 3 min, followed by incubation at 72°C for 10 min. Template DNA was purified by electrophoresis of the PCR reaction on a 0.6% agarose gel, removing the gel fragment containing the desired amplified DNA and spinning the DNA from the gel

fragment at 12 000 r.p.m. for 5 min in a microcentrifuge. The DNA-containing liquid was phenol and chloroform extracted and then ethanol precipitated. DNA sequencing was performed using ABI's Ready Reaction sequencing kit and resolved on an ABI 377 Automatic Sequencing Machine. DNA sequencing was performed with primers Seq1 (AACGTTCCCTTCAGGACC), Seq2 (ATGCCTGGCAGTTCCT), 5S-M (CGCCGTAGCGCCGATGGTAG), 1 and B.

Genomic Southern blots

Chromosomal DNA was isolated by pelleting 1.5 ml of an overnight culture and resuspending the pellet in 350 μ l of 0.01 M Tris, pH 7.6, 0.005 M EDTA, 0.5% SDS. Protease K was added to 50 μ g/ml and heated for 1.5 h at 65°C. The solution was then extracted twice with phenol, once with chloroform, digested with RNase A and ethanol precipitated. Chromosomal DNA was digested with *Bam*HI and *Pst*I and the resulting fragments separated on a 0.6% agarose gel and transferred to a nylon membrane (Hybond N⁺). Probe 5S-M (complementary to the deleted portion of the 5S rRNA gene) was end-labeled with γ -³²P using polynucleotide kinase (Promega) and purified on a BioRad -10 Bio-Spin column. Hybridizations were performed at room temperature in hybridization solution (5 \times Denhardt's solution, 5 \times SSPE, 0.1% SDS). Membranes were washed in 6 \times SSC at 36°C for ~2 h.

Compensatory plasmid pC5S

Plasmid pC5S was used to compensate for the loss of 5S rRNA in the deletion strains. This plasmid contains a partial *rrnB* operon (including an intact 5S rRNA gene and promoter region) cloned into the low copy number plasmid pCL1920 (11). Plasmid pC5S was constructed by restricting the *Bam*HI-*Eco*RI fragment containing the partial *rrnB* operon from plasmid pKK 5-1 (12), blunting the fragment ends with mung bean exonuclease and ligating to plasmid pCL1920 that had been digested with *Sma*I. Orientation of the insert, relative to pCL1920, was confirmed by restriction digestion. The 5S rRNA gene in pC5S is oriented such that 5S rRNA expression proceeds in the same direction as plasmid replication.

Growth rate determination

In all cases, 60 ml cultures of YT medium were inoculated with 100 μ l of an overnight culture also grown in YT medium. Bacterial growth rates were determined by monitoring the optical density of the culture at 600 nm (OD₆₀₀) with respect to time. For strains EMG2 B, BD BDH and (B)DHE, two growth trials were performed in duplicate, giving a total of four growth measurements. Both trials were performed at 36°C. One milliliter samples were removed (every ~15 min for the first trial and 30 min for the second trial) for OD₆₀₀ determination. For compensation determination, strains BDH/pC5S, EMG2/pC5S and BDH were tested in triplicate at 37°C. Samples were taken every 15 min for OD₆₀₀ determination. As a control experiment, the growth rate effect of the base plasmid pCL1920 was tested on the BDH and EMG2 strains. Growth trials of EMG2, BDH, EMG2/pCL1920 and BDH/pCL1920 were performed in duplicate at 36°C. Samples were taken every 15 min for OD₆₀₀ determination. The log of OD₆₀₀ versus time was plotted for the exponential portion of the growth curve. The plotted points were subjected to a linear regression analysis and the

