THE SYNTHESIS OF RIBOSOMES IN *E. COLI*

II. Analysis of the Kinetics of Tracer Incorporation in Growing Cells

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ABSTRACT Equations are derived representing the flow of radioactive tracer in a sequence of reactions. The conditions under which the equations are applicable are defined. A function ϕ, representing the newly synthesized fraction is defined, and its use in the analysis of precursor-product relationships is discussed.

A. INTRODUCTION

One of the great advantages of radioactive tracers in biological experimentation is the possibility of directly demonstrating in a complex system, for example a growing cell, that a particular sequence of reactions occurs. A radioactive compound may be chosen which enters into only a limited set of reactions; thus only a small number of cellular components become radioactive. These components may be separated from each other by much simpler procedures than those required for complete purification. By measuring the rate of entry of radioactivity into these intermediates and into the final products, proof of a sequential relationship can be obtained.

This paper examines the assumptions underlying such a proof, and presents a convenient method for analysis. The specific purpose is to introduce in a coherent form the arguments and method utilized in the following paper which reports studies of the stages of synthesis of ribosomal particles. Therefore in this discussion, although the analysis is entirely general, complicated and vague expressions have been avoided by referring almost entirely to the synthesis of RNA.

B. DERIVATION OF EQUATIONS REPRESENTING FLOW OF TRACER

1. *Restrictive Conditions.* In order to derive relatively simple equations representing the time course of passage of radioactivity through a sequence of intermediate objects, a number of severely restrictive conditions must be met.

(*a*) The relative quantity of intermediates should not change with time. This

statement is equivalent to a requirement for steady exponential growth of the culture.

(*b*) From a mathematical point of view each cell should obey condition (*a*) and the population should be homogeneous. However this is not achievable since growth normally occurs as a result of replication, followed by cell division. It will be presumed that the cells in the population are randomly phased and that any effects of variation of processes with time during the division cycle will be lost, since the samples of the population analyzed are always random.

(*c*) The specific radioactivity and rate of utilization of the tracer must be constant.

(*d*) It will also be assumed that the reactions which cause the transfer of tracer from one intermediate state to another are unidirectional. The failure of this condition does not necessarily lead to greater complexity, but such a case must be separately considered.

(*e*) Finally, it will be presumed that any molecule representative of a particular intermediate stage has a constant chance of being transferred to the next stage regardless of the length of time it has been present.

In a given experiment the proof that these conditions are actually met may not be available, or it may be known that one or more of them fail. In such a case careful analysis must be carried out in order to evaluate the type and magnitude of error that may result.

If the first precursor to be considered happens to be a pool of low molecular weight intermediates, it will most often be true that the specific radioactivity of the pool will rise with time as $1 - e^{-\alpha t}$. If there is no exchange between the external tracer and unlabeled pool compounds, the equations given in sections B and C will be valid as they stand. If exchange occurs, the equation for ϕ_T will not be valid and the time constant for the first precursor will be shorter than that expected from the size of the precursor pool. If this is taken into account, the remaining equations are still useful.

2. *Definition of Symbols.*

τ Time after addition of tracer; units such that $\tau = 1$ when the cells have grown by a factor e; $Q = Q_o e^{\tau}$ gives the growth of the cells or any component

μ Effective specific radioactivity of tracer; units such that the specific radioactivity of the RNA will approach μ after a long period of growth at a constant tracer concentration

X Quantity of a component, in general

X^* Its radioactivity

μ_X Its specific radioactivity

T The total of all components

M, N The quantities of RNA in the precursor and product, section 3

$$\phi_X = \frac{\mu_X}{\mu}\frac{X}{T} = \frac{X^*}{\mu T}$$

3. *Calculation of the Time Course of Labeling of a Single Precursor and Product.* From the steady exponential growth of each component:

$$M = M_0 e^{\tau}, \qquad N = N_0 e^{\tau}, \qquad T = T_0 e^{\tau}$$

The total RNA synthesized after $\tau = 0$ is:

$$T - T_0 = T_0(e^{\tau} - 1)$$

The newly synthesized fraction of the total RNA:

$$\phi_T = \frac{T_0(e^{\tau} - 1)}{T_0 e^{\tau}} = 1 - e^{-\tau} \tag{1}$$

The rate of change of the radioactivity of the precursor, M:

$$\frac{dM^*}{d\tau} = \mu \frac{dT}{d\tau} - \mu_M \frac{dN}{d\tau} = \mu T - \mu_M N$$

and

$$\frac{dM^*}{d\tau} = \frac{d}{d\tau}(\mu_M M) = \mu_M M + M \frac{d\mu_M}{d\tau}$$

therefore since $T = M + N$:

$$\frac{d\mu_M}{d\tau} = \frac{T}{M}(\mu - \mu_M)$$

Integrating and using the condition that $\mu_M = 0$ when $\tau = 0$:

$$\mu_M = \mu(1 - e^{-(T/M)\tau})$$

The newly synthesized fraction of the total RNA which is present in the precursor:

$$\phi_M = \frac{M}{T}\frac{\mu_M}{\mu} = \frac{M}{T}(1 - e^{-(T/M)\tau}) \tag{2}$$

Finally, since $\phi_T = \phi_M + \phi_N$ the newly synthesized fraction of the total RNA which is present in the product:

$$\phi_N = 1 - e^{-\tau} - \frac{M}{T}(1 - e^{-(T/M)\tau}) \tag{3}$$

Fig. 1 shows the three functions ϕ_T, ϕ_M, and ϕ_N calculated from equations (1), (2), and (3) for the specific case where $M/T = 0.10$. The interpretation of the log-log plot will be discussed below; however, in this mathematical section will be given the proof that ϕ_M and ϕ_N have the form at early times indicated on Fig. 1.

