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Macromolecular Composition During Steady-State Growth of *Escherichia coli* B/r

PATRICK P. DENNIS[1] AND HANS BREMER
The University of Texas at Dallas, Richardson, Texas 75080

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By using *Escherichia coli* B/r, the cellular amounts of ribonucleic acid (RNA) and protein were determined as a function of the steady-state growth rate (0.67 to 2.40 doublings per h) by a method which combines measurements of the RNA to deoxyribonucleic acid (DNA) ratio and the differential rate of ribosomal protein synthesis with the Cooper and Helmstetter theory of DNA replication. The results indicate that the ratios RNA/DNA, RNA/protein, and protein/DNA give linear relationships with the growth rate (above 1.2 doublings per h), whereas RNA/cell and protein/cell show a more complex growth rate dependency. The significance of these relationships is discussed. Finally, a detailed description of the growth parameters and composition of *E. coli* B/r is presented.

In bacteria, the cellular mass and amounts of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein were observed by Schaechter, Maaloe, and Kjeldgaard to increase exponentially with the bacterial growth rate (16, 24). Thus, rapidly growing cells are larger, have more DNA, and presumably have more chromosomes than slowly growing cells. Furthermore, whereas protein was found to increase in proportion to the DNA such that the ratio of protein/DNA is independent of the growth rate, cell mass and RNA were found to increase more rapidly such that the ratios RNA/DNA and RNA/protein increased exponentially with the growth rate. (Actually, these ratios were not determined, but the ratio of two exponential functions must be again an exponential function.) These relatively simple relationships suggested simple regulatory principles.

According to the model of Helmstetter and Cooper (3, 12), the function that describes the amount of DNA per cell at various growth rates, $G(\tau)$ (G = genome equivalents of DNA; τ = doubling time), depends upon two constant parameters: the time for replication of the bacterial chromosome, C (= 41 min); and the time between completion of a round of replication and cell division, D (= 22 min):

$$G(\tau) = \frac{\tau}{C \ln 2}[2^{(C+D)/\tau} - 2^{D/\tau}] \quad \text{for } \tau < C + D = 63 \text{ min} \quad (1a)$$

[1] Present address: Institute for Enzyme Research V, University of Wisconsin, Madison, Wis. 53706.

At slower growth rates, C and D increase such that

$$G(\tau) = G(63 \text{ min}) \text{ for } \tau > 63 \text{ min} \quad (1b)$$

According to this equation, the amount of DNA per cell increases in a nearly exponential manner with the growth rate μ ($\mu = 60/\tau$, in doublings per h), in good agreement with empirical DNA determinations (3, 24).

By combining Cooper and Helmstetter's model with the Schaechter empirical relationship between cell mass and growth rate, Donachie (8) showed that the cell mass per chromosome origin at the time of initiation of chromosome replication is nearly independent of the growth rate, suggesting that the mechanism regulating DNA replication is in some way directly related to the cell mass. Furthermore, this idea provides a rational explanation for the exponential relationship between cell mass and growth rate observed by Schaechter et al.

Using the bacterium *Escherichia coli* B/r, we have reexamined the growth rate dependency of the bacterial RNA and protein content and the invariance of the protein/DNA ratio. For this purpose, we evaluated results obtained previously from experiments on the control of the synthesis of ribosomal RNA (rRNA), transfer RNA (tRNA), and ribosomal proteins (r-proteins) (1, 4-7) and new data on the RNA/DNA ratio and cell mass. Combining these data with the Cooper and Helmstetter relationship (equation 1) permits us to calculate the amounts of RNA and proteins per cell and in addition a number of other growth parameters

of interest (e.g., messenger RNA [mRNA] per cell, number of translations per mRNA molecule, etc.) as a function of growth rate. This analysis provides useful insights into the problem of how macromolecular synthesis rates and overall cellular composition relate to the steady state growth rate of the bacterium.

MATERIALS AND METHODS

The bacterial strain used was *E. coli* B/r (ATCC 12407). All cultures were grown at 37 C in minimal medium C (12) containing various supplements as described previously (5, 7). To obtain exponential-phase growth, fresh overnight cultures were diluted at least 1,000-fold, and the bacterial mass was monitored as absorbance at 460 nm (A_{460}) by using a Gilford spectrophotometer with a 1-cm light path. The number of cells per milliliter of culture was measured by using a model B Coulter counter.

The measurements of the differential rate of ribosomal protein synthesis, α_r (rate of ribosomal protein synthesis/rate of total protein synthesis), are described elsewhere in detail (7). The RNA/DNA ratio in nucleotides/nucleotide was measured after labeling of stable nucleic acids with radioactive uracil as described previously (4). Briefly, total stable nucleic acid is measured as acid-insoluble radioactivity, whereas DNA is measured as alkali-resistant acid-insoluble radioactivity. The RNA/DNA ratio is calculated as the product 1.16 × [(radioactivity in stable nucleic acids − radioactivity in DNA)/(radioactivity in DNA)]. The factor 1.16 results from the 43 and 50% pyrimidine content of stable RNA and DNA, respectively. References to other measured parameters are given in the text and accompanying tables.

RESULTS

Ratios RNA/DNA, RNA/protein, and protein/DNA. The RNA/DNA ratio R_s/D (R_s = stable RNA; D = DNA; see Appendix for additional definitions) is seen to increase exactly in proportion to (i.e., not exponentially with) the growth rate (Fig. 1, middle panel).

$$\frac{R_s}{D} = 4.2\,\mu \text{ (nuc/nuc) for } \mu > 0.67 \quad (2)$$

The RNA/protein ratio was determined indirectly from the differential rate of r-protein synthesis, α_r (defined as the ratio of the synthesis rates of ribosomal protein to total protein). It can be shown, using theoretical considerations (Appendix, equation 3), that the ratio R_s/P (P = protein) is proportional to α_r:

$$\frac{R_s}{P} = 0.86\,\alpha_r \quad (3)$$

Direct, although less accurate, measurements of stable RNA and protein in exponentially growing cells corroborate this relationship (7). Using

