JOURNAL OF BACTERIOLOGY, Apr. 2007, p. 3237–3245 Vol. 189, No. 8
0021-9193/07/$08.00+0 doi:10.1128/JB.01686-06

Performance of the Translational Apparatus Varies with the Ecological Strategies of Bacteria▽

Les Dethlefsen† and Thomas M. Schmidt*

Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, Michigan 48824

Received 31 October 2006/Accepted 23 January 2007

Protein synthesis is the predominant activity of growing bacteria; the protein synthesis system accounts for more than one-half the cell's dry mass and consumes most of the cell's energy during rapid growth. Translation has been studied extensively using model organisms, and the translational apparatus is qualitatively similar in terms of structure and function across all known forms of life. However, little is known about variation between organisms in translational performance. Using measurements of macromolecular content in a phylogenetically diverse collection of bacteria with contrasting ecological strategies, we found that the translational power (the rate of protein synthesis normalized to the mass of the protein synthesis system) is three- to fourfold higher among bacteria that respond rapidly to nutrient availability than among bacteria that respond slowly. An analysis of codon use in completely sequenced bacterial genomes confirmed that the selective forces acting on translation vary with the ecological strategy. We propose that differences in translational power result from ecologically based variation among microbes in the relative importance of two competing benefits: reducing the biomass invested in the protein synthesis system and reducing the energetic expense of protein synthesis.

Protein synthesis is an ancient and essential cellular function. It is also expensive. Four high-energy phosphate bonds are consumed for each amino acid added to a polypeptide during ribosomal protein synthesis, whereas only two such bonds are used to add a nucleotide to either RNA or DNA. Since a bacterial cell is comprised mostly of protein, protein synthesis is responsible for a large fraction of all energy consumption in a growing bacterium; it accounts for nearly two-thirds of the energy consumption in *Escherichia coli* using glucose as a sole carbon and energy source (59, 69). The expense of protein synthesis is also reflected in the fraction of cell mass devoted to the protein synthesis system. This system includes ribosomes, tRNA, mRNA, and translation factors that are directly involved in protein synthesis; it also includes the RNA polymerases, transcription factors, RNA-modifying enzymes, and tRNA synthetases that synthesize and process RNA (44). The protein synthesis system can comprise well over one-half of the dry mass of a rapidly growing bacterial cell and about one-quarter of the dry mass even during slow growth (3, 24).

Selection tends to favor evolutionary changes that reduce the cost of operating the protein synthesis system or that reduce the amount of resources needed to build the protein synthesis system itself. To clearly differentiate between these two selective pressures, we use the terms "translational yield" for the amount of functional protein synthesized per unit of energy consumed in protein synthesis and "translational power" for the rate of functional protein synthesis per unit of biomass invested in the protein synthesis system (12). Selection for power and yield are distinct; high power is more important when the fitness of an organism depends primarily on rapid growth, whereas high yield becomes important when fitness is determined by the ability to grow or survive during periods of resource scarcity.

For a culture in balanced growth, translational power can be quantified as the ratio of the rate of protein synthesis to the mass of RNA, which is an easily measured proxy for the mass of the entire protein synthesis system: translational power = $\mu P/R$, where μ is the exponential growth rate and P and R are the protein and RNA contents, respectively (12). We avoid the older terms "ribosome efficiency" and "translational efficiency" that have been applied to this ratio (3, 16, 29, 37, 49, 65), because "efficiency" implies a measurement of waste obtained by comparing the output of a process to the input, without considering its rate (12). Mathematical manipulation of the ratio demonstrates that translational power is proportional to the product of the translation rate and the fraction of ribosomes that are active in translation (12).

Our analysis of selective pressures on the protein synthesis system is based on a large body of work by Charles Kurland, Måns Ehrenberg, and their colleagues at the University of Uppsala. Many insights of the Uppsala School follow from a model for the allocation of biomass to the various metabolic pathways of the cell, using growth rate as an optimizing criterion (16). These workers found that the growth rate-maximizing arrangement of the protein synthesis system (or any other metabolic pathway) varies with the fraction of the cell's total metabolic flux that passes through the pathway (16, 36). In particular, keeping the ribosomes saturated with substrates is prohibitively expensive during poor growth conditions; instead, the translation rate approaches its maximum value only asymptotically as growth conditions improve and the cell approaches

* Corresponding author. Mailing address: Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI 48824-4320. Phone: (517) 355-6463, ext. 1606. Fax: (517) 353-8957. E-mail: tschmidt@msu.edu.

† Present address: Department of Microbiology and Immunology, Stanford University, Palo Alto, CA 94304.

▽ Published ahead of print on 2 February 2007.

its maximal growth rate (16, 36). Although in conflict with the prevailing wisdom of the time that maximal translation rates should be favored under all conditions (24, 44), this theoretical result is consistent with both macromolecular data (3, 11) and metabolic shift-up experiments (33, 34). Empirical and theoretical results of the Uppsala School have also demonstrated that there are complex interactions between the rate, accuracy, and processivity of translation (1, 13, 25, 26, 35, 37) and that the translational rate is both variable and responsive to selection among strains of *E. coli* (38, 48, 49).

The ability of the environment to select for different translational phenotypes within a species suggests that microbes adapted to different ecological conditions may differ in their translational performance. We are aware of four such studies, and in each of these studies a single slowly growing species was compared to laboratory-adapted *E. coli* (5, 18, 55, 63). In every case, the translational output per ribosome or per RNA molecule was lower in the slowly growing species than in *E. coli* (5, 18, 55, 63). An analysis of previously published macromolecular data is consistent with the direct comparisons; translational power is lower in slowly growing microbes than in rapidly growing microbes, and the differences range from 2.5- to 6-fold (12). However, the results of the literature review must be interpreted cautiously. Colorimetric assays of macromolecular content are quite sensitive to the experimental conditions and standards employed, so comparisons are most reliable within a single study. Furthermore, the phylogenetic breadth of these comparisons is limited; most macromolecular data that have been published involve *E. coli* or closely related strains.

Recognition of an apparent relationship between the ecological traits of bacteria and translational performance, which is generally considered a routine housekeeping function, prompted us to make a direct comparison of translational power among recently isolated soil bacteria. Previous work established that soil bacteria capable of responding rapidly to nutrient availability have more copies of the rRNA (*rrn*) operon per genome than strains which respond only slowly to nutrients have (31). High *rrn* copy number (which ranges from 1 to 15 in bacteria) is adaptive for strains that exploit episodes of resource abundance in fluctuating environments, because it enables a rapid increase in the growth rate (shift-up) when nutrient availability increases and rapid, sustained growth while resources remain abundant (8, 31, 68). We refer to strains adapted to this ecological strategy as rapid responders. However, multiple *rrn* operons can impose a metabolic cost during slow growth or stasis when the basal level of transcription exceeds the cell's demand for ribosomes (18, 19, 31, 67). The fitness of strains that are not rapid responders could depend primarily on their ability to grow when resources are scarce or on their ability to survive when resources are absent. Given this uncertainty, we refer to such strains simply as slow responders, without meaning to suggest that a slow response to nutrient availability is a beneficial ecological strategy per se. We selected phylogenetically diverse rapid and slow responders from our collection of soil bacteria and measured their macromolecular content in laboratory cultures to test the hypothesis that translational power is higher among the rapid responders than among the slow responders. We also analyzed codon use in 76 bacterial genomes to confirm that the strength of selection for translational power varies with ecological strategy.

MATERIALS AND METHODS

Strain selection. Four phylogenetically diverse strains were chosen to represent each end of a spectrum of ecological strategies found in a previously described collection of soil bacteria (31). The "rapid responder" strategy was defined operationally by the ability to form a visible colony within 2 days during initial isolation and was confirmed by demonstrating that the strains possessed four or more copies of the *rrn* operon per genome. "Slow responders" required a week or more to form visible colonies during the initial isolation and were confirmed to have only one or two *rrn* copies per genome. Isolates were grown in the laboratory only long enough to ensure purity before −80°C freezer stocks were prepared. *E. coli* strain REL607 (a B/r derivative, obtained from R. Lenski [40]) and *Sphingopyxis* (formerly *Sphingomonas*) *alaskensis* RB2256 (18) (obtained from R. Cavicchioli and M. Ostrowski [15, 53]) were included in this study as well-characterized representatives of organisms with the rapid and slow response strategies, respectively.

