Metabolic Growth Rate Control in *Escherichia coli* May Be a Consequence of Subsaturation of the Macromolecular Biosynthetic Apparatus with Substrates and Catalytic Components

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INTRODUCTION

Bacteria such as *Escherichia coli* live as single cells in changing surroundings and the growth rate increases when the nutritional supply becomes "richer." For instance, *E. coli* grows more rapidly with glucose as the sole source of carbon and energy than with acetate and still more rapidly when the glucose minimal medium is supplemented with amino acids, nucleotide precursors, vitamins, etc. Concomitant with the changes in the growth rate, the composition of the cells is altered in a characteristic way: the individual cells become larger, when they grow fast, and they contain more replication forks on the chromosome. The RNA/mass ratio increases in parallel with the growth rate, primarily because more ribosomes are formed per unit mass in the fast-growing cells, while the DNA/mass ratio and the protein/mass ratio are fairly constant parameters (60, 61, 84; reviewed by Bremer and Dennis [7]).

Intimately connected with the growth rate control is the so-called stringent response (3), i.e., the cessation of stable RNA accumulation (90), when the bacteria are exposed to a nutritional downshift provoked, for instance, by removing amino acids from the medium or by changing the carbon source supply from glucose to acetate (reviewed by Cashel and Rudd [10]). In addition to rRNA synthesis, tRNA synthesis (76) and the synthesis of several proteins which function in the transcription or translation machinery (6, 18,

77) are subject to stringent control. For the ribosomal proteins the growth rate regulation (25, 28) and the stringent response appear to be mediated at the level of mRNA synthesis (19, 25, 62), although a combination of feedback regulation on the translation process and changes in the mRNA half-life may explain some or all of these previous results (13, 14, 64). A noncoordinate regulation of several RNA species during amino acid starvation has also been suggested (24), indicating that the stringent response is not completely coordinated at the transcriptional level, a view also supported by in vitro transcription experiments (50).

"Magic spot" (ppGpp) accumulates to very high levels immediately following a downshift (9). Subsequently, it decreases to a level only slightly higher than the preshift concentration. The ppGpp synthesis is a codon-specific response to charged tRNA deficiency (37, 49, 71), and it seems that the rapid ppGpp accumulation is responsible for the stringent arrest of stable RNA synthesis since *relA* mutants, defective in ppGpp synthesis, only slowly reduce the rate of stable RNA synthesis to the new steady state. This level, however, is the same for wild-type and *relA* strains, which contain identical ppGpp pools (7, 10).

The promoters for stable RNA transcription have been extensively characterized (reviewed in reference 10). Common "operator" sequences, which could explain the stringent regulation, have not been found, although these promoters do have common features (92). This indicates that RNA polymerase itself may be directly involved in the stringent response, a view supported by the isolation of

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mutants defective in this regulation, due to alterations in either the RNA polymerase genes (4, 59, 70) or the promoter regions for rRNA (20, 27).

Previously, the chain elongation rates during transcription and translation were considered to be invariable (39, 60) and, hence, unable to contribute to any regulation. Here we argue that this is incorrect. In fact, our model for growth rate control centers around the proposition that these elongation reactions are indeed variable, because the high maximal rates for protein and RNA biosyntheses exceed the capacity of the cellular metabolism to provide enough material to saturate the polymerization reactions. Consequently, the macromolecular biosynthetic activities drain the pools of substrates and unengaged catalytic molecules (i.e., free RNA polymerase and ribosomes) down to such low levels that the chain elongation reactions become subsaturated with precursors, the promoters become subsaturated with RNA polymerase, and the ribosome binding sites on the mRNA chains become subsaturated with ribosomes.

We assume that there is a kinetically determined coupling between the rate of protein chain elongation and the rate of RNA chain elongation. Mechanistically, this coupling may involve nucleotide and ppGpp pool adjustments, secondary structures in the RNA chains, the levels of charged tRNAs in complex with EF-Tu and GTP, and possibly the codon choice in the mRNA. Starved ribosomes will stall at codons where there is shortage of substrate and begin to synthesize large quantities of ppGpp which, in turn, will reduce the speed of both stable RNA and mRNA chain elongation. Consequently, the rate of RNA chain termination and, therefore, the concentration of free RNA polymerase will fall rapidly.

Our major proposal is that the stringently controlled promoters preferentially will lose activity due to the competition with other promoters because the stringent promoters depend on a high concentration of free RNA polymerase to perform near their maximal rate. Therefore, initiation at these promoters is particularly sensitive to the concentration of free polymerase. When the synthesis of new ribosomes stops in response to ppGpp, the concentration of active ribosomes decreases. As the ribosomes become fewer to share the limited amount of substrates that can be supplied in the new growth condition, they will gain speed and synthesize less ppGpp. This will tend to increase the elongation speed of RNA chain synthesis and to increase the amount of free RNA polymerase able to make new rRNA. Thus, the steady state is suspended as a compromise between the number of ribosomes and their speed.

Below, we shall elaborate more on the different aspects of the model and present a short critique of some experiments claimed to indicate constant and invariable elongation rates during RNA and protein syntheses.

MEDIUM, METABOLISM, AND GROWTH

The E. coli cell is considered as a system in which the capacity to use the activated precursors and catalytic components exceeds the capacity of the intermediary metabolism and the medium to provide these precursors. In other words, we consider the steady states in different media as being analogous to the growth condition in a chemostat, in which the bacterium intrinsically is able to grow faster than it is allowed to by the pump. Furthermore, we propose that all of the different macromolecular biosynthetic activities, at each level in gene expression (for example, the translation of different mRNA species in the total mRNA pool), compete

with each other for activated precursors (charged tRNAs in complex with GTP and EF-Tu) and catalytic structures (ribosomes) because they consume material from common pools. In this way, the different specific cellular activities have been mutually adjusted by evolution to "behave socially," since the intensity of no specific process can be altered without affecting other cellular activities. Ultimately, all biosynthetic activities consume the medium via metabolism, and since the cell is designed for rapid growth, it becomes unsaturated with precursors for macromolecular biosynthesis.