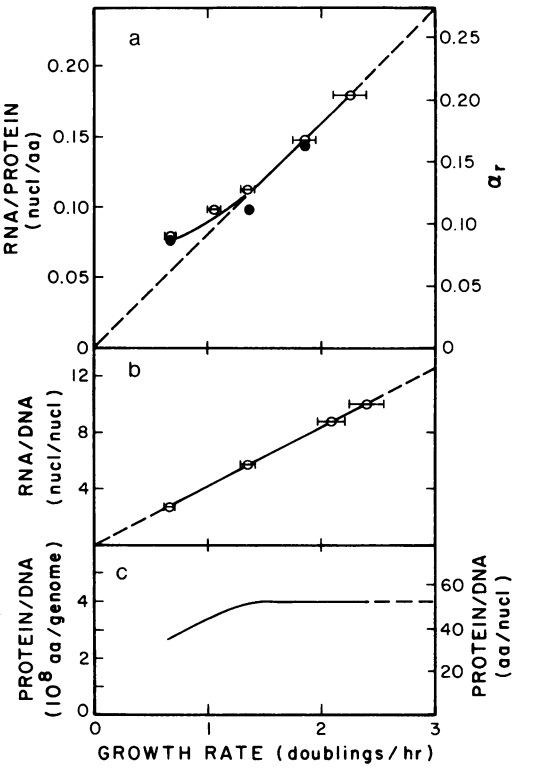

FIG. 1. *Ratios of RNA/protein, RNA/DNA, and protein/DNA as a function of the steady state growth rate. (a) The RNA/protein ratio in nucleotides/amino acid was calculated from the differential rate of ribosomal protein synthesis, α_r (ribosomal protein synthesis rate/total protein synthesis rate) using equation 3. The values of α_r were determined by using radioactive leucine (○) and proline (●), respectively (see reference 7 for details). Each point represents the average from four to six experiments. The horizontal bars indicate the uncertainty in estimating the steady state growth rates. (b) The RNA/DNA ratio, in nucleotides/nucleotide was measured by using radioactive uracil (see Materials and Methods and reference 4). The standard deviation of the ratio determination is less than ±5% (4), whereas the uncertainty in the estimation of the steady state growth rates are illustrated with the horizontal bars. (c) The protein/DNA ratio in amino acids/genome and amino acids/nucleotide (7.6×10^8 DNA nucleotide/genome; reference 3) was calculated as the quotient of the RNA/DNA and the RNA/protein ratio.*

this relationship and our previously determined values for α_r ($\alpha_r = 0.09\,\mu$ for $\mu > 1.2$; Fig. 1, right ordinate of top panel), we find for rapidly growing bacteria ($\mu > 1.2$) that the ratio R_s/P (like α_r) is also proportional to μ:

$$\frac{R_s}{P} = 0.86 \times 0.09\,\mu = 0.077\,\mu \text{ (nuc/aa)} \quad (4)$$

Below 1.2 doublings per h, however, R_s/P no longer follows this relationship, but shows increasingly positive deviations with slower growth rates.[2]

The quotient of the two ratios $(R_s/D)/(R_s/P)$ gives the ratio of protein per DNA, P/D. This P/D ratio is constant in rapidly growing bacteria (for $\mu > 1.2$: $P/D = 4 \times 10^8$ aa/G), but decreases at lower growth rates (Fig. 1, lower panel).

Amounts of DNA, RNA protein, and mass per cell. From these ratios (Fig. 1) and Cooper and Helmstetter's equation 1, the amounts of DNA, RNA, and protein per cell were calculated (Table 1) and plotted on an exponential scale versus the growth rate (Fig. 2). Qualitatively, the resulting relationships agree with previous measurements using *Salmonella* (13, 16, 24), although the relationships do not appear to be simple exponential functions. (The R_s/cell curve, for example, cannot be exponential if the R_s/D curve of Fig. 1 is linear.) The cell mass (determined as A_{460} units per Coulter count) also appears to increase nonexponentially with growth rate.

Other growth parameters. Finally, from the ratios R_s/D, and R_s/P (or α_r, respectively) and from our previously determined values for the RNA chain growth rates (2, 5) and the relative rate of stable RNA synthesis (1, 6), we have calculated a number of cellular parameters for *E. coli* B/r growing at 0.67, 1.36, and 2.10 doublings per h (Table 2 and Fig. 3). For example, the amount of unstable RNA per genome (presumably mRNA) increases approximately twofold over this range, whereas the percentage of total RNA that is unstable decreases from 4.5 to 2.8% (Table 2). It is also seen that the ribosome density on the mRNA and the number of translations per mRNA molecule increase at fast growth rates. These values, however, are subject to certain assumptions, namely, that all unstable RNA is mRNA and that the average life of mRNA is constant over this range of bacterial growth rates. The first assumption seems to be essentially correct, since our values for the relative rate of stable RNA synthesis agree with those from Nierlich (21), whose method (analysis of base composition of pulse-labeled RNA) does not depend on this assumption (Fig. 3, lower panel, O). Also,

[2] The R_s/P values of Fig. 1 would also fit to a straight line in a semilog plot; however, it can be shown that the quotient $(R_s/P)/\mu$ is inversely proportional to the ribosome efficiency (see Discussion). The ribosome efficiency is expected to approach a maximum (i.e., constant) value with increasing growth rate; an exponentially increasing R_s/P ratio implies an initial increase (as observed), but then a decrease of the ribosome efficiency with growth rate (see equation 5, Appendix), which is implausible.