Culture conditions. All species were grown in batch culture in R2BV medium, which contains (per liter) 0.5 g of glucose, 0.5 g of peptone, 0.5 g of yeast extract, 0.5 g of Casamino Acids, 0.5 g of soluble starch, 0.3 g of KH_2PO_4, 0.3 g of sodium pyruvate, 0.05 g of $MgSO_4$, and 1 ml of a vitamin solution. The vitamin solution contains (per liter) 200 mg of i-inositol and 100 mg of each of the following vitamins: biotin, choline-Cl, folic acid, lipoic acid, nicotinamide, pantothenate, *para*-aminobenzoic acid, pyridoxal HCl, riboflavin, and thiamine HCl. Most strains were also grown in PVY/10 medium, which contains (per liter) 0.71 g of Na_2HPO_4, 0.68 g of KH_2PO_4, 0.66 g of $(NH_4)_2SO_4$, 0.12 g of $MgSO_4$, 0.5 g of peptone, 0.05 g of yeast extract, 1 ml of the vitamin solution, and 1 ml of a trace element solution. The trace element solution contains (per liter) 6.03 ml of 11.6 N HCl, 2.085 g of $FeSO_4 \cdot 7H_2O$, 143.6 mg of $ZnSO_4$, 89.1 mg of $MnCl_2 \cdot 4H_2O$, 6.2 mg of H_3BO_3, 190.3 mg of $CoCl \cdot 6H_2O$, 1.7 mg of $CuCl_4 \cdot 2H_2O$, 23.8 mg of $NiCl_2 \cdot 6H_2O$, and 48.4 mg of $Na_2MoO_4 \cdot 2H_2O$. *E. coli* and soil isolates EC2, HS5, and LC9 did not grow adequately in PVY/10, so the second medium used for these strains was R2B-GCS, which is identical to R2BV except that it does not contain glucose, Casamino Acids, soluble starch, or vitamins.

Cultures of each strain-medium combination were started from freezer stock, grown at 25°C on an orbital shaker at 200 rpm, and transferred to fresh medium during exponential growth. Growth was monitored by determining the turbidity at 420 nm, which was chosen in order to maximize the sensitivity at low cell densities. Following the second transfer, at an optical density that was no more than one-half the optical density at which deviation from an exponential increase in the turbidity was detected in previous cultures of the strain-medium combination, biomass was collected by centrifugation (10,000 × *g*, 10 to 30 min, 4°C) from three or four aliquots each of at least three replicate cultures that had nearly identical growth curves. Cell pellets were frozen at −80°C until they were analyzed to determine the macromolecular content. At the time that biomass was harvested, culture aliquots were also collected for determinations of the culture density and biovolume. Specific growth rates were calculated for each culture by determining the slope of a linear regression of the natural logarithm of the last four optical densities at 420 nm with time, and the values were averaged to obtain a single estimate of the growth rate for each strain-medium combination. All growth rates were expressed as specific growth rates [$\mu = \ln(2)g^{-1}$, where g is the generation time].

Culture density. For determination of the number of cells per volume of culture we used standard protocols for counting bacterial cells stained with 4′,6-diamidino-2-phenylindole (DAPI) by epifluorescence microscopy (27, 28). Cells were fixed in formaldehyde (final concentration, 2%) at least overnight, stained for 5 to 7 min with 0.5 μg/ml (final concentration) DAPI in the filter funnel, and drawn onto a 25-mm-diameter, 0.2-μm-pore size Anodisc filter (Whatman) with a 25-kPa partial vacuum. Two or more filters were prepared for each culture vessel within 48 h of harvest and were stored at −20°C until the cells were counted with an Axioscop 2 (Carl Zeiss) equipped with a 100-W Hg lamp and a ×100 Plan-NeoFluar objective. Filters were prepared with 20 to 100 cells per optical grid, and at least 20 fields on two transects of the filterable area were counted per filter. Estimates of the number of cells ml^{-1} optical density $unit^{-1}$ were averaged by culture vessel to provide a single estimate of culture density for each strain-medium combination.

Biovolume. Cell sizes were determined by analyzing digital photomicrographs of unfixed bacteria immobilized on agarose-coated slides. The slides were scanned immediately after preparation (at the time of biomass harvest) in order to find areas with the optimum contrast between the cells and the background, which occurred just as the cells became immobilized. These areas were photo-

graphed in eight-bit grayscale with a Spot 2 cooled charge-coupled device camera (Diagnostic Instruments) mounted on an Axioskop 2 (Carl Zeiss) using a ×100 Plan-Neofluar objective with phase-contrast illumination with a green filter; 5 to 10 such images, each containing 20 to 200 mostly nonoverlapping cells, were obtained for each culture vessel. The highest-quality images from each strain-medium combination were processed interactively with Photoshop v6.0 (Adobe) to produce binary images containing a total of at least 300 nontouching cells. Shape-dependent measurement features available in the CMEIAS software package (42) were used with these binary images to produce a single estimate of average cell biovolume for each strain-medium combination. Actual dimensions of the cells were calibrated from a digital photomicrograph of a stage micrometer visualized under identical conditions.

Macromolecular content. Cell pellets were thawed in cold lysis buffer (10 mM Tris, 1 mM EDTA, 2% [vol/vol] ethanol, 0.05% [vol/vol] Igepal CA-630 detergent, 200 mg/liter sodium deoxycholate; pH 7.0) and lysed by sonication using a tapered probe with a 0.125-in. tip in a Sonifier model 450 (Branson) at half-maximum power with a 25% duty cycle for 90 s. Cell pellets from gram-positive strains EC5 and PX3.15 were subjected to five freeze-thaw cycles using a dry ice-ethanol bath and a 95°C water bath prior to addition of the lysis buffer. Triplicate aliquots of lysed cell material from each cell pellet were immediately diluted 1:10 (or more as necessary) into TE buffer (10 mM Tris, 1 mM EDTA) for nucleic acid assays; duplicate aliquots from the same pellet were immediately added to trichloroacetic acid (TCA) (final concentration, 10%) for protein extraction.

DNA and RNA contents of cell samples were determined using the proprietary fluorescent dyes PicoGreen and RiboGreen (Molecular Probes) in conjunction with standard curves for pure DNA and RNA. Both PicoGreen fluorescence and RiboGreen fluorescence were measured at three different dilutions of each of the three replicate aliquots of a single lysed cell pellet; triplicate standard curves for each dye were obtained by using five-point threefold dilution series of *E. coli* rRNA (maximum concentration, 1 μg/ml) and λ phage DNA (maximum concentration, 500 ng/ml) that had been sonicated and diluted like the cell samples. Fluorescence was measured in black 96-well plates with flat, optically clear bottoms (Costar) using a Storm 860 fluorescence scanner (Molecular Dynamics) and blue laser excitation with 100-μm resolution and 1,000-V photomultiplier tube edges of the 96-well plates were removed prior to sample loading so the well bottoms rested directly on the glass surface of the scanner. Images were analyzed to determine the amount of fluorescence per well using the ImageQuant 5.0 software (Molecular Dynamics). Since RiboGreen fluoresces in the presence of both DNA and RNA, the amount of RiboGreen fluorescence predicted from the DNA content of a cell sample (as determined by PicoGreen fluorescence) was subtracted from the total amount of RiboGreen fluorescence to obtain the residual amount of RiboGreen fluorescence attributable to RNA.