The growth rate of a bacterium increases when the medium is supplemented with amino acids, nucleosides and bases, and vitamins, because the metabolic energy and the carbon atoms can be used to form more activated precursors for macromolecular biosynthesis when not used for synthesis of the building blocks. However, even in a minimal medium, the presence of different carbon sources (e.g., glucose or acetate) results in different growth rates. Thus, glucose supports a much higher growth rate of E. coli than acetate and must, therefore, be able to support the formation of activated precursors at a higher rate than acetate (39). This may be because the energy cost of pumping one (ionic) molecule of acetate into the cell is at least as high as that of transporting one molecule of glucose. However, one molecule of acetate contains only two combustible carbon atoms, while glucose contains six. This means that a substantial part of the energy released from combusting the transported carbon atoms is consumed for the very purpose of making the acetate available for metabolism. When the amount of energy needed to maintain ion balances, membrane potentials, etc., is subtracted, much less energy and carbon are left for growth purposes with acetate as the carbon source than with glucose. In accordance with this view, it was observed that one unit mass of E. coli consumes an equal amount of energy per time unit, when the bacterium grows with widely different doubling times on a variety of carbon-energy sources (2). Evidently, it will not be possible for the bacterium to improve the pathways for acetate utilization such that this compound becomes as good an energy source as glucose. Therefore, equal growth rate on acetate and glucose can only be obtained when the bacterium sacrifices the highly efficient pathways for glucose utilization.

The growth behavior of E. coli may be considered analogous to an enzymatic reaction that shows saturation kinetics. The maximal growth rate, which reflects limitations from the internal parameters of the cell, can never be reached. It can only be approached at the cost of energy and material needed to accumulate high precursor pools. However, one important difference between E. coli and an enzyme is that the activated precursor pools are not arbitrarily fixed by an experimenter, but are instead determined as a compromise between the rates of precursor formation and utilization.

An enzyme may require high concentrations of the substrate for saturation either if it binds the substrate poorly or if it is a very efficient catalyst such that the catalytic reaction is completed very quickly after proper binding of the substrate. This is illustrated in Fig. 1, which shows the saturation behavior for three different enzymes that carry out the same reaction: substrate (S) converted to product (P). They all obey Michaelis-Menten kinetics, but are designed with different binding affinities and maximal reaction velocities. It is clear from Fig. 1 that the enzymes whose reaction velocity is most indifferent to variations in the substrate concentration over a broad range are those which bind the substrate avidly, but are very poor catalysts. With the substitutions
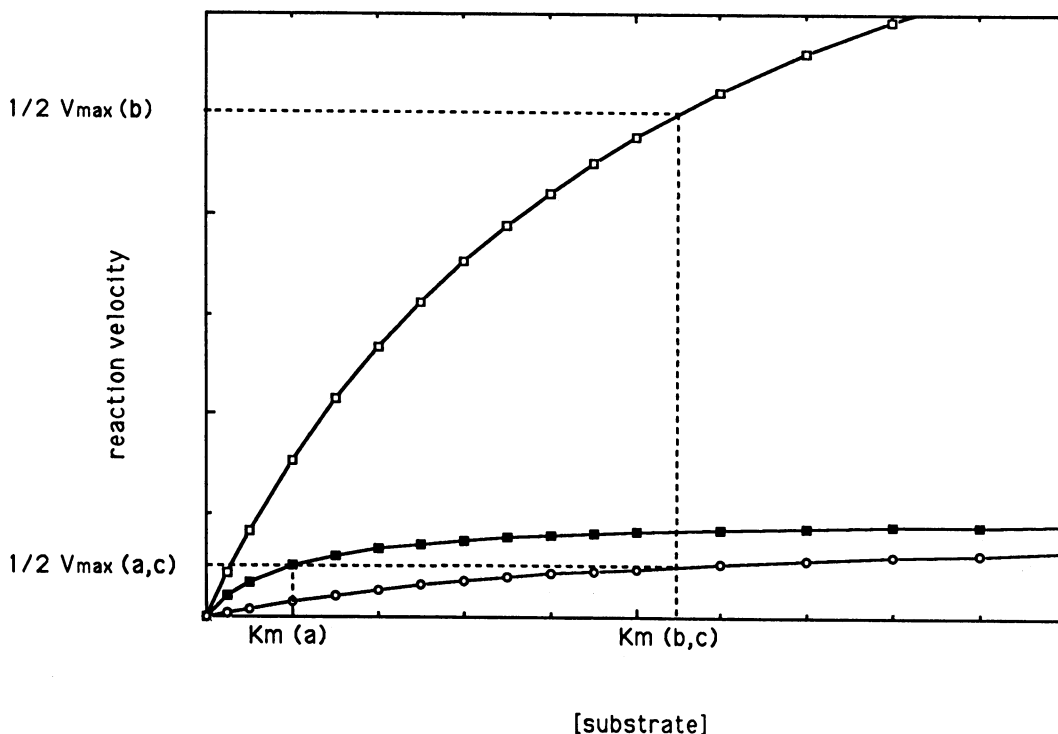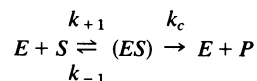
[substrate]

FIG. 1. Contribution from high maximal reaction rates and from weak binding of the substrate to the saturation properties of an enzyme. Shown are the saturation kinetics for three different enzymes (E; a [■], b [□], and c [○]), which all carry out the same enzymatic reaction, in which the substrate, S, is converted to the product, P, by the reaction scheme:

$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} (ES) \overset{k_c}{\rightarrow} E + P$$

Two of the enzymes (a and b) bind the substrate with equal affinity ($k_{+1} = 1$; $k_{-1} = 1$), but enzyme a is designed with a low maximal reaction velocity ($k_c = 1$), while enzyme b has a higher maximal reaction velocity ($k_c = 10$). The third enzyme c has the same low maximal reaction velocity as enzyme a (i.e., $k_c = 1$), but it binds the substrate less well, since it is designed with a greater tendency to lose the substrate in the nonproductive dissociation reaction, i.e., $k_{-1} = 10$ and $k_{+1} = 1$. The points shown are calculated on the basis of the indicated rate constants. The half-saturation constant K_m is equal to ($k_c + k_{-1})/k_{+1}$. Therefore, it is clear that enzyme a will be more easily saturated with the substrate ($K_m = 2$) than the other two enzymes, for which the $K_m = 11$. This means that both enzymes b and c change their reaction velocity considerably over a much broader range of substrate concentrations than enzyme a.

that E means a promoter, S means RNA polymerase, the rate constants k_{+1} and k_{-1} describe the binding of RNA polymerase to the promoter in the closed complex, and k_c describes the clearing of the promoter region to make it ready for binding of a new RNA polymerase, these considerations also apply to the saturation of various promoter types with RNA polymerase.

We propose that *E. coli* has been selected in such a way that it is able to exploit rich medium for rapid growth. This means that the genes whose products contribute most to the rapid growth rate in the rich medium, i.e., the components in the macromolecular apparatus for protein and RNA syntheses, have been selected for very high maximal rates of gene expression. However, this design has by itself made the macromolecular biosynthetic machinery highly dependent on the accumulation of large pools of activated precursors and free catalytic components in order to achieve maximal rates. In many situations, particularly in a minimal medium, these large precursor pools cannot build up and the biosynthetic reactions, which may take place very rapidly in the rich medium, lose speed in the minimal medium according to the degree of subsaturation of macromolecular synthetic processes.