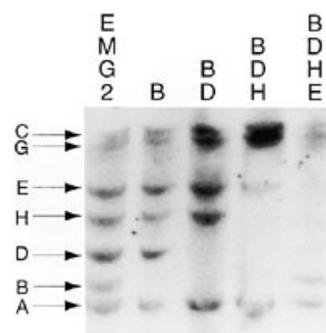


Figure 2. Autoradiogram of a *Pst*I and *Bam*HI double digest of chromosomal DNA from deletion strains B, BD, BDH, (B)DHE and base strain EMG2. The genomic blot was hybridized with Oligo 5S-M (complementary to the deleted nucleotide sequence in 5S rRNA genes). The seven operons (A, B, D, G, H, E and C) are identified with their specific restriction fragments.

slope of the resulting line was defined as the growth constant (k). Calculated doubling time (d) was determined by $d = \log_2/k$.

Restriction analysis of operon B in (B)DHE strain

The FRT site used in this study contains an internal *Xba*I site. Chromosomal DNA from strains (B)DHE, EMG2 and BDH was amplified with primers 1 and B. Amplification conditions were 30 cycles of 95°C for 30 s, 60°C for 30 s, 72°C for 2 min, followed by incubation at 72°C for 10 min. The resulting amplification fragments were digested with *Xba*I. These digestions were separated on a 1% agarose gel and visualized by staining with ethidium bromide.

RESULTS

Deletion of 5S rRNA genes

A major portion of the 5S rRNA gene (between primers 2 and 3) from operons B, D, H and E was deleted. The resulting strains were B, BD, BDH and (B)DHE, where the letters denote the operons in which the deletions occurred. Deletion of 5S rRNA gene sequences was confirmed by Southern hybridization analysis of genomic DNA utilizing oligonucleotide probe 5S-M, specific for the deleted 5S rRNA gene sequences. Figure 2 illustrates that strains B, BD and BDH contain the expected nucleotide deletions. However, along with the expected loss of 5S rRNA gene sequences in operons D, H and E, strain (B)DHE gave a hybridization band for a 5S rRNA gene in operon B. These results suggested that the deleted 5S rRNA gene sequences in operon B had been regained in strain (B)DHE. Further analysis of operon B was therefore performed on this strain.

Analysis of operon B in (B)DHE

The deletion process utilized leaves a *Xba*I site-containing FRT site in place of the deleted 5S rRNA gene (Fig. 1). Thus, a DNA fragment, PCR amplified across a deletion site, should contain a *Xba*I site. Figure 3 illustrates that the *Xba*I site found in operon B of BDH was no longer present in (B)DHE. This result suggested that the apparent acquisition of a 5S rRNA gene in operon B coincided with the loss of the FRT site, consistent with a gene conversion event. If gene conversion did occur then the 5S

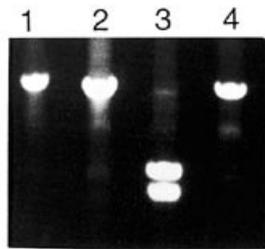


Figure 3. Evidence for reacquisition of a 5S rRNA gene in operon B of strain (B)DHE by a gene conversion event. An ethidium bromide stained agarose gel of DNA from selected strains using primers 0 and B. Primer 0 amplifies from any of the operons, whereas primer B is specific for operon B. Lane 1, EMG2 digested with *Xba*I; lane 2, BDH undigested; lane 3, BDH digested with *Xba*I; lane 4, (B)DHE digested with *Xba*I.

rRNA gene present in operon B must have originated from one of the remaining 5S rRNA genes (e.g. operons G, C or A). A PCR-amplified fragment of (B)DHE genomic DNA representing the 5S rRNA region of operon (B) was subjected to nucleotide sequence analysis. Three informative sites were observed where the nucleotide sequence showed variation among the seven rRNA operons. For the purposes of discussion, positions are numbered relative to the first nucleotide of 5S rRNA (T of TGCC...), which is arbitrarily considered to be position 0. Site 1 (a G, at position -81) was specific for operons A and E. Site 2 (a T at position +91) was specific for operon A. Site 3 (a G at position +144) was specific for operons A and D. Together, these informative sites strongly suggest that concurrent with the deletion of the 5S rRNA gene in operon E of strain BDH, a gene conversion event occurred in which the 5S rRNA gene from operon A was copied onto the 5S rRNA site of operon B.

