Metabolic Regulation of β -Galactosidase Synthesis in *Escherichia coli*

A TEST FOR CONSTITUTIVE RIBOSOME SYNTHESIS

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The rate of differential synthesis of β -galactosidase (α_{lac}) was measured in maximally induced cultures of *Escherichia coli* B/r with 0.01M-inducer and 0.01M-cyclic AMP. The value of α_{lac} decreases with growth rate (60% between 0.67 and 2.1 doublings/h) and after a nutritional shift-up. This decrease is presumed to reflect a decrease in the intracellular concentration of free active RNA polymerase after a shift-up, which implies that the increase in ribosome synthesis after a shift-up is due to an active induction of the ribosomal components.

To test the suggestion that ribosomal proteins are synthesized constitutively (Maaløe, 1969), we measured the synthesis of the enzyme β -galactosidase under conditions that were used previously for measurement of the synthesis of ribosomal components in *Escherichia coli* B/r (Dennis & Bremer, 1974a,b), and where the expression of the *lac* operon is presumed to be physiologically constitutive (high concentrations of inducer and cyclic AMP). In a strict sense, *lac*-gene expression is not constitutive under these conditions since it is controlled by the cyclic AMP acceptor-protein factor. However, after saturation of the cyclic AMP acceptor-protein factor with cyclic AMP and inactivation of the *lac* repressor by inducer, transcription of the *lac* operon is assumed to be limited by the intracellular concentration of free active RNA polymerase, a property which is used here to define constitutivity.

The expression of constitutive genes may be modulated, reflecting the effect of non-gene-specific factors, when bacteria are grown under different physiological conditions. One such non-gene-specific factor is the concentration of free active RNA polymerase. Although the promoters of different constitutive genes may respond unequally to changes in the concentration of free RNA polymerase, at least the direction of response (increase or decrease) should be the same. Hence, if ribosomal proteins were synthesized constitutively, their rates of synthesis should vary with growth rate (or after nutritional shift-up) in a similar manner as the rate of synthesis of any other constitutive gene product. The results obtained here suggest that constitutive genes and ribosomal genes respond in opposite directions to a nutritional shift-up and therefore

the results do not support the idea of constitutive ribosome synthesis.

Since these results may be influenced by catabolite-repression effects, they are not a final proof for active ribosome control; however, the results obtained here are qualitatively consistent with results obtained previously on expression of the constitutive *trp* gene at different growth rates (Rose & Yanofsky, 1972). Together, these observations suggest that active ribosome control is occurring.

Materials and Methods

Bacterial strain and growth medium

Cultures of *E. coli* B/r (A.T.C.C. 12407) were grown at 37°C in minimal C medium (Helmstetter, 1967) supplemented with 0.2% of either succinate, glycerol or glucose and with or without a mixture of 20 synthetic L-amino acids, each to a final concentration of 20 μ g/ml. Experimental cultures were inoculated with a fresh stationary-phase culture in the same medium (at least a 500-fold dilution).

β -Galactosidase induction and assay

β Galactosidase synthesis was induced by adding isopropyl- β -D-thiogalactopyranoside (Sigma Chemical Co., St. Louis, Mo., U.S.A.) to a final concentration of 0.01M when the culture had reached a turbidity of 0.3 E_{460} unit; cyclic AMP was added 15 min before induction to a concentration of 0.01M. These concentrations give maximal induction (Fig. 1).

The amount of enzyme was assayed by the method of Kepes & Beguin (1966). Samples (0.1 ml) of the induced culture were added to 0.7 ml of C medium

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Fig. 1. Dependence of β -galactosidase synthesis on the concentration of inducer and cyclic AMP

(a) A culture growing in glucose-amino acids medium was induced at an E_{460} of 0.30 with various concentrations of isopropyl β -D-thiogalactopyranoside either with (■) or without (●) cyclic AMP (0.01 M, added 15 min before induction). Samples (0.1 ml) were taken after 20 min and assayed for β -galactosidase as described in the Materials and Methods section, except that the assay time was 2 h. (b) A culture growing exponentially in succinate minimal medium was treated with various concentrations of cyclic AMP and after 15 min, at an E_{460} of 0.44, was induced. Half of the cultures were supplemented with glucose and amino acids at the time of induction (●, ■), the other cultures were given no further growth supplements (○, □). Samples were taken at 8 min (○, ●) or 16 min after induction respectively and assayed as above.