TRANSCRIPTION

Promoter Competition

As a part of the general subsaturation of gene expression, we believe that the promoters are, intrinsically, able to sequester and engage more RNA polymerases in transcription elongation than are actually present in the cell, a view which has gained some experimental support (11). Therefore, the individual promoters compete with each other for a limited amount of free RNA polymerase and, hence, the frequency of transcription initiation at a given promoter depends on its ability to compete with the other promoters.

Accordingly, we consider the ribosomal promoters, as well as other stringently controlled promoters, as belonging to a class of promoters which are difficult to saturate with RNA polymerase and hence require high concentrations of free RNA polymerase to perform near their maximal rate for RNA chain initiation. Other promoters may require only little free RNA polymerase to become saturated, and we regard the nonstringently controlled promoters as belonging to this category. This implies that the activity of the rRNA promoters, and of other stringently controlled promoters,
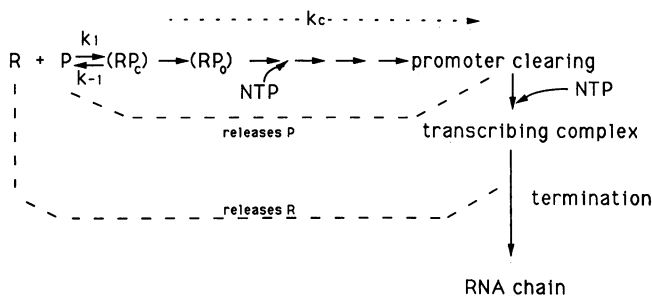
FIG. 2. Saturation of a promoter with RNA polymerase. The saturation of a promoter (P) may be considered analogous to the saturation of an enzyme, but the substrate is RNA polymerase (R). The catalytic rate constant (k_c) is a measure of the time required for formation of the open complex (RP_o) and for clearing the promoter to make it ready for a new RNA polymerase binding event. For very fast promoters, the high catalytic rate constant contributes to make the promoter difficult to saturate with RNA polymerase, as is also the case for promoters with lower catalytic rates but which lose the RNA polymerase before initiation by the nonproductive dissociation reaction (k_{-1}). Thus, both types of promoters need a high concentration of free RNA polymerase to be able to initiate transcription at a level near their maximal rates, since otherwise they tend to be limited by the association rate $(= k_{+1}[RNA\ polymerase])$. Therefore, their "strength" in vivo depends strongly on the concentration of free RNA polymerase in the cell (see Fig. 1). These promoters will also be stringently controlled by our model, which implies that the stringent response causes a decrease in the cellular concentration of free RNA polymerase (see text).

will change considerably if the cellular concentration of free RNA polymerase changes, while the activity of many mRNA promoters will remain relatively constant. This view is consistent with the few existing measurements of the saturation parameters of E. coli promoters with RNA polymerase (29, 40, 50).

The ribosomal rrn operons contain two strong promoters, P1 and P2, in front of their structural genes. The growth rate control appears to be associated primarily with the upstream promoter, P1 (33, 82). The activity of this promoter, in contrast to P2, was shown in vitro to be strongly stimulated by high concentrations of RNA polymerase relative to promoter DNA (29, 40).

Elongation Rate as a Determinant of Free RNA Polymerase Concentration

When a molecule of RNA polymerase is engaged in the process of transcription elongation, it is occupied with this specific purpose for a considerable period of time until the RNA chain is completed and the enzyme released in free form is able to initiate a new transcription cycle (Fig. 2). Thus, the rate of RNA chain elongation is a very critical parameter in determining the concentration of free RNA polymerase and, if the elongation rate suddenly falls, more RNA polymerase molecules will become sequestered at elongation, less will be released per time unit, and the concentration of free RNA polymerase will fall rapidly. Consequently, the frequency of transcription initiation will decrease suddenly, particularly at those promoters which are difficult to saturate with RNA polymerase, i.e., the stable RNA promoters and other stringently controlled promoters, according to our model.

Determinants of RNA Elongation Rate

RNA polymerase consumes nucleoside triphosphates (NTPs) during transcription elongation, and the enzyme has

very high K_ms for the NTP substrates in vitro. In particular, the K_ms for UTP and GTP are of a similar size as the intracellular concentrations of these compounds (43, 46, 53), indicating that RNA polymerase is unsaturated with the substrates in vivo and moves at submaximal and, thus, variable speed during elongation. This notion is supported by the finding that some pyrimidine nucleotide biosynthetic genes are controlled by an attenuation mechanism modulated by changes in the concentration of the (NTP) substrates for RNA polymerization (44, 75, 79). Moreover, even small concentrations of the magic spot compound, ppGpp, similar to basal level concentrations in vivo, were shown to lower the RNA chain elongation rate during in vitro transcription by potentiating RNA polymerase pausing during the synthesis of both mRNA and rRNA chains (52, 53). This is particularly relevant since ppGpp is synthesized by the ribosomes when they are subsaturated with the substrates (37, 49, 71). If the inhibition of RNA chain elongation by ppGpp does indeed take place in vivo, this implies that an increased ppGpp pool will reduce the concentration of free RNA polymerase and, thereby, cause a fall in transcription initiation at the stringently controlled promoters as it was, in fact, observed for the rrnA promoter by Sarubbi et al. in vivo (83). However, this observation could also be interpreted to mean that ppGpp controls the activity of the rrnA promoter directly, as suggested by Baracchini and Bremer (3).

During in vitro transcription of a truncated rrnB template, Glaser et al. (29) observed that ppGpp severely inhibited the P1 promoter, while leaving P2 relatively unaffected. In addition, it was observed that ppGpp induced transcriptional pauses at elongation. However, since the pause sites were all found in the common part, i.e., after promoter P2, it was concluded that ppGpp selectively inhibited transcription from P1 by direct interference with the specificity of the RNA polymerase-promoter recognition reaction, not by affecting RNA chain elongation (29). In our opinion, this conclusion is not valid, since the in vitro system involved reinitiating rounds of transcription. Thus, the transcriptional pauses induced by ppGpp may well have sequestered more RNA polymerase in the elongation phase and lowered the concentration of the free enzyme. Since promoter P1 is more difficult to saturate with RNA polymerase than promoter P2 (29, 40, 50), an inhibition of RNA chain elongation by ppGpp may well affect the activity of P1 more severely than that of P2.