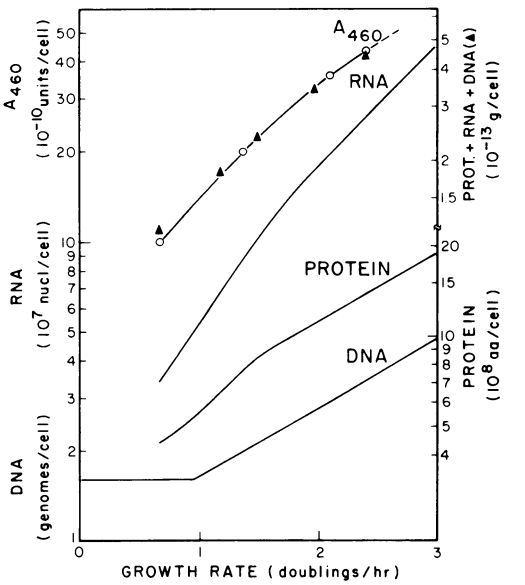

FIG. 2. *Cellular composition of E. coli B/r as a function of the steady state growth rate. The DNA per cell curve was obtained directly from the relationship of Cooper and Helmstetter (references 3 and 12; equations 1a and 1b of Introduction). The protein per cell was calculated as the quotient of the protein/DNA relationship from Fig. 1 and the DNA/cell relationship. Similarly, the RNA per cell curve was calculated as the quotient of the RNA/DNA ratio from Fig. 1 and the DNA/cell relationship. The relationship between cell number and A_{460} (assumed to be proportional to bacterial mass) was determined as described in Materials and Methods.*

the second assumption appears to be essentially correct since we detect only minor differences in the average lifetime of β-galactosidase mRNA in bacteria grown in succinate or glucose-amino acids medium (unpublished data).

DISCUSSION

Nutritional limitations and balanced growth. Low growth rates are obtained by either employing a growth medium of poor nutritional quality (= balanced growth) or by maintaining the concentration of an essential component in the medium (such as the carbon source) at a constant suboptimal, and therefore growth-limiting, level (such as chemostat growth). When the growth rate is varied by reducing the concentration of the growth-limiting component, the changes in the macromolecular composition of the bacteria are less than those observed at different rates of balanced growth (24). In this paper we have only dealt with balanced growth conditions. In *E. coli* B/r, we operationally define balanced growth at 37 C

TABLE 1. *Macromolecular composition of E. coli B/r at different steady-state growth rates*

τ (min)	μ (doublings/h)	G/cell[a]	R_s/cell[b] (nuc × 10^{-7})	α_r[c]	R_s/P[d] (nuc/aa)	P/cell[e] (aa × 10^{-8})	P/G[f] (aa × 10^{-8})	a_i[g]	$P(a_i)$/cell[h] (aa × 10^{-8})	$O(a_i)$[i]	$P(a_i)/O(a_i)$ (aa × 10^{-8})
90	0.67	1.61	3.4	0.089	0.075	4.5	2.8	0	3.2	1	3.2
60	1.00	1.65	5.3	0.107	0.094	5.6	3.4	0.95	7.8	2	3.9
50	1.20	1.83	7.1	0.122	0.107	6.6	3.6	0.74	7.9	2	4.0
40	1.50	2.13	10.4	0.137	0.121	8.6	4.0	0.43	8.3	2	4.2
30	2.00	2.77	17.8	0.182	0.160	11.2	4.0	0.90	15.0	4	3.8
25	2.40	3.43	26.3	0.218	0.192	13.8	4.0	0.48	14.0	4	3.5
20	3.00	4.74	45.5	0.272	0.239	19.0	4.0	0.85	24.8	8	3.1

[a] G/cell, Genome equivalents of DNA per average cell = $[\tau/(C \cdot \ln 2)] \times [2^{(C+D)/\tau} - 2^{D/\tau}]$; C, replication time = 41 min, D, time between completion of replication and cell division = 22 min (references 3,12).

[b] R_s, Stable RNA; R_s/cell = $(R_s/D) \cdot (D/G) \cdot (G/\text{cell})$; R_s/D = RNA nucleotides/DNA nucleotides = $4.2 \cdot \mu$ (Fig. 1); D/G, DNA nucleotides per genome equivalent of DNA = 7.6×10^8.

[c] α_r from Fig. 1 (reference 7).

[d] R_s/P = RNA nucleotides in stable nucleic acids/amino acid residues in protein = $0.88 \cdot \alpha_r$ (reference 7).

[e] P/cell, Protein per cell = $(R_s/\text{cell})/(R_s/P)$.

[f] P/G, protein per genome equivalent of DNA = $(P/\text{cell})/(G/\text{cell})$.

[g] a_i, Cell age at initiation of DNA replication = $[(n\tau) - 63]/\tau$, where n = integer number, such that $63 + \tau > n\tau > 63 = C + D$ (footnote *a*).

[h] $P(a_i)$/cell, Protein per cell at the age of initiation of DNA replication = $(P/\text{cell}) \cdot 2^{(a_i - a_{av})}$; a_{av}, age at which cells contain an average amount of protein = $[\ln(2 \cdot \ln 2)]/\ln 2 = 0.47$, calculated from the age distribution $n(a) = \ln 2 \cdot 2^{1-a}$ (reference 27) under the assumption that the protein per cell increases exponentially with age, thus $P(a) \sim 2^a$.

[i] $O(a_i)$, Number of chromosome origins immediately before initiation of DNA replication (reference 3).