(minus supplements) containing 50 μ l of toluene-Triton X-100 (0.3 μ l of Triton X-100/ml of toluene) at 0°C and mixed vigorously. After 30–60 min at 0°C the toluene-treated samples were incubated overnight at 30°C to evaporate the toluene. Then 0.8 ml of substrate mixture [0.125 M-sodium phosphate buffer, pH 7.0, 0.05 M-2-mercaptoethanol, 2 mg of *o*-nitrophenyl β -D-galactopyranoside/ml (Sigma)] was added, and after 1 h (unless stated otherwise) at 30°C, the reaction was stopped with 1.6 ml of 1 M- Na_2CO_3 . The E_{420} was read within 4 h; samples with an E_{420} greater than 0.8 were diluted 1:4 before reading.

Determination of the differential rate of enzyme synthesis

The differential rate of enzyme synthesis is calculated from the slope of the graph of the induction kinetics. To correct for the growth of the culture during an experiment, the induction kinetics were plotted with an expanded time-scale (McCarthy & Britten, 1962); the expanded time-value is given by

$$t' = \frac{D}{\ln 2} (2^{t/D} - 1)$$

where D is the doubling time, t' is chosen such that the abscissa values (t') are proportional to the increase in the rate of total protein synthesis (per ml of culture) between zero time and the sampling time. For steady-state growth the rate of protein synthesis in a culture is also proportional to the cell mass (turbidity of the culture), such that the slope of the graph of the induction kinetics corresponds to the enzyme activity per cell mass or enzyme activity per total protein content (i.e. specific activity or differential rate of enzyme synthesis; see the Discussion section).

After a nutritional shift-up, the rate of protein synthesis increases within 5 min with the post-shift steady-state rate constant (Schleif, 1967); therefore after a shift-up, the same expansion factor was used as for steady-state growth in the post-shift medium.

The slope of the straight portion of the induction kinetics curve ($t > 5$ min) was measured by using the units of time for $t \rightarrow 0$ (i.e. by ignoring the scale expansion). The slope defined in this way corresponds to the rate of enzyme synthesis in E_{420} units/min in a culture with a turbidity equal to that of the culture at zero time (induction or shift-up time).

The differential rate of β -galactosidase synthesis (α_{lac}^*) for steady-state growth is then found by using the relationship:

$$\alpha_{lac}^* = \frac{\text{slope } (E_{420} \text{ units/min}) \text{ per } E_{460} \text{ of culture at induction time}}{\text{rate of protein synthesis per } E_{460} \text{ (amino acids incorporated/min per } E_{460} \text{ unit)}}$$

Table 1. Doubling time, rate of protein synthesis and ribosome efficiency for *E. coli* B/r growing in different media

For details see the Materials and Methods section. The rate of protein synthesis is given as $10^{15} \times$ amino acids/min per E_{460} unit of culture (Dennis & Bremer, 1974c; H. Bremer & P. P. Dennis, unpublished work). The relative ribosome efficiency is the rate of protein synthesis per average ribosome, normalized to the ribosome efficiency in succinate-grown bacteria, which is set at unity (Dennis & Bremer, 1974a).

Growth medium	Doubling time (min)	Rate of protein synthesis	Relative ribosome efficiency
Succinate	90	3.60	1.00
Glycerol	55	5.28	1.17
Succinate-amino acids	46	6.10	1.41
Glucose	45	6.40	1.41
Glycerol-amino acids	38	6.90	1.41
Glucose-amino acids	20	8.10	1.41

