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Transcription in Bacteria at Different DNA Concentrations

GORDON CHURCHWARD,† HANS BREMER,* AND RY YOUNG‡

The University of Texas at Dallas, Richardson, Texas 75080

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The effect of changing the DNA concentration on RNA synthesis, protein synthesis, and cell growth rate was studied in *Escherichia coli* B/r. The DNA concentration was varied by changing the replication velocity or by changing replication initiation in a thymine-requiring strain with a mutation in replication control. The results demonstrate that changes in DNA concentration (per mass) have no effect on the cell growth rate and the rates of synthesis (per mass) of stable RNA (rRNA, tRNA), bulk mRNA, or protein or on the concentration of RNA polymerase (total RNA polymerase per mass). Thus, transcription in *E. coli* is not limited by the concentration of DNA, but rather by the concentration of functional RNA polymerase in the cytoplasm. Changing the DNA concentration does, however, affect fully induced *lac* gene activity, here used as a model for constitutive gene expression. The magnitude of the effect of DNA concentration on *lac* gene activity depends on the distribution of replication forks over the chromosome, which is a function of the replication velocity. Analysis of these data reinforces the conclusion that transcription is limited by the concentration of functional RNA polymerase in the cytoplasm.

Is the rate of transcription initiation in bacteria limited by the concentration of DNA or by the concentration of functional RNA polymerase? Furthermore, is the rate-limiting step the binding of RNA polymerase to the promoter or the formation of an active initiation complex? These questions are important for a quantitative understanding of bacterial gene expression, especially for the class of genes that are constitutively expressed. The growth rate-dependent variation in the expression of such genes has been called "metabolic regulation" (35).

It has been argued that such regulation results wholly, or in part, from a limitation by the DNA concentration (9, 16, 27) or, alternatively, by functional RNA polymerase (3, 6, 32). The question has eluded experimental solution because of the difficulty in manipulating the two parameters in vivo without changing additional parameters that affect transcription (see below).

We have previously studied the properties of a mutant of *Escherichia coli* B/r, strain TJK16, which has a lower DNA concentration than its wild-type parent due to a mutationally altered control of initiation of chromosome replication (10, 12). TJK16 is auxotrophic for thymine, and its DNA concentration can be further lowered by growing it at low thymine concentrations (12). Therefore, this strain is useful for studying the effects of varying DNA concentration on the rate of in vivo transcription. The results suggest that in both B/r and TJK16, DNA is transcribed under conditions of DNA excess; i.e., transcription is limited by the concentration of active RNA polymerase rather than by DNA. In *E. coli* only 20 to 30% of the total RNA polymerase enzyme is actively engaged in transcription (RNA chain elongation) at any given time, whereas 70 to 80% of the RNA polymerase appears to be inactive (39). Since the results here indicate that DNA is not limiting the rate of transcription, the inactivity of RNA polymerase must be caused by other factors rather than a low DNA concentration.

† Present address: Université de Genève, Departement de Biologie Moleculaire, CH-1211 Genève 4, Switzerland.

‡ Present address: Texas A&M University, Department of Medical Biochemistry, College Station, TX 77843.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The bacteria used were *E. coli* B/r A (ATCC 12407; 22) and *E. coli* TJK16, which is a *thyA deoB* derivative of *E. coli* B/r A obtained from J. Kwoh (M.S. thesis, University of Texas at Dallas, 1975). NF955 *ilv thr leu thi* (λ *b*515 *b*519 *c*I857 *S*7 λ *dilv*5), used to prepare λ *dilv* DNA for hybridization studies, was kindly provided by N. Fiil. Growth conditions were as described previously (23, 39).

Determination of cell mass, DNA, protein, and RNA. Cell mass (units of optical density at 460 nm [OD_{460}] per 10^9 cells) and DNA (colorimetric assay) were determined as described previously (7, 12). There was no significant difference between the plating titer and the particle concentration for either strain used. To determine protein and RNA, 5-ml samples were precipitated with 1 ml of 3 M trichloroacetic acid, filtered through glass fiber filters (Reeve Angel; 984H),

washed with tap water, dried, and placed in glass vials. After hydrolysis with 2.0 ml of 0.2 N NaOH at 23°C for 20 h, 0.5 ml of the hydrolysate was removed for estimation of the protein content by a modification of the method of Lowry et al. (7, 28). The protein assays were incubated for 20 h at 23°C. Under these conditions an absorbancy at 750 nm/OD_{460} ratio of 1 corresponds to 9.1×10^{17} amino acids per OD_{460} unit of culture (calibration with bovine serum albumin). For RNA, the remaining alkaline hydrolysate (after removal of the sample for estimation of protein) was treated with an equal volume of ice-cold 0.5 M perchloric acid to precipitate protein and DNA and filtered through a Bact-T-Flex membrane filter (Schleicher & Schuell Co.; 0.45-μm pore size). The absorbancy at 260 nm of the filtrate was determined. An absorbancy at 260 nm/OD_{460} ratio of 1 corresponds to 4.5×10^{16} RNA nucleotides per OD_{460} unit of culture (calculated from the molar extinction of *E. coli* RNA at acid pH). The reproducibility of the colorimetric assays for DNA, RNA, and protein is shown in Table 1.