Nucleotide sequence analysis of deletion strains

23S rRNA is chromosomally located proximally 5' of the 5S rRNA gene. Therefore, the same nucleotide sequence of a particular, but unknown, 23S rRNA gene was utilized in all four recombination cassettes as part of region B (Fig. 1). A sequence analysis of the corresponding genomic 23S rRNA gene region in the deletion strains was therefore undertaken in order to determine if any variation in 23S rRNA sequence occurred as a result of the deletion process. Results indicated that the 23S rRNA gene in operons B, H and E had the wild-type sequence. However, with nucleotide position 1 being defined as the first G of the 23S rRNA gene sequence (GGTTAA...), operon D contains a T→C transition at position 2552 and an A→G transition at position 2826. Additionally, the 23S rRNA genes from all seven operons can be divided into two groups based on naturally occurring sequence variation located between nucleotide positions 2794 and 2802 within region B of the recombination cassette. Operons G, B and D contain the sequence CCTTTAAGG at this position (variant 1) and operons H, E, A and C contain the sequence TCCTTGAGA (variant 2). Sequence analysis of the 23S rRNA genes from operons D, H and E in the deletion strain (B)DHE and operon B in strain BDH showed that all carried sequence variant 2, indicating that region B of the recombination cassette was derived from one of the variant 2 genes.

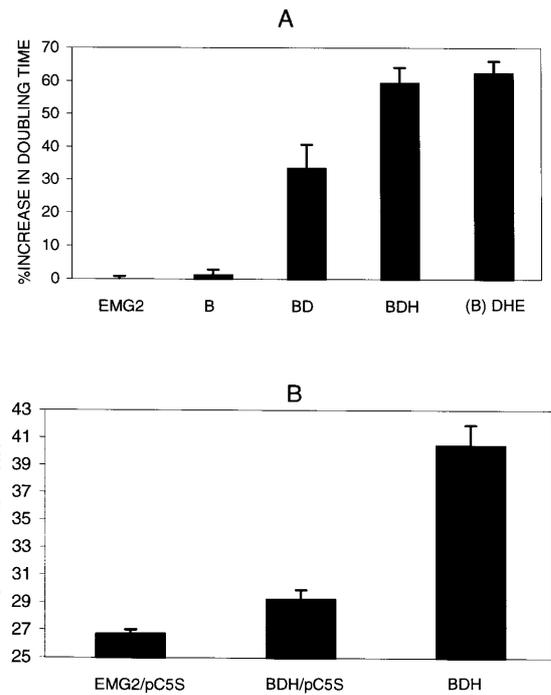


Figure 4. (A) Doubling times of 5S rRNA deletion strains B, BD, BDH and (B)DHE presented as percent increase in doubling time relative to the base strain EMG2 (27.6 ± 0.6 min). (B) Doubling times of the base strain EMG2 grown with the compensatory plasmid pC5S (representing the maximum growth rate), strain BDH (representing the minimum growth rate) and strain BDH with compensatory plasmid pC5S. Values represent the average growth rate for multiple trials. The standard deviation between the trials is shown as an error bar.

Effects of 5S rRNA loss on growth rate

Figure 4A illustrates that there is an increase in doubling time with increasing numbers of 5S rRNA gene deletions. The average doubling times of EMG2, B, BD, BDH and (B)DHE were 27.6 ± 0.6 , 27.6 ± 0.9 , 36.8 ± 1.2 , 44.0 ± 0.7 and 44.8 ± 0.5 min, respectively. An increase in doubling time was not readily apparent in strain B, however, a strong increase in doubling time was observed between strains B and BD and again when an additional 5S rRNA gene was removed from strain BD, giving BDH. As would be predicted, there was not an appreciable difference in doubling time between strains BDH and (B)DHE, both of which have four functional 5S rRNA genes.