The values for the rate of protein synthesis per E_{460} unit of culture in different media are listed in Table 1. If the assay reaction time was different from 1 h, then the slope was divided by the actual assay time (in h). An example of this calculation is taken from Fig. 3: the slope of the straight portion of the induction kinetics (measured in the units of time for $t \rightarrow 0$) corresponds to an increase of 0.023 E_{420} unit per min. This slope is normalized for 1 h assay time (division by 0.5) and for an initial bacterial density of 1 E_{460} unit/ml (division by 0.322), corresponding to $0.023/(0.5 \times 0.322) = 0.144$ E_{420} unit of enzyme activity/min per E_{460} unit of bacteria (rate of enzyme synthesis). The rate of total protein synthesis in this medium is 8.1×10^{15} amino acid residues/min per E_{460} unit of bacteria (Table 1); the quotient (α_{lac}^*) 'rate of enzyme synthesis/rate of total protein synthesis' is $0.144/8.1 \times 10^{15}$ or 1.8×10^{-17} (E_{420} units/amino acid residue). This value is plotted in Fig. 4 as one of the five experimental points at μ (growth rate) = 2.0.

After a shift-up, the total rate of protein synthesis (per E_{460} unit) of the pre-shift medium was used, multiplied by the increase in the ribosome efficiency (e_r , rate of protein synthesis per average ribosome; see Table 1) owing to the shift-up. (Note that if the ribosome efficiency or the peptide chain-elongation rate increases while the differential rate of enzyme synthesis remains unchanged, then the absolute rate of enzyme synthesis or the slope of the induction kinetics respectively must increase by the same factor.) Thus after a shift-up:

$$\alpha_{lac}^* = \frac{\text{slope } (E_{420} \text{ units/min}) \text{ per } E_{460} \text{ unit of culture at shift-up time}}{\text{rate of protein synthesis per } E_{460} \text{ unit in pre-shift medium} \times e_r}$$

Fig. 2. Sodium dodecyl sulphate-polyacrylamide-gel electrophoresis of ^3H -labelled proteins from an uninduced culture (----) and ^{14}C -labelled proteins from a lac-induced culture (—)

See the Materials and Methods section for details. The stained gel slab was dried on filter paper and cut into 2 mm sections. Each section was oxidized and the radioactive isotopes (in water and CO_2) were counted in separate vials. The total acid-precipitable radioactivity electrophoresed was 1.38×10^5 and 1.19×10^5 c.p.m. of ^3H and ^{14}C respectively.

Determination of the absolute rate of β -galactosidase synthesis

The absolute rate of β -galactosidase synthesis was determined for bacteria grown in succinate medium from the radioactivity in β -galactosidase protein found in the electrophoretic distribution of a radioactively labelled bacterial lysate. For this purpose an exponentially growing succinate culture was divided into two portions; one portion was induced in the presence of cyclic AMP as described above, the other portion was not induced. At 15 min after induction [^3H]leucine (8.3 nmol/ml, 4.8×10^7 c.p.m./nmol) from Schwarz Bioresearch, Mount Vernon, N.Y., U.S.A. was added to the induced culture and [^{14}C]leucine (160 nmol/ml, 6.25×10^4 c.p.m. per nmol; Schwarz) to the uninduced culture. After a further 15 min both cultures were concentrated 12:1 by centrifugation and resuspension in lysing medium (Laemmli, 1970), heated for 1.5 min to 100°C and then kept at room temperature until electrophoresis. For electrophoresis the ^3H - and ^{14}C -labelled lysates were first mixed and then electrophoresed through a 5–10% (w/v) polyacrylamide gradient slab gel containing 0.1% sodium dodecyl sulphate [the

method of Margolis & Kendrick (1968) was used with the buffer system of Laemmli (1970)]. The β -galactosidase was found between 2.6 and 3.0 cm (Plate 1, Fig. 2); this region also contained two other proteins visible as bands in the stained gel from an uninduced culture. Throughout most of the gel the radioactive isotope ratio was close to unity (the ratio in the total mixture); however, some exceptions were found, particularly in the RNA polymerase $\beta\beta'$ region (around 2 cm). These deviations probably reflect cyclic AMP effects. The difference in the relative amount of radioactive isotopes between 2.6 and 3.0 cm corresponded to 0.97% of total radioactivity. The reproducibility of this value was approximately within 10%. Thus for succinate-grown bacteria, the differential rate of β -galactosidase synthesis, is assumed to be 0.009. The relative differential rate, α_{lac}^* (succinate), was found to be 4×10^{-17} (E_{420} units of enzyme/amino acid residue incorporated into total protein) (see the Results section), such that:

