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Regulatory State of Ribosomal Genes and Physiological Changes in the Concentration of Free Ribonucleic Acid Polymerase in *Escherichia coli*

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The concept of promoter efficiency is introduced as frequency of RNA chain initiation at a given promoter normalized to the intracellular concentration of free (but functional) RNA polymerase. Previous observations from this laboratory on the synthesis of ribosomes and β-galactosidase are used to show that during a nutritional shift-up from succinate minimal to glucose–amino acids medium (3-fold increase in steady-state growth rate) the concentration of free (active) RNA polymerase decreases to one-quarter of the pre-shift value and the promoter efficiencies of the genes for ribosomal RNA and ribosomal proteins increase 9- and 6-fold respectively. This extent of control of ribosomal genes is much greater than expected on the basis of the increase in the rate of ribosome synthesis (3-fold).

Concepts

Free active RNA polymerase

The RNA polymerase molecules in a growing bacterium may be grouped into three classes: active transcribing RNA polymerase, active (functional) free RNA polymerase (enzyme molecules that have just finished catalysis of an RNA chain and are ready to bind to another promoter) and inactive polymerase (i.e. an inactive modification). The number of transcribing RNA polymerase molecules has been determined for *Escherichia coli* B/r from the number of growing RNA chains (quotient synthesis rate/chain-growth rate; Dennis & Bremer, 1974*c*). The total number of RNA polymerase molecules in *E. coli* has been estimated from the amount of β and β′ RNA polymerase (subunit) protein; it was found to be more than twice as much as the number of transcribing RNA polymerase molecules (Matzura *et al.*, 1971; Dalbow, 1973; Iwakura *et al.*, 1974). Although these estimates may be subject to considerable experimental error, both Matzura *et al.* (1971) and Dalbow (1973) concluded that the calculated excess of non-functioning RNA polymerase is real. Their conclusion is supported by the recent finding that DNA-free bacterial 'minicells' contain β and β′ subunits of RNA polymerase, but no RNA polymerase activity (Rogerson & Stone, 1974).

The difference, total minus transcribing RNA polymerase, must be equal to the sum of free active plus inactive polymerase. It is not known how much is free, and how much is inactive. The following evaluation of previously published results from this laboratory suggests that the number of free active RNA polymerase molecules is very small compared with the number of transcribing RNA polymerase molecules.

Promoter efficiency

The regulatory state of different genes may be measured by comparing the rates of RNA chain initiation at the promoters of those genes. Such initiation rates, however, become an inadequate measure for gene control in the case of gross changes in the activity of the genome such as occur in bacteria during a nutritional shift-up. The reason for this is the change in the concentration of free (active) RNA polymerase which accompanies such gross changes in genome activity. Let us assume (the hypothetical case) that all genes of the bacterial genome were half repressed, i.e. half the time their promoters are blocked, the remaining time their promoters are open for transcription. Let us further assume that the total rate of transcription is mainly limited by active RNA polymerase (i.e. not by DNA), for example, 99% of the active polymerase may be transcribing at any time, and 1% is free (the inactive polymerase can be ignored). Now we assume that, owing to some regulatory event, all genes become fully de-repressed. Although each gene is now potentially twice as active as before, the transcription of each gene can increase maximally (without increase in total active RNA polymerase) only by 1%. However, the fraction of free RNA polymerase will decrease twofold (to about 0.5%). (This may be quantitatively shown by applying the mass-action law† to the reaction 'free

† The application of the mass-action law is formally correct, although the meaning of the terms is different from standard enzyme–substrate reactions, since, in the case of transcription, there is no normal reversibility: the free promoter is liberated immediately when the polymerase begins transcription, but the free polymerase is liberated only after termination of transcription.

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polymerase+promoters → transcribing polymerase' and setting the 'concentration of promoters' first to one-half and then to unity.) Thus, in this example, the fraction of transcribing RNA polymerase, and the transcription rate of each gene, will increase only insignificantly (from 99 to 99.5%) in spite of a two fold induction of each gene.

Several conclusions can be drawn from this consideration.

(1) The quotient transcription rate (RNA chain initiations per min at a given promoter) over the concentration of free (active) RNA polymerase (polymerase molecules per ml of intracellular space) can be used as a measure for the regulatory state of a given gene and is defined here as promoter efficiency. In the hypothetical example above, the promoter efficiency of all genes increased twofold.