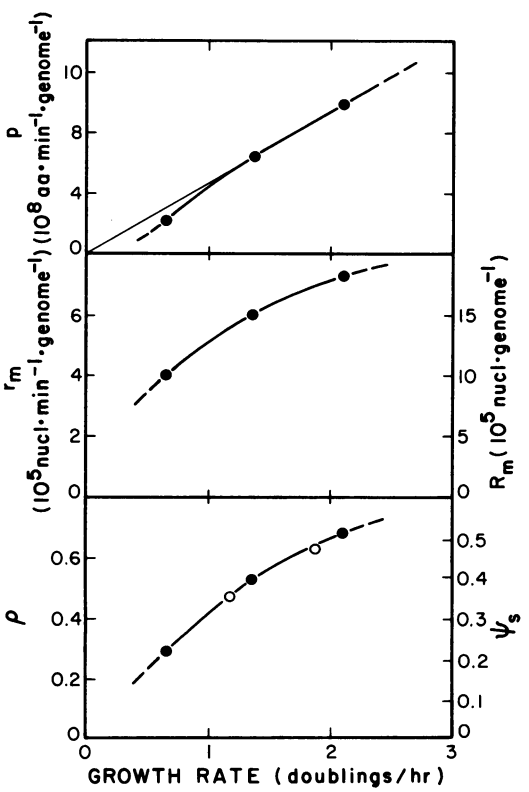

FIG. 3. *Growth parameters of E. coli B/r as a function of the steady-state growth rate. Lower panel, left ordinate: Relative rate of stable RNA synthesis, ρ = fraction of the instantaneous rate of RNA synthesis that is rRNA and tRNA synthesis. Symbols: ●, data from reference 6; ○, data from Nierlich (reference 21), who used E. coli ML 30. Right ordinate: Calibration showing the fraction of functioning RNA polymerase transcribing rRNA and tRNA genes, ψ_s (for the conversion see Table 2, or reference 1). Middle panel, left ordinate: Rate of mRNA synthesis, r_m, calculated from ρ (lower panel of this figure) and from the ratio R_s/D (Fig. 1, middle panel), using the following equation (obtained by combining the equations given in Table 2): $r_m(\mu) = k \cdot D \cdot [R_s/D]_\mu \cdot [(1 - \rho)/\rho] \cdot \mu$, or after substituting $k = \ln 2/60$, $D = 7.6 \cdot 10^8$ DNA nucleotides/genome, and $R_s/D = 4.2\ \mu$ (Fig. 1): $r_m(\mu) = 3.67 \cdot 10^5 \cdot [(1 - \rho)/\rho] \cdot \mu^2$ (mRNA nucleotide/min/genome. Right ordinate: Amount of mRNA, R_m, calculated from r_m: $R_m = r_m \cdot \tau_m$ (mRNA nucleotides/genome), where τ_m is the average lifetime of mRNA, assumed to be 2.5 min. Upper panel: Rate of protein synthesis, p, calculated from R_s/D (Fig. 1) and the differential rate of ribosomal protein synthesis, α_r (data from reference 7; see Table 2), using the equation (obtained again by combining the equations provided in Table 2): $p(\mu) = k \cdot D \cdot f_r \cdot [(aa/rib)/(nuc/rib)] \cdot [R_s/D] \cdot \{\mu/[\alpha_r(\mu)]\}$. Substituting $k = \ln 2/60$, $D = 7.6 \cdot 10^8$ (DNA nucleotide/genome), $f_r = 0.85$ (reference 4), aa/rib = 6,300 (amino acids per 70S ribosome; reference 7), nuc/rib = 4,620 (rRNA nucleotides per 70S ribosome), and $R_s/D = 4.2\ \mu$ (Fig. 1) gives: $p = 4.25 \cdot 10^8 \cdot [(\mu^2)/(\alpha_r)]$ (for $0.67 < \mu < 1.2$), or for $\mu > 1.2$, when $\alpha_r = 0.09\ \mu$ (reference 7 or Fig. 1 upper panel) $p = 4.7 \cdot 10^8 \cdot \mu$(aa/min/genome).*

TABLE 2. *Growth parameters for E. coli B/r*

Parameter[a]	Units	Symbol[b]	Glu-aa[c]	Glu[c]	Succ[c]	Equation or reference[d]
Growth rate*	Doublings/h	μ	2.14	1.36	0.67	
Cells per mass unit*	Coulter counts $\times 10^{-8}$/A_{460} nm	C	2.6	4.6	10.0	
Genomes per cell	Cells^{-1}	$\bar{G}$	2.95	2.02	1.61	1 genome = $2.5 \cdot 10^9$ daltons = D = $7.6 \cdot 10^8$ nucleotides (3)
Stable RNA per DNA*	RNA nucleotide/DNA nucleotide	(R_s/D)	8.99	5.71	2.81	$R_s/D = 4.2\,\mu$ (Fig. 1)
Stable RNA	RNA nucleotide $\times 10^{-7}$/genome	R_s	6.83	4.34	2.14	$R_s = 7.6 \cdot 10^6 \cdot (R_s/D)$
Stable RNA synthesis rate	RNA nucleotide $\times 10^{-5}$/min/genome	r_s	16.89	6.82	1.65	$r_s = R_s(\ln 2/60) \cdot \mu$
Relat. rate of stable RNA synthesis*	Fraction of stable/total RNA synthesis	ρ	0.68	0.53	0.29	(reference 1,6; Fig. 3)
Total RNA synth. rate	Nucleotide $\times 10^{-5}$/min/genome	r	24.83	12.87	5.71	$r = r_s/\rho$
Unstable RNA synthesis rate	Nucleotide $\times 10^{-5}$/min/genome	r_m	7.94	6.04	4.05	$r_m = r - r_s$ (Fig. 3)
Unstable RNA[f]	Nucleotide $\times 10^{-6}$/genome	R_m	1.99	1.51	1.01	$R_m = r_m \cdot \tau_m$ (Fig. 3) τ_m = 2.5 min = avg. life of mRNA
	% of total RNA		2.83	3.37	4.52	$R_m(\%) = 100\, R_m/(R_m + R_s)$
Ribosomal RNA	RNA nucleotide $\times 10^{-7}$/genome	R_r	5.80	3.69	1.81	$R_r = f_r \cdot R_s$ $f_r = \dfrac{\text{rRNA}}{\text{rRNA} + \text{tRNA}} = 0.85$ (reference 4)
Transfer RNA	RNA nucleotide $\times 10^{-7}$/genome	R_t	1.02	0.65	0.32	$R_t = R_s - R_r$
Stable RNA chain growth rate*	Nucleotide $\times 10^{-3}$/min	c_s	6.3	5.2	4.4	Reference 5
RNA polymerase synthesizing stable RNA	Fraction of total functioning RNA polymerase	ψ_s	0.50	0.39	0.22	$\psi_s = \dfrac{\rho}{\rho + (1 - \rho)c_s/c_m}$ c_m = mRNA chain growth rate = 3,000 nucleotides/s (references 2, 5)
Number of functioning RNA polymerase	Nascent RNA chains/genome	N_{pol}	533	333	173	$N_{pol} = r_s/(c_s \cdot \psi_s)$
Number of ribosomes	70*S* ribosomes $\times 10^{-3}$/genome	N_r	12.6	7.99	3.93	$N_r = R_r/4620$
Different rate of ribosomal protein synthesis*	Fraction ribosomal protein/total protein synthesis rate	α_r	0.193	0.122	0.085	Reference 7, Fig. 1