Compensation of 5S rRNA loss by plasmid expression of 5S rRNA

We sought to verify that the substantial decrease in growth rate exhibited by strain BDH was primarily caused by loss of 5S rDNA. In order to do this, a compensatory plasmid was utilized that could produce additional 5S rRNA without addressing other possible effects of 5S rRNA gene deletion, e.g. minor sequence changes in 23S rRNA. Figure 4B illustrates that when the EMG2 base strain was grown with the compensatory plasmid pC5S, a growth rate of 26.7 ± 0.3 min was obtained, representing the maximum growth rate. A growth rate of 40.4 ± 1.5 min was obtained for strain BDH, representing the minimum possible growth rate. Strain BDH with compensatory plasmid pC5S gave

a growth rate of 29.2 ± 0.7 min, a value closely approximating the maximum growth rate expected of 26.7 ± 0.3 min. In a control experiment, the growth rate effect of the base plasmid pCL1920 was tested on the BDH and EMG2 strains. In this experiment, the growth rate of the EMG2 strain was 29.2 min, changing very little when carrying the control plasmid pCL1920 (27.8 ± 0.7 min). In the case of the BDH strain carrying pCL1920, doubling time was 51.7 ± 3.4 min as compared with BDH alone, which had a doubling time of 47.5 ± 1.9 min. These results indicate that, by itself, plasmid pCL1920 does not alter the growth rate of either EMG2 or the deletion strains within experimental error.

DISCUSSION

Regulation of rRNA genes in *E. coli* is largely dependent on the translational potential of the cell (5). *Escherichia coli* can up- and down-regulate the rRNA operons to meet the cells' translational needs. It is not surprising then that when rRNA levels, and thus the cells translational potential, are artificially reduced by the deletion of rRNA genes (16S and 23S), rRNA operons are up-regulated in order to compensate for the deficiency (1,6). It is reasonable to expect that deficiencies in cellular 5S rRNA levels would exhibit a similar effect. With respect to this, we have investigated the effect of 5S rRNA deficiencies on cell fitness through the sequential deletion of 5S rRNA genes. Unexpectedly, our findings indicate that the cell's innate ability to up-regulate rRNA operons did not compensate for 5S rRNA deficiencies in a manner similar to that previously observed for 16S and 23S rRNAs (3).

Sequential deletions of 5S rRNA genes from operons B, D, H and E were sought. As was found in prior studies with 16S and 23S rRNA, we observed that the loss of 5S rRNA synthesis from one operon had very little effect on the cells' fitness as measured by its doubling time. This is not surprising because an extra 5S rRNA gene naturally occurs in operon D such that there are eight copies of 5S rRNA as compared with seven copies for 16S and 23S rRNA. Thus, removal of the 5S rRNA gene from operon B should still leave the cell with roughly stoichiometric levels of all three rRNAs. However, when additional genes were deleted (Fig. 4A), we encountered a strong and increasing drop in growth rate. Moreover, the decline is much more severe than that observed previously for 16S and 23S rRNAs. For example, deletion of all but four of the 16S and 23S rRNA genes resulted in an ~25% increase in doubling time (3). In contrast, when five 5S rRNA genes were left the decrease was already 33% and when only four remained a 60% decrease was observed. Furthermore, when we attempted to delete a fifth 5S rRNA gene (E) from a BDH mutant, we observed (along with the deletion of the 5S rRNA gene in operon E) the concurrent addition of a 5S rRNA gene in operon B. Such an event is probably not common. This observation is perhaps indicative of a severely impaired growth rate imparted on the cell resulting from the loss of the fifth 5S rRNA gene and suggests that the loss of five genes was highly detrimental to the cell under the conditions utilized.