Determination of β-galactosidase activity. Enzyme activity in fully induced cultures was determined by using standard methods (15), except the reaction was carried out at 23°C for 30 min.

Determination of the fractional rate of stable RNA synthesis. Samples (0.5 ml) of culture were removed and labeled with 5 μl of [5-^{3}H]uridine (Schwarz/Mann; 2.5 μCi/5 μl, 21 Ci/mmol) for 1 min. The pulse was terminated by the addition of lysing medium (5), and the sample was immediately placed in a boiling water bath for 1 min. The fraction of label in rRNA was determined by hybridization to λ *ilv5*, which carries an rRNA gene (13). Phage DNA was prepared after induction of NF955 by the method of Miller (31) and loaded onto Bact-T-Flex filters (0.45-μm pore size, 10 to 25 μg/filter) after alkaline denaturation. Hybridization was carried out in scintillation vials containing 1 ml of 2× SSC (1× SSC is 0.15 M NaCl–0.015 M sodium citrate), 0.5% sodium dodecyl sulfate, 0.02 M EDTA, at 67°C for 18 h. Each vial contained two DNA filters, a blank filter without DNA or with heterologous λ or T5 DNA, approximately 0.05 μg of pulse-labeled RNA, and approximately 0.005 μg of purified (18) ^{14}C-labeled rRNA as a standard to determine the hybridization efficiency. In control experiments it was found that nonspecific hybridization to filters loaded with λ or T5 DNA was indistinguishable from binding to blank filters. After corrections for background and hybridization efficiency the fraction of pulse label in rRNA (l_r/l_{tot}) was determined. The fractional rate of synthesis of stable RNA, r_s/r_{tot}, was obtained from l_r/l_{tot} by using the formula: $r_s/r_{tot} = (l_r/l_{tot})/\{(l_r/l_{tot}) + [0.86 - (l_r/l_{tot})] \times 0.86\}$. This formula takes into account the different mole fractions of pyrimidines in stable and mRNA (0.43 and 0.5, respectively; ratio, 0.86) and the fraction of stable RNA that is rRNA (0.86). The synthesis rate of stable RNA, r_s, includes the spacer material in the stable RNA precursor. The relative rate of mRNA synthesis, r_m/r_{tot}, is equal to the difference $1 - r_s/r_{tot}$; r_{tot} is the total instantaneous rate of RNA synthesis, including mRNA and unstable spacers in stable RNA precursors.

Determination of RNA polymerase. Determination of RNA polymerase was as described previously (39) from the amount of β and β′ RNA polymerase subunit protein found after sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

RESULTS

Two ways of changing the DNA concentration. The strain TJK16 is a derivative of *E. coli* B/r which, in addition to the *thyA deoB* markers conferring a low-thymine requirement, has a third mutation which increases its "initiation mass" (10, 12). Since TJK16 has a greater cell mass per replication origin, it has less DNA per mass, i.e., a lower DNA concentration, in comparison with the B/r parent at any growth rate (Fig. 1a). At high thymine concentration (>10 μg/ml), the DNA chain elongation in this strain is indistinguishable from that in the parent strain

TABLE 1. Reproducibility of colimetric assays for DNA, RNA, and protein

Day[a]	DNA		RNA		Protein	
	A_{600}[b]	Deviation from avg (%)	A_{260}[b]	Deviation from avg (%)	A_{750}[b]	Deviation from avg (%)
1	0.337	−2.0	1.244	−2.0	0.486	−8.0
	0.354	+3.0	1.252	−1.3	0.507	−4.1
	0.355	+3.3	1.258	−0.9	0.517	−2.2
	0.360	+4.7	1.277	+0.6	0.510	−3.5
4	0.333	−3.1	1.221	−3.8	0.543	+2.7
	0.340	−1.1	1.270	+0.1	0.574	+8.6
5	0.335	−2.6	1.284	+1.2	0.514	−2.7
	0.355	+3.3	1.310	+3.2	0.527	−0.3
6	0.331	−3.7	1.260	−0.7	0.553	+4.6
	0.338	−1.7	1.314	+3.6	0.554	+4.8
Avg or σ[c]	0.344	σ = 3.2%	1.269	σ = 2.3%	0.529	σ = 5.7%

[a] Ten samples (10 ml each for DNA and 5 ml each for RNA and protein) were taken simultaneously from the same culture, chilled on ice (B/r in glucose minimal medium, τ = 43 min, OD_{460} = 0.98), precipitated with trichloroacetic acid, and collected on glass fiber filters immediately, but further processed on different days as indicated.

[b] Blank values subtracted.

[c] Standard deviation, $\sigma\ (\%) = \sqrt{\Sigma\Delta^2/(n-1)}$; Δ, deviation from average in percent; *n*, number of samples (10).