(2) An increase in the promoter efficiency of a gene is equivalent to an induction of that gene. For constitutive (i.e. unregulated) genes, the promoter efficiency is a constant property of the promoter, independent of the physiological state of the bacteria.

(3) A change in the promoter efficiency of any one gene due to some (gene-specific) regulatory event causes a change in the concentration of free RNA polymerase and a corresponding change in the transcription rate of all other genes, but no change in the promoter efficiency of the other genes. The effects on the free polymerase may cancel if regulatory events at different promoters have opposite directions (i.e. if some genes are repressed and others de-repressed).

(4) If the promoter efficiency increases n-fold of one gene or of a small number of genes [whose summed promoter efficiencies is only a small fraction of the total (summed) promoter efficiency of all genes], then the free RNA polymerase will not decrease significantly and these induced genes will become essentially n-fold more active. If, however, the promoter efficiency of a large number of genes or of a small number of very active genes (e.g. rRNA genes) increases n-fold, then the free RNA polymerase will decrease significantly and the activity of the induced genes will increase less than n-fold.

(5) The intracellular concentration of free active RNA polymerase is proportional to the intracellular concentration of active (transcribing plus free) RNA polymerase and inversely proportional to the summed promoter efficiencies of all genes of the genome.

(6) The promoter efficiency is also a measure for the probability (per unit of time) that an RNA polymerase molecule will initiate transcription at the promoter considered when the concentration of free polymerase is unity. For that reason the promoter efficiency is related to the 'affinity' of the promoter for RNA polymerase; however, the promoter efficiency includes the regulatory state of the promoter; i.e. only for a fully de-repressed or for a constitutive gene are 'affinity' and 'promoter efficiency' identical. (For positive gene control, the 'affinity' cannot be defined in a meaningful way as long as we do not know the exact mechanism of positive control.) For negative control, promoter efficiency may be regarded as the product 'affinity times the relative proportion of time the repressor is dissociated from the operator'. The latter factor has previously been designated ε (Maaløe, 1969).

In the following, these concepts* will be applied to the changes in the regulatory state of ribosomal genes and in the concentration of free RNA polymerase occurring when bacteria are subjected to a nutritional shift-up.

Derivation of the Promoter Efficiency from Measurable Physiological Parameters

Neither the concentration of free RNA polymerase nor the promoter efficiency of a given gene (x) can be measured directly. These parameters can be derived from the following measurable parameters: ψ_m is the fraction of transcribing RNA polymerase engaged in the synthesis of mRNA; α_c is the differential synthesis rate of a gene product from a constitutive gene (quotient constitutive protein/total protein); α_x is the differential synthesis rate of gene x protein (quotient gene x protein/total protein).

Additional parameters need to be defined but not be measured since they cancel when the calculation considered below is applied to a nutritional shift-up: Pol_x is the number of RNA polymerase molecules transcribing gene x (in arbitrary units, for example per unit of culture volume, or per genome equivalent of DNA); Pol_t is the total number of transcribing RNA polymerase molecules (same units as for Pol_x); ψ_x is the fraction of total transcribing RNA polymerase molecules transcribing gene x: $\psi_x = Pol_x/Pol_t$; G_x is the number of genes x (same units as for Pol_x); f_x is the frequency of RNA chain initiation at the promoters of gene x (RNA chain initiations/min per promoter); t_x is the transcription time of gene x (min), i.e. the time it takes for an RNA polymerase molecule to transcribe the gene considered.

The wanted parameters are:

Pol_f/ml = intracellular concentration of free active RNA polymerase (number of free RNA polymerase molecules per ml of intracellular space contained in one unit used to measure Pol_x)

p_x = promoter efficiency of gene x, defined by $p_x = f_x/(Pol_f/ml)$

* Although we have developed these concepts from our previously defined fractions of RNA polymerase engaged in the transcription of stable and of mRNA (Bremer *et al.*, 1973), it is clear that they were also implied in an earlier analysis of bacterial growth by Maaløe (1969).