Ribosome efficiency[e]	Amino acids/min/70*S* ribosome	e_r	809	809	574	$e_r = \frac{\text{aa/rib ln2} \cdot \mu}{\alpha_r \cdot 60 \text{ min/h}}$ aa/ribosome = 6,300 amino acids[e] per 70S ribosome
Protein synthesis rate[e]	Amino acids $\times 10^{-6}$/min/genome	p	10.16	6.46	2.25	$p = N_r \cdot e_r$ (Fig. 3)
Protein[e]	Amino acids $\times 10^{-3}$/genome	P	4.11	4.11	2.91	$P = \frac{p \cdot 60 \text{ min/h}}{\mu \cdot \ln 2}$
Avg no. of[e] translations per mRNA molecule	70*S* ribosomes	$\bar{N}$	38	32	17	$\bar{N} = \frac{p \cdot 3 \text{ nuc/aa}}{r_m}$ (reference 7)
Distance of ribosomes[f] on mRNA	mRNA nucleotides/ribosome	D_r	198	237	322	$D_r = \frac{R_m}{\beta_r \cdot N_r}$ (reference 7) β_r = fraction of active ribosomes = 0.8

[a] Parameters marked with asterisks were measured; the others were calculated.

[b] In general, capital letters denote amounts and numbers, small letters denote rates, greek letters denote fractions.

[c] Growth medium.

[d] In these calculations we have used experimentally determined values from *E. coli* B/r. The growth parameters can be recalculated with alternative values for these parameters by using the equations provided.

[e] The values given for e_r, p, P, and $\bar{N}$ are based on the assumption that a 70*S* ribosome contains 6,300 amino acid residues which may be a minimal estimate (7). These values would increase by 20% if a maximal estimate were used.

[f] Values are based on the assumption that mRNA has 2.5 min of average lifetime (1.7 min half-life), which may be 20 to 30% overestimated.

by the relationship $R_s/D = 4.2\ \mu$ (Fig. 1) for $\mu > 0.67$. At much lower growth rates, this relationship is not expected to hold (see below).

Cell composition at low growth rates. The R_s/D curve in Fig. 1 suggests that very slowly growing cells ($\mu < 0.67$) contain very small amounts of stable RNA (since DNA/cell remains finite, equation 1b) and thus very few ribosomes. On the other hand, the R_s/P ratio extrapolates to a positive value for $\mu \rightarrow 0$, which implies (together with the R_s/D curve) that, with decreasing growth rate, the cellular protein content decreases even faster than the RNA content. Obviously, there must be a minimal cell size (probably not much below the size of succinate-grown bacteria); therefore, below 0.67 doubling per h, the R_s/D and P/D curves must have reduced slopes and approach positive values for $\mu \rightarrow 0$. The point at which the R_s/D curve begins to deviate from linearity may represent a minimal rate of balanced growth below which some cellular reaction rate (such as the rate of uptake of the carbon source from the medium) becomes growth limiting and thus produces a physiological state comparable to chemostat growth.

The idea of Schaechter et al. (24) that RNA, protein, and mass per cell increase exponentially with growth rate (see above) implies a minimal cell size (since exponential curves extrapolate to positive values for $\mu \rightarrow 0$). Therefore, there was no need for Schaechter et al. to assume a deviation at low growth rates from the laws observed at fast growth and, hence, to entertain the possibility of a minimal rate of balanced growth.

Measurement of macromolecular ratios: (i) stable RNA to protein ratio. We observed that the stable RNA/protein ratio is proportional to growth rate ($R_s/P = 0.077\mu$) above 1.2 doublings per h, but greater than $0.077\ \mu$ in slowly growing bacteria. This was established by using two different methods. First, RNA and protein were measured separately (RNA as A_{260} of acid-precipitable, alkali-labile material; protein as acid-precipitable ^{35}S in cultures grown with $(^{35}SO_4)^{-2}$ as the only source of S). Secondly, we measured the differential rate of r-protein synthesis (α_r = r-protein/total protein = P_r/P) which is proportional to the ratios rRNA/protein and stable RNA/protein (assuming in the latter ratio that the proportions of rRNA and tRNA are growth rate independent; see below). Both methods gave nearly identical results (7). The methods used for the direct determination of RNA and protein are not subject to obvious experimental errors; i.e., there is no loss of RNA as a result of fractionation, and the prepared RNA hydrolysate is not contaminated by DNA or protein hydrolysis products. For protein, 5 to 10% of the cold acid-precipitable ^{35}S is alkali soluble, which is an upper error limit (e.g., due to S-containing tRNA bases). The α_r method is independent of the quantitative recovery of any bacterial fraction, since it is based on isotope ratio measurements (7).

In previous studies, the ratio R_s/P was found to increase linearly with, but not proportionally to, growth rate (Table 3-2 of reference 16; 19, 23). However, the ratio R_r/P (rRNA to protein) for rapidly growing *Salmonella* and *E. coli* TAU-bar was found to be proportional to growth rate (10; Table 3-2 of reference 16). Proportionality of R_r/P, but not of R_s/P indicates a growth rate dependency of the proportions of rRNA and tRNA in the total stable RNA.

According to most investigators, the proportions of rRNA and tRNA in stable RNA are nearly constant between 0.6 and 2.4 doublings per h if expressed as rRNA/(rRNA + tRNA) = R_r/R_s (f_r in Table 2). Maximally, a 7% decrease of f_r (from 0.88 to 0.82)[3] was found in this range of growth rates (*Aerobacter:* constant = 0.86, [reference 19] or 0.88 to 0.84 [reference 23]; *Salmonella:* 0.85 to 0.82 [reference 23] or 0.88 to 0.82 [reference 9]; *E. coli:* constant = 0.85 [reference 23] or 0.87 to 0.83 [reference 4; P. P. Dennis, unpublished data]). Originally, Maaloe and Kjeldgaard found smaller values and greater changes with μ (13, 16, 17), which were later shown to reflect an overestimate of tRNA, particularly at low growth rates, due to the presence of DNA hydrolysis products in their tRNA (9). This explains why they found the rRNA/protein ratio but not the stable RNA/protein ratio proportional to μ.