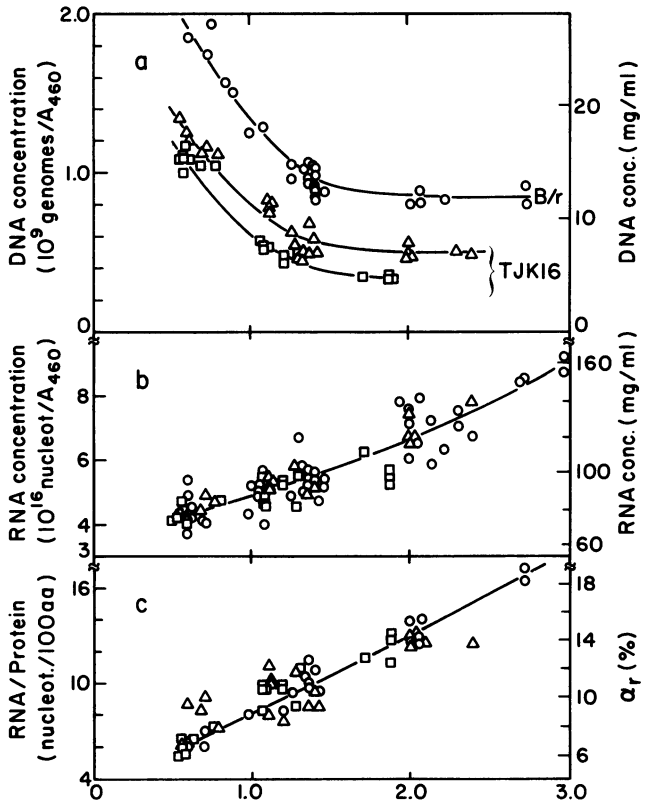

FIG. 1. Concentration of DNA and RNA in *E. coli* B/r and TJK16. Comparison of the concentrations of DNA (DNA/OD_{460} unit, panel a), RNA (RNA per OD_{460} unit, panel b; RNA per protein, panel c) in *E. coli* B/r (○) and TJK16 growing in 20 μg of thymine per ml (△) or 1 μg of thymine per ml (□). Each point represents the average of four values from one culture (two duplicate samples taken a doubling time apart). For estimation of the absolute DNA concentration it was assumed that 1 OD_{460} unit corresponds to 0.3 μl of cell volume (12). For conversion of the RNA/protein ratio into the fractional rate of synthesis of ribosomal protein, α_r (24), it was assumed that 83% of the bacterial RNA is rRNA (14% tRNA [17, 41] and 3% mRNA [26, 33]), and that a 70S ribosome contains 6,300 amino acid residues in protein and 4,720 nucleotide residues in rRNA.

(11), i.e., the *thyA deoB* mutations have no effect. This implies (14) that at high thymine levels the distribution of replication forks on the chromosome and the relative gene dosages (number of gene copies per genome equivalent of DNA) are the same in B/r and TJK16. Only the effects of the initiation mutation are then apparent under these conditions, i.e., the concentration of all genes in TJK16 is reduced by the same factor.

In a "step-up" experiment the velocity of replication forks is increased by increasing the concentration of thymine in the medium from an initial low concentration (34). This changes the distribution of replication forks over the chromosome, i.e., the chromosomes become less branched because rounds of replication are completed faster. As a result, genes far from the replication origin increase in concentration, but genes near the origin do not (2, 9). Figure 1a shows that average gene concentrations (DNA/OD_{460} unit) in TJK16 increased with increasing thymine concentration.

The concentration of DNA in bacteria is also a function of growth rate (9, 21); in *E. coli* B/r and in the Thy^- initiation mutant TJK16, the DNA concentration decreases with growth rate (observed above 0.6 doublings per h) and reaches a minimum level above 1.5 doublings per h (Fig. 1a). Assuming that 1 OD_{460} unit of cells has a volume of about 0.3 μl (12), intracellular concentrations of DNA are estimated to vary between 5 and 25 mg/ml depending on the growth rate and replication velocity (Fig. 1a).

RNA polymerase concentration and activity. The amount of RNA polymerase β and β′ subunit protein was determined for *E. coli* B/r and TJK16 growing in glucose-amino acids medium at high and low thymine concentration (Table 2). Assuming that each subunit was part of a complete polymerase core enzyme, the fractional rate of accumulation of RNA polymerase, α_p,

TABLE 2. Accumulation of RNA polymerase (α_p = RNA polymerase protein per total protein) in *E. coli* B/r and TJK16 growing in glucose-amino acids medium and, in the case of TJK16, supplemented with 20 or 1 μg of thymine per ml as indicated

Strain	Thymine concn (μg/ml)	Doubling time (min)	Protein concn[a] (mg/ml)	α_p[b] (%)	Avg α_p
B/r	0	25	2.36	1.28	
		27	1.59	1.29	1.29
		27	1.89	1.30	
TJK16	20	30	2.22	1.39	
		30	2.08	1.16	1.35
		28	2.13	1.49	
TJK16	1	30	2.74	1.25	
		31	2.44	1.31	1.34
		28	1.94	1.46	
B/r	0	25	2.36	[1.33][c] [1.36]; [1.26] [1.22]; [1.29] [1.22]	1.28

[a] Protein concentration in each sample used for electrophoresis was determined by the method of Lowry calibrated with bovine serum albumin.