Measurements of RNA Elongation Rate

The elongation rate of the RNA polymerase has been measured and found to be constant and independent of the growth rate (8, 65, 66, 80, 81). The reason for this result may reside in the nature of the experiments made to determine this parameter. In all cases, but one (8), these determinations involved measurements of the time needed to complete the synthesis of an RNA chain after blocking new transcription initiation with rifampin, while adding a radioactively labeled nucleotide precursor simultaneously with the drug (65, 66, 80, 81). The stop of transcription initiation results in a gradual, but immediate, decrease in the rate of NTP consumption, as the transcribing RNA polymerases run off their templates. We have investigated the size of the nucleotide pools in this situation (Fig. 3). A rapid accumulation of NTPs is observed, because the synthesis of these compounds exceeds consumption in the presence of rifampin. Furthermore, the concentration of ppGpp decreases (data not shown), probably because the overall rate of protein synthe-
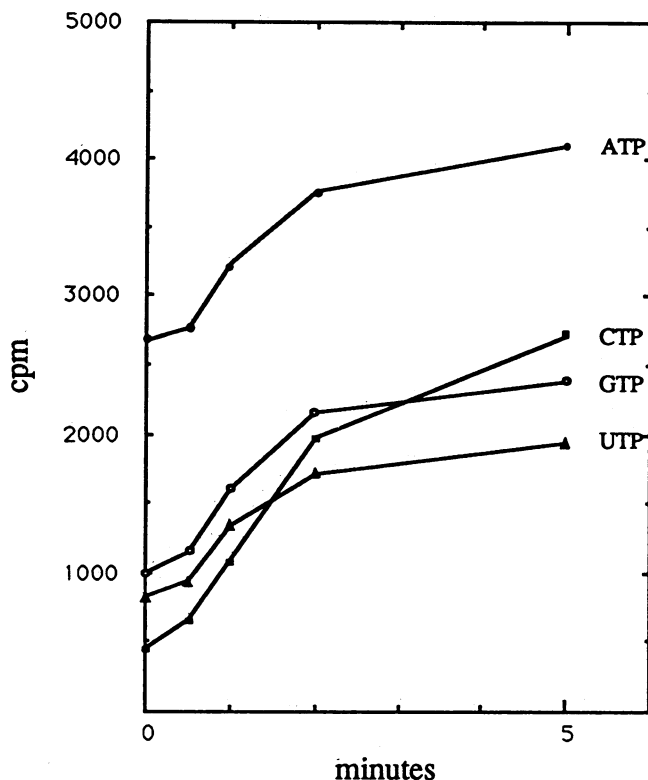
FIG. 3. Rapid increase in intracellular NTP pools after addition of rifampin to a culture of *E. coli*. Bacterial strain NF1133, highly permeable to rifampin (48), was grown exponentially for one generation in a glucose-Casamino Acids medium at 37°C in the presence of ^{32}P while samples (20 μl) were withdrawn for determination of nucleotide pools (45). Rifampin (50 μg/ml) was added at an optical density at 436 nm of 0.5 at time zero. The ordinate represents radioactivity in the individual NTPs per 20-μl culture.

sis now becomes limited by the availability of mRNAs and the ribosomes, therefore, become fully saturated with substrates. Thus, the RNA chain growth rate may well increase substantially after the addition of rifampin and become overestimated in such experiments. The published time course of the nucleotide incorporation into RNA can indeed be taken as evidence for such an acceleration of RNA polymerase movement early after rifampin addition, since the incorporation curves were concave upwards until they broke abruptly, but this was ascribed to a slow labeling of the NTP pools (65, 66). However, even if not corrected for the presumed acceleration, the data obtained in these experiments are best in agreement with a 20 to 30% variation in elongation rate between the slowest and the fastest medium (65, 80). The only experiment in which rifampin was not used and which indicated constant elongation rate is that of Bremer and Yuan (8). They, however, studied only two growth conditions which supported rather similar growth rates and used an elaborate centrifugation procedure to separate RNA chains according to size, which resulted in, especially at the faster growth rate, only a few points being taken. All together, we think that the previous conclusion from these experiments, namely, that RNA chain growth rate is constant, is true in the sense that the variations are small compared with the changes in growth rate, but we believe that to say that there is no variation at all is a too rigid interpretation of the data.

An overestimation of the RNA chain growth rate will necessarily lead to an underestimation of the amount of RNA polymerase actively engaged in transcription, since this parameter is calculated by dividing the gross rate of nucleotide incorporation into RNA by the (erroneously high) elongation rate (7, 63). In turn, this has led to the supposed existence of a large pool of free RNA polymerase in *E. coli*, particularly at the low growth rates. However, there is another reason for this supposition, which is that the new, high rate of RNA synthesis is acquired so shortly after an amino acid upshift that new RNA polymerase molecules could not possibly have been made by new protein synthesis. Hence, it was concluded that vast amounts of idle RNA polymerase molecules were present before the upshift (69). We propose that this increase in rRNA synthesis is produced by polymerases that accelerate because the ribosomes speed up and stop forming ppGpp. This leads to a rapid increase in the pool of free RNA polymerases which preferentially initiate transcription at the stringent promoters since these were the least saturated with RNA polymerase before the upshift. Thus, it is likely that there is only little free RNA polymerase at any time in *E. coli*. In agreement with this, it was found (81) that the amount of RNA polymerase in complex with the DNA is higher than the fraction of RNA polymerase previously calculated to be active from the total RNA synthesis under the assumption of an invariant elongation rate.

TRANSLATION

Subsaturation of Ribosome Binding Site with Ribosomes

As another aspect of the general subsaturation of gene expression, we consider the cellular mRNA pool as being intrinsically able to engage more ribosomes in protein synthesis than are actually present. Thereby, the ribosome concentration, rather than the mRNA concentration, becomes limiting for protein synthesis. This is supported by the calculations of Bremer and Dennis (7), who found that the individual mRNA chains, on average, are translated more times in rapidly growing cells with a high concentration of ribosomes than in slowly growing cells with few ribosomes and by the observations that the rate of total protein synthesis correlated better with the amount of ribosomes than with the amount of total mRNA (68). In addition, Pedersen et al. (74) observed an increase in the absolute synthesis rate for some proteins in the first few minutes after rifampin addition. This increase was most pronounced for the α and β subunits of the RNA polymerase, but also seen for EF-TuA and EF-G (74) and several other proteins encoded by relatively stable mRNA chains (S. Pedersen, unpublished observations). As rifampin in the very same experiment was shown to prevent initiation of new transcription virtually instantaneously, the most simple explanation for this result is that the ribosome binding sites on these mRNA chains must have the capacity to engage more ribosomes than they normally do. This shows up after rifampin addition when the general mRNA pool decays and more ribosomes become available for initiation purposes. In the case of the synthesis of the β and β' subunits, a rifampin-induced change in activity of the attenuator in front of these genes has been suggested to be involved also (26).