The protein contents of cell samples were determined using the bicinchoninic acid assay (MicroBCA kit; Pierce Chemical) following hot TCA extraction (10) of lysed cell material. The protein pellets obtained from TCA extraction were suspended in 50 μl of alkaline sodium dodecyl sulfate (5% [vol/vol] sodium dodecyl sulfate in 0.1 N NaOH) with shaking for 1 h and then were diluted in 1.00 ml of a sodium chloride solution (0.9%, wt/vol). The protein extracted from each of the duplicate aliquots of a single cell pellet was analyzed at two different dilutions (three wells each) in untreated, clear, flat-bottom, 96-well plates (Nunc). Protein standard curves were obtained by using five-point twofold dilution series (maximum concentration, 1 μg/ml or 500 ng/ml) of bovine serum albumin (Pierce Chemical) that had been sonicated and TCA extracted like the cell samples; duplicate curves were prepared for each of two independent TCA extractions of the standard. The plates were incubated at room temperature for 90 min before the absorbance at 562 nm was determined with a Biokinetics EL312e plate reader (Bio-Tek Instruments). Protein concentrations in the sample wells were estimated by using a quadratic relationship between absorbance and protein concentration of the standard; TCA-extracted and unextracted reagent blanks were used to control for the small difference in absorbance attributable to residual effects of the TCA extraction.

rRNA gene sequences and phylogenetic analysis. Nearly full-length sequences of the small-subunit (16S) rRNA genes were obtained for the eight recent soil isolates in our collection and *S. alaskensis.* Genomic DNA was extracted from pure cultures of each strain using either an UltraClean soil DNA kit (MoBio Laboratories) or a Bactozol kit (Molecular Research Center) as directed by the manufacturer. The genomic DNA was used as a template for amplification of the 16S rRNA gene by PCR with standard bacterial primer 8F (AGAGTTTGATC CTGGCTCAG) paired with either 1492R (GGTTACCTTGTTACGACTT) or 1540R (AAGGAGGTGATCCARCCGCA), using the following parameters: 5 min at 95°C, followed by 30 or 35 cycles of 95°C for 30 s, 65°C for 30 s, and 72°C for 30 s and then 5 min at 72°C. Reaction mixtures were purified with either Wizard PCR Preps (Promega) or Microcon centrifugal filters (Millipore), and amplicons were sequenced directly using Big Dye terminator chemistry (Applied Biosystems) with an ABI 377 or ABI 3700 gene sequencer (Applied Biosystems) and sequencing primers that provided an average of >3-fold coverage over the length of the amplicon.

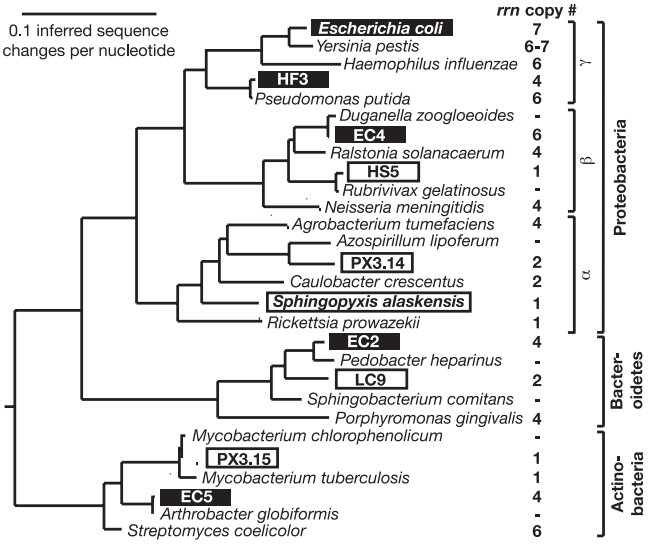

FIG. 1. Phylogenetic relationships among the experimental bacteria, inferred by maximum likelihood analysis of 16S rRNA gene sequences as described in Materials and Methods. Species closely related to the experimental strains are included for reference. At least four evolutionary transitions between the rapid response (solid boxes) and slow response (open boxes) ecological strategies must have occurred. The numbers of *rrn* operons per genome (where known) (31, 32) and the taxonomic affiliations of the strains are indicated on the right.

16S rRNA gene sequences from fully sequenced microbial genomes (operon A or operon 1 in the case of multiple operons), as well as sequences from our isolates, were imported into an Arb database (43) and aligned automatically with full-length aligned sequences obtained from release 8.0 of the Ribosomal Database Project (7). The initial alignments were optimized manually with reference to secondary structure information available at the Comparative RNA Website (www.rna.icmb.utexas.edu) (4). In addition to the sequences of our strains, we analyzed a set of high-quality, full-length or nearly full-length 16S rRNA sequences representing close relatives of our isolates, microbes whose genomes have been sequenced, and organisms representing the remaining major bacterial lineages; several diverse archaeal lineages were included as outgroups. A mask was created to exclude positions at which the alignment was ambiguous, which left 1,250 columns for phylogenetic inference using the FastDNA-ML maximum likelihood algorithm implemented within Arb. The resulting tree containing 166 rRNA sequences is the basis for the phylogenic tree shown in Fig. 1 and the phylogenetic hypothesis incorporated into the likelihood ratio tests of codon bias.

Codon bias and tRNA gene copy number. Codon usage and tRNA gene copy number data were obtained for all completely sequenced bacterial genomes available from the Comprehensive Microbial Resource of The Institute for Genome Research (58) in June 2003. The frequency of each sense codon was compiled for the entire genome (all predicted genes of the original annotation) and a set of highly expressed genes. The highly expressed gene set included five ribosomal protein genes (*rpsA* encoding S1, *rpsB* encoding S2, *rpsI* encoding S9, *rplA* encoding L1, and *rplM* encoding L13) and three elongation factor genes (*tuf* encoding EF-Tu, *tsf* encoding EF-Ts, and *fus* encoding EF-G). When multiple copies of these genes were found in a single genome, all copies were included. Our measure of the codon bias due specifically to translational selection was derived from Novembre's N'_C (51), which is itself an extension of Wright's effective number of codons (N_C) (70). Wright's N_C measures codon bias relative to uniform use of all synonymous codons. Novembre's N'_C extends this approach to measure biased codon usage relative to any specified set of expected codon frequencies. The scale of both N_C and N'_C ranges from 20 to 61, with lower

TABLE 1. Cell and culture traits of experimental bacteria

Species or strain	Taxonomic position[a]	Reference	No. of *rrn* genes[b]	Culture medium	Specific growth rate (h^{-1})	Cell vol (fl)	Culture density (10^7 cells ml^{-1})[c]
Rapid responders							
E. coli	γ-*Proteobacteria*, *Enterobacteriaceae*	40	7	R2BV	0.67 ± 0.015	6.4 ± 0.4	2.9 ± 0.1
				R2B-GCS	0.65 ± 0.006	4.9 ± 0.2	3.4 ± 0.1
HF3	γ-*Proteobacteria*, *Pseudomonas*	20	4	R2BV	0.710 ± 0.008	5.7 ± 0.5	1.8 ± 0.1
				PVY/10	0.645 ± 0.007	3.2 ± 0.2	2.9 ± 0.3
EC4	β-*Proteobacteria*, *Oxalobacteriaceae*	31	4	R2BV	0.546 ± 0.005	5.3 ± 0.5	3.6 ± 0.1
				R2B-GCS	0.482 ± 0.003	5.7 ± 0.4	3.4 ± 0.2
EC2	*Bacteroidetes*, *Sphingobacteriaceae*	31	6	R2BV	0.431 ± 0.002	2.4 ± 0.1	7.1 ± 0.6
				PVY/10	0.236 ± 0.002	1.5 ± 0.1	12 ± 1.8
EC5	*Actinobacteriaceae*, *Arthrobacter*	31	4	R2BV	0.545 ± 0.004	2.4 ± 0.1	5.6 ± 0.2
				PVY/10	0.470 ± 0.008	2.3 ± 0.2	6.2 ± 0.3
Slow responders							
S. alaskensis	α-*Proteobacteria*, *Sphingomonadaceae*	61, 62	1	R2BV	0.217 ± 0.002	1.9 ± 0.2	9.1 ± 0.4
				PVY/10	0.130 ± 0.002	0.8 ± 0.1	16.5 ± 0.7
PX3.14	α-*Proteobacteria*, *Rhodospirillaceae*	30	2	R2BV	0.144 ± 0.001	2.8 ± 0.1	3.4 ± 0.1
				PVY/10	0.126 ± 0.001	2.9 ± 0.1	2.9 ± 0.1
HS5	β-*Proteobacteria*, *Comamonadaceae*	20	1	R2BV	0.081 ± 0.001	3.5 ± 0.2	4.8 ± 0.3
				R2B-GCS	0.067 ± 0.001	2.3 ± 0.1	11.2 ± 0.3
LC9	*Bacteroidetes*, *Sphingobacteriaceae*	31	2	R2BV	0.239 ± 0.003	1.3 ± 0.2	12.1 ± 0.4
				R2B-GCS	0.118 ± 0.005	0.9 ± 0.1	9.7 ± 0.6
PX3.15	*Actinobacteriaceae*, *Mycobacterium*	30	2	R2BV	0.106 ± 0.001	1.5 ± 0.1	2.1 ± 0.2
				PVY/10	0.040 ± 0.003	1.3 ± 0.1	2.2 ± 0.1

[a] Taxonomic positions of soil isolates at the family or genus level based on 16S RNA gene sequence analysis.
[b] Number of copies of the *rrn* operon per genome from references 30 and 31.
[c] Culture density at an optical density at 420 nm of 0.1.

values (fewer effective codons) indicating greater bias (i.e., a greater deviation of the observed codon frequencies from the expected values) (51). Using the genome codon frequencies as expected values and observed highly expressed codon frequencies, our measure ($\Delta N'_C = 61 - N'_C$) ranged (theoretically) from 0 to 41, with larger values indicating increased codon bias and stronger evidence of translational selection.

