CONTROL MECHANISMS IN BACTERIAL CELLS

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ABSTRACT

The existence of self-regulating mechanisms in complex organisms is common knowledge. The single cell, the unit from which more complex organisms are built up, also possesses remarkable powers of adjustment. Detailed quantitative studies of the growth of bacteria in various environmental conditions have given an insight into the control mechanisms and various models have been set up. The cases when reproduction in a given environment can be described by autocatalytic-type reactions are treated, as well as conditions on transfer of the organisms into a fresh medium of identical composition or into a medium of different type. The self-regulating mechanisms are explained on the basis of simple kinetic models. The importance of the role of molecular biology in explaining the process of reproduction is stressed.

The principal object of microbial engineering is production and as has been aptly remarked[1], ... 'as a productive machine the living cell is a miracle of ingenuity, flexibility and variety. A single-celled bacterium produces a multiplicity of chemical substances, some simple, some complex, such that the catalogue of its products would bear comparison with, and in some respects surpass, that of the most skilled manufacturer of fine chemicals'. This is achieved often at the expense of simple and varied starting materials alone, by integrated unit processes, governed by enzymes which, themselves, must be regulated by control mechanisms. Intricate machines can be studied in two ways both equally valuable. They can either be taken to pieces or they can be observed in action in various conditions and general propositions set up to explain the behaviour. The latter method has been used extensively in our laboratory.

It is well known that in a constant environment, such as is obtained in continuous culture, a bacterial cell reproduces all its parts according to the relationship

$$dx/dt = kX \text{ or } X = X_0 e^{kt} \qquad (1)$$

This comes about by the interplay of enzyme reactions. Enzymes in isolation do not, however, increase autocatalytically but nevertheless it is easily established that substances which are by no means self-replicating in their own right can increase according to the law of autocatalysis which makes

them appear autosynthetic. For example, if we have two substances X and Y whose rates of formation are mutually interdependent such that

$$dX/dt = \alpha Y \text{ and } dY/dt = \beta X \tag{2}$$

then it is easily shown that the solutions of the equations are

$$X = A_1 e^{kt} + B_1 e^{-kt} \text{ and } Y = A_2 e^{kt} + B_2 e^{-kt} \tag{3}$$

where $A_1 + B_1 = X_0$ and $A_2 + B_2 = Y_0$. X_0 and Y_0 are the amounts of X and Y at zero time and it follows that

$$X = \frac{1}{2}\left(X_0 + \frac{\alpha}{k} Y_0\right) e^{kt} + \frac{1}{2}\left(X_0 - \frac{\alpha}{k} Y_0\right) e^{-kt} \tag{4a}$$

$$Y = \frac{1}{2}\left(Y_0 + \frac{k}{\alpha} X_0\right) e^{kt} + \frac{1}{2}\left(Y_0 - \frac{k}{\alpha} X_0\right) e^{-kt} \tag{4b}$$

where $\alpha\beta = k^2$. When growth has continued for a long time the ratio X/Y settles down to

$$\left(X_0 + \frac{\alpha}{k} Y_0\right) \Big/ \left(Y_0 + \frac{k}{\alpha} X_0\right) = \alpha/k.$$

If a portion of the system is now isolated and used as the starting point of a new system, $X_0/Y_0 = \alpha/k$ and equations 4a and 4b reduce to $X = X_0 e^{kt}$ and $Y = Y_0 e^{kt}$. Each separate component then increases with time as though formed in accordance with the simple autocatalytic law given in equation 1.

When three interdependent components are present i.e.

$$dX/dt = \alpha Y, \qquad dY/dt = \beta Z, \qquad dZ/dt = \gamma X \tag{5}$$

differentiation gives $d^3X/dt^3 = \alpha\beta\gamma X = k^3 X$, with similar expressions in Y and Z, and the solutions are of the form

$$X = A_1 e^{kt} + B_1 e^{\theta_1 kt} + C_1 e^{\theta_2 kt}$$

where $A_1 + B_1 + C_1 = 0$ and 1, θ_1 and θ_2 are the three cube roots of unity ($\theta_1 = -\frac{1}{2} + i(\sqrt{3})/2$ and $\theta_2 = -\frac{1}{2} - i(\sqrt{3})/2$). On evaluating the constants we have

