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An Analysis of Bacterial Growth

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1. THE SYSTEM

A growing bacterium is a self-contained system whose dominating activity is protein synthesis. Between four-fifths and nine-tenths of the carbon assimilated, and a similarly large fraction of the energy consumed serve this need. As the mediators of protein synthesis the ribosomes play a key role, and it was therefore not surprising to find that mechanisms exist which permit bacteria to adjust the number of ribosomes they produce in accordance with the environment they grow in. But it was both unexpected and gratifying to see that, by and large, this adjustment follows a simple, almost "sensible" rule, namely, in a given environment, no more ribosomes are produced than can be engaged with high efficiency in protein synthesis. This observation allows some of the major syntheses in these cells to be described in relatively simple terms, and the model to be presented is an attempt to account for a mass of data which are suggestive enough, I think, to warrant this exercise.

2. THE COLLECTION OF DATA

For years our chief interest has been to study the control mechanisms which determine and stabilize the growth rate of bacteria characteristic of a particular medium. To analyze the "fine adjustments" necessary to maintain a steady state of growth, data had to be obtained without perturbing the system by manipulating the cultures. With this restriction, the only variable left to work with was the growth rate itself, and, fortunately, this rate can be varied over a wide range by choosing among different media. Our present model is therefore based critically on measurements of the relative quantities of protein, RNA, and DNA in samples drawn from steady state cultures. The degree to which this ideal state was approximated in our experiments is discussed in a recent monograph (Maaløe and Kjeldgaard, 1966).

Supplementary, but very valuable, information has come from experiments involving shifts between media. By far the simplest experiment of this type is a shift-up which does not impose new syn-

thetic activities on the cells. The best example of such a "gratuitous" shift is one in which amino acids and nucleosides are added to a steady-state culture in a minimal medium with glucose as the only carbon and energy source. The cells immediately increase their growth rate, probably as a direct result of multiple repressions and feedback inhibitions and a corresponding, drastic reduction of a considerable number of synthetic activities. The reverse shift is difficult to interpret. After the shift, the capacity of the cells for synthesizing their own amino acids, etc., is extremely low, and before growth at the definitive postshift rate can be established, the enzyme equipment of the cells has to be readjusted. So far, this slow and gradual process is not well understood.

For later reference the main results of the various measurements are numbered and listed here. If nothing else is said, all quantities and numbers of molecules are normalized to DNA; the reference unit chosen is one genome equivalent of DNA (written: per genome). Standard abbreviations are used throughout, except that the "r" which stands for "ribosomal" in rRNA is also used in the combination r-protein, and as subscript; e.g., in α_r, which designates the r-protein as fraction of all protein. The growth rate, μ, is expressed in doublings per hour.

2.1 The total protein, per genome, has been measured in *Salmonella typhimurium* (see Maaløe and Kjeldgaard, 1966) and in strain TAU-bar of *Escherichia coli* (Forchhammer and Lindahl, in preparation). In both organisms the quantity of protein, per genome, represents 4 to 5 $\times$ 10^8 amino acids. Within experimental error, *this figure is independent of* μ (between $\mu \simeq 0.2$ and $\mu = 2.5$).

2.2 The number of ribosomes, per genome, has been calculated from measurements of total RNA after subtraction of tRNA. The original figures for *S. typhimurium* (Maaløe and Kjeldgaard, 1966) have been corrected for errors in the estimates of tRNA (Kjeldgaard, 1967), and the agreement between measurement on different organisms and in different laboratories is now reasonably good. At relatively high growth rates, the number of ribosomes, per genome, is proportional to μ; but for values of $\mu_{37°}$ much below unity the number of ribosomes, per genome, is somewhat higher than would be expected on the basis of strict proportionality. Details about these important relationships are given by Rosset *et al.* (1966), Kjeldgaard (1967), and Forchhammer and Lindahl (in preparation); extensive measurements by R. Lavallé (personal communication)

agree with the published data. To indicate the actual numbers obtained it may suffice to state that strain TAU-bar contains close to 10^4 ribosomes, per genome, at $\mu_{37°} = 1.5$.

It should be noted that proportionality between μ and the *number* of ribosomes imply that the *rate* of ribosome synthesis is proportional to μ^2. To illustrate this, compare two steady states with the growth rates μ and $\mu/2$, respectively. The corresponding numbers of ribosomes then are n_r and $n_r/2$, and the rates of synthesis are consequently $n_r\mu$ and $(n_r/2) \times (\mu/2)$. Thus, to a factor 2 between the μ values corresponds a factor 4 between the rates of ribosome synthesis.

Measurements of α_r (Schleif, 1967a, b, 1968) strongly support the notion of proportionality between ribosome number and μ (see Fig. 1).

2.3 As mentioned above, tRNA has been measured as a fraction of the total RNA in several laboratories. The total quantity of tRNA varies little with μ, and a representative number of tRNA molecules, per genome, is 2×10^5.

2.4 The mRNA activity, per milligram of extracted RNA can be measured *in vitro*, and this technique has been used extensively by J. Forchhammer and collaborators in our laboratory. The validity of this assay has been discussed by Forchhammer and Kjeldgaard (1967).

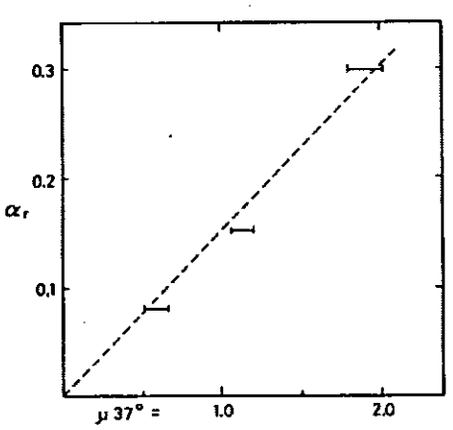

FIG. 1. The α_r and μ values were measured several times in minimal media with succinate or glucose as sole carbon sources, and in a glucose minimal medium enriched with casamino acids, etc. (Schleif, 1967a). The bars illustrate the ranges of growth rates observed.