A priori, it seems plausible that the ribosome binding site on an mRNA chain is difficult to saturate with ribosomes, since the formation of a functional initiation complex in-

volves the interaction of several macromolecular structures with each other and since the ribosomes, once bound, are likely to take off for protein synthesis very rapidly. Therefore, rather than being determined by the intrinsic binding constant, the saturation of the translational start site on an mRNA chain is likely to be governed by the association rate, dependent on the concentration of free ribosomes, and by the rate by which the ribosomes clear the binding site to make it ready for a new initiation event. The latter process is intimately connected with the polypeptide chain growth kinetics in the first part of the protein chain and may, therefore, also depend on the codon usage in the 5' end of the mRNA (57, 73) as well as on the supply of charged aminoacyl-tRNAs in complex with EF-Tu and GTP.

The ribosomes consume aminoacyl-tRNAs when they make proteins and if, as has been suggested by the measurements of Yanofsky et al. (98), the concentrations of aminoacyl-tRNAs (and EF-Tu or GTP) are lower in a poor medium than in a rich medium, the implication being that the elongation of the protein chains should occur more slowly in the poor medium. This has been shown by direct measurements (73) and is also accepted as part of the mechanistic basis for the function of the amino acid biosynthetic operon attenuators as regulatory elements (55).

However, the differences between the protein chain growth rates in the various media are relatively small when compared with the differences between the corresponding growth rates, and it can be calculated that the average ribosome incorporates approximately 1.6 times as many amino acid residues per second in a protein chain when $E. coli$ grows with a doubling time of 24 min than it does during growth with a doubling time of 100 min (7, 73).

We propose that the maintenance of a relatively constant elongation rate in the different media (39, 60) is due to the adjustment of the ribosome concentration in the cell (28, 85). This is because fewer translating ribosomes can move faster (individually) than many ribosomes can, as the supply of substrates limits the total rate of amino acid incorporation. Indeed, we would expect considerable changes in the polypeptide chain growth rate to occur as a function of the medium if this adjustment of the ribosome number did not take place, as observed in the early phase following an energy source downshift of a relA strain (48) in which the rate of rRNA synthesis is only slowly adjusted to the new steady-state level (66). Thus, the steady state seems to be a compromise between the number of translating ribosomes and their reaction speed since the total number of peptide bonds that can be formed per time unit per unit cell mass is dictated by the medium. Still, we regard the elongation reactions as being the primary sensors that mediate the global cellular adjustments of macromolecular biosynthesis in relation to the medium and the growth rate.

Thus, the high polypeptide chain growth rate in a rich medium has two regulatory functions. It makes the ribosomes spend less time at elongation, and this contributes to increasing the concentration of free ribosomes. This leads to an increase in the frequency by which mRNAs are translated. Moreover, due to the coupling between transcription and translation and the fact that saturated ribosomes make less ppGpp than unsaturated ribosomes do (37, 71), the fast performance of the ribosomes will tend to increase the RNA chain growth rate. Thereby, it contributes to increasing the concentration of free RNA polymerase and the capacity for new transcription initiations, particularly at rRNA promoters and other stringently controlled promoters.

Measurements of Translation Elongation Rate

The rate of protein chain elongation was considered constant and growth rate independent for a very long time (12, 22, 39). This led to the conclusion that there is an excess of free, unengaged ribosomes in slowly growing cells (39), since the number of active ribosomes was calculated from the total amino acid incorporation in proteins under the assumption of a constant protein chain elongation rate.

These results have always been in conflict with other results: indirect calculations from measurements of total protein synthesis and ribosome content (23), measurements of the induction lag for β-galactosidase synthesis (16), or, recently, measurements of the elongation rate on several individual mRNAs (73) in which it was found that the protein chain elongation rate falls with decreasing growth rate.

Engbæk et al. (22) determined the kinetics of appearance of N-terminal threonine in purified β-galactosidase after induction and used the induction lag as a measure for the translation time. A close examination of their data shows that the translation rate was about 33% higher in glucose medium than in acetate medium, but as the rate in a broth medium appeared similar to the rate in acetate, it was concluded that the translation rate was independent of the growth rate. However, this result from the broth medium was troubled by a high isopropyl-β-D-thiogalactopyranoside-independent background of N-terminal threonine in other proteins and by the few samples examined from broth cultures. The study by Koch and co-workers (12) used chemostats to achieve very slow growth rates and measured the induction lag for β-galactosidase. Their data are compatible with an up to 50% variation in peptide elongation rate between the fastest and the slowest succinate cultures, but it was concluded that this variation was negligible compared with the >10-fold variation in growth rate. This was the major issue at that time.

By the same type of experimentation, Jacobsen (cited in reference 39) concluded that the peptide chain elongation rate was constant at different growth rates. However, we shall point out that the determination of the low background level of β-galactosidase is very critical for the induction lag estimation. Perhaps also, as argued by Sørensen (M. A. Sørensen, Ph.D. thesis, University of Copenhagen, Copenhagen, Denmark, 1988), this method gives more weight to the rate of the most rapid ribosomes in the population which are likely to be less affected by changes in the substrate supply than the average ribosome since these ribosomes found the substrates in the shortest time.

COUPLING BETWEEN TRANSCRIPTION AND TRANSLATION

The degree of coupling between transcription and translation is expected to vary as a function of the concentrations of the activated precursors for both processes, if these are indeed subsaturated with their substrates. On the one hand, this must be the case, since the transcription attenuators, reacting to variations in the distance between RNA polymerase and the first coupled ribosome, are able to control the expression of both amino acid biosynthetic operons and nucleotide biosynthetic genes in response to the supply of the ribosomes with aminoacyl-tRNAs and of RNA polymerase with NTPs, respectively (43, 44, 55). On the other hand, it seems unlikely that the cell will allow the RNA polymerase just to move ahead, independently of the ribosomes, since such a condition of decoupling creates transcriptional polarity in many genes (1).

TABLE 1. Adjustment of transcription elongation rate
to speed of the ribosomes[a]

Pyrimidine added	16 amino acids per s			5 to 6 amino acids per s		
	UTP pool (μmol/g)	Sp act (U/mg of protein)		UTP pool (μmol/g)	Sp act (U/mg of protein)	
		ATCase (*pyrBI*)	OPRTase (*pyrE*)		ATCase (*pyrBI*)	OPRTase (*pyrE*)
Uridine	3.1	27	37	3.0	3	2
Uracil	2.9	34	40	2.7	5	3
None	2.4	75	60	1.4	69	33

[a] Strain SØ3829 (*rpsL*+) has ribosomes moving at a rate of ca. 16 amino acids per s, while strain SØ3831 (*rpsL*) has ribosomes that work at a speed of 5 to 6 amino acids per s. The two strains were grown at 37°C in the presence of different pyrimidine supplements. Cells were harvested for determination of NTP pools and the level of pyrimidine biosynthetic enzymes. The data presented were rearranged from reference 42.