[b] α_p, was calculated as micrograms of core RNA polymerase × 100 per microgram of total protein; average of six values (two slots on three gels) for each culture (one horizontal line). Each culture growth was repeated three times resulting in slightly different doubling times. Each gel also contained two slots with a known amount of bovine serum albumin for calibration.

[c] The reproducibility of the estimate from gel to gel is shown by the six values which resulted in the average α_p value of 1.28%. The brackets indicate the two slots from one gel.

was found to be about 1.33% (range, 1.29 to 1.35% [percentage of total protein that is RNA polymerase]) for both strains and independent of thymine concentration. Since mRNA and stable RNA synthesis per OD_{460} unit were the same for both strains (see below), it seems likely that also the concentrations of actively transcribing RNA polymerase molecules are the same.

The observation that RNA polymerase synthesis was independent of DNA concentration is important for models of RNA polymerase gene (*rpoBC*) control; it means that either the concentration of the presumed control factor (37) is in great excess over its binding sites on the DNA (see below), or the activity of this factor itself is controlled.

RNA concentration and stable RNA synthesis. The concentration of RNA (amount of total RNA per OD_{460} unit) increased with growth rate in both strains; at a given growth rate, the RNA concentrations were not significantly different in B/r and TJK16 (Fig. 1b). Most (~97%) of the bacterial RNA is stable (26, 33); under conditions of exponential growth and for doubling times of less than 60 min, this stable RNA is 86% rRNA and 14% tRNA (17, 41). Thus, at a given growth rate, the rates of rRNA and tRNA synthesis per OD_{460} unit were independent of DNA concentration. However, the synthesis rates per gene did depend on DNA concentration: in TJK16, with all genes (including rRNA genes) at lower concentrations as compared with B/r, the transcription per gene was correspondingly higher (Fig. 2). The transcription of rRNA genes was also a strong function of growth rate, increasing from 3 to 30 (B/r) or 12 to 50 (TJK16) transcriptions per rRNA gene per min over a two- to threefold range in growth rate (Fig. 2).

Ribosome synthesis and function. The amount of RNA per protein (ratio R/P, proportional to the number of ribosomes per amount of protein) is an indicator of ribosome synthesis and function (Fig. 1c; α_r = ribosomal/total protein); obviously the denominator of the ratio R/P represents the product of ribosome function (ribosomes make protein), whereas the numerator is essentially a measure of the number of ribosomes. RNA per protein was found to increase with growth rate in an identical manner in wild-type *E. coli* B/r (Fig. 1c) and in TJK16, both at high and at low levels of exogenous thymine (Fig. 1c). This means that ribosome synthesis and function are independent of DNA replication, at least within the limits represented by wild-type B/r and the replication mutant TJK16.

mRNA synthesis. The relative proportions of stable and mRNA synthesis were the same in B/r and TJK16 (Table 3); thus, bulk mRNA synthe-

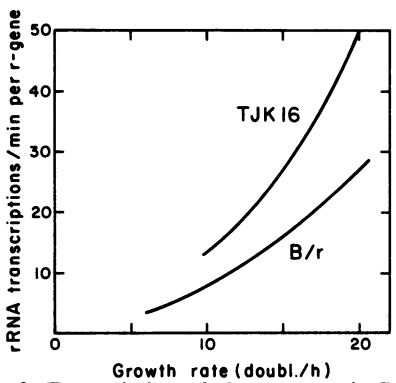

FIG. 2. Transcription of rRNA genes in B/r and TJK16 (at any thymine concentration), calculated from the rate of rRNA synthesis in nucleotides per minute per genome [(drRNA/dt)/genome = RNA/genome × 0.83(ln2/τ); RNA/genome is the ratio of curves in Fig. 1b and a; 0.83 is the fraction of total RNA that is rRNA, assuming 14% tRNA (17, 41) and 3% mRNA (26, 33); τ is the doubling time] and from the number and map locations of the seven rRNA genes (19), using the formula for the relative gene dosage from reference 2 and from the number of nucleotides per ribosome (4,720).

TABLE 3. Relative proportions of stable and mRNA synthesis in *E. coli* B/r and TJK16 growing in glucose minimal medium (TJK16 supplemented with 20 or 1 μg of thymine per ml, as indicated)

Strain	Thymine concn (μg/ml)	Doubling time (min)	Total cpm in hybridization mix		Hybridization efficiency[c] (%)	Relative proportions of:		
			^3H[a]	^{14}C[b]		Stable RNA synthesis		mRNA synthesis (avg %)
						%[d]	Avg %	
B/r	0	48	5616	258	64	52	54	46
		46	6029	258	34	51		
			6029	258	50	55		
		45	6573	290	22	57		
TJK16	20	45	5735	380	82	49	48	52[e]
		46	5963	368	84	46		
	1	45	2638	380	85	55	52	48
		46	3125	368	92	48		

[a] ^3H-pulse-labeled RNA.

[b] [^{14}C]rRNA (purified) added to hybridization mixture for determination of the hybridization efficiency.