The large increase in doubling time shown for the 5S rDNA deletion mutants is presumed to result from the deletion of 5S rDNA. However, the introduction of two nucleotide changes in the 23S rRNA gene sequence of operon D during the deletion process introduces the possibility that some of the observed slowing in growth rate was independent of 5S rDNA loss. Similarly, homogenization of the 23S rRNA genes in the deletion

operons with one of the two naturally occurring 23S rRNA sequence variants could also have contributed to a slowing in the growth rate. However, in either instance, any contribution to the decline in the growth rate was probably negligible at best. For instance, mutant BD is already deficient in 5S rRNA and thus has a relative excess of 23S rRNA. Thus losing product from one 23S rRNA gene should not functionally represent a loss to the cell in terms of functional ribosomes, beyond that which already exists. Similarly, the 23S rRNA variant cannot be considered between mutant strains BD and BDH or (B)DHE because both operons H and E naturally contain the variant found in the deletion construct and therefore there was not a change in 23S rRNA sequence with respect to the variant site. Thus, the observed increase in doubling times going from one to three 5S rRNA gene deletions is probably due solely to the loss of 5S rDNA. In order to experimentally test this conjecture, the effect of a plasmid-borne 5S rRNA gene was examined. This compensatory plasmid was extremely effective, returning the growth rate to nearly the wild-type level. This demonstrates that essentially all of the growth rate difference is due to the loss of chromosomal 5S rDNA and not due to other things, such as the above-mentioned mutations in 23S rDNA. In addition, it would appear that this type of compensatory plasmid might allow the construction of strains in which all the genomic 5S rRNA genes are damaged.

Current thinking on rRNA regulation predicts that if the translational potential of a cell is decreased there is an up-regulation of the rRNA operons as part of a general response mechanism. In fact a general up-regulation of rRNA operons has been shown to occur in diverse cases that impair the cells' translational ability (14). It would appear that such an up-regulation could also produce additional 5S rRNA and circumvent the problem of 5S rRNA gene loss. Why then is the increase in generation time of 5S rRNA mutants far more pronounced than those observed in similar studies for 16S and 23S rRNAs? One possibility is that the regulatory machinery is not able to detect the existence of a problem. Alternatively, it is possible that the effects of a 5S rRNA deficit are not repairable by additional expression alone. For example, relatively lower cellular levels of 5S rRNA, as compared with 16S and 23S rRNAs, could conceivably have a detrimental effect on the cell's growth rate. One might envision a relative deficiency in 5S rRNA resulting in the accumulation of defective, 5S rRNA-deficient 50S ribosomal subunits that compete with their functional counterparts. A general up-regulation of rRNA operons may bring 5S rRNA levels to normal levels but would not change the ratio of functional to defective 50S subunits and thus could not compensate. However, *in vitro* reconstitution experiments have previously been reported in which 5S rRNA-deficient 47S particles have been generated and shown to be very inefficient in comparison with 50S particles in the formation of 70S couples (15). Alternatively, it is also conceivable that synthesis of defective 50S particles, coupled with the cell's reduced translational potential, is capable by itself of energetically taxing the cell sufficiently to cause the slower growth rates.

Our findings suggest that *E. coli* may be highly sensitive to changes in the number of functional 5S rRNA genes and that the loss of more than one 5S rRNA gene is highly detrimental to the cell. It is therefore logical to consider to what extent 5S rRNA levels must be deficient in order to invoke a fitness drop in the wild. Although not necessarily indicative, it is interesting to note that *E. coli* naturally carries two 5S rRNA genes in operon D, a situation that presumably enables the cell to keep slightly higher levels of 5S rRNAs than 23S rRNAs.

ACKNOWLEDGEMENTS

We thank Dr Michael Benedik for many helpful discussions. This work was supported by grants from the Environmental Protection Agency (R825354-01-O), the Texas Advanced Research Program (003652-978) and the University of Houston Shell Scholars Program.

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