Our calibration of the α_r determinations as R_s/P ratio (Fig. 1) includes the factor $f_r = R_r/R_s$ (equation 5, Appendix; assumed to be constant = 0.85 between 0.6 and 2.4 doublings per h). For the rRNA/protein ratio, we find for $\mu > 1.2$ doublings per h the relation $R_r/P = 0.065\ \mu$ (i.e., $0.85 \times R_s/P = 0.85 \times 0.077\ \mu$). Maaloe and Kjeldgaard observed $R_r/P = 0.15\ \mu$ (g/g) for *Salmonella* (Table 3-2 of reference 16) or, taking into account the molecular weights of nucleotide and amino acid residues, $R_r/P = 0.057\ \mu$ (aa/nuc). They used the Lowry assay for protein and ^{32}P labeling and phenol extraction followed by sedimentation analysis for assay of rRNA.

[3] Although the fraction of rRNA in stable RNA varies little (0.88 to 0.82), the fraction of tRNA varies relatively more (maximally 0.12 to 0.18), but for our purpose only the fraction R_r/R_s matters.

Considering the difference in methods, the values 0.065 and 0.057 for the proportionality factor are in good agreement. Rosset et al. (22) found for both R_r/P and R_s/P a nonproportional increase with μ (see below).

(ii) Stable RNA/DNA ratio. We observed that the ratio stable RNA/DNA increases proportionally to growth rate ($R_s/D = 4.2\ \mu$). Our method employs labeling of bacterial nucleic acids with [^{14}C]uracil for about 20 min, during which time the label is exhausted from the medium. The bacteria are then grown further for about three generations in the presence of an excess of nonradioactive uracil. At various times during this incubation period, samples are taken and the radioactivity in cold acid-precipitable material is determined either directly (=label in RNA + DNA) or after alkali treatment (=label in DNA). Except for a small correction in the different mole fractions of pyrimidines in DNA and RNA, this method is probably the most direct and simplest one which has been used for this purpose (see below). An incidental result of these studies was that the ratio stable RNA/DNA remained constant during the postlabeling incubation period (4), which indicates that rRNA and tRNA are as stable as DNA. If RNA turnover were greater, label from RNA should have been "chased" into DNA, and the ratio R_s/D would have decreased. This does not mean that all rRNA and tRNA synthesized is stable; in fact, some breakdown might have occurred during and immediately after the labeling (20). Rather, it implies that, if breakdown occurs, RNA behaves heterogeneously: only a fraction of RNA is degraded immediately after its synthesis, whereas another fraction (presumably the RNA incorporated into ribosomal particles and mature tRNAs) escapes degradation and is stable (i.e., as stable as DNA). Thus, the ratio R_s/D reflects the ratio of the accumulation or net synthesis rates of stable RNA and DNA rather than the ratio of the synthesis rates.

Previously, the ratio of rRNA to DNA (R_r/D) was found to be proportional to the growth rate (section 2.2 of reference 17; Fig. 3-6 of reference 16). The value observed for *Salmonella* is numerically equal to the value obtained here for *E. coli* B/r; Maaloe and Kjeldgaard found R_r/D = 250/30 (g/g) = 8.3 at μ = 2.4 (Table 3-2 of reference 16); our relationship $R_s/D = 4.2\ \mu$ (Fig. 1) with $R_r/R_s = 0.85$ (see above) gives $R_r/D = 0.85 \times 4.2 \times 2.4 = 8.6$.

In contrast to R_r/D, the ratio R_s/D was found to increase exponentially (13; equation 3-6 of reference 16; 24) or linearly (10; Table 3-2 of reference 16; 23) with growth rate. Except for those of Rosset et al. (23), these deviations from the proportionality relationship can now all be explained as a result of an overestimate of tRNA (see above).

Rosset et al. (23) found that the proportions of rRNA and tRNA for *E. coli* were growth-rate independent above 0.4 doublings per h (and nearly independent for *Salmonella*), which implies that the ratios R_r/D and R_s/D must have the same growth rate dependencies. According to these authors, the functions R_r/D and R_s/D of μ, although linear, extrapolate to positive values for $\mu \rightarrow 0$. Rosset et al. used colorimetric assays for total RNA, DNA, and protein. Their protein/DNA ratio agrees with the previous measurements, whereas only their RNA/DNA and RNA/protein measurements are different. It seems likely that their RNA determination contains a systematic (growth rate-dependent) error, probably related to the colorimetric assay or to the hot trichloroacetic acid extraction they employed before the assay.

In conclusion, our R_s/D values agree with those observed previously if the overestimate of tRNA in some of the earlier studies is taken into account. The only apparent discrepancies are the values of Rosset et al. (23).

(iii) Protein/DNA ratio. We have not measured protein and DNA but, rather, calculated the ratio P/D as a quotient of the ratios R_s/P and R_s/D. The result we obtained, i.e., the constancy of the protein/DNA ratio at high growth rates, appears to be undisputed (10, 13, 16, 17, 23, 24), and our value of 4.1×10^8 amino acids per genome (Fig. 1) agrees with previous estimates of 4×10^8 to 5×10^8 for both *Salmonella* and *E. coli* (17).

The deviation from constancy of the P/D ratio at low growth rates ($\mu < 1.2$) has not been observed previously. This deviation is formally the result of a reduced ribosome efficiency (increased R_s/P ratio) at low growth rates (i.e., the increased R_s/P value in the denominator of the equation $(R_s/D)/(R_s/P) = P/D$ results in a decreased P/D value). A reduced ribosome efficiency at low growth rates has also been observed previously (10, 19), although other data suggested a constant ribosome efficiency (17, 25). The constancy of the ribosome efficiency (between 0.7 and 1.2 doublings per h) was inferred from measurements of α_r, the differential rate of r-protein synthesis (25). Reasons for the difference in the earlier values (25) and in our recent α_r values have been discussed (7).