$$X = \frac{1}{3}\left(X_0 + \frac{\alpha}{k} Y_0 + \frac{\alpha\beta}{k^2} Z_0\right) e^{kt} + \frac{1}{3}\left(X_0 + \theta_2 \frac{\alpha}{k} Y_0 + \theta_1 \frac{\alpha\beta}{k^2} Z_0\right) e^{\theta_1 kt} + \frac{1}{3}\left(X_0 + \theta_1 \frac{\alpha}{k} Y_0 + \theta_2 \frac{\alpha\beta}{k^2} Z_0\right) e^{\theta_2 kt} \tag{6a}$$

$$Y = \frac{1}{3}\left(\frac{k}{\alpha} X_0 + Y_0 + \frac{\beta}{k} Z_0\right) e^{kt} + \frac{1}{3}\left(\theta_1 \frac{k}{\alpha} X_0 + Y_0 + \theta_2 \frac{\beta}{k} Z_0\right) e^{\theta_1 kt} + \frac{1}{3}\left(\theta_2 \frac{k}{\alpha} X_0 + Y_0 + \theta_1 \frac{\beta}{k} Z_0\right) e^{\theta_2 kt} \tag{6b}$$

$$Z = \frac{1}{3}\left(\frac{k^2}{\alpha\beta}X_0 + \frac{k}{\beta}Y_0 + Z_0\right)e^{kt} + \frac{1}{3}\left(\theta_2\frac{k^2}{\alpha\beta}X_0 + \theta_1\frac{k}{\beta}Y_0 + Z_0\right)e^{\theta_1 kt}$$

$$+ \frac{1}{3}\left(\theta_1\frac{k^2}{\alpha\beta}X_0 + \theta_2\frac{k}{\beta}Y_0 + Z_0\right)e^{\theta_2 kt} \quad (6c)$$

In this example when t is large the terms containing $e^{\theta_1 kt}$ and $e^{\theta_2 kt}$ vanish and we again have constant ratios of the components. Thus $X/Y = \alpha/k$ and $X/Z = \alpha\beta/k^2$ and once more if we start a new system, in which X_0, Y_0 and Z_0 are in this proportion, each of the components follows equation 1, the terms in $e^{\theta_1 kt}$ and $e^{\theta_2 kt}$ vanishing since $\theta_1 + \theta_2 = -1$. This treatment is due to Hinshelwood[2] who further showed that the conclusions applied generally. Thus with r components a differential equation of the rth order, e.g. $d^rX/dt^r = kX$ is obtained for each and the solution is the sum of r terms of the type $A\,e^{\rho kt}$. ρ is the complex rth root of unity. The rth roots form a geometric series and when sufficient time has elapsed only the term in e^{kt} is of importance. On removing the imaginary terms from the non-steady state relationships sine and cosine terms appear. For example, the equations in series 6 become

$$X = \frac{1}{3}\left(X_0 + \frac{\alpha}{k}Y_0 + \frac{\alpha\beta}{k^2}Z_0\right)e^{kt} + \frac{1}{3}e^{-\frac{1}{2}kt}\left\{\left(2X_0 - \frac{\alpha}{k}Y_0 - \frac{\alpha\beta}{k^2}Z_0\right)\cos\frac{\sqrt{3}}{2}kt\right.$$

$$\left. + \sqrt{3}\left(\frac{\alpha}{k}Y_0 - \frac{\alpha\beta}{k^2}Z_0\right)\sin\frac{\sqrt{3}}{2}kt\right\} \quad (7)$$

with analogous expressions in Y and Z. The appearance of the sine and cosine terms predicts oscillatory behaviour in non-steady states whose relevance will become apparent later.

Subsequently Dean and Hinshelwood[3–6] developed ideas of the kind given above into a more elaborate theory of cell function again based on general physicochemical principles. The underlying assumptions were as follows:

(i) The chemical activity of the cell involves reactions split into a large number of stages, the elementary reactions being combinable in many different ways giving a variable reaction pattern.
(ii) There is an essential dependence of given reactions on specific cell constituents (proteins synthesized under the guidance of nucleic acids, nucleic acids dependent on enzymes for their production, some enzymes dependent on other enzymes and so on). The whole set of dependences forms a closed network to which a mathematical theorem (the network theorem) applies.
(iii) That the cell has some form of spatial organization.