$$\alpha_{lac} = 2.3 \times 10^{14} \alpha_{lac}^*$$

Results

Differential rate of β -galactosidase synthesis

The differential rate of β -galactosidase syntheses is defined as the quotient:

$$\alpha_{lac} = \frac{\text{rate of enzyme synthesis (amino acids incorporated/min)}}{\text{total protein synthesis rate (amino acids incorporated/min)}}$$

Throughout most of this work a relative measure, α_{lac}^* is used, and is obtained by normalizing the numerator and denominator of this quotient to the turbidity (E_{460}) of the culture and by expressing the amount of enzyme in terms of the colour intensity (E_{420}) of hydrolysis products of an enzyme activity assay rather than in amino acids:

$$\alpha_{lac} = \frac{\text{rate of enzyme synthesis } (E_{420}/\text{min per } E_{460} \text{ unit})}{\text{rate of total protein synthesis (amino acids incorporated/min per } E_{460} \text{ unit)}}$$

The rate of enzyme synthesis is the final slope of the induction-kinetics curve (Fig. 3; to correct for the exponential growth of the culture, an expanded time-scale was used; see the Materials and Methods section for details), divided by the E_{460} of the culture at the moment of induction. The total protein-synthesis rate per E_{460} unit was determined previously (Dennis & Bremer, 1974c; see the Materials and Methods section and Table 1).

The relative α_{lac}^* can be converted into the absolute value by using a conversion factor obtained from the

amount of radioactively labelled β -galactosidase in the electrophoretic distribution of a bacterial lysate (see the Materials and Methods section and Fig. 2):

$$\alpha_{lac} = 2.3 \times 10^{14} \alpha_{lac}^*$$

In this way it was found that 0.9% of protein is β -galactosidase for maximally induced bacteria growing in succinate medium.

β -Galactosidase synthesis at different growth rates

The differential rate of β -galactosidase synthesis has been measured in bacteria growing in six different media (Fig. 4a) obtained by using three different carbon sources (succinate, glycerol or glucose) either with (closed symbols) or without (open symbols) a mixture of 20 amino acids. In all cases the measurements were made in the presence of saturating amounts of inducer and cyclic AMP (see the Materials and Methods section and Fig. 1). Cyclic AMP was found to have no effect on the bacterial growth rate (cell titre measured with a Coulter counter over two doublings) and hence it has presumably no effect on the synthesis of ribosomes.

The differential rate of enzyme synthesis is seen to generally decrease with growth rate, although there is no strict correlation between growth rate and α_{lac} . However, if the amino acid-supplemented

media and minimal media are considered separately (Fig. 4b), the correlation is strict. The results can then be interpreted by assuming that the presence of amino acids increases the differential rate of synthesis of β -galactosidase, as would be expected if repression of a significant fraction of very active genes (those involved in amino acid biosynthesis) causes some

increase in the intracellular concentration of free (active) RNA polymerase and thus greater activity of constitutive genes.

β -Galactosidase synthesis after a nutritional shift-up

When bacteria growing in succinate minimal medium are first induced and 10–15 min later nutritional supplements are added (shift-up), the rate of β -galactosidase synthesis is found to decrease after the shift-up (Figs. 5 and 6, circular

EXPLANATION OF PLATE I

Sodium dodecyl sulphate-polyacrylamide-gel electrophoresis pattern of stained protein from a bacterial lysate induced for β -galactosidase

Gel 1, purified *E. coli* RNA polymerase (β' , β , σ , α subunits) for comparison; gel 2, uninduced culture; gel 3, 20 min-induced culture.

symbols). In a variation of this experiment, inducer was added 3 min after a shift-up from succinate minimal medium (Fig. 6, triangular symbols). In all these experiments the differential rate of enzyme synthesis a few minutes after the shift is about as