The significance of correlations between the number of *rrn* operons per genome and either $\Delta N'_C$ or tRNA gene copy number and estimates of the associated correlation coefficients were obtained from likelihood ratio comparisons as implemented in the Continuous software program (54). The observed values for each trait for each bacterial genome were input into the program along with a 16S rRNA-based phylogeny relating the genomes (obtained as described above). The Continuous program was used to estimate the maximum likelihood model of trait evolution over the specified phylogeny that resulted in the observed distribution of traits, either if the traits were not correlated or if the traits had some unspecified degree of correlation. In the latter case, the maximum likelihood estimate of the correlation coefficient was also provided by the software. The ratio of the likelihood values for the best models for uncorrelated and correlated trait evolution provided the probability that the traits were not correlated (*p* values in Fig. 4); the square of the estimated correlation coefficient provided the coefficients of determination (R^2 values in Fig. 4).

Nucleotide sequence accession numbers. The assembled sequences determined in this study have been deposited in the GenBank database under accession numbers AY337597 to AY337605.

RESULTS

The ecological strategies and *rrn* copy numbers of the strains examined in this study and their phylogenetic relationships (inferred from 16S rRNA gene sequence analysis) are shown in Fig. 1. From a previously described collection of recently isolated soil bacteria (31), we chose both a rapidly responding isolate and a slowly responding isolate belonging to the *Actinobacteria*, to the *Bacteroidetes*, and to the β-*Proteobacteria*. We also included a rapidly responding member of the γ-*Proteobacteria* and a slowly responding member of the α-*Proteobacteria*, but for these two strains our collection did not contain a matching strain of the contrasting strategy in the same phylogenetic subdivisions for these two strains. Nonetheless, the eight strains that we examined must represent at least four evolutionary transitions between the two ecological strategies, so any correlation between traits of the organisms and their ecological strategies cannot be explained simply by the shared inheritance of an ancestral phenotype. The γ-*Proteobacteria* strain *E. coli* REL607 and the α-*Proteobacteria* strain *S. alaskensis* RB2256 were included as well-characterized representatives of bacteria with the rapid and slow response strategies, respectively; these strains are laboratory-adapted strains derived originally from the human gut (40) and oligotrophic ocean water (15).

The taxonomic affiliation of each strain and its growth rate, biovolume, and culture density in two media are shown in Table 1. As expected, the rapidly responding bacteria grew faster than the slow responders; in fact, the growth rates of strains with different strategies barely overlapped under the conditions examined. The range of growth rates obtained in the two media was greater for the slow responders (mean, 1.7-fold difference) than for the rapid responders (mean, 1.2-fold difference). The biovolume generally increased with the growth rate for a strain (as expected), although the relationship between the biovolume and the growth rate varied considerably between strains.

The macromolecular contents per cell and per biovolume are shown for each strain-medium combination in Table 2. As expected, the rank order of macromolecular abundance was protein > RNA > DNA for all strains except the slowly responding mycobacterium strain PX3.15, for which the RNA and DNA contents were not statistically distinct. The macromolecular content per cell was quite variable among the

TABLE 2. Macromolecular contents of experimental bacteria

Species or strain	Culture medium	DNA concn		RNA concn		Protein concn	
		Per cell (fg cell^{-1})	Per biovolume (fg fl^{-1})	Per cell (fg cell^{-1})	Per biovolume (fg fl^{-1})	Per cell (fg cell^{-1})	Per biovolume (fg fl^{-1})
Rapid responders							
E. coli	R2BV	31 ± 0.7	4.9 ± 0.4	162 ± 8	26 ± 2	428 ± 33	69 ± 8
	R2B-GCS	24 ± 0.7	4.9 ± 0.2	123 ± 7	25 ± 1	307 ± 33	63 ± 8
HF3	R2BV	54 ± 2.2	9.7 ± 0.9	249 ± 6	45 ± 3	582 ± 35	104 ± 8
	PVY/10	16 ± 3.0	4.9 ± 0.9	59 ± 5	19 ± 2	306 ± 9	97 ± 2
EC4	R2BV	29 ± 0.9	5.6 ± 0.5	54 ± 1	10 ± 1	330 ± 26	64 ± 8
	R2B-GCS	29 ± 3.2	5.3 ± 0.9	54 ± 9	10 ± 2	366 ± 42	66 ± 11
EC2	R2BV	13 ± 0.3	5.3 ± 0.1	37 ± 1	15 ± 1	157 ± 8	65 ± 4
	PVY/10	11 ± 0.3	7.3 ± 0.5	18 ± 1	12 ± 1	118 ± 3	81 ± 4
EC5	R2BV	5 ± 0.5	2.0 ± 0.3	23 ± 3	10 ± 1	94 ± 9	39 ± 5
	PVY/10	5 ± 0.6	2.1 ± 0.2	29 ± 2	13 ± 1	130 ± 6	58 ± 4
Slow responders							
S. alaskensis	R2BV	8 ± 0.5	4.3 ± 0.4	23 ± 1	12 ± 1	93 ± 4	49 ± 4
	PVY/10	5 ± 0.3	6.6 ± 0.7	9 ± 0.7	12 ± 2	51 ± 2	68 ± 8
PX3.14	R2BV	15 ± 1.0	5.3 ± 0.6	38 ± 1	13 ± 1	198 ± 7	71 ± 5
	PVY/10	17 ± 1.0	6.0 ± 0.4	52 ± 1	18 ± 1	254 ± 18	89 ± 7
HS5	R2BV	11 ± 0.8	3.2 ± 0.4	16 ± 1	4.7 ± 0.4	157 ± 5	46 ± 3
	R2B-GCS	12 ± 0.5	5.0 ± 0.3	16 ± 1	6.9 ± 0.2	92 ± 2	39 ± 2
LC9	R2BV	9 ± 0.2	7.4 ± 1.6	22 ± 1	19 ± 4	80 ± 1	66 ± 13
	R2B-GCS	12 ± 0.7	13.2 ± 1.5	20 ± 2	21 ± 1	105 ± 1	111 ± 7
PX3.15	R2BV	17 ± 1.2	11.5 ± 0.8	15 ± 1	10 ± 1	155 ± 7	101 ± 5
	PVY/10	17 ± 0.5	13.1 ± 0.4	16 ± 1	12 ± 1	139 ± 8	108 ± 7

strains, primarily reflecting the differences in cell size. The macromolecular content per biovolume was more consistent across the strains.

Our measurements for the protein and RNA contents per cell at a given growth rate for *E. coli* were comparable to those reported by Bremer and Dennis (3), once a correction was made for the expected 2.3-fold difference in growth rate due to incubation at 25°C instead of 37°C (17). Our temperature-corrected values for DNA content per cell for *E. coli* were about 1.7-fold higher than those of Bremer and Dennis (3), which could have been due to differences in the strains examined. However, we noted that an increase in the DNA content per cell at lower growth temperatures was also found in three of the five media tested by Schaechter et al., who performed the original study that established the conventional wisdom that temperature influences bacterial growth rates without altering the macromolecular content (60).