The mathematical development of these ideas suggested (a) that living cells should be adaptable to changes in environment, developing the capacity to utilize unfamiliar nutrients with optimum efficiency; (b) that this plasticity should reveal itself in changes in cell composition and in particular in the proportions of enzymes; (c) that enzymes no longer necessary in a new

environment would diminish while those specially needed would increase: (d) that the rate of establishment of a steady state after transfer to a new environment would be very variable, and in particular that a pattern of reactions once established might prove highly persistent even though essentially unstable, and (e) that the general laws of growth forced upon the cell a tendency to divide as its volume increased beyond a certain point. Basic equations of the type $dX_J/dt = \alpha_J . X_{J+1}$ were set up. For example, a closed and unbranched network of mutual dependences was expressed as

$$dX_1/dt = \alpha_1 X_2, \qquad dX_2/dt = \alpha_2 X_3, \ldots, \qquad dX_J/dt = \alpha_J X_{J+1}, \ldots,$$

and finally to close the cycle $dX_n/dt = \alpha_n X_1$ (8)

The X terms represent parts of the cell in which a structural specificity resides such as nucleic acids, enzymes and possibly polysaccharides. They do not refer to the concentration of medium constituents or metabolites. These concentrations can influence the values of the α coefficients which may also vary from one medium to another and it should be emphasized that the treatment has little or nothing in common with a conventional mass-action treatment of chemical reactions.

It is evident from the simpler examples already given that the short term solution of the equations in set 8 is very complicated. Eventually, however, when the values of $X_1, X_2, \ldots, X_J, \ldots$ are large compared to their initial levels simple relationships again prevail viz:

$$dX_1/dt = kX_1, \qquad dX_2/dt = kX_2, \ldots, \qquad dX_J/dt = kX_J, \text{ etc.}$$

and k, the overall growth rate constant, is the geometrical mean of the α coefficients (i.e. $k^n = \alpha_1 . \alpha_2 \ldots \alpha_n$). The steady state ratios of the various components are given by equations of the type

$$X_J/X_{J+1} = \alpha_J/k \tag{9}$$

and once more, as in the simpler examples already given, if some of the material is transferred to another supply of the same medium each component increases with time in accordance with the simple autocatalytic law equation 1

$$X_1 = (X_1)_0 e^{kt}, \qquad X_2 = (X_2)_0 e^{kt}, \text{ etc} \tag{10}$$

It is also easily shown that a few of the α terms can become quite small, as might occur on transfer to a new kind of growth medium, without lowering the overall growth rate very much. For example, it one of them, say α_J, was reduced to one-tenth in a network containing 40 terms, k would be reduced in the ratio $(0.1)^{0.025}$ or by about 5.6 per cent. Initially the rate of formation of X_J would also be reduced since $dX_J/dt = \alpha_J X_{J+1}$ but in the new steady state X_{J+1}/X_J will be equal to k/α_J as before and since α_J is only one-tenth of its original value the ratio X_{J+1}/X_J will have automatically increased in proportion to restore the balance. It is also apparent that if any one of the α coefficients becomes zero then growth is impossible in a network of the kind shown in equation 8. However, the actual situation must be much more complex, the network of mutual dependencies containing numerous branchings and rejoinings. The quantitative treatment of branched mutual-

dependence cycles predicts behaviour of considerable biological relevance and the essential features can be illustrated by considering a single branch as in the example given by Dean and Hinshelwood[4].

The basic premises are that X_J in the closed cycle given in equation 8 is formed not only under the influence of X_{J+1}, as previously, but also under that of Y_1 i.e., $dX_J/dt = \alpha_J X_{J+1} + \beta_J Y_1$. The relevant part of the cycle can then be expressed as

$$\ldots X_{J-1} \rightarrow X_J \begin{cases} \rightarrow X_{J+1} \\ \rightarrow Y_1 \end{cases} \rightarrow X_{J+2} \rightarrow X_{J+3} \ldots X_n, \tag{11}$$