Fig. 3. Determination of the differential synthesis rate of β -galactosidase, α_{lac}^*

An exponential culture in glucose-amino acids medium was induced at $t=0$ at an E_{460} of 0.322 (0.01 M-cyclic AMP was added 15 min before induction). Samples were assayed (30 min assay time) for β -galactosidase activity and the results (E_{420} of hydrolysis products) plotted by using an expanded time-scale on the abscissa which corrects for the growth of the culture (see the Materials and Methods section for details). The constant slope of the induction kinetics implies a constant rate of β -galactosidase synthesis per total protein synthesis. —, Basal level plus background (spontaneous substrate hydrolysis).

great as during steady-state growth in the post-shift medium (Fig. 6; steady-state range indicated by dashed lines). If the same culture is induced before or at various times after a shift-up, identical differential rates of enzyme synthesis are observed in the post-shift medium (Fig. 7; same final slopes of kinetics), indicating that the differential rate decreases 'stepwise' to the post-shift steady-state value within a few minutes after the shift-up. Since neither the *lac* gene concentration (*lac* genes per cell mass) nor the gene dosage (*lac* genes per total amount of DNA) changes rapidly after a shift-up, the change in the differential rate of enzyme synthesis cannot be due to gene concentration or gene-dosage effects (see the Discussion section).

Discussion

Physiological constitutivity

In the following discussion it is assumed that the high inducer and cyclic AMP concentrations used render the *lac* operon physiologically constitutive. We considered the use of other 'constitutive' enzymes, whose syntheses does not depend on external effectors; however, the existence of internal effectors cannot be excluded, therefore it is better to use a regulated enzyme when the regulation mechanism is well known. Besides, the enzyme should have a simple, sensitive assay which does not require cell lysis and removal of a debris fraction. These considerations made β -galactosidase a first choice. Use of a cyclic

Fig. 4. Differential rate of synthesis of β -galactosidase at different steady-state growth rates

(a) Exponential cultures in succinate, glycerol and glucose minimal media (○) and in the same three media supplemented with amino acids (●) were maximally induced in the presence of cyclic AMP (see the Materials and Methods section for details) and the differential rate of synthesis of β -galactosidase was determined (see Fig. 3). (b) The results in (a) are interpreted as representing two groups (minimal and amino acid-supplemented media); within each group the differential rate of synthesis of β -galactosidase decreases with growth rate. ○ and ●, Average values; vertical bars and dashed lines, fluctuation range from (a).

AMP-acceptor protein mutant would have decreased the assay sensitivity; besides it would have been difficult to exclude the possibility of 'leakiness'

Fig. 5. β -Galactosidase synthesis after a nutritional shift-up ($\uparrow$) from succinate (O) to glucose-amino acids medium (●)

Solid lines are calculated corresponding to the straight portions from an expanded time-scale plot. (The shifted and unshifted cultures have different expansion functions.) —, Basal level plus background.

(i.e. the shift-up effect might still be due to catabolite repression).

The final level of the cyclic AMP-dependence curves in Fig. 1(b) may reflect (1) a maximum intracellular concentration of cyclic AMP (limitation of cyclic AMP uptake), (2) a limitation of gene activity by the intracellular concentration of cyclic AMP-acceptor protein, or (3) a limitation by free active RNA polymerase. The first possibility is unlikely if cyclic AMP uptake is passive as suggested by the high concentrations needed for its effect. The second possibility is unlikely because the differential rate of enzyme synthesis decreases to its post-shift steady-state value within a few minutes after a shift-up when the concentration of cyclic AMP-acceptor protein cannot have changed very much. Hence, the third possibility is the most plausible one.