It is observed that the *in vitro* mRNA activity, per ribosome equivalent of rRNA in the extracts, is nearly independent of μ. This is interpreted to mean that the quantity of mRNA, per ribosome, is nearly the same at all growth rates (Forchhammer and Kjeldgaard, 1968). Like rRNA, mRNA is thus roughly proportional to μ. Note, however, that to maintain this relation the unstable mRNA has to be synthesized at a *rate* which is also proportional to μ (*not* to μ^2, as in the case of the stable rRNA).

2.5. The distribution of the ribosomal material between subunits, single 70 S particles, and polysomes as function of μ has been determined in strain TAU-bar by Forchhammer and Lindahl (in preparation). In this strain, the 30 S and 50 S subunits together make up about 15% of the total at all growth rates. This fraction contains the mature, recycling subunits (Kaempfer *et al.*, 1968), but is probably contaminated with an unknown amount of subunit precursors. Most of the 70 S particles are in the polysome fraction. The *in vivo* distribution of these particles is uncertain since some breakdown of polysome material (and perhaps some runoff of 70 S units) occurs during extraction and preparation. The present estimate is that 70–85% of the total material is in polysomes *in vivo*.

3. NUMERICAL ANALYSIS

Consider first a bacterial culture in a steady state of growth with the growth rate, μ, defined by $M_t = M_0 \exp(c_1 \mu t)$ or, $dM/M = c_1 \mu dt$, where M is the mass per unit volume of any cell component, and t is time. Since we express μ in doublings per hour, and t in minutes, the constant $c_1 = \ln 2/60$. Let the system contain N amino acids in all of its protein, and $\alpha_r N$ in the r-proteins. The latter reside largely in mature ribosomes with approximately 10^4 amino acids per 7 S particle, and the number of ribosomes in the system is therefore $\simeq \alpha_r N \times 10^{-4}$. As a unit of protein synthesis we choose a "*ribosome-minute*," r', which is the average number of amino acids added per minute to a growing polypeptide chain; i.e., the average chain growth rate. This definition gives, $10^{-4} r' = (dN/dt)/(\alpha_r N)$ which, combined with the differential growth equation, yields

$$\mu = c_2 \alpha_r r' \qquad (3\text{-}1)$$

where the constant c_2 is approximately 8.7×10^{-3}.

As derived here on the basis of protein synthesis in a steady state of growth, equation (3-1) can be applied directly to Schleif's α_r

measurements (cf. 2.2). Figure 1 is a plot of α_r against corresponding values of μ, and the close proportionality between these parameters shows that r' is more or less constant over a considerable range of growth rates. This graphical analysis is rather insensitive to deviations from proportionality at low μ values. It should therefore be restated here that r' decreases significantly at low growth rates (cf. 2.2). This property of the system will be discussed in Section 5.2.

The chain growth rate of a specific protein, the β-galactosidase, has been measured by Lacroute and Stent (1968); they estimate r'_{37} at about 800–900. Equation (3-1) can therefore be reduced to

$$\mu \simeq c_3 \alpha_r, \tag{3-2}$$

where $c_3 = c_2 r'$ is approximately 8. A system depending on *r*-proteins only for its growth ($\alpha_r = 1$) would thus double about eight times per hour. A similar figure was quoted years ago by Leslie Orgel on the basis of data from Schaechter *et al.* (1958). As everybody knows, three doublings per hour is about the highest growth rate attainable at 37°C, and it seems that, to achieve this, as much as 35–40% of the cells protein must be r-protein.

Our general knowledge of the mechanism of protein synthesis indicates that a system which obeys equation (3-2) must have the following properties: (a) the production of rRNA must at least match that of r-protein; (b) a constant fraction of all ribosomes must be engaged in protein synthesis; and (c) the various components involved in this process, including the transcribing and activating enzymes, must supply enough mRNA and maintain adequate concentrations of amino acid charged tRNA's. In fact, these inferences are supported by independent, experimental evidence. Thus, Schleif (1968) has demonstrated that growing *E. coli* cells contain very little free r-protein; and we find that the polysomes are made up of a nearly constant fraction of all ribosomes, irrespective of growth rate (cf. 2.5). As regards (c) two points should be made: first, that the mRNA content of growing cells, *per ribosome*, is constant; i.e., the same piece, or length, of mRNA is available to a ribosome whether growth is fast or slow (cf. 2.4). Second, a shift-up from a glucose minimal medium to broth does not measurably increase r' (Maaløe and Kjeldgaard, 1966).

The significance of the last point should perhaps be explained. When broth is added to a minimal medium culture, all the amino acid pools swell, as evidenced by the fact that repression and feed-

back inhibition become effective at once and as shown directly for a few amino acids by Britten and McClure (1962). When a new amino acid is added to a growing polypeptide chain, it seems obvious that the trial and error process through which the right tRNA is fitted to its codon occupies most of the time involved in the overall reaction. The fact that r' remains unchanged after the shift indicates that the protein synthesizing machinery was fully primed with charged tRNA already *before* the shift; this in turn suggests that the r' value observed is about as high as the physical properties of the components of the system allow.

The relations between r', α_r, and μ were developed formally for an "average" r'. For theoretical reasons the same r' must be assumed to apply to *all* ribosome-mediated protein syntheses, and experiments indicate that r' does not vary during the bacterial division cycle. Thus the same induction kinetics apply for β-galactosidase when inducer is added at different times during the cycle (Cummings, 1965), and autoradiographic studies by Ecker and Kokaisl (1969) indicate that r' is constant in time.

The notion that r' is relatively independent of μ is not new. It was first suggested by Schaechter *et al.* (1958) on the basis of rather crude measurements of total RNA in cells growing at different rates. Since then this type of experiment has been considerably refined, and the data now available permit us to estimate relative as well as absolute values of r'.

First, let us examine the rather striking fact (a) that the total quantity of protein, per genome, is nearly the same at all growth rates. In one doubling time (t min) some 4 to 5 $\times$ 10^8 amino acids must therefore be built into protein, per genome, in the growing culture (cf. 2.1). We also note (b) that the quantity of rRNA and also the number of ribosomal particles, per genome, are more or less proportional to μ (cf. 2.2). Since $\mu = 60/t$, it follows from (b) that the number of ribosome-minutes, *per doubling time*, is nearly constant; taking (a) and (b) together it can be seen that this is true also of r'. It is an interesting property of this system that it always produces about the same quantity of protein, per genome, irrespective of the composition of this protein in terms of enzymes, r-protein, etc. This point will be discussed in some detail in the next sections.