Y_1 spanning the gap between X_J and X_{J+2} as an alternative to X_{J+1}. In the steady state all the components will increase in accordance with the exponential law and k, the overall growth rate constant, is given by

$$k^n = \alpha_1 \alpha_2 \ldots \alpha_{J-1}(\alpha_J \alpha_{J+1} + \beta_1 b_1)\alpha_{J+2} \ldots \alpha_n, \tag{12}$$

where b_1 is the coefficient for the formation of Y_1 under the influence of X_{J+2} in the relationship $dY_1/dt = b_1 X_{J+2}$. k is still essentially a geometric mean and again the effect of changes in a few of the individual coefficients will largely be damped out. In the steady state

$$\frac{1}{X_1} \cdot \frac{dX_1}{dt} = k \ldots \tag{13}$$

and at the branch, since $dX_J/dt = \alpha_J X_{J+1} + \beta_J Y_1$,

$$\frac{1}{X_J} \cdot \frac{dX_J}{dt} = k = \alpha_J \frac{X_{J+1}}{X_J} + \beta_J \frac{Y_1}{X_J} \tag{14}$$

The latter proves to be a very important relationship which predicts behaviour having a striking similarity to many biological phenomena.

For example, in a given set of conditions $\beta_J Y_1/X_J$ may be very small compared to $\alpha_J X_{J+1}/X_J$ since the branch containing Y_1 is little used relative to the alternative. On transfer to a new medium, however, the supply conditions may be such that α_J is very small or even zero. Initially, growth in the new environment will be very slow due to the low value of $\beta_J Y_1/X_J$ but as it proceeds a new steady state will eventually be established in which $\beta_J Y_1/X_J$ is equal to k'. As shown above k' need not be much smaller than k and hence Y_1 must have increased considerably relative to X_J. Such automatically-occurring adjustments suggest a direct response to a need and are reminiscent of the plasticity frequently observed when bacteria are exposed to new environments. Furthermore, on transfer back from the second medium to the original conditions, in which α_J is large and the active functioning of Y_1 is no longer necessary, the composition alters again so that $\alpha_J(X_{J+1}/\alpha_J) + \beta_J(Y_1/X_J)$ once more is equal to k and Y_1/X_J reverts to its original low value. This latter behaviour is very like that termed enzyme repression but depends not on an active repressing of synthesis but on the deprivation by the competition of a process giving more efficient overall growth.

These kinetic equations consider only the temporal organization of the cell. It is also organized in space as modern researches bear witness and it

seems reasonable to postulate that corresponding to an optimum reaction pattern there will coexist an optimum geometry of the cellular components in any given state. This is essential so that intermediates, often labile and capable of suffering alternative fates, are produced in the right place and at the right time. Moreover, the lengths of diffusion paths and other factors influencing the transfer of intermediary metabolites from one centre of synthesis to another must be consistent with the optimum values of the various rate constants in the kinetic equations. In this respect the values of the α coefficients are themselves functions of the spatial arrangement. These ideas are embodied in what has been called the concept of the 'spatial map' of the cell and are discussed in detail elsewhere.[5,6] For the present, however, a simple model will illustrate the basic ideas. Suppose that in one set of environmental conditions the association of a network N_1 with a geometry M_1 gives the optimum rate of autosynthesis. In other conditions, and for reasons which have already been given in the discussion of the relevant equations, a network N_2 and a spatial map M_2 may be the most suitable. We then have the change from N_1M_1 to N_2M_2 but the automatically-occurring adjustment $N_1 \rightarrow N_2$ should occur much more rapidly than the more profound changes inherent in the change in geometry from M_1 to M_2. The stages N_1M_1 followed by N_2M_1 and finally N_2M_2 can then be envisaged. An interesting situation arises if the cells are now returned to the original conditions since the more rapid adjustment of the reaction pattern compared to the spatial arrangement of the cellular components implies that on the return journey the stages are N_2M_2, N_1M_2, N_1M_1. In effect the loss of an adaptation, which has proceeded to the maximum extent, may not simply be the reversal of the process by which it was acquired. A sort of hysteresis effect may intervene in which the cells pass through a stage (i.e. N_1M_2) not encountered before. Induced reversal at any earlier stage, when the changes have only proceeded from N_1M_1 to N_2M_1, should accordingly be more rapid and depending on the number of stages involved, which is likely to be more than in our simple model, a variety of types of behaviour, ranging from easy and complete loss of adaptations, through slow and partial losses, to tenacious retention would be expected. This has been our experience.