[c] The differences in hybridization efficiency are mainly due to differences in the amount of λ *dilv* DNA loaded to the filters. In the B/r experiments, about 10 μg of DNA was used per filter; in the TJK16 experiments, about 25 μg of DNA was used.

[d] Each value given is the average from three hybridization vials.

[e] The slightly higher mRNA values for TJK16 in comparison with B/r mean that the rate of total RNA synthesis in TJK16 is 2 to 6% higher than in B/r (despite the lower DNA concentration in TJK16). This difference is experimentally not significant.

sis, like stable RNA synthesis per OD_{460} unit, was not affected by changes in DNA concentration.

To focus on a particular mRNA, we compared *lac* transcription in the two strains. Most mRNA synthesis is specifically regulated; to observe only the effects of gene concentration and polymerase availability, we used conditions where the *lac* operon was physiologically constitutive (i.e., maximally induced). Since the position of the *lac* genes is midway in the replication path (1), there is always about 1 *lac* gene per genome equivalent, regardless of growth rate. (This is not true for genes near the origin or the terminus of replication.) Thus, measuring the relative amount of DNA per OD_{460} unit is the same as measuring the relative concentration of *lac* genes. The synthesis of *lac* mRNA was determined indirectly from the synthesis of total mRNA and from the synthesis of β-galactosidase enzyme (see below). The results depended on the kind of experimental condition that was used to change the DNA concentration, i.e., whether it was due to a change in replication velocity or due to a change in replication initiation. These two conditions are examined separately below.

(i) Gene activities during a step-up. A replication velocity step-up was found to have no effect on the rate of synthesis (per mass) of stable RNA (Fig. 3b), bulk mRNA (Fig. 4b), and protein (Fig. 3b). Constitutive β-galactosidase synthesis per mass increased by 40%, compared with the control culture (Fig. 4a), in agreement with previous observations (9). Since the synthesis per OD_{460} unit of β-galactosidase is essentially the fractional rate of *lacZ* transcription per total mRNA transcription, the apparent increase in *lac* expression per OD_{460} unit actually stemmed from a constant rate of transcription per *lac* gene and an increased number of *lac* genes per mass (see below) (Table 4).

Since the genes for rRNA are clustered near the origin of chromosome replication (1), and since a step-up does not affect replication of the origin (4, 34), the concentration of rRNA genes and the rate of rRNA synthesis per rRNA gene remain unaltered by the step-up (Fig. 2; the curve for TJK16 is valid for any thymine concentration). Thus, changes in DNA concentration brought about by changes in replication velocity affect neither rRNA nor constitutive *lac* gene activities (Table 4).

(ii) Gene activities after a change in replication initiation. In Fig. 5, the ratios of *lac* gene concentrations in B/r and TJK16 are shown for high thymine levels when the only difference between the two strains was in the control of replication initiation. The mutant had from 50 to 70% of the *lac* gene concentration of the wild type, depending on the growth rate. The β-galactosidase synthesis per OD_{460} unit was nearly equal in the two strains, although the synthesis in the mutant relative to the parent B/r decreased somewhat with increasing growth rate. By dividing the enzyme synthesis per OD_{460} unit by the number of *lac* genes per OD_{460} unit (~DNA/OD_{460}), one obtains a measure for the relative transcription rate per *lac* gene (see below). At all growth rates, constitutive transcription per *lac* gene was

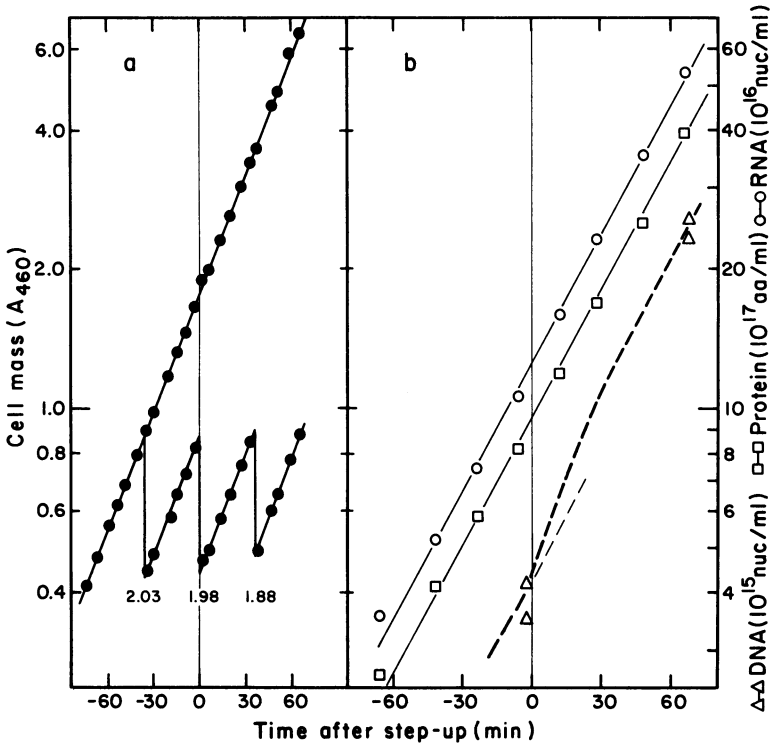

FIG. 3. Transcription in TJK16 (growing in glucose-amino acids medium + 1 mM IPTG) during a step-up in the replication velocity brought about by an increase in thymine concentration from 1 to 20 μg/ml. (a) Mass accumulation; dilutions as indicated (dilution factors). (b) Accumulation of DNA, RNA and protein; DNA accumulation (---) calculated (4).