Assuming that all ribosomes are active in protein synthesis, a minimum value can be assigned to r'. Estimates of the number of

ribosomes necessary for the actual calculations have been obtained from measurements of rRNA, r-protein, and whole particles, respectively (cf. 2.2 and 2.5).

The minimum values obtained for *S. typhimurium* and given by Maaløe and Kjeldgaard (1966), have been slightly adjusted in the light of the improved tRNA measurements referred to in 2.2. The present conclusion is that the chain growth rate is constant, at about 16 amino acids sec^{-1} at 37°C, at medium and high growth rates ($\mu = 1.2$ and $\mu = 2.4$, respectively), and that it is reduced by about 40% at $\mu \approx 0.2$; thus r' drops from 900–1000 to about 600. With strain TAU-bar, Forchhammer and Lindahl (in preparation) obtained similar figures, and much the same decrease at low μ-values. The same general picture is reported by Rosset *et al.* (1966) except that the constancy at high growth rates is less pronounced, and in some strains r' decreased more or less continuously.

Lacroute and Stent (1968) estimated r' for β-galactosidase. Their figure of 800–900 is also a minimum estimate, since it is based on measuring the time it takes to synthesize the polypeptide chain of the enzyme plus the unknown time required to fold the chain and produce the active tetramere. An r' of about 800 was calculated for the r-proteins by Schleif (1967a, b).

All the minimum estimates based on participation in protein synthesis of all the ribosomes should be corrected so as to express r' in terms of actively engaged ribosomes. According to 2.5 the minimum estimates should be increased by approximately 25%.

4. GENETIC ANALYSIS

We have seen that a steady state of growth is characterized by an α_r value which uniquely defines the growth rate μ as long as r' remains unchanged. Schleif originally used an α to indicate the fraction of all protein which is ribosomal. In the present description of the system, I have called this fraction α_r, to distinguish it from a general α_i, where the subscript refers to any nonribosomal species of protein in the cell. By definition the sum of all α values, including α_r, is unity. A numerical equivalent to α_i is the number of amino acids, per genome, residing in protein "i"; thus an $\alpha_i = 0.01$ means that the corresponding protein species contain a total of 4 to 5×10^5 amino acids (1% of the total).

Our problem is then to understand how the α_r which defines a given steady state is reached, and maintained. The central idea of

the model developed here is that α_r is determined by a multivariable function, namely *the entire set of repressions prevailing in the growing cell* (operon-specific as well as catabolite repressions; induction, of course, is viewed as a decrease in degree of repression). A short and incomplete version of the model was published in a note two years ago (Maaløe, 1968).

The concept of a set of repressions requires elaboration. The cistrons representing the different *E. coli* proteins map singly or in groups, corresponding to functionally related enzymes, and transcription is controlled by highly specific effectors (Jacob and Monod, 1961) or by less selective, catabolite effectors (reviewed by Anderson and Wood, 1969). If we focus on a particular unit of control, an operon O_i, we can therefore ask about the probability, P_i, that the next act of transcription in the cell takes place in this rather than any other segment of the genome. If we assume, as is often done tacitly, that all messenger cistrons yield the same average number of protein molecules, then, for a cistron of average length, $P_i = \alpha_i$; i.e., the relative frequency of transcription equals the relative abundance of the final product. Of course, polypeptide chains of very different molecular weight are produced, but the multivariable function we consider represents a large number of separately controlled cistrons. As a good approximation we can therefore think in terms of an "average" cistron producing an "average" quantity of protein.

Focusing again on the operon O_i, we note that three parameters (at least) are involved in determining the yield of the corresponding protein(s). First, the activity of the *operator* which we assume depends on the concentration in the cell of the operator-specific effector, and of the less specific, catabolite effectors; second, the affinity of the *promotor* site for the transcribing polymerase; and, third, the average *gene-dose*. As described here, the DNA structure in the promotor region of the operon determines the efficiency with which a colliding polymerase molecule attaches. This efficiency is thus a permanent, individual property of an operon. The gene-dose is relevant to the model mainly because the multifork pattern of replication, characteristic of rapidly growing cells, accentuates the difference in gene-dose between early and late replicating cistrons.

Of the three parameters described, only the operon control is of immediate interest to us now. Consider first the state of an operon in terms of the fraction, ϵ_i, of total time it is open, i.e., derepressed. While the operon is in this state, transcription will be initiated with

a frequency which is limited by the concentration of various cytoplasmic elements (cf. Section 5.3), and modulated by the structural properties of the promotor. The wide range over which ϵ_i can be varied is typically shown by inducing the synthesis of β-galactosidase in a cryptic (permease-deficient) strain using several concentrations of a specific inducer (Cohen and Monod, 1957). In this way the steady state of enzyme synthesis can be set anywhere between a maximum, at which some 5% of all newly made protein is β-galactosidase, and a background level, yielding maybe 10^3 times less enzyme. At saturating inducer concentrations, the lac operator probably is totally derepressed and therefore transcribed as frequently as the overall system permits. When the inducer is removed and repression takes over, our index of derepression, ϵ_{lac}, may thus drop from a value near unity to about 10^{-3}. In experiments of this kind all other control indices presumably remain practically unchanged, and the growth rate is in fact changed very little by inducing the cells to produce about 5% of their protein in the form of a dispensable enzyme (Novick and Weiner, 1957).

In a steady state of growth, each protein, and indeed any cell component, increases its mass at the growth rate μ of the culture. Applied to individual cells it is, of course, only meaningful to think of ϵ_i, and of gene-dose, as time averages. However, in the population as a whole a well defined ϵ_i, and gene-dose, can be ascribed to each genetically controlled unit. Finally, if the promotor activity is taken into account, an actual transcription index, $\bar{\epsilon}_i$, can be assigned. This index, taken as a fraction of the sum of all indices, is the probability P_i. The multivariable function referred to above can now be identified as the set of $\bar{\epsilon}$ values, and it can be seen that this set constitutes the *partition-function* according to which the individual species of protein are represented in a steady-state culture. This function has two important properties: (1) it is independent of the *intensity* of transcription, i.e., the partitioning is the same whether the probability of initiating an act of transcription at an open site is high or low; and (2), conversely, the elements of the set can be multiplied by a common factor without changing the partitioning, provided $\bar{\epsilon}_i < 1$ applies throughout (see Fig. 2 and legend).