We propose that *E. coli* possesses mechanisms to prevent too much decoupling between transcription and translation and that these mechanisms make up a central part of the metabolic growth rate control. Thus, the distance between the transcribing RNA polymerase and the first coupled ribosomes in the *pyrBI* and *pyrE* attenuator regions was found to be almost identical in wild-type *E. coli*, where the ribosomes propagate the protein chains by an average rate of 16 amino acids per s, and in an *rpsL* mutant, where the ribosomes move by only 5 to 6 amino acids per s (42) (Table 1). Moreover, Jaquet and Kepes (41) observed that completion of the *lacZ* mRNA chain in vivo required a longer time when the ribosomes were inhibited.

This adjustment of RNA polymerization to the speed of the ribosomes may reside in the structure of the genes themselves and may also involve the concentration of substrates and inhibitors of RNA polymerase activity. Thus, transcription elongation in vitro appears generally to consist of intervals when RNA polymerase moves very rapidly, alternating with other intervals when RNA polymerase pauses at specific positions. The pause sites are closely spaced along the template (51, 52), and they often coincide with regions where the RNA chain has a potential to form secondary structures that interfere with the base pairing between the transcript and the DNA template strand in the transcription bubble (55, 88, 95). The duration of the transcriptional pauses generally increases as a function of either decreasing substrate (NTP) concentrations (51, 93) or increasing concentrations of ppGpp, which, thereby, becomes an inhibitor of RNA chain elongation in general (52, 53). In addition, the NusA protein, which actually may be an elongation-specific subunit of RNA polymerase (34, 38), inhibits RNA chain elongation in vitro in a manner dependent on the concentrations of the RNA polymerase substrates (51, 86, 88).

The ribosomes must be able to "iron out" secondary structures in the mRNA chains, since the translation rate in vivo is unaffected by the presence of even very stable secondary structures in the *lacZ* mRNA (89) and since this "ironing" function is part of the mechanistic basis for control of gene expression by attenuation (55). Specifically, the ribosomes were shown to be able to inactivate a terminating hairpin structure in the *pyrBI* mRNA (78) and to release RNA polymerase from a paused transcription complex at a secondary structure in the *trp* leader (54).

The translating ribosomes move slowly when they are short of substrates. Such a condition arises when there are too many ribosomes relative to the capacity of the medium to supply them with their substrates. When starving, the ribosomes possess an occupied P site and an uncharged tRNA in the A site and synthesize considerable quantities of ppGpp (37, 71). Moreover, a decrease in the ribosome propagation speed, caused by a reduction in the substrate supply, will increase attenuation at some of the pyrimidine nucleotide biosynthetic genes and, thereby, reduce the formation of the NTP substrates for RNA polymerization on a long time scale. The point is that these mechanisms all tend to adjust the transcription elongation rate during both stable RNA and mRNA chain syntheses in response to changes in the performance rate of the ribosomes. The potential physiological importance of this is outlined below.

STRINGENT RESPONSE AND CONTROL OF RIBOSOME SYNTHESIS

Stringent Response and Synthesis and Effects of ppGpp

When amino acids are removed from the medium or when wild-type *E. coli* is exposed to an energy-carbon source downshift, e.g., by addition of α-methylglucoside to the culture, the rate of stable RNA (and, thus, ribosome) synthesis is abruptly reduced. Moreover, the individual ribosome loses polymerization speed and ppGpp accumulates transiently to very high levels (48, 66), indicating subsaturation with the substrates, because the ppGpp synthetase (the *relA* gene product) is activated in the starved ribosomes (37, 71). As the ppGpp pool falls again to the new postshift level, the synthesis of stable RNA chains resumes at its new steady-state velocity and, within a few minutes following the downshift, the ribosomes acquire an elongation speed which is only slightly lower than the preshift rate (48).

In *relA* mutants, no such rapid accumulation of ppGpp takes place, and stable RNA synthesis adjusts only slowly to the new and lower steady-state level which is the same as for wild-type (*relA*+) cells. Furthermore, in *relA* strains, the downshift causes a long period of time (1 to 2 h) in which the ribosomes operate at reduced speed (48), indicating a long period of starvation of the ribosomes for the activated aminoacyl-tRNA substrates. For a similarly long period of time, a strong polarity is observed for the transcription of *lacZ* (48), indicating that RNA polymerase runs away from the leading ribosomes in *relA* strains following the downshift. In the wild-type (*relA*+) strains, the downshift causes no such long period of polarity in *lacZ* gene expression (48), in accordance with the finding that ppGpp inhibits RNA chain elongation in vitro (53) and with our postulate that a high concentration of ppGpp prevents RNA polymerase from running significantly away from the ribosomes.

It appears that the decrease of stable RNA accumulation in the wild-type strain during amino acid starvation takes place at the synthesis level (72) as a control of transcription initiations at the promoters, at least for the *rrnA* operon (83). As a mechanistic basis for this, Baracchini and Bremer (3) have proposed that ppGpp binds to RNA polymerase and divides it into two forms with different specificity in the promoter recognition reaction. Thus, the ppGpp-complexed form of RNA polymerase should be unable to initiate transcription at stable RNA promoters, while the ppGpp-free RNA polymerase was considered able to bind at all promoters (3).

We prefer to consider ppGpp as an inhibitor of the RNA chain elongation reaction because this is a well-documented effect of ppGpp on transcription in vitro at concentrations similar to the steady-state pools of ppGpp in vivo (50 to 200

μM) (30, 52, 53). Accordingly, the ppGpp binding site on RNA polymerase need not control the promoter selection directly, as suggested by Baracchini and Bremer (3). Furthermore, our hypothesis may explain why several RNA polymerase β- and β'-subunit mutations appear to be defective in the stringent response and the control of ribosome synthesis (59, 67, 70), since such mutations may well have altered the general RNA chain elongation kinetics. Thus, we explain the ppGpp effects on transcription initiation as being due to a general inhibition of RNA chain elongation, which causes a greater fraction of RNA polymerase to become sequestered in the elongation processes and, in turn, lowers the concentration of free RNA polymerase available for initiation purposes. This affects the stringently controlled promoters in a very differentiated pattern. In accordance with this, the consensus promoter regions of the gene for tRNA$_1$^{Leu} and of the rrnB operon were shown to contain all information necessary for growth rate control and stringent regulation (21, 32, 33). Actually our model implies that all promoters are inhibited during the stringent response, but to varying degrees. The least affected, or most "persistent," promoters will be those which bind RNA polymerase avidly, but initiate transcription at a low frequency (Fig. 1).