Significance of macromolecular ratios: (i) stable RNA/protein ratio. The significance of the R_s/P ratio lies in its close relation to the R_r/P ratio (rRNA/protein), which is a measure

for the fraction of metabolic activity the cell devotes to ribosome synthesis. This is expressed in two related equations (see Appendix):

$$\frac{R_r}{P} = \frac{\text{nuc/rib} \cdot k \cdot \mu}{e_r} \quad (5a)^4$$

$$\frac{P_r}{P} = \frac{\text{aa/rib} \cdot k \cdot \mu}{e_r} \quad (10)^4$$

(nuc/rib and aa/rib are the nucleotides [nuc] or amino acid [aa] residues in structural RNA and protein of a 70*S* ribosome [rib]; $k = \ln 2/60$; e_r = ribosome efficiency [in amino acid residues per minute]; see Table 2 or Appendix for additional definitions of symbols). These equations say that the ratios rRNA/protein and r-protein/total protein are proportional to growth rate and inversely proportional to the ribosome efficiency. They express the intuitively plausible principle: the greater the ribosome efficiency, the less ribosomes are needed to achieve a given growth rate. They also express Maaloe and Kjeldgaard's theorem (section 3-4 of reference 16): "Together, the analysis of the purified RNA and that of whole particles indicate that the number of ribosomes is proportional to the rate of growth and of protein synthesis." These two equations were derived previously and have been used to determine the ribosome efficiency (e.g., reference 25).

Equation 5a implies that the growth rate dependency of the ratio R_r/P, and thus also of R_s/P in Fig. 1 directly specifies the ribosome efficiency. The linear portion of the R_s/P curve in Fig. 1 ($R_s/P = 0.077\ \mu$ for $\mu > 1.2$) reflects a constant ribosome efficiency in rapidly growing bacteria (e_r = 810 aa/min/rib, see Table 2). The reduced slope of the R_s/P curve below 1.2 doublings per h reflects a reduced ribosome efficiency.

Equations 5 and 10 are implied by the definitions of the parameters used (see Appendix), and contain no assumptions except that the number of ribosomes is determined by the amount of rRNA (for equation 5) or r-protein (for equation 10), respectively; i.e., rRNA and r-protein must be matched. The matching of ribosomal components must be verified for these equations to hold. In our measurements of the differential rate of r-protein synthesis (ratio P_r/P of equation 10) which we used to calculate R_s/P we did verify that virtually all rRNA is in particles, and we measured only r-protein associated with rRNA.

(ii) Stable RNA/DNA ratio. It is frequently assumed that the amount of DNA in bacteria is limiting for RNA synthesis (which implies that the RNA synthesis rate per DNA is constant); for example, Koch based a theory on the control of ribosome synthesis on the assumption that the stable RNA synthesis rate is proportional to DNA (which is equivalent to a constant rate per DNA) (equation 7 of reference 14). We observed that the amount of stable RNA per DNA is proportional to growth rate ($R_s/D = 4.2\ \mu$), which implies that the stable RNA synthesis rate per DNA is proportional to the square of the growth rate (Table 2: $r_s \sim 4.2\ k\mu^2$), i.e., not constant. Even if one takes into account the increasing number of rRNA genes per genome with increasing growth rate (due to multiple fork replication), the rRNA synthesis rate per rRNA gene is still found to increase with growth rate (5). Also, an increased turnover of stable RNA at low growth rates, such that the actual synthesis rate (but not the net synthesis rate) of stable RNA per DNA is constant, does not occur (see above; Fig. 3). Thus, the R_s/D relationship with μ cannot be explained in terms of a limitation of RNA synthesis by DNA. According to the following argument, the R_s/D relation appears to be fortuitous.

The R_s/D ratio might be determined by the mechanism that controls the amount of stable RNA or the amount of DNA per cell. The rate of stable RNA synthesis depends upon the activity of the genes for stable RNA (rRNA and tRNA), and of the gene(s) for RNA polymerase. The activity of these genes is subject to restrictions, because the rate of rRNA synthesis must be balanced to the rate of r-protein synthesis. At a given growth rate, the differential rate of r-protein synthesis (α_r) and, therefore, also the rate of stable RNA accumulation are given (Appendix, equation 10). Consequently, the R_s/D ratio (at a given μ) must be determined by a mechanism relating to DNA synthesis. For example, if the rate of DNA synthesis were doubled and μ, α_r, and the rate of stable RNA synthesis were unchanged, the DNA per cell would double and the R_s/D and P/D ratios would be 50% reduced. Since DNA replication is probably not linked to the accumulation of stable RNA but rather to protein (see below), it is assumed that the linearity of the R_s/D curve (Fig. 1) is the fortuitous result of the growth rate dependency of the ribosome efficiency (R_s/P) and of the mechanism linking DNA synthesis to protein synthesis (P/D).

[4] Equations 5 and 10 can be used for any exponentially growing living tissue or culture of any species or mutant as long as matching of ribosomal components is taken into account. Furthermore, cell division or DNA replication are not required for these equations to hold, if the growth rate is defined by the doubling time of the amount of protein, not of cell number or DNA.

(iii) Protein/DNA ratio. It may be thought that the constant *P/D* ratio implies that protein synthesis is indirectly limited by DNA, i.e., the more DNA, the more mRNA and thus the more protein. This idea is not supported by observations: neither is mRNA synthesis limited by DNA (Table 2: r_m/G is not constant), nor is protein synthesis limited by mRNA (Table 2: protein synthesis rate per amount of mRNA increases with growth rate).