It now remains to discuss actual values of the $\bar{\epsilon}$ index. We have seen that $\bar{\epsilon}_{lac}$ can be varied over a 10^3-fold range and that enormous amounts of β-galactosidase are produced if the partition function remains more or less unchanged, except that $\bar{\epsilon}_{lac}$ is raised to a high

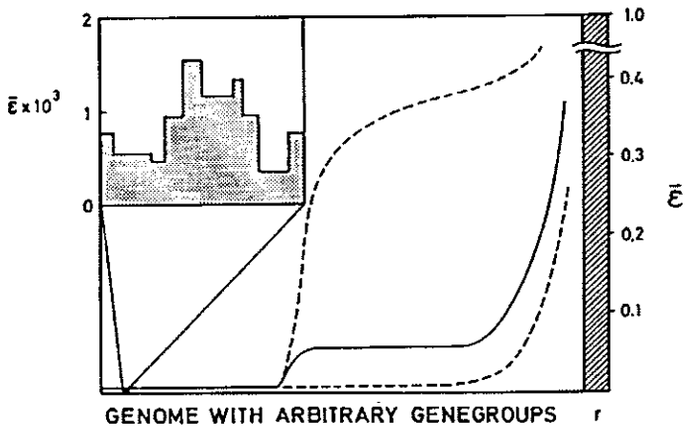

FIG. 2. This graph illustrates the partition-function described in the text as the set of derepression indices $\bar{\epsilon}_i$.

Ideally each polypeptide encoded in the genome should be represented on the abscissa with a segment corresponding to its molecular weight. From left to right are shown: first the class of enzyme always produced in small quantities (very low $\bar{\epsilon}$ value); then the class of biosynthetic enzymes with typical $\bar{\epsilon}$-values around 0.05 in glucose minimal medium (solid curve); followed by a relatively small segment representing proteins assumed to have higher or very high $\bar{\epsilon}$ values. The r-proteins are represented on the extreme right by the hatched column which, to be clearly visible, has been *increased* five times in width; to be true to scale it should cover about 1% of the genome.

The curves show, qualitatively, the expected shape of the function. The top and center curves represent minimal medium with a "poor" carbon source and with glucose, respectively; the bottom curve shows the maximally reduced partition function expected in a rich medium.

The inset illustrates what a small segment of the curve might look like if the actual molecular weights and $\bar{\epsilon}$ values were known. As indicated, the segment is chosen from the left-hand part of the graph.

value. Very similar data exist for another inducible enzyme, alkaline phosphatase.

In the light of these examples let us examine some biosynthetic enzymes about which it is known that the activity assays commonly used measure actual quantities of the specific proteins. The three cases I have chosen are: the aspartic transcarbamylase, ACTase, studied particularly by Gerhart and Schachman (see, e.g., Gerhart and Holoubek, 1967); the ornithine transcarbamylase, OTCase, extensively studied by Gorini and his associates (see, e.g., Jacoby and Gorini, 1969); and the tryptophan-synthesizing enzymes, ana-

lyzed by Yanofsky's group (see, e.g., Yanofsky and Ito, 1966). Wild-type organisms growing in a minimal medium produce all these enzymes in *small* quantities compared to those produced by mutants in which little or no repression is exerted.

The ACTase normally constitutes 0.1 to 0.2% of the cell protein, but strains exist in which this percentage is more than 20 times higher (J. C. Gerhart, personal communication). In the case of OTCase a similar difference has been demonstrated between wild-type and repression-defective mutants; in the wild type, further, and almost complete suppression of enzyme synthesis is caused by adding arginine to the medium (see Fig. 3 and legend). Finally, a special case recently analyzed by C. Yanofsky (personal communication) should be mentioned. The enzymes of the trp operon constitute about 0.4% of the cell protein in a wild-type, minimal medium culture, but a strain which produces about 15 times more has been isolated. This mutant carries asparagine instead of one of the glycines in the B protein, and this substitution seriously affects the activity of the enzyme. This case is particularly clear, because it shows how the normal, unimpaired control system reacts by derepressing the trp operon, because the efficiency of one of its enzymes is greatly reduced.

The three cases discussed here indicate that enzymes which carry heavy biosynthetic loads, as a rule, are produced under considerable internal repression. In other words, during growth with a single car-

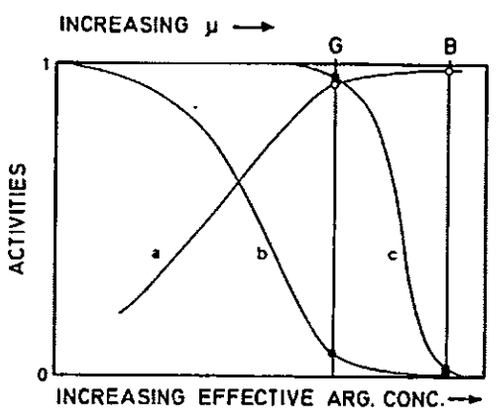

FIG. 3. The three curves illustrate the dependency on the intracellular arginine concentration of (*a*) the charging of $tRNA_{arg}$; (*b*) the degree of derepression of the arginine-synthesizing enzymes; and (*c*) the activity of these enzymes as affected by end-product inhibition. The construction of the curves is described in the text.

bon source the cells seem to maintain pool levels of arginine and tryptophan which cause strong repression. The data indicate that ϵ_{arg} and ϵ_{trp} are reduced to about 1/20, or less, of their maximum values (see Fig. 3).

The low ϵ-values which seem be characteristic of many biosynthetic enzymes are pertinent to this analysis in two ways: on the one hand, here is a group of at least 100 enzymes which make up a large fraction of the total protein in a minimal medium culture, and the synthesis of which can be drastically reduced by amino acids, purines, and pyrimidines added from outside. On the other hand, the same group of enzymes would continue to be synthesized, *in more or less the same proportions*, if all the relevant ϵ values were multiplied by a common factor. This factor could be fairly large without violating the condition that the individual ϵ_i remains below unity. The effect of such a change on the relative abundance of proteins whose ϵ_i did *not* change is discussed in Section 5.2.