It is important to notice that this model allows a ppGpp-independent regulation of stable RNA synthesis exerted via the concentration of the NTP substrates for RNA polymerization. Indeed, we have recently found that both ppGpp and the rate of stable RNA synthesis decrease during partial pyrimidine starvation (U. Vogel, S. Pedersen, and K. F. Jensen, unpublished data).

Apart from the biochemical analyses described by Kingston et al. (52, 53), there are other reasons to focus attention towards ppGpp acting as a transcription elongation inhibitor in vivo. These are the fact that relA mutants have long been known to be defective in the derepression of amino acid biosynthetic operon expression when amino acids are left out of the medium (91), combined with the observation that the ribosomes regain their elongation speed more rapidly following a nutritional downshift in wild-type bacteria than they do in relA strains (48). Both of these phenomena may be explained by the inhibitory effect of ppGpp on RNA chain elongation, since the attenuators of many amino acid biosynthetic operons may be trapped in a "hyperterminating" configuration, if RNA polymerase is not prevented from running away from the leading ribosomes when these lose speed due to a general shortage of charged tRNAs. The high concentration of ppGpp that accumulates in wild-type cells during aminoacyl-tRNA shortage is a likely candidate for promoting this coupling between transcription and translation, because many amino acid biosynthetic operon attenuators contain ppGpp-sensitive transcriptional pause sites in their leader regions (10, 55).

Our model in which ppGpp acts as an inhibitor of RNA chain elongation, thereby reducing the concentration of free RNA polymerase, cannot explain a stimulation of any promoter activity by ppGpp, as suggested (for example) for the his promoter by Shand et al. (87). What the model does allow, however, is an apparent stimulation for promoters that bind RNA polymerase avidly and, thereby, are relatively unaffected by the decreased concentration of free RNA polymerase induced by the ppGpp accumulation during the stringent response. Thus, what was observed by Shand et al. (87) was an approximately twofold increase, at a reduced growth rate, in the differential rate of β-galactosidase synthesis from a his-lacZ fusion in a relA$^+$ strain that was treated with serine hydroxamate and accumulated

ppGpp, combined with a strongly decreased rate of β-galactosidase synthesis after a similar treatment with serine hydroxamate of a relA strain in which the ppGpp pool decayed to half its original size. The latter observation may be due to the well-known polarity that arises in the lacZ gene when ribosome movement is inhibited without concomitant ppGpp accumulation (36, 48) and perhaps to misincorporation of amino acids into β-galactosidase in the starved relaxed strain (35). Thus, the very large difference in his-lac expression between relaxed and stringent strains treated with serine hydroxamate (87) does not prove that ppGpp stimulates his promoter activity: absolute synthesis rates were not measured.

Ribosome Synthesis

The rate of ribosome synthesis is limited by the formation of rRNA chains because the mRNAs for the ribosomal proteins carry sequences that resemble the binding sites for the ribosomal proteins of the rRNA chains. Thus, the ribosomal proteins are made as long as there are vacant binding sites for the proteins on new rRNA chains. However, when present in excess over rRNA, the proteins bind to their corresponding mRNAs and prevent their own synthesis (reviewed by Nomura et al. [69] and Lindahl and Zengel [58]).

Ribosomal Feedback Regulation

Nomura and co-workers have conducted a series of experiments which show that the synthesis of rRNA, and hence of ribosomes, is almost independent of gene dosage in E. coli. Thus, Jinks-Robertson et al. (47) observed that the synthesis of rRNA only increased by ca. 11% when the gene dosage was doubled by the presence of plasmids carrying the intact rrnE or rrnB operon. This indicated the existence of a feedback regulation of the (free, unengaged) ribosomes on rrn transcription, since the rRNA products, not the tRNAs, were responsible for the autoregulation (31) and since ribosomal operons with internal deletions were also inactive (47). By measuring also transcription from the tRNA genes, it was concluded (47) that the expression from the chromosomal rrn operons was repressed to compensate for the existence of the extra, plasmid-encoded rrn operons, but importantly, the transcription of tRNA genes outside the chromosomal rrn operons was repressed equally as much by the plasmids as were the tRNA genes inside rrn operons. Similarly, a repression by about 25% was also observed for the chromosomal rrn operons (97) and ribosomal protein mRNAs (cited in reference 97) 20 min after induction of an rrn operon.

Furthermore, two lines of evidence (15, 96) suggest that the apparent autoregulation of rRNA synthesis is due to ribosomes actively engaged in translation. First, shortage of initiation factor IF2, created by bringing the infB expression under lac-promoter-operator control, resulted in the accumulation of idle ribosomes without concomitant repression of rRNA synthesis (15). Second, a plasmid-encoded rrn operon with a poor matching to the Shine-Dalgarno sequence of E. coli mRNAs also caused no repression of chromosomal rrn operon expression (96).

This is consistent with our model, simply because the unengaged ribosomes do not use any aminoacyl-tRNAs and thus do not compete with the active ribosomes for substrates. In addition, the inactive ribosomes do not produce any ppGpp, since this reaction requires an occupied P site

and an uncharged tRNA in the A site (37, 71). Accordingly, we explain the results of Nomura and co-workers (15, 47, 68) as follows.

An increased gene dosage of *rrn* operons, as created by Jinks-Robertson et al. (47) or by Yamagishi et al. (97), does tend to increase ribosome synthesis (11% was observed in the steady state; 1.6-fold was seen in the induction experiment). This small increase in the ribosome number causes a corresponding decrease in the saturation of the ribosomes with their substrates and a corresponding rate loss at elongation, since the number of peptide bonds that can be formed per time unit per unit mass is dictated by the medium. The partially starving ribosomes synthesize increased amounts of ppGpp and tend also to reduce the pyrimidine NTP pools via effects on the attenuators. The increased ribosome number will, thus, reduce the rate of RNA chain elongation and, thereby, lower the concentration of free RNA polymerase and the frequency of transcription initiation at stringently controlled promoters, including the *rrn* promoters and the promoters for tRNA genes outside *rrn* operons.

Moreover, Cole et al. (15) increased the level of IF2 such that the idle ribosomes that had accumulated when there was a shortage of IF2 were now allowed to initiate translation. This resulted in an immediate stop of new rRNA synthesis which lasted until the ribosome number per cell was adjusted to be near the steady-state concentration characteristic for wild-type cells. We propose that the ribosomes, which suddenly become active and begin to consume substrates because of an adequate supply of IF2, cause a strong decrease in the saturation level of all ribosomes with their substrates and provoke a situation in the cells which resembles a nutritional downshift, when the ribosomes lose speed due to a change in the medium (see above). Thus, we expect that the ribosomes also in this case (15) will lose speed, because there are too many relative to the number which can be supported by the medium, and begin to produce more ppGpp. This sequesters RNA polymerase at elongation and thereby lowers the concentration of free RNA polymerase and the initiation frequency for transcription from (all) stringently controlled promoters. However, such general side effects of the experiment were not analyzed.