Thus far only the total masses of the various components have been considered and the possibility of maintaining constant conditions assumed. A division condition is easily introduced into kinetic treatments by assuming that it occurs when some constituent (e_μ) of the cell reaches a critical amount (or what proves to be equivalent, when the concentration of something within the cell reaches a critical level). A proportionality will then exist between the number of cells (n) and e_μ so that $n = \beta e_\mu$, where β is a constant. Nevertheless, it is instructive to consider the necessity for division. As a cell grows the ratio of its surface to its volume decreases rendering the access of nutrients to the interior more difficult and impeding the loss of metabolic products. The potential energy of the integrated system of cellular components increases, diffusion paths lengthen and the relative rates of chemical processes are altered. Clearly for a steady state of autosynthetic growth these variations must only occur between restricted limits about a more or less constant average value and in actual fact this is achieved by regular cell division.

The steady state of growth, where each component increases in accordance with the exponential law, then demands that the division mechanisms are operating in a regular manner, that the cellular components are present in the required stable ratios and that the concentrations of diffusible intermediates (which participate in the linking of the various cellular reactions) have reached their appropriate levels. These various conditions are not necessarily all satisfied simultaneously or in a regular manner during the nonsteady states which accompany the transfer of bacteria to new conditions or the transfer to a fresh supply of the same medium of organisms which have been allowed to age. Accordingly and depending on the circumstances, lag phases followed by multiplication at optimal or less than optimal rates, the onset of division preceding or lagging behind any increase in cell substance and the periodic waxing and waning in the rates of increase of various cellular components (compare equation 7) are all, in principle, possible before steady state conditions are again established. Such phenomena have all been observed experimentally (see for example, References 6–9).

Other seemingly diverse bacterial properties such as lysogeny and virulence in phage–bacteria systems[10–12], 'thymine-less death'[13], 'substrate-accelerated death'[14,15] and synchrony[16,17] can also be brought within the framework of the mathematical models if allowance is made for the reversibility of some of the stages[18]. It is not proposed to discuss these phenomena here but rather to point out that the 'turnover' of cellular components is similarly easily accommodated. If, for example, X_{J+1} in equation 11 is assumed to be an enzyme which can degrade component X_{J+2} then the rate of production of X_{J+2} is given by

$$dX_{J+2}/dt = \alpha_{J+2} \cdot X_{J+3} - \gamma X_{J+1} \tag{15}$$

Whether γX_{J+1} is significant or not depends on the circumstances: in actively-growing cells it is likely to be very small or even zero but might be appreciable in 'resting' cells.

The question now arises as to the relation of these assumptions and general propositions to the picture presented by molecular biology. In this picture nucleic acids are the repositories of structural information in terms of which messenger molecules are formed and travel as 'messengers' about the cell in parts of which they mediate protein synthesis[19,20]. Certain nucleotide structures bring about the incorporation of specific amino acids into proteins[21,22]. Enzymes of course play their traditional roles in all these processes[23]. In most ways the more specific and the more general kinds of treatment are in no sort of opposition. The general kinetic propositions do not ignore the essential genetic determination of the cell properties. The X terms in the equations represent various structural elements in the cell and might as well refer to nucleic acids as to anything else.

Next we come to the matter of messengers. The kinetic treatment postulates systems somehow organized in space, and envisages the occurrence of the overall synthetic reactions in very large numbers of relatively simple unit processes. This picture involves the diffusion of the products of one step to the place where the next occurs. No theory which postulates the formation of nucleic acids, proteins and other cellular components at different parts of a cell can possibly dispense with such a general postulate. Messengers,

then, are always needed whether they are given the quite specific task of mediating between DNA and the ribosomes, or assigned the more general functions which from the nature of a spatially heterogeneous, multistage chemical system must be filled. If, then, in a living cell the structure of what is present determines what is formed, if the total process is split into stages as in the common chemical rule, and if the whole has organization in space (without which the very concept of the cell is meaningless), it follows that codes, messengers, reading and transcription are necessities. The merit of molecular biology lies in the fact that it has begun to identify the chemical structures and reactions involved in these processes. It can fairly be said that the kinetic models could not ever yield information about specific interactions of a structural kind. Similarly, they do not predict gene repression. Nor do they deny it and, in principle, conditions for it might be written into the kinetic schemes. As the latter stand, however, they suggest that repression-like effects, brought about by competitive deprivation, are a common characteristic of living cells. Moreover, the theorems predict a wide range of circumstances in which such effects should be observed and generally speaking this is where they would be expected.

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