Calculations of macromolecular content per cell or per biovolume facilitate comparisons with previously published work, but they necessarily introduce additional experimental uncertainty by incorporating empirical estimates of culture density and biovolume. To avoid such errors, for our calculations for all quantities involving macromolecular ratios we used measurements for the protein, RNA, and DNA contents for the same cell pellet. The standard errors of estimates of such ratios were obtained from independent estimates for three or four replicate cultures of each strain-medium combination. Figure 2 shows the proportional content of each macromolecule (DNA, RNA, or protein mass divided by the total mass) for all strains and media as a function of the growth rate. The expected trends (an increasing proportion of RNA and a decreasing proportion of protein with increasing growth rate) are apparent across all strains and within most strains (3). However, the

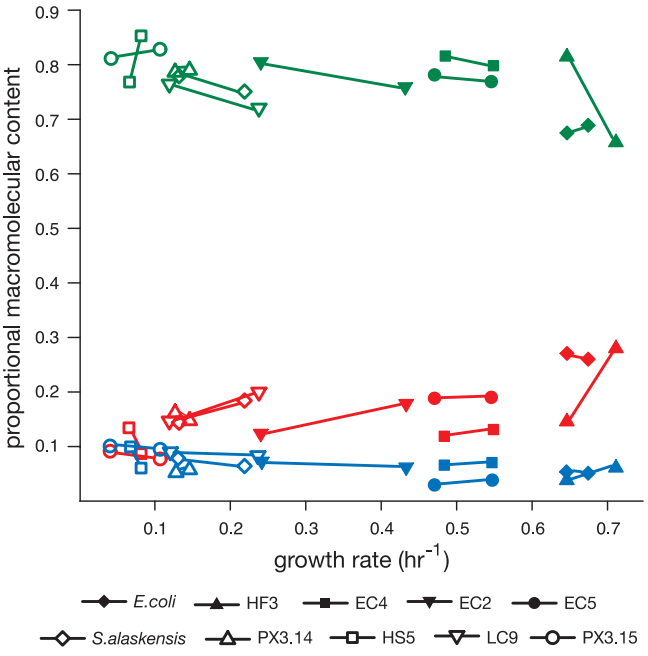

FIG. 2. Proportional contents (individual macromolecular masses divided by the total for all three macromolecular masses) of protein (green), RNA (red), and DNA (blue) plotted as a function of the growth rate for rapidly responding (solid symbols) and slowly responding (open symbols) bacteria. Symbols connected by a line represent the same strain growing in different media. The growth rates of the rapid and slow responders barely overlap under the conditions examined. A trend toward lower protein content and higher RNA content with increasing growth rate is evident across all strains and within most individual strains, but the macromolecular contents are fairly similar across all organisms. Each data point represents the mean of at least three independent culture replicates; error bars are not shown for clarity, but the average standard errors were less than 6% of the mean for individual macromolecules and less than 1.5% of the mean for the growth rate.

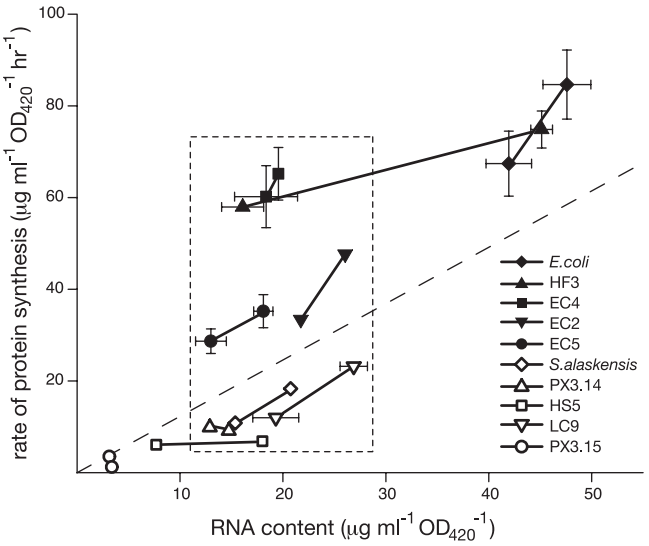

FIG. 3. Rate of protein synthesis (growth rate × protein content) plotted as a function of RNA content for rapidly responding (solid symbols) and slowly responding (open symbols) bacteria. Points with higher translational power are located toward the top and toward the left; any straight line through the origin connects points of equal translational power. In the range of RNA contents represented by both ecological strategies (area in the box), the translational power of rapid responders is 3.6-fold higher than that of slow responders. Symbols connected by a line represent the same strain growing in different media. The data points and error bars are the means and standard errors of measurements for at least three independent culture replicates; error bars smaller than the symbols are not shown. OD_{420}, optical density at 420 nm.

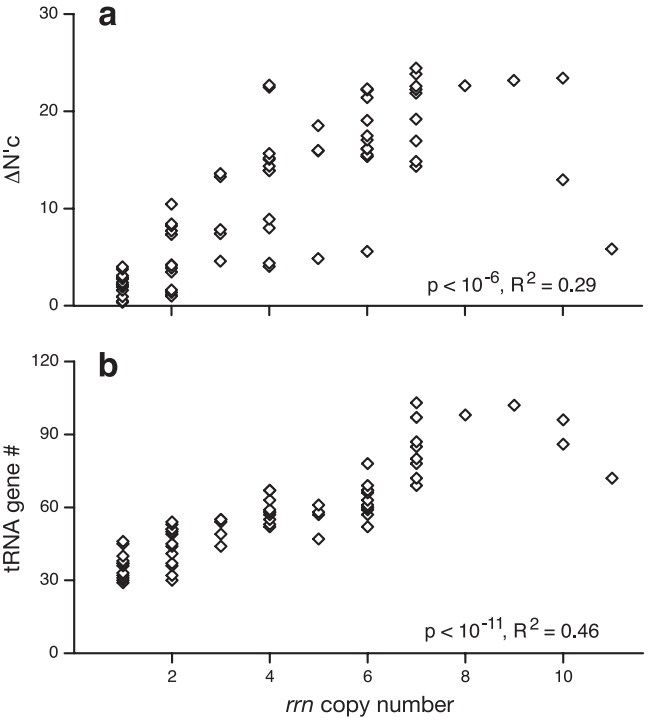

FIG. 4. Strength of translational selection measured by codon use (a) (see Materials and Methods for the definition of $\Delta N'C$) or tRNA gene copy number (b) correlated with *rrn* copy number, an index of adaptation to the rapid response ecological strategy. Each data point represents a sequenced bacterial genome ($n = 76$); the strength and significance of the correlations account for different degrees of relatedness among bacteria, as described in Materials and Methods.

macromolecular compositions of all bacteria are generally similar regardless of the ecological strategy or the growth rate, with protein comprising 65 to 85% of the total macromolecular mass, RNA comprising 8 to 28% of the total macromolecular mass, and DNA comprising 3 to 10% of the total macromolecular mass.

The rate of protein synthesis (μP) is plotted against the RNA content for all strain-medium combinations in Fig. 3. The *y* axis represents the numerator of translational power, while the *x* axis represents the denominator, so higher translational power is represented by points toward the top left in Fig. 3, and any straight line through the origin connects points of equivalent translational power. As predicted, the translational power is higher for the rapid responders and lower for the slow responders. In the region where the RNA contents of the rapid responders and slow responders overlap, the rate of protein synthesis is three- to fourfold higher for the rapid responders, consistent with an analysis of previously published data for bacterial macromolecular contents (12).

Codon analysis provides corroborating evidence that the importance of translational power for bacterial fitness varies with the ecological strategy, using *rrn* copy number as an index of ecological strategies ranging from slow responders (low *rrn* copy number) to rapid responders (high *rrn* copy number). Translational selection for biased codon use is based on the contribution of rapid exponential growth to bacterial fitness (22, 39, 65), so it ought to be stronger among rapidly responding bacteria with high *rrn* copy numbers. However, to test this hypothesis, the effects of translational selection on codon use must be distinguished from the effects of mutation bias, which is the major source of codon use variation across genomes (6). We used the average codon frequencies across all genes in a genome to establish the expected frequencies due to the mutational bias of a bacterial strain, and we measured translational selection as the deviation of codon use in highly expressed genes from the expected values, quantified as $\Delta N'_C$ (see Materials and Methods). The total number of tRNA genes per genome provides an independent measure of translational selection that does not depend on codon analysis, because the benefit of using a preferred codon depends in large part on the abundance of its cognate tRNA (2), which is related in turn to tRNA gene dosage (14).