Finally, I want to mention a somewhat ill-defined but important class of proteins, namely, those that seem never to be present in the cell in more than a few copies. All the repressor proteins probably belong in this class, and a fair number of enzymes which, in contrast to those discussed above, carry very light biosynthesis loads, may be included (e.g., the enzymes catalyzing the synthesis of the B vitamins). It is convenient to imagine that very "tough" promotors exist, which, without the intervention of other control elements, would keep the frequency of transcription of the cistrons corresponding to this class of proteins very low. In the case of the repressor protein of the lac operon, one mRNA produced per doubling time would seem to be quite adequate (Gilbert and Müller-Hill, 1967). In the terminology used here, the cistron coding for this protein would be characterized by an ϵ value of unity, *and* an ε value of, say, 10^{-3} or 10^{-4}.

5. THE MODEL

5.1. *General Properties*

About ten years ago our work on bacterial growth could be summarized in this way:

> The sketch of the growing bacterium presented here is based essentially on the idea of exchange of information between different molecular levels of organization in the cell. A flow of information is assumed to descend from a linear, genetic specification on a DNA strand, *via* RNA and protein, and to give to a small molecule, such as an amino acid, its three-dimensional individuality. Equally specific information is believed to pass from the level of the small molecules back in the

> direction of the nucleus. This feedback of information, which produces the phenomenon of repression, is thought to be responsible for one of the remarkable properties of the cell: its ability to adjust the size and activity of the different synthetic systems to the set of nutrients present in the medium; an adjustment which results in the establishment of a definite partitioning of energy and matter among the synthetic systems, to which corresponds a definite growth rate and cell composition. (Maaløe, 1960.)

When this was written, the mechanism of protein synthesis, and the built-in controls, were poorly understood, and many statements were necessarily vague. Today a more detailed analysis can be made. However, our general attitude to the problem has not changed. It still seems necessary to me to treat the bacterial cell as a unit system, and it is attractive to consider the controls of the synthesis of all the individual proteins as equivalent; i.e., as contributing in identical manner, but with different weight, toward determining the growth rate. The present model thus further develops the old idea of describing the unit system in terms of "flow of information."

Much of the data used here are relatively new, and I shall therefore begin the description of the model by restating the basic facts for which it has to account:

(a) At medium and high μ values the number of ribosomes is proportional to μ. This implies that the *rate* of synthesis of the ribosomes is proportional to μ^2. At low growth rates the ribosome numbers are somewhat higher than expected on the basis of strict proportionality (cf. 2.2).

(b) The quantity of mRNA, per ribosome, is nearly constant (cf. 2.4). These unstable RNA molecules do not accumulate as ribosomes do, and if we assume that the messenger half-life is more or less independent of μ, mRNA must be synthesized at a rate proportional to the ribosome number. With the restriction mentioned in (a), the rate of mRNA synthesis is therefore proportional to μ.

(c) The synthesis of rRNA closely matches that of the r-proteins, and the cells maintain very small pools of free rRNA, and of free r-protein (cf. 2.2).

Very briefly, the model accounts for these observations in the following way: the partition-function described in Section 4, and illustrated in Fig. 2, is thought *by itself* to generate α_r, and the balance between ribosomes and mRNA on the one hand, and between r-protein and rRNA on the other, are thought to be maintained by separate feedback mechanisms. These basic elements of the model will now be discussed one by one.

5.2 *Passive, or Indirect Control of* α_r

The full weight of generating α_r can be put on the partition function by having the r-protein segment(s) of the genome always derepressed, i.e., subject to promotor activity, and to the effect of changes in gene dose, but with an $\epsilon_r = 1$. This situation is illustrated in Fig. 2, where the column on the right-hand side represents the ribosomal proteins, and α_r is the area of this column as fraction of the total area under one of the curves (see legend). There can be no doubt that this total area (and thereby α_r) *changes* from one growth condition to another, but it is perhaps not obvious that it changes in the right direction, let alone that it changes sufficiently to account for the observed variations in α_r.

To examine this problem, consider first growth in glucose minimal medium. In *E. coli* and many other organisms, glucose or one of several related compounds, are the carbon and energy sources on which these microorganisms grow fastest. Thus, flow rates of carbon and energy can be established via the glucose pathway(s), which are higher than those obtainable through pathways utilizing other substrates. I emphasize this because it means that growth rates higher than $\mu_{glucose}$ can be obtained only by supplying ready-made building blocks (e.g., amino acids) in the medium, whereas rates below $\mu_{glucose}$ can be realized with carbon sources which are converted less efficiently than glucose (similarly, μ can be reduced by replacing ammonia by other nitrogen sources, but we have made little use of this alternative). The steady state of growth with glucose as the sole carbon and energy source is characterized by an $\alpha_r \simeq 0.15$, and a $\mu_{37^\circ} \simeq 1.2$; in Fig. 2 the corresponding partitioning between ribosomal and nonribosomal protein is indicated by the solid curve.

In broth, α_r and μ are typically twice as high as in glucose minimal medium. Two factors seem to contribute independently to this increase. In the first place, production of the biosynthetic enzymes is more or less totally repressed, and in view of the reduced energy requirement (per milligram of protein produced) other enzyme systems may be reduced in size. Secondly, the r-protein (at least the "30 S-proteins") seem to map in the early replicating segment of the genome, and the gene-dose would therefore increase from about 1.4 in the glucose culture to about 1.9 in the rich medium (assuming 2 gene copies per 1.4 genome equivalents of DNA in the glucose culture, and 4 copies per 2.1 equivalents in rapidly growing cells; see e.g., Helmstetter *et al.*, 1968). This effect on the gene dose would

raise the α_r of a glucose culture from 0.15 to 0.20, and to reach the value of 0.30 observed in the rapidly growing culture, repression would have to reduce the sum of the $\bar{\epsilon}_{glucose}$ values by about 40%. Considering that the biosynthetic enzymes alone probably account for 20–40% of the protein in a glucose culture, this does not sound unreasonable.