The fraction of RNA polymerase molecules transcribing gene x (ψ_x) is a subfraction of the polymerase molecules transcribing total mRNA (ψ_m). If gene x-mRNA were translated as frequently as average mRNA, then the ratio ψ_x/ψ_m would be equal to the ratio of gene x protein to total protein (α_x). However, since gene x-mRNA may be, by a certain factor (k_x), more or less efficient as template for translation than average mRNA, one can write

$$\psi_x = k_x \cdot \alpha_x \cdot \psi_m \qquad (1)$$

(Note that α_x and ψ_m are measurable parameters.) By definition, ψ_x is also the ratio of the numbers of RNA polymerase molecules transcribing gene x to the total number of transcribing RNA polymerase

$$\psi_x = \frac{Pol_x}{Pol_t} \qquad (2)$$

Further, the number of RNA polymerase molecules transcribing gene x (at any given instant) is related to the initiation frequency at promoter x (f_x), the gene number (G_x) and the transcription time of mRNA for gene x (t_x):

$$Pol_x = G_x \cdot f_x \cdot t_x \qquad (3)$$

Combining these three equations and resolving for f_x gives the frequency with which a given gene x is transcribed:

$$f_x = \frac{Pol_t \cdot k_x \cdot \alpha_x \cdot \psi_m}{G_x \cdot t_x} \qquad (4)$$

and the promoter efficiency of gene x, by definition:

$$p_x = \frac{f_x}{(Pol_f/ml)} \qquad (5)$$

Analysis of a Nutritional Shift-Up

Change in the concentration of free RNA polymerase

Now the promoter efficiency of a constitutive gene (p_c) is considered, shortly before and shortly after a nutritional shift-up (at zero time, $t = 0$), i.e. p_c for $-t \to 0$ (pre-shift value) and for $+t \to 0$ (post-shift value), designated $p_c(-0)$ and $p_c(+0)$. Only the change in this value, the ratio $p_c(+0)/p_c(-0)$ is needed.

Using eqns. (4) and (5) and assuming the following parameters to be constant during the few minutes after the shift-up: number of genes (G_c), synthesis time (t_c), mRNA-translation efficiency relative to the efficiency of average mRNA translation (k_c), and total number of transcribing RNA polymerase molecules (Pol_t) (such that these parameters cancel), one obtains:

$$\frac{p_c(+0)}{p_c(-0)} = \frac{\alpha_c(+0) \cdot \psi_m(+0)/\alpha_c(-0) \cdot \psi_m(-0)}{Pol_f(+0)/Pol_f(-0)} \qquad (6)$$

On the other hand, constitutive genes are unregulated, such that their transcription rate depends only on the intrinsic promoter properties and on the concentration of free RNA polymerase, i.e. the promoter efficiency of constitutive genes does not change during a shift-up:

$$p_c(+0) = p_c(-0) \qquad (7)$$

Thus by combining eqns. (6) and (7), the change in free RNA polymerase can be obtained:

$$\frac{Pol_f(+0)}{Pol_f(-0)} = \frac{\alpha_c(+0) \cdot \psi_m(+0)}{\alpha_c(-0) \cdot \psi_m(-0)} \qquad (8)$$

We have previously measured the differential synthesis rate of β-galactosidase (α_{lac}) before and after a shift-up from succinate minimal to glucose–amino acids medium under conditions which were assumed to render the *lac* operon physiologically constitutive (high concentrations of inducer and cyclic AMP) and found α_{lac} to decrease to 0.4 of the pre-shift value within a few minutes after the shift (Dalbow & Bremer, 1975). Similarly, ψ_m was found to decrease within 5 min after a shift-up from 0.78 to 0.50 (Dennis & Bremer, 1974*b*). Substituting these values into eqn. (8) gives the change in the concentration of free RNA polymerase:

$$\frac{Pol_f(+0)}{Pol_f(-0)} = \frac{0.4 \cdot 0.50}{1.0 \cdot 0.78} = 0.26$$

i.e. the intracellular concentration of free RNA polymerase decreases to about one-quarter of the pre-shift concentration within minutes after the shift-up.

We have previously observed that the number of transcribing RNA polymerase molecules does not significantly change immediately after a shift-up [$Pol_t(+0) = Pol_t(-0)$ (Dennis & Bremer, 1974*b*); this was also implied for the derivation of eqn. (6)]. If the free polymerase decreases, but total transcribing RNA polymerase does not significantly increase, it implies that the number of free polymerase molecules is much smaller than the number of transcribing RNA polymerase molecules [$Pol_f(\pm 0) \ll Pol_t(\pm 0)$], i.e. most of the active RNA polymerase is engaged in transcription at any time. This means that the rate of bacterial RNA synthesis is essentially determined by the amount of active RNA polymerase and is not limited by DNA.