30% higher in the mutant. A different extent of catabolite repression in the two strains was not involved; the ratio of the rates (not the absolute rate) was independent of the presence of 0.01 M exogenous cyclic AMP (data not shown), shown in previous work (15) to be in excess of the amount necessary to maximally reduce catabolite repression. Thus, *lac* gene transcription increases when the concentration of all genes is reduced proportionately. Clearly, DNA concentration does not limit *lac* gene expression in balanced growth.

DISCUSSION

Effects of DNA concentration on the total rate of RNA synthesis. In the past, the DNA concentration in bacteria was found to vary with the growth rate or with the replication velocity (9, 21), but in these cases the accompanying gene regulation and changes in replication fork pat-

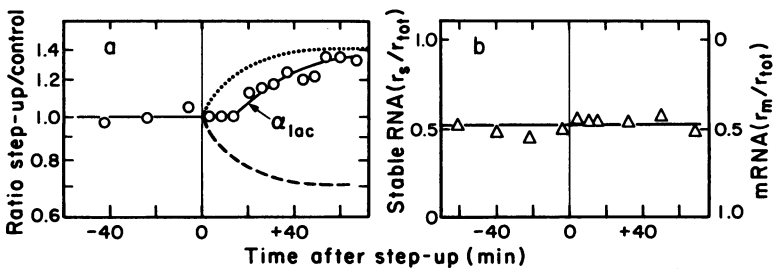

FIG. 4. *lac* gene expression and mRNA synthesis after a stepwise increase in the replication velocity (same experiment as in Fig. 3). (a) Change in the fractional synthesis of β-galactosidase (α_{lac}), compared with changes in mRNA synthesis per genome (---) (calculated from Fig. 3b and 4b) and in the *lac* gene concentration (*lac* genes/OD_{460} unit) (· · ·). (The number of *lac* genes per OD_{460} unit is equivalent to genome equivalents per OD_{460} unit from Fig. 3a and b.) (b) Changes in the relative synthesis rates of stable and mRNA, r_s/r_{tot} and r_m/r_{tot}.

TABLE 4. Effect of changing DNA concentration on macromolecular synthesis rates and cell composition in *E. coli* B/r growing in glucose-amino acids medium at 2 doublings per h

DNA concn changed by:	*n*-fold change in:											
	Concn				Activity		Rate per mass			Mass doubling time[b]	RNA/ genome[a]	Protein/ genome[a]
	DNA[a]	RNA polymerase[b]	rRNA gene[c]	*lac* gene[d]	rRNA gene[e]	*lac* gene[f]	Bulk mRNA[g]	rRNA, tRNA[a]	Protein[a]			
Initiation control	0.6	1.0	0.6	0.6	1.7	1.3	1.0	1.0	1.0	1.0	1.7	1.7
Replication velocity	0.7	1.0	1.0	0.7	1.0	1.0	1.0	1.0	1.0	1.0	1.5	1.5

[a] Data from Fig. 1.
[b] Data from Table 2.
[c] rRNA gene concentration changes as much as DNA concentration (0.6-fold) by initiation control, and does not change (1.0-fold) by changes in replication velocity (see text).
[d] *lac* gene concentration changes as much as DNA concentration (see text).
[e] rRNA gene activity changes as the quotient of (change in rRNA synthesis per mass)/(change in rRNA gene concentration).
[f] Data from Fig. 3, 4, and 5.
[g] Data from Table 3.

terns have obscured the effect of DNA concentration on transcription. In temperature-sensitive DNA initiation mutants, the DNA concentration can be altered by growing the bacteria at semipermissive (i.e., intermediate between fully permissive and nonpermissive) temperatures (20) without changes in replication fork patterns, but the change in temperature affects the rate of transcription, again making the results difficult to interpret. In the current work, we have utilized a strain with a non-temperature-sensitive DNA initiation mutation in which the DNA concentration is reduced in comparison to its wild-type parental strain without concomitant changes in growth rate or in replication fork patterns (12). This mutant is thus ideally suited to study the effects of altered DNA concentration on the rate of transcription. The data in Table 4 demonstrate that total RNA synthesis in *E. coli* per unit mass is not affected by a reduction in DNA concentration; we conclude that transcription in *E. coli* is not limited by the concentration of DNA (i.e., by the availability of free promoters). Under such conditions, free functional RNA polymerase would be increased by a decrease in total DNA. The concentration of free RNA polymerase can be monitored by the transcription of constitutive genes; the expression of maximally induced *lacZ* genes observed here at different DNA concentrations qualitatively agreed with this expectation and thus reinforced the conclusion that transcription in *E. coli* is limited by the concentration of active RNA polymerase rather than by DNA.