Translational selection in 76 fully sequenced bacterial genomes (58), expressed as $\Delta N'_C$, is positively correlated with the number of *rrn* operons per genome, as predicted (Fig. 4a). Although $\Delta N'_C$ measures the difference in codon use between highly expressed genes and all genes of a genome, the number of *rrn* operons is not related to the degree of codon bias in either set of genes individually (Fig. 5). Thus, ecological strategies are related specifically to the strength of translational selection, not to mutational bias or other influences on codon use. The same correlation with ecological strategy is found when translational selection is measured as the number of tRNA genes per genome (Fig. 4b). Although Fig. 4 and 5 show each bacterial genome as a separate data point, in reality the

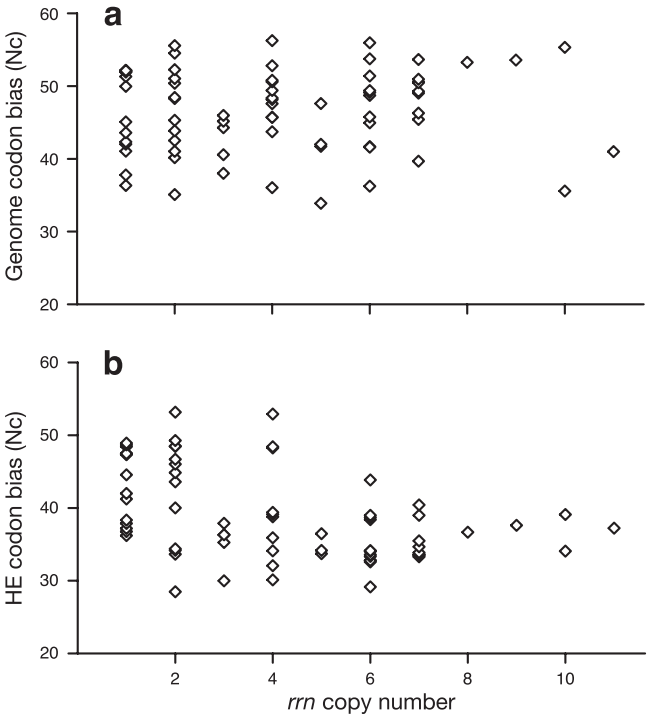

FIG. 5. Codon bias measured as the effective number of codons (70) of all predicted genes of a genome (a) or across a set of highly expressed (HE) genes (b) (genes listed in Materials and Methods) is not significantly related to *rrn* copy number ($P > 0.1$), demonstrating that the correlations shown in Fig. 4 are due specifically to translational selection and not to other sources of codon bias. Each data point represents a sequenced bacterial genome ($n = 76$); correlations were tested as described for Fig. 4.

bacteria have shared evolutionary histories to a greater or lesser extent and thus are not statistically independent. Hence, the significance and strength of these correlations were evaluated using maximum likelihood methods and ratio tests that accounted for the evolutionary history of the organisms (54).

DISCUSSION

The macromolecular data summarized in Fig. 3 confirm that the translational power is higher for rapidly responding bacteria than for slow responders, as suggested by previous results (5, 12, 18, 55, 63). This study extended previous pairwise comparisons of *E. coli* with other strains by including phylogenetically diverse representatives of both ecological strategies. Furthermore, measuring the macromolecular content of multiple species in a single study eliminated the possibility that the conclusions were influenced by methodological differences between laboratories. Although the generality of the link between translational power and bacterial ecological strategy has previously escaped notice, the result is consistent with analyses of codon usage bias across many species, which indicate that the strength of translational selection is correlated with *rrn* copy number and hence ecological strategy (Fig. 4) (64).

Paul Sharp and his colleagues have long argued that differences among bacteria in the strength of translational selection reflect, in part, ecological variation in the contribution of rapid exponential growth to fitness (22, 39, 65). Sharp et al. recently published an estimate of the fitness benefit conferred by preferred codon use in 80 bacterial species, based on four amino acids that have twofold codon degeneracy and the same preferred codon in all bacteria (64). As predicted, these authors found that the benefit of codon bias was correlated with *rrn* copy number, showing that selection for translational power is stronger in strains adapted to the rapid response strategy. We took a less precise but more inclusive approach, relying on an index of translational selection calculated for all 18 amino acids with multiple codons, and reached the same conclusion.

Could differences in codon usage across bacterial species be a sufficient explanation for the observed variation in translational power? Preferred codons are translated more quickly than nonpreferred synonyms are translated (9, 57), so a greater bias toward preferred codons in rapidly responding bacteria than in slowly responding bacteria should contribute to the high translational power of the former organisms. Unfortunately, the data required to quantify the effect of codon bias on the average translation rate are incomplete even for *E. coli* and are not available for other bacteria. Nonetheless, we have published a mathematical model comparing the translation rates of *E. coli* and a hypothetical bacterium that differs from it only by the absence of codon bias, bridging gaps in the data with assumptions that deliberately overestimate the effect of bias (12). This model shows that codon bias alone can accelerate translation no more than 0.6-fold in *E. coli* (12). We concluded that most of the three- to fourfold variation in translational power between rapidly and slowly responding bacteria must be attributed not to codon bias, but to differences in the performance of the translational apparatus itself.

Translational power differences cannot be explained by a complete absence of selection for high translational power among slowly responding bacteria. Codon bias is only weakly sensitive to natural selection for increased power, because any individual preferred codon has at most a small effect. Nonetheless, Sharp et al. detected translationally selected codon bias in 10 of 23 bacterial genomes with a single *rrn* operon (64). Selection for high translational power in other slow responders could be too weak to maintain hundreds of preferred codons and thus generate detectable bias, but it could still be strong enough to select for single mutations that directly influence the translation rate or the active fraction of ribosomes. Furthermore, the Ehrenberg-Kurland model shows that selection for high translational power is proportional to the fraction of cell mass invested in the protein synthesis system, but rapid and slow responders with similar levels of investment in protein synthesis have dramatic differences in translational power (Fig. 3) (5, 12, 18, 55, 63). Hence, we suggest that the observed differences in translational power are explained not only by a reduced benefit of high power for slow responders but also by a cost of high power that is particularly acute for these strains. Specifically, we propose that there is an evolutionary tradeoff between translational power and translational yield.

All known organisms consume four high-energy phosphate bonds per amino acid during ribosomal protein synthesis, so we expect the variation between strains in the translational yield of functional protein to be due entirely to variation in translational error rates. Kurland and his colleagues have argued that missense errors, which generally produce proteins with at least

partial functionality, are much less costly to a cell than ribosomal processivity (i.e., movement) errors, which most often produce completely nonfunctional polypeptides (35, 37). Processivity errors include both frameshifts and dropoff events, the accidental release of peptidyl-tRNA from the ribosome at a sense codon (35, 37). Dropoff is much more frequent than frameshifting, at least for laboratory-adapted strains of *E. coli*, the only organism in which they have been measured (25, 37). Estimates obtained with different experimental approaches have suggested that the average dropoff rate is roughly once per 2.5×10^4 codons, implying that about 10% of the ribosomes initiating translation on a typical gene fail to reach the end (37). The resulting dropoff products can be hydrolyzed to recover the constituent amino acids, but the energy spent on partially synthesized polypeptides is lost.

The possibility of a power-yield tradeoff mediated by dropoff errors is consistent with studies of wild-type and mutant ribosomes that have shown connections between the rate, accuracy, and processivity of translation (1, 13, 25, 26, 35, 37). Selection in the lab for high translational power in natural *E. coli* strains also reduces their tolerance for starvation, which could be a direct result of reduced translational yield (38, 48, 49). One mechanistic basis for the tradeoff could be the affinity of the ribosome for peptidyl-tRNA, which affects both the translation rate and the dropoff frequency (26, 37, 41). A second potential mechanism relates dropoff events to the fraction of translationally active ribosomes: the same factors that recycle post-termination ribosomes to the active pool also promote dropoff events (23, 56).

The waste of energy due to translational errors may not matter to rapid responders growing in resource-rich environments; there is little evidence that energy limits the growth of *E. coli* or other fast-growing strains in typical laboratory media (46, 50, 59). In nature, however, resource levels often fluctuate around the minimum thresholds for bacterial growth or viability, thresholds which are determined in part by the resources required for essential protein synthesis (21, 52, 66). In this situation, selection favors strains that use resources efficiently enough to grow or at least survive, while competitors remain in stasis or die. The spatial structure of environments such as soil could also result in selection for resource use efficiency and high translational yield, if the resources spared by efficient growth are not shared among all competitors but are used primarily by the more efficient cell and its descendants. In contrast to the fitness of rapid responders, which depends primarily on the rate at which the cells convert resources into progeny, we believe that the fitness of slow responders depends primarily on the number of viable progeny generated from a given allocation of resources.

Thus far, dropoff rates have been estimated only for laboratory-adapted strains of *E. coli* (25, 45, 47). Such measurements have to be extended to phylogenetically diverse microbes with both rapidly and slowly responding ecological strategies in order test our hypothesis that dropoff errors mediate an evolutionary tradeoff between translational power and translational yield. Whether or not this hypothesis is confirmed as an explanation of low translational power in slowly responding bacteria, the results reported here show that translational power varies among bacterial species in a manner that reflects ecological factors and not phylogeny. Recognition that the translational apparatus responds to selection has the potential to increase our understanding of translation itself, via comparisons of the structures and functions of the translational apparatus among microbes adapted to different ecological conditions. Furthermore, the adaptive nature of translational performance is likely to be an important factor contributing to the diversity and distribution of microbial life on our planet.