The decrease in α_r and μ observed with "poorer" carbon sources may also be accounted for by changes in the partition pattern. To explain a decrease in α_r from 0.15 to, say, 0.05 the sum of the $\bar{\epsilon}_{glucose}$ values must increase threefold. Part of this increase would come from derepressing the synthesis of enzyme systems required by the new carbon and energy source. Since, by definition, this compound is converted less efficiently than glucose, the overall system would be expected to compensate by producing large quantities of the required enzymes, i.e., to derepress the relevant operon(s) more or less completely. However, even complete derepression of a few operons can hardly be imagined to increase the sum of the $\epsilon_{glucose}$ values threefold. It is therefore important that the biosynthetic enzymes represent a large number of operons which can be derepressed to considerable degrees without much change in the partition pattern in that sector (see description of the partition function in Section 4). As we have seen, $\epsilon_{glucose}$ values around 0.05 seem to be typical of enzymes of this class, and the entire threefold increase in the sum of the $\epsilon_{glucose}$ values could probably be achieved by raising all the 5% values to about 50%.

When equations (3-1) and (3-2) were developed, we noted that, for values of $\mu_{37°}$ much below unity, α_r decreases less than predicted by the simplified equation, $\mu = c_3\alpha_r$. Figure 3 is constructed to illustrate how this trend can be understood in terms of the amino acid pool-levels in the cells. The three curves represent: (a) the degree of charging of tRNA, (b) the level of *de*repression, and (c) the level of enzyme activity; all three, of course, are functions of the effective, intracellular concentration of an amino acid (in this case, arginine). The shapes of these curves are largely unknown, but their relative positions can be deduced as follows. In a glucose culture (position G on the abscissa), the arginine concentration is sufficient to repress OTCase synthesis to about 5% of its maximum value; at the same position little, if any, end-product inhibition is exerted (one point on curve b, and one on curve c). The $tRNA_{arg}$ must be more or less fully charged at position G, since the pool level can be

raised (by adding arginine from outside) without significantly increasing r' (one point on curve a). In a broth culture (B on the abscissa) intense repression and end-product inhibition prevail (low points on curves b and c), and the charging of the tRNA is, if anything, more complete than before (second point on curve a). At concentrations below position G, all three arginine-dependent effects must fade out gradually.

According to the model, α_r decreases as a consequence of general derepression, i.e., as the sum of the ϵ values increases. Thus, low α_r and μ values are supposed to reflect low concentrations of intracellular amino acids, and other quantitatively important effectors. At some point along this line the degree of charging of tRNA must also begin to decrease significantly. The effect of this will be to reduce r', because the average time it takes to add a new amino acid to a growing polypeptide chain will increase. The equation $\mu = c_2\alpha_r r'$ (3-1), which must now be applied, shows that when r' begins to decrease, μ must be expected to decrease more sharply than α_r. This, I believe, is the reason why the proportionality between α_r and μ breaks down at low growth rates (cf. 2.2 and 5.1).

As discussed in Section 4, the amino acid pools definitely increase between positions G and B in Fig. 3; but, we do not yet know to what extent, nor how generally, these pools are reduced at lower growth rates. At the moment more is known about the riboside-triphosphate concentrations which have been shown to decrease more or less linearly with μ (see below). One of them, UTP, acts as corepressor of ATCase synthesis and thus belongs in the group of "other important effectors.".

So far our arguments show that the partition function might account for the known variations in α_r without invoking active or direct control of the synthesis of the r-proteins. However, before this main aspect of the model is accepted as a serious hypothesis two critical questions should be examined: (a) Would passive control exerted by the collective of repressions confer stability on the system? (b) Can more or less obvious alternatives be excluded?

The first question can be answered by considering a cell with the ideal composition corresponding to a particular steady state of growth. At division, let the two sister cells receive $(n_r/2) \pm \Delta n_r$ ribosomes, respectively, everything else being evenly distributed. In the cell with excess ribosomes the tendency will be to raise the rate of protein synthesis above the average; this will cause excessive drain on the pools of amino acids, etc., lower their concentrations,

and relieve internal repression to some extent. The net result will therefore be to *reduce* α_r temporarily, and thus to bring the ribosome number down. By the same reasoning it can be seen that α_r will increase temporarily in the sister cell. This is the kind of "fine adjustment" referred to in Section 2, where the principles of data collecting were discussed.

The second question cannot be answered definitively, and only one plausible alternative has occurred to me. Suppose the observed changes in α_r could *not* be accounted for without including in the model a control acting directly on $\bar{\epsilon}_r$. To introduce specific repression of the synthesis of the r-proteins (i.e., to allow ϵ_r to assume values below unity) implies the existence of an effector which would contribute to the setting of α_r. We know that the metabolic pattern changes greatly with the carbon source used, and one therefore has to look for an effector whose concentration nevertheless would vary in a monotonic manner with μ.

J. Neuhard (personal communication) has shown that the riboside triphosphate concentrations increase more or less linearly with μ, probably reflecting a parallel increase in the rate at which energy is consumed. One of these triphosphates, or a derivative thereof, could therefore be the effector we look for. However, I think this possibility can be excluded, because experiments show that α_r can be increased and, at the same time, the concentrations of all the riboside triphosphates drop. This situation obtains after a shift from glucose minimal to a rich medium, when α_r actually overshoots its definitive value (Schleif, 1967a), while the triphosphates are temporarily reduced to about a third of their preshift concentrations (unpublished data of J. Neuhard and J. Ingraham, discussed by Maaløe and Kjeldgaard, 1966).

5.3 *The Balance between mRNA and Ribosomes*

The feedback mechanism thought to maintain this balance was first suggested by Stent (1964). The simplest version of Stent's idea is that a ribosome, or one of its subunits, is required to initiate the synthesis of any mRNA molecule. This view is supported by *in vitro* studies of RNA synthesis (Shin and Moldave, 1966); and the work of Revel *et al.* (1968) suggests that the 30 S subunit is the ribosomal element involved in the act of initiation. It is therefore important to recall that the free subunits constitute a fixed fraction (about 15%) of all the ribosomal material (cf. 2.5).

Our present model specifically states that all classes of mRNA, in-

cluding the r-protein messenger, are synthesized by the same polymerase, *and* that the initiation mechanism is the same. The frequency of initiation is supposed to be proportional to the number of ribosomes at all values of μ; i.e., neither shortage of polymerase, nor queueing along the DNA template is allowed to affect the frequency. In a system with these properties, the initiation frequency will be proportional to the ribosome number, and so, of course, will the number of mRNA molecules produced per minute. Finally, to produce the observed almost constant ratio between the *quantity* of mRNA and the number of ribosomes, the messenger half-life must be assumed to be more or less independent of μ.