Differential rate of enzyme synthesis, gene dosage and gene concentration

Within a few minutes after a nutritional shift-up, the synthesis of stable RNA increases, mainly at the expense of the synthesis of mRNA (Pato & von Meyenburg, 1970; Nierlich, 1972; Dennis & Bremer, 1974b). Accordingly, the synthesis rate and the amount of mRNA decrease temporarily after a shift-up. The synthesis rate of protein, on the other hand, does not change and may even increase after a shift-up (Schleif, 1967; Dennis & Bremer, 1974a). These observations have two implications. (1) The rate of protein synthesis in bacteria is not limited by the amount or by the concentration of mRNA. This is consistent with the inference that the rate of protein synthesis at any instant after a shift-up is completely

Fig. 6. β -Galactosidase synthesis after a shift from succinate to other minimal media (a) including a 'no-shift' succinate to succinate control, or amino acid-supplemented media (b) as a function of the post-shift growth rate

O and ●, Shift-up 10 min after induction; Δ and ▲, shift-up 3 min before induction; ----, range of values for steady-state growth from Fig. 4.

Fig. 7. β -Galactosidase synthesis induced at various times ($\downarrow$) before and after a shift-up ($t = 0$) from succinate medium to glucose-amino acids medium

The equal post-shift slopes of the induction kinetics indicate that the differential rate of β -galactosidase synthesis becomes constant a few minutes after the shift-up. Induction time 10 min before ($\bullet$) or 3 ($\blacktriangle$), 15.5 ($\blacksquare$) and 31 ($\blacktriangledown$) min after the shift-up.

determined by the number of ribosomes and by the ribosome efficiency (Scheif, 1967; the ribosome efficiency is defined as the rate of protein synthesis per average ribosome). (2) The differential rate of synthesis of α -galactosidase (i.e. the ratio β -galactosidase protein to total protein) is independent of the absolute amount or of the concentration of *lac* mRNA, but is determined by the ratio of the amounts of *lac* mRNA to total mRNA.

The ratio *lac* mRNA/total mRNA in maximally induced bacteria is determined by the gene dosage (*lac* genes per total DNA) and by the regulatory state of the whole genome. In *E. coli*, the map location of the *lac* gene with respect to the location of the origin of DNA replication is such that the number of *lac* genes per genome equivalent of DNA is virtually independent of the growth rate, and, therefore, gene-dosage effects need not be considered here. The observed decrease in the differential rate of fully induced β -galactosidase synthesis after a shift-up (Figs. 5–7) reflects, therefore, a change in the regulatory state of other genes.

Pritchard (1974), in order to explain the rate of synthesis of β -galactosidase during the cell cycle and after a DNA synthesis velocity-jump experiment, proposed that 'the differential rate of synthesis by a gene in a fully derepressed state will be proportional to the gene concentration (gene:mass ratio) and independent of its relative dosage'. Here we observed the differential rate of synthesis of β -galactosidase to decrease within a few minutes after a shift-up to its post-shift steady-state value (Fig. 7). Immediately after a shift-up all gene concentrations are still the same as during pre-shift steady-state growth;

later, during post-shift growth, all gene concentrations (DNA/mass) decrease (Pritchard, 1974). This shows that the gene concentration does not affect the differential rate of synthesis of maximally induced β -galactosidase. At this time we cannot explain the contradiction between our conclusion and Pritchard's. Direct measurements of the differential rate of synthesis of β -galactosidase after DNA synthesis velocity jumps may resolve this question.

Comparison of constitutive *lac*- and *trp*-gene expression

Rose & Yanofsky (1972) measured the specific activity (enzyme activity/total protein content; the specific activity is proportional to the differential rate of enzyme synthesis if the turnover rates of enzyme and total protein are equal or negligibly small) or tryptophan synthetase for *trpR*₂ mutants growing in different media. They observed specific activity values of 18.3, 26.0, 19.5, 6.8 and 5.4 for growth in proline (doubling time 400 min), glycerol (120 min), glucose (70 min), glucose-amino acids (38 min) and broth (28 min) media respectively. Thus except for the value for proline medium, they also observed a decrease in the differential rate of constitutive enzyme synthesis with growth rate. For another enzyme of the *trp* operon (anthranilate synthetase) they find continuously decreasing specific activity with growth rate; the corresponding values are 2.3, 1.7, 1.6, 0.48 and 0.38 respectively.