ACKNOWLEDGMENTS

We thank Joel Klappenbach, Tsutomu Hattori, Martin Ostrowski, Richard Cavicchioli, and Rich Lenski for providing the bacterial strains used in this work and Frank Dazzo for access to the CMEIAS software. We also thank our labmates and colleagues at Michigan State University for numerous fruitful discussions, and we gratefully acknowledge our intellectual debt to Charles Kurland, Måns Ehrenberg, and their colleagues for their research into the selective forces acting on the protein synthesis system.

This work was supported by an MSU Distinguished Graduate Fellowship and an EPA STAR Fellowship to L.D. and by the National Science Foundation through the LTER program and grant IBN 987254 to T.M.S.

REFERENCES

1. **Andersson, D. I., H. W. Vanverseveld, A. H. Stouthamer, and C. G. Kurland.** 1986. Suboptimal growth with hyper-accurate ribosomes. Arch. Microbiol. **144:**96–101.
2. **Andersson, S. G. E., and C. G. Kurland.** 1990. Codon preferences in free-living microorganisms. Microbiol. Rev. **54:**198–210.
3. **Bremer, H., and P. P. Dennis.** 1996. Modulation of chemical composition and other parameters of the cell by growth rate, p. 1553–1569. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. A. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed., vol. 2. ASM Press, Washington, DC.
4. **Cannone, J., S. Subramanian, M. Schnare, J. Collett, L. D'Souza, Y. Du, B. Feng, N. Lin, L. Madabusi, K. Muller, N. Pande, Z. Shang, N. Yu, and R. Gutell.** 2002. The Comparative RNA Web (CRW) site: an online database of comparative sequence and structure information for ribosomal, intron, and other RNAs. BMC Bioinformatics **3:**2.
5. **Chant, J., I. Hui, D. Dejongwong, L. Shimmin, and P. P. Dennis.** 1986. The protein synthesizing machinery of the Archaebacterium *Halobacterium cutirubrum*—molecular characterization. Syst. Appl. Microbiol. **7:**106–114.
6. **Chen, S. L., W. Lee, A. K. Hottes, L. Shapiro, and H. H. McAdams.** 2004. Codon usage between genomes is constrained by genome-wide mutational processes. Proc. Natl. Acad. Sci. USA **101:**3480–3485.
7. **Cole, J. R., B. Chai, T. L. Marsh, R. J. Farris, Q. Wang, S. A. Kulam, S. Chandra, D. M. McGarrell, T. M. Schmidt, G. M. Garrity, and J. M. Tiedje.** 2003. The Ribosomal Database Project (RDP-II): previewing a new autoaligner that allows regular updates and the new prokaryotic taxonomy. Nucleic Acids Res. **31:**442–443.
8. **Condon, C., D. Liveris, C. Squires, I. Schwartz, and C. L. Squires.** 1995. Ribosomal RNA operon multiplicity in *Escherichia coli* and the physiological implications of *rrn* inactivation. J. Bacteriol. **177:**4152–4156.
9. **Curran, J. F., and M. Yarus.** 1989. Rates of aminoacyl-tRNA selection at 29 sense codons *in vivo*. J. Mol. Biol. **209:**65–77.
10. **Daniels, L., R. S. Hanson, and J. A. Phillips.** 1994. Chemical analysis, p. 512–514. *In* P. Gerhardt, R. G. E. Murray, W. A. Wood, and N. R. Krieg (ed.), Methods for general and molecular bacteriology. ASM Press, Washington, DC.
11. **Dennis, P., and H. Bremer.** 1974. Macromolecular composition during steady-state growth of *Escherichia coli* B/r. J. Bacteriol. **119:**270–281.
12. **Dethlefsen, L., and T. M. Schmidt.** 2005. Differences in codon bias cannot explain differences in translational power among microbes. BMC Bioinformatics **6:**3.
13. **Dong, H. J., and C. G. Kurland.** 1995. Ribosome mutants with altered accuracy translate with reduced processivity. J. Mol. Biol. **248:**551–561.
14. **Dong, H. J., L. Nilsson, and C. G. Kurland.** 1996. Co-variation of tRNA abundance and codon usage in *Escherichia coli* at different growth rates. J. Mol. Biol. **260:**649–663.
15. **Eguchi, M., T. Nishikawa, K. MacDonald, R. Cavicchioli, J. C. Gottschal, and S. Kjelleberg.** 1996. Responses to stress and nutrient availability by the marine ultramicrobacterium *Sphingomonas* sp. strain RB2256. Appl. Environ. Microbiol. **62:**1287–1294.
16. **Ehrenberg, M., and C. G. Kurland.** 1984. Costs of accuracy determined by a maximal growth rate constraint. Q. Rev. Biophys. **17:**45–82.
17. **Farewell, A., and F. C. Neidhardt.** 1998. Effect of temperature on in vivo protein synthetic capacity in *Escherichia coli*. J. Bacteriol. **180:**4704–4710.