Recent measurements of the RNA chain growth rate (Bremer and Yuan, 1968; Manor *et al.*, 1969) show that the number of codons transcribed per second agrees reasonably with the number of amino acids added to a polypeptide chain in the same time. Transcription and translation *could* therefore be intimately coupled. In fact, Manor *et al.* show that, like our r', the RNA chain growth rate is moderately reduced in slow-growing cells.

As an alternative, the frequency of transcription could be imagined to be governed by the polymerase concentration. If the polymerase cistrons were exempt from repression, as we imagine the r-protein cistrons to be, polymerase and ribosomes would always be produced in the same relative amounts. The reason I do not think this scheme would be satisfactory is that a single polymerase seems to be responsible for the synthesis of all three classes of RNA [a point mutation can make the polymerase resistant to rifamycin *in vitro* (R. Schleif, personal communication); and this drug inhibits both mRNA and stable RNA synthesis *in vivo* (B. Watson, personal communication)]. We know that at reasonably high growth rates, $d(\text{mRNA})/dt$, and $d(\text{tRNA})/dt$ are proportional to μ, whereas $d(\text{rRNA})/dt$ increases as μ^2. This means that the total number of growing RNA chains in a cell, which equals the number of polymerase molecules engaged, increases *more* than linearly with μ. If the polymerase were to limit the frequency of transcription at all growth rates, its concentration would therefore have to increase *more* than does the number of ribosomes. This could not be achieved simply by exempting the polymerase cistrons from repression.

This discussion of mRNA synthesis shows how badly we need good measurements of mRNA half-life, and of polymerase concentration at different, and especially at low growth rates.

5.4 *The Balance between Ribosomal Protein and rRNA*

This part of the model rests quite heavily on Schleif's data (1967a,b, 1968). They are given this weight for two reasons: first, the technique unambiguously separates r-protein from all other proteins in the cell; and, second, Schleif shows that the protein associated with the excess rRNA produced in the presence of chloramphenicol is largely nonribosomal. Together with the kinetic data, this demonstration makes clear that it was wrong to conclude from the existence of the so-called chloramphenicol particles that normally growing cells maintain a large pool of free ribosomal proteins (Kurland and Maaløe, 1962).

To explain the production of matching quantities of r-protein and rRNA at all growth rates, a completely *ad hoc* feature has been introduced into the model: it is assumed that one of the r-proteins acts as an inducer of rRNA synthesis. The hypothetical inducer is thus constantly being introduced into the cytoplasm and removed again by incorporation into new ribosomes.

This scheme obviously serves the purpose for which it was invented, and thereby overcomes a difficulty which may not be too apparent. The extreme diversity among the r-proteins (Moore *et al.*, 1968), plus the evidence that rRNA maps apart from at least some of the r-proteins (experiments by Atwood, communicated by S. Spiegelman), makes it more and more unlikely that nascent rRNA serves as messenger for more than a few, if any, of the r-proteins. An ordinary mRNA molecule yields some 50–100 protein molecules per cistron, and applying this figure to the synthesis of the r-proteins it appears that for each mRNA produced a fairly large number of rRNA molecules are required to balance the protein yield. Even allowing for a four- to sixfold duplication of the rRNA cistrons, it seems that the promotor activity would have to be ten or more times higher in the rRNA than in the r-protein cistrons, and it is conceivable that matching quantities of their products could be assured simply by choosing the proper ratio between the promotor activities (an extra variable with no strings attached can do almost anything for you).

The proposed induction scheme was preferred because it adds a desirable property to the model: *the synthesis of rRNA, but not of mRNA, is put under stringent control.* It has been argued that if a single polymerase carried out all transcription, the three classes of RNA might be affected more or less equally (coordinately) under amino acid starvation (Maaløe and Kjeldgaard, 1966; Edlin and

Maaløe, 1966; Friesen, 1966). There is now strong evidence that mRNA is produced in considerable quantities in amino acid-starved cells (Edlin *et al.*, 1968; Lavallé and De Hauwer, 1968; Morris and Kjeldgaard, 1968; Stubbs and Hall, 1968), and at least qualitatively, this noncoordinate regulation of RNA synthesis agrees with the model. The quantitative aspect is difficult to assess (see Section 6).

6. CONSEQUENCES OF THE ANALYSIS

To construct the skeleton model just described, it was not necessary to decide whether the mechanism thought to balance r-protein and rRNA synthesis represented positive or negative control; nor did we have to know in detail about the coupling between ribosomes and mRNA synthesis. Both mechanisms can probably best be tested *in vitro* by adapting and refining already existing systems for RNA and protein synthesis.

The strength of the model is that it accounts for a large body of quantitative measurements made on *cells in well defined states of growth*. Its weakness is that it is hard to test its main thesis, because the complexity of the system renders the interpretation of even simple *in vivo* experiments ambiguous. Alternative models can almost certainly be constructed; however, it would be difficult to consider such a model seriously unless it included an account of our data, or questioned their validity.

The assembly of repressions, which is described here by the multivariable partition function, must figure in any analysis of bacterial growth. In the present model, this function is assigned special properties with regard to determining α_r, and stabilizing it during steady-state growth. These properties are such that the α_i of a protein generated from a nonrepressible cistron must always, even through a shift experiment, be proportional to α_r (except for possible changes in the relative gene-doses, as discussed in Sections 4 and 5.2). The model would be strongly supported if this prediction were verified. However, the critical test is complicated by catabolite repression. It is a simple matter to select a strain lacking a specific repressor protein, and thus exempt from specific repression of a given operon; but it is difficult to prove that the same operon is insensitive to catabolite repression. Moreover, it may be necessary to insist on *total* insensitivity, since even considerably reduced sensitivity could obliterate relatively small but critical differences. At the moment, the most promising material for this kind of experiment

would seem to be strains of the types described by Silverstone *et al.* (1969) which carry mutations assumed to reduce or abolish the sensitivity of the lac-promotor region to catabolite repression. In fact, some of the results reported in their paper point in the direction predicted by our model; others would have to be interpreted in terms of residual, slight sensitivity to catabolite repression.

A less stringent test would be to compare, say, OTCase synthesis in the wild type and in repression-defective mutants at different growth rates. If the mutants chosen were truly nonrepressible, the enzyme level in the wild type would be expected to approach that in the mutants as the growth rate decreased. However, the degree to which the difference would be reduced cannot be predicted (see discussion of growth with "poor" carbon sources in 5.2).