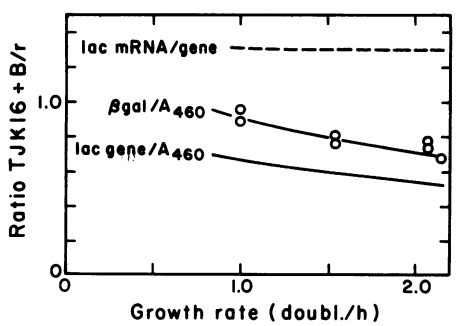

FIG. 5. Comparison of *lac* gene expression in *E. coli* B/r and TJK16 growing at 20 μg of thymine per ml. (—), *lac* gene concentration; the number of *lac* genes per OD_{460} unit was assumed to be equal to the number of genome equivalents of DNA per OD_{460} unit (see text) and was obtained from the DNA measurements of Fig. 1. (○), β-galactosidase per OD_{460} unit, each point represents the ratio of the β-galactosidase concentrations obtained from two identical cultures, B/r and TJK16, maximally induced for β-galactosidase. (---), *lac* mRNA per gene, the ratio *lac* gene activity in TJK16/*lac* gene activity in B/r corresponds to the ratio of the lower two curves (see text).

This conclusion apparently contradicts the results obtained by Seeburg et al., using an in vitro transcription system with purified *E. coli* RNA polymerase and defined DNA fragments of phage fd containing only one promoter, these workers observed that 20 s to 3 min (half time) was required for formation of an active initiation complex after the initial RNA polymerase binding (38). Using these data, Maaløe estimated that a concentration of two to three free RNA polymerase molecules per cell would suffice to saturate the bacterial promoters with polymerase; at higher enzyme concentrations, the rate of formation of the active RNA chain initiation complex would be rate limiting (30). Since free functional RNA polymerase in bacteria has not

been directly measured, the estimated value of enzyme concentration required for promoter saturation does not help to answer the question of whether bacterial transcription is limited by DNA or by RNA polymerase. The observation (Fig. 2) that rRNA promoters showed no signs of saturation and in fact underwent initiation up to once per second, indicates that in vivo the formation of active initiation complex is at least 1 order of magnitude faster than under the in vitro conditions studied by Seeburg et al. (38), which supports our conclusion that DNA concentration is not limiting the rate of transcription in *E. coli*.

It is important to note the units used in these analyses. If RNA synthesis were measured with other reference units rather than per unit mass, e.g., as rate per cell or rate per genome (29), changes in DNA concentration would affect RNA synthesis indirectly. This reflects the effects of DNA replication on these reference units, i.e., on DNA and cell number, rather than effects on RNA synthesis. Such units must be avoided in a consideration of general transcriptional regulation.

RNA polymerase activity. In the condition of DNA excess, free polymerase should be a small fraction of total enzyme, and the major portion of the enzyme should be engaged in transcription. By quantitating RNA polymerase subunits in DNA-free minicells, Shepherd (Ph.D. thesis, The University of Texas at Dallas, 1980) found that the concentration of cytoplasmic RNA polymerase was indeed a small fraction of the total enzyme. Yet only 20 to 30% of all RNA polymerase in *E. coli* B/r is active in transcription at any given time (39). If RNA synthesis is not limited by DNA, the inactivity of a large proportion of bacterial RNA polymerase must have other reasons than a low DNA concentration. It is conceivable that the RNA polymerase activity is limited by the availability of sigma factor. The molar amount of sigma per cell is about 30% of that of core enzyme (24, 25). However, since sigma factor is only required for RNA chain initiation and is released from the transcription complex immediately after initiation (8), it is not obvious why a sigma/core ratio of 0.3 should not be sufficient to activate all RNA polymerase, i.e., the ratio of free sigma to free core enzyme is presumably much greater than 1. Another indication that factors other than DNA concentration may limit the RNA polymerase activity is the observation that the RNA polymerase activity rapidly responds to changes in the intracellular level of guanosine tetraphosphate (36).

Kawakami et al. (25) observed that up to 100% of the total *E. coli* RNA polymerase could be recovered as nucleoid-bound enzyme when the nucleoids were prepared from exponential bacteria at a KCl concentration of 0.2 M; this fraction decreased during the transitional period between exponential growth and the stationary phase. The fact that enzyme was DNA bound in vitro does not necessarily mean that it is also bound in vivo, where ionic conditions are different. Even if most polymerase enzyme were DNA bound in vivo, which would be consistent with the minicell data, this would not mean that the enzyme must be actively engaged in transcription. Thus, the data of Kawakami et al. are not inconsistent with a low polymerase activity of 20 to 30%.

Expression of individual genes after changes in DNA concentration. If the expression of specific genes is considered rather than total RNA synthesis, changes in DNA concentration can be expected to produce more subtle effects since it then becomes important to consider the location of the gene on the chromosome with respect to the origin of replication and the particular manner by which the DNA concentration was changed; changes in DNA replication velocity alter relative gene dosages, depending on the gene location, whereas changes in replication initiation have no such effect. Further, mass action, just as it predicts changes in free RNA polymerase as a result of changes in DNA concentration, also predicts changes in the concentration of free positive or negative control factors that bind to DNA. These effects, as they are relevant for the interpretation of the data obtained here, will be discussed below.