18. **Fegatella, F., J. Lim, S. Kjelleberg, and R. Cavicchioli.** 1998. Implications of rRNA operon copy number and ribosome content in the marine oligotrophic ultramicrobacterium *Sphingomonas* sp. strain RB2256. Appl. Environ. Microbiol. **64:**4433–4438.
19. **Gausing, K.** 1977. Regulation of ribosome production in Escherichia coli: synthesis and stability of ribosomal RNA and of ribosomal protein messenger RNA at different growth rates. J. Mol. Biol. **115:**335–354.
20. **Gorlach, K., R. Shingaki, H. Morisaki, and T. Hattori.** 1994. Construction of eco-collection of paddy field soil bacteria for population analysis. J. Gen. Appl. Microbiol. **40:**509–517.
21. **Groat, R. G., J. E. Schultz, E. Zychlinsky, A. Bockman, and A. Matin.** 1986. Starvation proteins in *Escherichia coli*: kinetics of synthesis and role in starvation survival. J. Bacteriol. **168:**486–493.
22. **Grocock, R. J., and P. M. Sharp.** 2002. Synonymous codon usage in *Pseudomonas aeruginosa* PA01. Gene **289:**131–139.
23. **Heurgue-Hamard, V., R. Karimi, L. Mora, J. MacDougall, C. Leboeuf, G. Grentzmann, M. Ehrenberg, and R. H. Buckingham.** 1998. Ribosome release factor RF4 and termination factor RF3 are involved in dissociation of peptidyl-tRNA from the ribosome. EMBO J. **17:**808–816.
24. **Ingraham, J. L., O. Maaløe, and F. C. Neidhardt.** 1983. Growth of the bacterial cell. Sinauer, Sunderland, MA.
25. **Jorgensen, F., and C. G. Kurland.** 1990. Processivity errors of gene expression in *Escherichia coli*. J. Mol. Biol. **215:**511–521.
26. **Karimi, R., and M. Ehrenberg.** 1996. Dissociation rates of peptidyl-tRNA from the P-site of *E. coli* ribosomes. EMBO J. **15:**1149–1154.
27. **Kepner, R. L., and J. R. Pratt.** 1994. Use of fluorochromes for direct enumeration of total bacteria in environmental samples—past and present. Microbiol. Rev. **58:**603–615.
28. **Kirchman, D., J. Sigda, R. Kapuscinski, and R. Mitchell.** 1982. Statistical analysis of the direct count method for enumerating bacteria. Appl. Environ. Microbiol. **44:**376–382.
29. **Kjeldgaard, N. O., and C. G. Kurland.** 1963. The distribution of soluble and ribosomal RNA as a function of growth rate. J. Mol. Biol. **6:**341–348.
30. **Klappenbach, J. A.** 2001. Ecological and evolutionary implications of ribosomal RNA gene copy number in heterotrophic soil bacteria. Ph.D. dissertation. Michigan State University, East Lansing, MI.
31. **Klappenbach, J. A., J. M. Dunbar, and T. M. Schmidt.** 2000. rRNA operon copy number reflects ecological strategies of bacteria. Appl. Environ. Microbiol. **66:**1328–1333.
32. **Klappenbach, J. A., P. R. Saxman, J. R. Cole, and T. M. Schmidt.** 2001. *rrndb*: the Ribosomal RNA Operon Copy Number Database. Nucleic Acids Res. **29:**181–184.
33. **Koch, A. L.** 1980. Inefficiency of ribosomes functioning in *Escherichia coli* growing at moderate rates. J. Gen. Microbiol. **116:**165–171.
34. **Koch, A. L., and C. S. Deppe.** 1971. *In vivo* assay of protein synthesizing capacity of *Escherichia coli* from slowly growing chemostat cultures. J. Mol. Biol. **55:**549–562.
35. **Kurland, C. G., and M. Ehrenberg.** 1985. Constraints on the accuracy of messenger RNA movement. Q. Rev. Biophys. **18:**423–450.
36. **Kurland, C. G., and M. Ehrenberg.** 1987. Growth-optimizing accuracy of gene expression. Annu. Rev. Biophys. Biophys. Chem. **16:**291–317.
37. **Kurland, C. G., D. Hughes, and M. Ehrenberg.** 1996. Limitations of translational accuracy, p. 979–1004. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. A. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed., vol. 1. ASM Press, Washington, DC.
38. **Kurland, C. G., and R. Mikkola.** 1993. The impact of nutritional state on the microevolution of ribosomes, p. 225–237. *In* S. Kjelleberg (ed.), Starvation in bacteria. Plenum Press, New York, NY.
39. **Lafay, B., J. C. Atherton, and P. M. Sharp.** 2000. Absence of translationally selected synonymous codon usage bias in *Helicobacter pylori*. Microbiology **146:**851–860.
40. **Lenski, R. E., M. R. Rose, S. C. Simpson, and S. C. Tadler.** 1991. Long term experimental evolution in *Escherichia coli*. 1. Adaptation and divergence during 2,000 generations. Am. Nat. **138:**1315–1341.
41. **Lim, V. I., and J. F. Curran.** 2001. Analysis of codon:anticodon interactions within the ribosome provides new insights into codon reading and the genetic code structure. RNA **7:**942–957.
42. **Liu, J., F. B. Dazzo, O. Glagoleva, B. Yu, and A. K. Jain.** 2001. CMEIAS: a computer-aided system for the image analysis of bacterial morphotypes in microbial communities. Microb. Ecol. **41:**173–194.
43. **Ludwig, W., O. Strunk, R. Westram, L. Richter, H. Meier, Yadhukumar, A. Buchner, T. Lai, S. Steppi, G. Jobb, W. Forster, I. Brettske, S. Gerber, A. W. Ginhart, O. Gross, S. Grumann, S. Hermann, R. Jost, A. Konig, T. Liss, R. Lussmann, M. May, B. Nonhoff, B. Reichel, R. Strehlow, A. Stamatakis, N. Stuckmann, A. Vilbig, M. Lenke, T. Ludwig, A. Bode, and K.-H. Schleifer.** 2004. ARB: a software environment for sequence data. Nucleic Acids Res. **32:**1363–1371.
44. **Maaløe, O.** 1979. Regulation of the protein-synthesizing machinery—ribosomes, tRNA, factors, and so on, p. 487–542. *In* R. F. Goldberger (ed.), Gene expression, vol. 1. Plenum Press, New York, NY.
45. **Manley, J. L.** 1978. Synthesis and degradation of termination and premature termination fragments of β-galactosidase *in vitro* and *in vivo*. J. Mol. Biol. **125:**407–432.
46. **Marr, A. G.** 1991. Growth rate of *Escherichia coli*. Microbiol. Rev. **55:**316–333.
47. **Menninger, J. R.** 1976. Peptidyl transfer RNA dissociates during protein synthesis from ribosomes of *Escherichia coli*. J. Biol. Chem. **251:**3392–3398.
48. **Mikkola, R., and C. G. Kurland.** 1991. Is there a unique ribosome phenotype for naturally-occurring *Escherichia coli*? Biochimie **73:**1061–1066.
49. **Mikkola, R., and C. G. Kurland.** 1992. Selection of laboratory wild-type phenotype from natural isolates of *Escherichia coli* in chemostats. Mol. Biol. Evol. **9:**394–402.
50. **Neijssel, O. M., M. J. T. De Mattos, and D. W. Tempest.** 1996. Growth yield and energy distribution, p. 1683–1692. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. A. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed., vol. 2. ASM Press, Washington, DC.
51. **Novembre, J. A.** 2002. Accounting for background nucleotide composition when measuring codon usage bias. Mol. Biol. Evol. **19:**1390–1394.
52. **Nystrom, T., K. Flardh, and S. Kjelleberg.** 1990. Responses to multiple-nutrient starvation in marine *Vibrio* sp. strain CCUG 15956. J. Bacteriol. **172:**7085–7097.
53. **Ostrowski, M., R. Cavicchioli, M. Blaauw, and J. C. Gottschal.** 2001. Specific growth rate plays a critical role in hydrogen peroxide resistance of the marine oligotrophic ultramicrobacterium *Sphingomonas alaskensis* strain RB2256. Appl. Environ. Microbiol. **67:**1292–1299.
54. **Pagel, M.** 1999. Inferring the historical patterns of biological evolution. Nature **401:**877–884.
55. **Pang, H. L., and H. H. Winkler.** 1994. The concentrations of stable RNA and ribosomes in *Rickettsia prowazekii*. Mol. Microbiol. **12:**115–120.
56. **Pavlov, M. Y., D. V. Freistroffer, J. MacDougall, R. H. Buckingham, and M. Ehrenberg.** 1997. Fast recycling of *Escherichia coli* ribosomes requires both ribosome recycling factor (RRF) and release factor RF3. EMBO J. **16:**4134–4141.
57. **Pedersen, S.** 1984. *Escherichia coli* ribosomes translate *in vivo* with variable rate. EMBO J. **3:**2895–2898.
58. **Peterson, J. D., L. A. Umayam, T. Dickinson, E. K. Hickey, and O. White.** 2001. The comprehensive microbial resource. Nucleic Acids Res. **29:**123–125.
59. **Russell, J. B., and G. M. Cook.** 1995. Energetics of bacterial growth: balance of anabolic and catabolic reactions. Microbiol. Rev. **59:**48–62.
60. **Schaechter, M., O. Maaloe, and N. O. Kjeldgaard.** 1958. Dependency on medium and temperature of cell size and chemical composition during balanced growth of *Salmonella typhimurium*. J. Gen. Microbiol. **19:**592–606.
61. **Schut, F., E. J. Devries, J. C. Gottschal, B. R. Robertson, W. Harder, R. A. Prins, and D. K. Button.** 1993. Isolation of typical marine bacteria by dilution culture: growth, maintenance, and characteristics of isolates under laboratory conditions. Appl. Environ. Microbiol. **59:**2150–2160.
62. **Schut, F., J. C. Gottschal, and R. A. Prins.** 1997. Isolation and characterisation of the marine ultramicrobacterium *Sphingomonas sp*. strain RB2256. FEMS Microbiol. Rev. **20:**363–369.
63. **Shahab, N., F. Flett, S. G. Oliver, and P. R. Butler.** 1996. Growth rate control of protein and nucleic acid content in *Streptomyces coelicolor* A3(2) and *Escherichia coli* B/r. Microbiology **142:**1927–1935.
64. **Sharp, P. M., E. Bailes, R. J. Grocock, J. F. Peden, and R. E. Sockett.** 2005. Variation in the strength of selected codon usage bias among bacteria. Nucleic Acids Res. **33:**1141–1153.
65. **Sharp, P. M., M. Stenico, J. F. Peden, and A. T. Lloyd.** 1993. Codon usage—mutational bias, translational selection, or both? Biochem. Soc. Trans. **21:**835–841.
66. **Siegele, D. A., and L. J. Guynn.** 1996. *Escherichia coli* proteins synthesized during recovery from starvation. J. Bacteriol. **178:**6352–6356.
67. **Stevenson, B. S., and T. M. Schmidt.** 1998. Growth rate-dependent accumulation of RNA from plasmid-borne rRNA operons in *Escherichia coli*. J. Bacteriol. **180:**1970–1972.
68. **Stevenson, B. S., and T. M. Schmidt.** 2004. Life history implications of rRNA gene copy number in *Escherichia coli*. Appl. Environ. Microbiol. **70:**6670–6677.
69. **Stouthamer, A. H.** 1979. The search for correlation between theoretical and experimental growth yields, p. 1–47. *In* J. R. Quayle (ed.), International review of biochemistry and microbial biochemistry, vol. 21. University Park Press, Baltimore, MD.
70. **Wright, F.** 1990. The effective number of codons used in a gene. Gene **87:**23–29.