These specific activity values are not exactly comparable with the α_{lac} values obtained here because of differences in the bacterial strain and doubling times; further, Rose & Yanofsky (1972) assumed total protein content to be proportional to the turbidity of the culture (for deviations from this expectation see Table 1) and showed only one experiment for each growth medium (Fig. 4 shows a 20% fluctuation range for α_{lac}). Also, destruction of some enzyme activity during sonication, or variable loss of enzyme in the debris fraction may have occurred, effects which do not have to be considered in the β -galactosidase assay.

Cooper & Magasanik (1974) measured, at three different growth rates, the transcription of the constitutive *trp* operon by DNA-RNA hybridization. They concluded that 'in cells whose rate of bulk RNA synthesis is widely different, the rate of synthesis of the unregulated *trp*-specific RNA is proportional to the rate of synthesis of bulk RNA'. This means that the ratio of the rates of synthesis of *trp* mRNA to bulk RNA is growth-rate-independent. It is not clear whether 'bulk' RNA means 'total RNA' (rRNA + tRNA + mRNA) or only 'stable RNA' [rRNA + tRNA, comprising about 97% ('bulk') of the total amount of cellular RNA but a much smaller fraction of the instantaneous rate of synthesis of total RNA]. According to our results either ratio of the synthesis

rates, i.e. *trp* mRNA/total RNA or *trp* mRNA/stable RNA, is expected to decrease with growth rate, although not in the same manner as α_{lac} (Fig. 4), which is assumed to parallel the ratio *trp* mRNA/total mRNA (see preceding section of the Discussion). However, a quantitative analysis of Cooper & Magasanik's hybridization data indicates a qualitative agreement between those results and ours. [Cooper & Magasanik's (1974) results allow one to calculate the radioactivity in unregulated *trp* mRNA, incorporated during a 3-min pulse, per μg of stable RNA. Those data also allow one to calculate the specific radioactivity of *lac* mRNA (quotient radioactive *lac* mRNA per μg of stable RNA/relative amount of *lac* mRNA per μg of stable RNA). Assuming the specific activities of the simultaneously labelled *trp*- and *lac*-mRNA molecules to be equal or at least very similar, one can calculate the relative amounts of unregulated *trp* mRNA/ μg of stable RNA at three different growth rates. These ratios of the amounts can then be converted into the ratios of the rates of synthesis of *trp* mRNA/stable RNA, or if the ratio mRNA synthesis/total RNA synthesis is known, to the ratio of *trp* mRNA synthesis to total RNA synthesis. Both these ratios decrease with growth rate, in qualitative agreement with our evaluation of enzyme-activity measurements (Fig. 4).]

Changes in free RNA polymerase after a shift-up

Changes in the rate of transcription of constitutive genes are probably paralleled by changes in the intracellular concentration of free active RNA polymerase. The concentration of RNA polymerase inside the cell is not directly measurable. It can be shown, however, that the concentration of polymerase is proportional to the product of two measurable parameters: the differential rate of synthesis of a constitutive enzyme and the fraction of RNA polymerase engaged in the synthesis of mRNA (Bremer & Dalbow, 1975). Since either one of these two parameters decreases after a shift-up (Figs. 5–7; Pato & von Meyenburg, 1970; Nierlich, 1972; Dennis & Bremer, 1974b), their product, corresponding to the concentration of free active RNA polymerase, must also decrease after the shift-up, probably reflecting the surge of polymerase to the ribosomal genes.

Control of ribosome synthesis

If both β -galactosidase (under the conditions used here) and ribosomal proteins were synthesized

constitutively, their rates of synthesis after a nutritional shift-up should change to the same extent, reflecting the changes in the intracellular concentration of free RNA polymerase (transcription frequency) and in the translation frequency per mRNA molecule (Dennis & Bremer, 1974c). In a shift from succinate medium to glucose–amino acids medium, the differential rate of ribosomal protein synthesis (α_r) increases 2.1-fold (Dennis & Bremer, 1974a); at the same time α_{lac} was seen to decrease to 0.4 of the pre-shift value (Figs. 5 and 6). If the α_{lac} values observed here are not grossly underestimated due to catabolite repression, this difference (2.1 and 0.4) implies that ribosomal proteins, and for the same reason rRNA, are controlled by active induction.

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