The strain TJK16 has all genes in a lower concentration than its wild-type B/r parent, and since the rate of total transcription per OD_{460} unit is the same in TJK16 as in B/r (Table 4), the genes producing the bulk of stable RNA and mRNA must be more active in TJK16 than in B/r. This is interpreted as a reflection of the increase in the concentration of free (cytoplasmic) RNA polymerase (3) due to the decreased DNA concentration. The fact that the relative proportions of rRNA and mRNA synthesis were not affected by changes in DNA concentration (Table 3) suggests that any differences in individual gene activities do not reflect a guanosine tetraphosphate-dependent control of RNA polymerase conformation (40).

Chandler and Pritchard (9) observed an increase in *lac* gene output (α_{lac}, β-galactosidase per total protein, or, similarly, β-galactosidase per OD_{460} unit), after an increase in replication velocity, in agreement with our data (Table 4). Since this increase occurred in the absence of an increased relative gene dosage (*lac* genes per genome), they concluded that relative gene dosage is not a factor in determining constitutive gene expression. On the other hand, since the

concentration of *lac* genes (number of *lac* genes per OD_{460} unit) increased after a step-up to about the same extent as α_{lac} (Fig. 3), they suggested that the increase in gene output after a step-up is due to an increase in *lac* gene concentration and concluded that gene expression is limited by the gene concentration, which appears to be at variance with our conclusion that DNA concentration does not limit the rate of transcription. It is important to note that the gene expression measured as specific enzyme activity (α_{lac}) depends not only on the synthesis of *lac*-mRNA, but also on total mRNA synthesis, since ribosomes must compete for different mRNA molecules. We suggest the use of the term gene activity, defined as rate of transcription per given gene (rate of initiation of mRNA chains at the promoter considered) instead of the term gene output. The transcription rate per (constitutive) *lac* gene did not change by the step-up in replication velocity (Table 4).

The unaltered *lac* gene activity after the replication velocity step-up means that also the concentration of free (cytoplasmic) RNA polymerase (in the conformation that binds to *lac* promoters) remains unaltered. The law of mass action would predict a decreased concentration of free RNA polymerase by the step-up (3). Only in two circumstances would this not be the case. (i) If most transcription would come from particularly active genes near the replication origin, the number of polymerase binding sites (per mass) would actually not increase since the concentration of genes near the origin is not affected by changes in replication velocity. (b) If, for most regulated genes, an increase in copy number were compensated by a corresponding repression, the summed affinities of all promoters for RNA polymerase would not be significantly altered. The first effect, i.e., the clustering of active genes near the origin of replication, may be more important, since only this would explain the difference in *lac* gene expression after changes in initiation control (Table 4).

Effects on gene control. If control factors are not in great excess over their binding sites on the DNA, the lower DNA concentration in TJK16 would be expected to bring about an increased concentration of free (not DNA-bound) positive and negative control factors, and activation in the case of positive factors or repression of the corresponding genes in the case of negative factors. The disproportionate change in constitutive *lac* transcription and DNA concentration observed here (Table 4) might be an effect of such changes in gene control.

APPENDIX

Determination of *lac* gene transcription. Transcription of constitutive *lac* genes was found from the fractional synthesis of β-galactosidase and total mRNA synthesis in the presence of excess inducer and cyclic AMP (15). The fractional synthesis, α_{lac}, is defined as: α_{lac} = (synthesis of *lac* protein)/(synthesis of total protein) = (synthesis of *lac* mRNA)/(synthesis of total mRNA) × C. The constant C reflects the differences in the functional life and ribosome loading efficiency of *lacZ* mRNA relative to average or bulk mRNA; the factor is presumed to be invariable under the conditions compared.

To find the transcription per gene, the quotient is expanded as follows: $\alpha_{lac} \sim$ [(*lac* genes per genome) × (*lac* mRNA synthesis per *lac* gene)]/(total mRNA synthesis per genome). α_{lac} and total mRNA synthesis per genome was observed and *lac* genes per genome (relative gene dosage) can be calculated from the replication velocity and the map location of the *lac* gene (1). In the particular case of *lac*, the location halfway between the origin and terminus of replication results in about one *lac* gene per genome at any replication velocity (*lac* genes per genome = 1).

Instead of using DNA as a reference unit (per genome), one may also use cell mass (per OD_{460} unit) without changing the equation since the reference units cancel in numerator and denominator: $\alpha_{lac} \sim$ [(*lac* genes per unit mass) × (*lac* mRNA synthesis per *lac* gene)]/(total mRNA synthesis per mass). This form is particularly convenient for comparison of conditions where total mRNA synthesis per mass (i.e., the denominator on the right side) remains unchanged, such that *lac* mRNA synthesis per lac gene ~ α_{lac}/(DNA/OD_{460}).

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