More indirectly the model can be tested by applying it to special cases. Two can be mentioned here:

(a) A relatively small number of different strains, and of different steady states have been carefully examined, and we therefore wanted directly to test one of the consequences of equations (3-1) and (3-2), namely, that for a given μ, the same α_r should obtain irrespective of the carbon and energy source (in minimal media). For this purpose strains were selected carrying a single mutation which greatly reduces the capacity for uptake of a variety of sugars and amino acids. In batch cultures of these strains μ is defined by the concentration of the carbon source, and steady states of growth can be maintained at quite satisfactory cell densities. With lactose as the sole carbon source μ values between 1.5 and 0.5 were established and the RNA:DNA ratios determined by chemical analyses. Throughout, the correspondence between this ratio and μ was almost exactly the same as that found by comparing *different* carbon sources, such as glucose, glycerol, succinate, and acetate. (This study, including the strain selection, was made by K. v. Meyenburg in our laboratory, and will be published elsewhere.)

(b) The second case is clearly pathological, and concerns a mutant analyzed by MacDonald *et al.* (1967). This strain is characterized by an abnormally high RNA:DNA ratio and it grows slowly in all media. The lesion affects the maturation of the 50 S ribosomal subunit, and large pools of precursor material and of free, apparently normal 30 S subunits are maintained during growth. It is obvious that, relative to the low growth rate, these cells contain very large total quantities of r-protein as well as rRNA. In terms of the equation $\mu = c_2\alpha_r r'$ (3-1),

α_r is abnormally high and r' correspondingly reduced. However, equation (3-1) presupposes that all, or a constant fraction of the ribosomal material is in active 70 S particles. Relative to the wild type, this condition is far from being realized in the mutant, and the conclusion about r' being reduced is therefore trivial. The case is brought up to emphasize that if, for any reason, r' is reduced, α_r must be correspondingly increased. This would seem to apply specifically to growth with restricted availability of an amino acid (or of some species of charged tRNA), because the primary effect of the restriction must be to increase the step time for the amino acid in short supply and thus reduce r'. Again, however, the complexity of the system makes it impossible to predict *how much* r', and α_r, would be affected.

7. DEFICIENCIES IN THE ANALYSIS

The phenomenon of "relaxedness" and the control of tRNA synthesis and of DNA replication have deliberately been left out of the main discussion. I shall briefly explain why, and try to relate each of these topics to the model.

It has been shown how stringent control of rRNA synthesis is built into the present model (as it should be). The "relaxed" (RC^-) mutants do not figure in the body of the text because I now believe that they represent a secondary defect in the RNA control mechanism, and tell nothing about its main principle of operation.

Little attention has been given to the fact that amino acid starvation, i.e., the condition in which the RC^- phenotype is revealed, greatly upsets the metabolism of the cells. During normal growth most of the carbon and energy are consumed in protein synthesis, and this flow is cut drastically when a required amino acid is withdrawn. As a result precursors and catabolites must accumulate. Cashel and Gallant (1969) have shown that rare, or abnormal nucleotides accumulate in RC^+ but not in RC^- cells. Unfortunately, neither the work of Gallant and his associates, nor the coupling scheme proposed here, appear to explain why chloramphenicol, and other antibiotics interfering with protein synthesis, uncouple rRNA production in RC^+ cells.

In retrospect, it was probably an error to imagine that studies of RC^- mutants (Stent and Brenner, 1961) and of the uncoupling effect of chloramphenicol (Kurland and Maaløe, 1962) would lead to an understanding of the *main* features of the control of RNA synthesis.

It has long been known that RC^+ and RC^- cells have identical growth characteristics in different media and respond the same way to a shift-up (Neidhardt, 1963). These facts are not readily explained by the old hypothesis which made uncharged tRNA the *main* effector in the control of RNA synthesis, and described RC^- cells as being relatively insensitive to its inhibitory effect on RNA synthesis. The present model allows RC^+ and RC^- cells to respond identically to fluctuations in the partition function, and thus to carry out the fine adjustments required to establish and stabilize a steady state of growth; the two types are thought to differ in a secondary feature of the control system, the coupling between the syntheses of r-protein and rRNA, and this difference is revealed only under conditions of metabolic congestion. In summary, the uncoupling of rRNA (and tRNA) synthesis in amino acid starved RC^- cells, *and* in RC^+ cells in the presence of chloramphenicol, etc., remains somewhat mysterious, despite the impressive amount of biochemical and genetic data now available (reviewed by Edlin and Broda, 1968).

The synthesis of tRNA and replication of DNA have an important characteristic in common: per genome, the same total quantity is produced, during one doubling time, at all growth rates. This is self-evident in the case of DNA, and since the tRNA:DNA ratio is more or less independent of μ it applies to tRNA as well (cf. 2.3).

We have seen that the structure of the whole system imposes the same rule on protein synthesis, i.e., that irrespective of its composition the total quantity of protein per genome is constant. The same could be true within the class of tRNA molecules, but one crucial piece of information is lacking, without which it is impossible to tackle the problem: it is not known whether the individual tRNA species are produced in constant molar ratios, or whether these ratios are subject to specific regulations. Constant molar ratios seem most reasonable to me, because the trial and error process by which the correct species of changed tRNA is selected at any step in polypeptide synthesis probably is governed by diffusion. It would therefore seem that the *concentrations* of the individual species cannot be allowed to change much with the growth rate. However, the problem of the ratios should be settled before speculating further.

Much has been learned in recent years about DNA replication and about its relation to bacterial growth. The pertinent work is well represented in Volume 33 of the Cold Spring Harbor Symposia (1968). In relation to the model, the most important fact is that, like

tRNA and total protein, the initiator(s) of replication must be produced in a fixed amount per genome and per doubling time. Very likely, one or more proteins are involved specifically in the initiation of replication, and these proteins must therefore be synthesized as a constant fraction of all the cells proteins, *irrespective of the composition of this assembly*. No simple mechanism for achieving this has suggested itself, and a link is therefore missing between the present model and the elegant scheme constructed by Helmstetter and Cooper to account for the pattern of replication at different growth rates (see Helmstetter *et al.*, 1968).

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