Autoregulation of RNA Polymerase Synthesis

The formation of RNA polymerase also seems to be autoregulated in the sense that the amount of enzyme does not increase in proportion to the gene dosage. Both transcriptional and translational controls seem to be involved (17). Bedwell and Nomura (5) have analyzed this by bringing the various genes for the RNA polymerase subunits under *lac*-operator-promoter control. They found that it was possible to overproduce the individual subunits considerably (8- to 13-fold) but that the functional RNA polymerase (core enzyme or holo-enzyme) could only be overproduced ca. twofold. Our model may explain the transcriptional part of this autoregulation since it predicts that the presence of an excess of RNA polymerase over the amount characteristic for the medium will result in a rate loss during RNA chain elongation. Thus, the fraction of free RNA polymerase and, hence, the initiation capacity will not increase in proportion to the total amount of RNA polymerase and, therefore, the amount of *rpo* mRNA will not increase in proportion to the gene dosage. Moreover, the excess RNA polymerase that (still) is formed is predicted to engage primarily in transcription from those promoters which were the least saturated in the unperturbed condition. This tends to increase the ribo-

some concentration and further enhance the rate loss during transcription elongation, as outlined in the preceding paragraph.

Nomura et al. (68) have used the constructions described above to analyze the consequences of varying the cellular content of RNA polymerase. When the isopropyl-β-D-thiogalactopyranoside concentration was lowered, a fall in the amount of RNA polymerase did indeed occur without initially affecting the growth rate or the rate of total protein synthesis. What appears to be in conflict with our model is that the total mRNA pool fell (slightly) before the synthesis of ribosomes, when the concentration of RNA polymerase was being reduced. However, this conflict is not necessarily real since, first, the synthesis of several mRNA chains may also be under stringent control like rRNA synthesis (19, 62) and, second, we expect that the reduced concentration of RNA polymerase will result in a higher rate of RNA chain elongation, in the same medium. Thus, the lowering of the amount of RNA polymerase could create transcriptional polarity in many mRNA genes due to the resulting decoupling between transcription and translation, while the stable RNA gene transcription may escape from this polarity, possibly due to the existence of antitermination mechanisms for these genes (56).

CONTROL OF PROTEIN SYNTHESIS AS A FUNCTION OF GROWTH RATE

At high growth rates, all proteins must accumulate more rapidly than they do in slow-growing cells just to keep their concentration constant. We propose that the increase in ribosome concentration as a function of the growth rate may contribute to ensure this metabolic control of protein synthesis. This is because the translation frequency per mRNA chain (see reference 7) will increase as a function of the ribosome concentration if the Shine-Dalgarno sequences on the mRNA chains are indeed subsaturated with the ribosomes, as suggested here. This might also apply to the synthesis of the proteins that trigger the initiation of a new round of DNA replication, when accumulated at a certain concentration (94). Thereby, the very increase in ribosome concentration as a function of the medium might contribute to control the frequency of DNA replication initiation as a function of the growth rate.

CONCLUSIONS

In this paper, we have attempted to describe the *E. coli* cell as an unsaturated system designed for rapid growth, but limited by the "feeder reactions" and the medium. We think that several growth-related phenomena, assumed by many to be regulated by specific mechanisms acting at the transcription initiation level, may be explained as consequences of medium-induced changes in the elongation kinetics during protein and RNA chain syntheses, because such changes interfere with the amount of free ribosomes and free RNA polymerases able to engage in new initiation reactions. Thereby, the chain elongation reactions, which directly consume the substrates, could be involved in determining the chain initiation reactions, which do not consume any substrates per se. This occurs by increasing or decreasing the strength of competition between the ribosome binding sites on different mRNA chains and between different promoters, whose primary sequences and abilities to perform in the competition are designed by evolution. Evidently, our model does not rule out the existence of additional control

mechanisms acting at the level of promoter function and refining the adjustment of the cell composition to the medium, but we believe that the principles in the model will be superimposed on the specific control mechanisms and apply to all gene expression in *E. coli*. Thus, the kinetics of the macromolecular chain elongation reactions and the coupling between translation and transcription (made possible by the absence of a nuclear membrane) are features worthy of consideration when experiments aimed to elucidate the growth physiology of bacteria are designed and interpreted.

Our postulate that the pattern of transcription from the bacterial chromosome is dictated, primarily, by competition between the promoters for the binding of a limited supply of free RNA polymerase is similar to Maaløe's ideas about a passive growth rate control of macromolecular biosynthesis (39, 60). However, as Maaløe considered the RNA chain elongation rate as invariable, he regarded the repression of biosynthetic gene activity in the richer media as the only source of increasing the amount of free RNA polymerase. Thus, Maaløe had difficulties in explaining the differences between the transcription patterns seen with different carbon sources in minimal media. However, a variation in the RNA chain growth rate seems to us as important as repression or derepression of biosynthetic gene activity for the concentration of free RNA polymerase in the cells. In all likelihood, both parameters contribute to determine the composition of *E. coli* as a function of the medium.

There are also formal similarities between our model and the model proposed by Bremer and co-workers in the sense that the steady-state pool of magic spot, ppGpp, is considered important for the control of stable RNA synthesis and that both models regard RNA polymerase as limiting for the total transcription initiation frequency. However, Baracchini and Bremer proposed (3) that ppGpp influences the transcription pattern by causing a partitioning of RNA polymerase in two forms: a ppGpp-bound form of RNA polymerase, which is unable to initiate transcription at stable RNA promoters while being able to produce mRNA chains; and a ppGpp-free form of RNA polymerase able to initiate transcription at all promoters. We prefer the idea that ppGpp acts by inhibiting RNA chain elongation, since this is a well-documented biochemical observation (52, 53) and because this hypothesis predicts that other perturbations of the transcription elongation kinetics (such as changes in the nucleotide pools or mutations in the RNA polymerase genes) analogously may regulate the global transcription pattern of the cell. Still, the equations (7) relating the ppGpp concentration to the cellular composition and the growth rate will apply to our model since we regard the "inactive" fraction of RNA polymerase, unable to transcribe the stable RNA genes (7), as being sequestered in the elongation phase of transcription. Thus, the two models are quite different at the mechanistic level and they suggest different experimental ways to solve the problem of the growth rate control and the stringent response. Our model focuses attention towards the macromolecular chain elongation reactions, as well as on the promoters, while the study of the promoters usually has dominated these considerations totally.

ACKNOWLEDGMENTS

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