Many investigators suggest that the constant *P/D* ratio is related to the control of DNA replication; it has been proposed that the initiation of DNA replication requires a specific "initiation protein," whose accumulation is roughly proportional to the accumulation of total protein (3, 12, 26). This idea is supported by the observation that a transient inhibition of protein synthesis by chloramphenicol in *E. coli* B/r causes a delay in the initiation of the next round of replication corresponding to the duration of the inhibition (22). In principle, such a mechanism can result in a constant *P/D* ratio. Constant *P/D* implies that the rate of DNA synthesis, and thus the rate of initiation of DNA replication (average number of initiations per unit of time in a nonsynchronized culture), is proportional to the rate of protein synthesis. (It should be noted that the rate of DNA synthesis is completely determined by the initiation rate, independent of the DNA chain elongation rate.) However, the exact timing of initiation of replication cannot be explained, since the amount of protein per chromosome origin at the time of initiation of DNA replication decreases by about 25% when the growth rate triples ($1 < \mu < 3$; Table 1). Moreover, recent observations indicate that initiation of replication can continue for up to 15 min in the absence of protein synthesis (11, 15, 18), indicating that the causal relationship between initiation of DNA replication and protein synthesis is less direct than implied by the accumulation-of-initiator-protein idea. Nevertheless, some relationship between DNA and protein synthesis must exist (i.e., the constant *P/D* ratio for $\mu > 1.2$ is probably not fortuitous) because initiation of replication ceases within 15 min of inhibition of protein synthesis when other metabolic reactions, including RNA synthesis, continue. Alternate models may propose (i) accumulation plus maturation. This implies that, in addition to accumulation of some protein (e.g., membrane protein), a "maturation" process not dependent on protein synthesis is required to trigger initiation, which may last up to 15 min. The reduced protein/origin ratio in fast-growing cells (Table 2) may then be due to a faster maturation. (ii) The effect of protein accumulation may indirectly increase the cell volume, which, in turn, may reduce a repressor concentration and thus trigger initiation (cell volume may continue to increase somewhat after inhibition of protein synthesis).

Any model which tries to explain the constant *P/D* ratio in fast-growing cells must also explain not only the reduced *P/D* in slowly growing cells, but also the observation that the magnitude of this reduction just compensates the reduction in the ribosome efficiency (R_s/P ratio), such that the R_s/D ratio is porportional to μ. Since the reduced ribosome efficiency has the effect of increasing the activity of the r-protein genes (i.e., increasing α_r), the possibility must be considered that the synthesis of a protein involved in the initiation of DNA synthesis (e.g., the membrane protein considered above) is coordinately controlled with r-protein.

Relationship between cell mass and growth rate. The idea that accumulation of protein rather than mass is related to DNA replication (see above) implies that the exponential increase of cell mass with growth rate (24) cannot be explained as suggested by Donachie (see Introduction). However, our measurements suggest that the mass/cell curve represents the sum of the major macromolecular cell components (weights of protein + RNA + DNA; Fig. 2, ▲).

Donachie's relationship between cell mass and initiation of replication (8) also suggests that a minimal cell mass exists corresponding to the mass at initiation of replication of cells growing at rates below 1 doubling per h. The fact that the R_s/D curve in Fig. 1 continues to decrease below 1 doubling per h indicates that the minimal mass of *E. coli* B/r is considerably smaller than Donachie's unit mass.

APPENDIX

The following equations (3, 5, and 10), mentioned in the text, are derived as follows.

Definitions:

P_r, P = amount of ribosomal and total protein, respectively (amino acid [aa]/genome [G])

p_r, p = synthesis rate of ribosomal and total protein, respectively, (aa/min/G)

R_s = amount of stable (ribosomal and transfer) RNA (nucleotides [nuc/G])

r_s = synthesis rate of stable RNA (nuc/min/G)

N_{rib} = number of ribosomes (per G)

aa/rib = amino acid residues per 70*S* ribosome = 6,300 (reference 7)

nuc/rib = rRNA nucleotides per 70*S* ribo-

some = 4,620

c_p = peptide chain elongation rate (aa/min)

c_s = stable RNA chain elongation rate (nuc/min)

α_r = $p_r/p = P_r/P$

β_r = functioning ribosomes/N_{rib}

e_r = ribosome efficiency = $\beta_r \cdot c_p$ (aa/min/ribosome)

f_r = R_r/R_s = rRNA/(rRNA + tRNA) = 0.85

k = ln2/60 (h/min) = 1.15×10^{-2} (h/min)

μ = growth rate (doublings/h)

(i) Relationship between the ratio stable RNA/protein and the differential rate of ribosomal protein synthesis. Setting

$$R_s = \frac{R_r}{f_r} = \frac{N_{rib}\,(\text{nuc/rib})}{f_r} \tag{6}$$

and

$$P = \frac{P_r}{\alpha_r} = \frac{N_{rib}(\text{aa/rib})}{\alpha_r} \tag{7}$$

and forming the ratio R_s/P gives

$$\frac{R_s}{P} = \frac{(\text{nuc/rib}) \cdot \alpha_r}{(\text{aa/rib}) \cdot f_r} = 0.86\,\alpha_r \tag{3}$$

(ii) Relationships between growth rate, differential rate of ribosomal protein synthesis, and ribosome efficiency. Using the definition

$$\alpha_r = \frac{p_r}{p}$$

and substituting

$$p_r = N_{rib}(\text{aa/rib}) \cdot k \cdot \mu \tag{8}$$

$$p = N_{rib} \cdot \beta_r \cdot c_p \tag{9}$$

gives

$$\alpha_r = \frac{(\text{aa/rib}) \cdot k \cdot \mu}{\beta_r \cdot c_p} = \frac{72.5 \cdot \mu}{e_r} \tag{10}$$

(iii) Relationship between ribosome efficiency and the R_s/P ratio. Substituting equation 10 into equation 3 gives

$$\frac{R_s}{P} = \frac{\text{nuc/rib}}{\text{aa/rib}\,f_r} \frac{(\text{aa/rib})\,k \cdot \mu}{e_r}$$

or after cancellation of aa/rib:

$$\frac{R_s}{P} = \mathrm{K} \cdot \frac{\mu}{e_r} \tag{5}$$

where $K = \dfrac{(\text{nuc/rib}) \cdot k}{f_r} = 62.5$

Similarly, the rRNA-to-protein ratio is

$$\frac{R_r}{P} = \frac{(\text{nuc/rib}) \cdot k \cdot \mu}{e_r} \tag{5a}$$

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