The conclusion, that at any time most of the active bacterial RNA polymerase is engaged in transcription while only a small fraction is free implies that the excess of β and β' RNA polymerase subunits found in bacteria (Dalbow, 1973; Matzura *et al.*, 1971) is in the form of inactive RNA polymerase, as mentioned above.

Change in the regulatory state of ribosomal genes

Applying eqn. (6) to ribosomal protein genes (denoted by the subscript r) and substituting $\alpha_r(+0)$ and $\alpha_r(-0)$ equal to 0.19 and 0.085 (Dennis & Bremer, 1974*a*); $\psi_m(+0)$ and $\psi_m(-0)$ equal to 0.50 and 0.78 (Dennis & Bremer, 1974*b*) and the change in free RNA polymerase [denominator in eqn. (6)] equal to 0.26 (see preceding section), gives the change in the promoter efficiency for ribosomal protein genes:

$$\frac{p_r(+0)}{p_r(-0)} = \frac{0.19 \cdot 0.50/0.085 \cdot 0.78}{0.26} = 5.5$$

Similarly, the change in the promoter efficiency of rRNA genes is found from the fraction $\psi_s(=1-\psi_m)$ of RNA polymerase molecules engaged in the transcription of stable RNA [rRNA plus tRNA; both rRNA and tRNA gene activities increase similarly after a shift-up, according to Dennis (1972)]:

$$\frac{p_{rRNA}(+0)}{p_{rRNA}(-0)} = \frac{\psi_s(+0)/\psi_s(-0)}{0.26} = \frac{0.50/0.22}{0.26} = 8.7^*$$

This corresponds to a 6- or 9-fold induction respectively, of the ribosomal protein and rRNA genes. [The lower degree of induction of ribosomal protein genes compared with rRNA genes is compensated by an increased translation of mRNA (Dennis & Bremer, 1974*c*).] These values are much greater than the actual increases in gene activity (frequency of RNA chain initiation); the difference is due to the decrease in the concentration of free RNA polymerase.

Effect of systematic experimental error

The calculation of the change in the concentration of the free RNA polymerase requires four experimental measurements, i.e. two ψ_m values and two α_{lac} values [eqn. (8)]. Of these four values, the ψ_m measurements have the greatest uncertainty, since they were obtained from a complex evaluation of experiments involving treatment of the bacteria with ethylenedinitrilotetra-acetic acid and rifampicin, and a correction for the difference in the chain-elongation rates of rRNA and mRNA (Bremer *et al.*, 1973; Dennis & Bremer, 1974*b*). However, the ψ_m values in eqn. (8) occur as ratios of the pre- and post-shift values, such that systematic errors in the absolute values cancel partially. For example, if the ψ_m values were not corrected for the difference in the chain-elongation rate (i.e. if one assumes that rRNA and mRNA chains grow with the same velocity), the concentration of free RNA polymerase would decrease, in a shift from succinate minimal medium to glucose–amino acids medium, to 18%, rather than to 26%, of the pre-shift value.

In the calculation of the change in the promoter efficiency of the genes for ribosomal proteins, all ψ_m values cancel [combination of eqns. (6) and (8)]. Still, this calculation requires four experimental measurements, i.e. two α_r values and two α_{lac} values. Both pairs of α values occur as ratios whose experimental error is estimated from the fluctuation of results in repeated measurements to be about 20% (Dennis & Bremer, 1974*a*; Dalbow & Bremer, 1975).

Apart from experimental errors, the estimates depend on two assumptions: (1) that there is no gene-specific regulation on the translational level in *E. coli* (no regulation that affects the translation of different mRNA species differently after a shift-up); (2) that high concentrations of cyclic AMP abolish catabolite repression (see the Discussion section in Dalbow & Bremer, 1975). Currently, all evidence speaks in favour of these two assumptions, but they are not proven.

*This calculation assumes that the promoters of all genes compete for the same type of RNA polymerase; i.e. that all control occurs at the level of the DNA and not at the level of polymerase.

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