Expansion of the exponential gives for equation (2):

$$\frac{M}{T}\left(1 - 1 + \frac{T}{M}\tau - \frac{T^2}{2M^2}\tau^2 \cdots\right) = \tau - \frac{T}{2M}\tau^2 \cdots$$

At early times when $T\tau/2M << 1$, $\phi_M = \tau$ and ϕ_M thus follows a straight line at 45° (slope 1) on Fig. 1.

Similarly, expansion of the exponentials in equation (3) gives:

$$\tau - \frac{\tau^2}{2} + \frac{\tau^3}{6} \cdots - \tau + \frac{T}{M}\frac{\tau^2}{2} - \frac{T^2}{M^2}\frac{\tau^3}{6} \cdots$$

Here the terms in τ cancel at early times and

$$\phi_N = \frac{N\tau^2}{2M}\left(1 - \frac{T+M}{3M}\tau \cdots\right). \tag{4}$$

ϕ_N has the form indicated on Fig. 1 at early times and as a matter of fact deviates from this by less than 10 per cent when $\tau = .03$. At this time the precursor has already reached one-third of its final radioactivity. Thus for a usefully long period the radioactivity of the product rises as τ^2 and follows a straight line of slope 2 on the log-log plot.

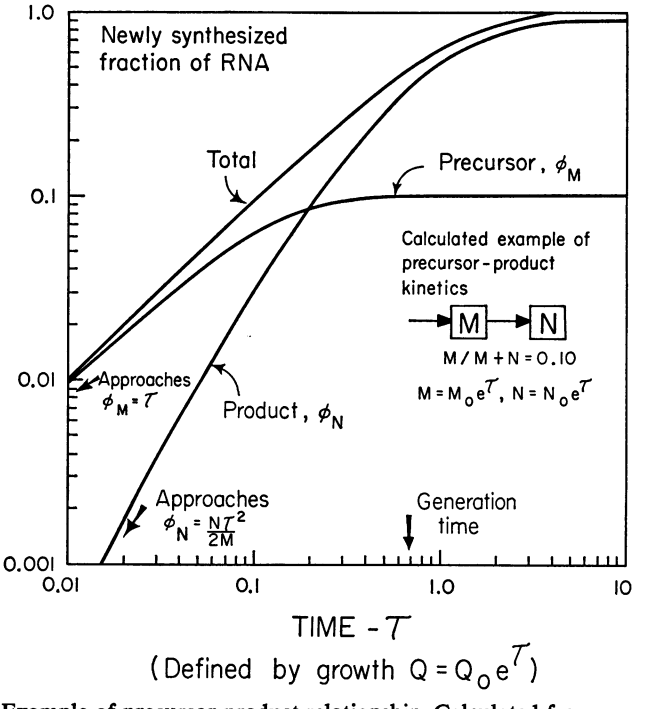

FIGURE 1 Example of precursor-product relationship. Calculated from equations (1), (2), and (3) for the case in which one tenth of the total RNA is in the precursor. The precursor levels off at 0.10 and reaches 63 per cent of this value when $\tau = 0.10$. Ordinate and abscissa scales defined in text.

4. *The Time Course of Labeling of Two Sequential Precursors and a Product.* Where the tracer enters sequentially into 3 objects ($\rightarrow E \rightarrow N \rightarrow R$) an analysis similar to that given in section 3 but involving one further integration yields the following equations:

$$\phi_T = 1 - e^{-\tau} \tag{5}$$

$$\phi_E = \frac{E}{T}(1 - e^{-(T/E)\tau}) \tag{6}$$

$$\phi_N = \frac{N}{T}\left(1 - \frac{E(R + N)e^{-(T/E)\tau} - TNe^{-(R/N)\tau}}{E(R + N) - TN}\right) \tag{7}$$

$$\phi_R = \phi_T - \phi_E - \phi_N \tag{8}$$

Analysis of the time course of labeling of an indefinite number of such sequential precursors may be easily carried out in a similar way. At early times the radioactivity of each of the objects in sequence will rise in proportion to τ, τ^2, τ^3, τ^4, etc. Without a detailed study of the approximations involved this may be easily seen from the following argument.

At early times the first precursor will contain essentially all of the radioactivity and its specific radioactivity will rise in proportion to τ. Since the amount of radioactivity in the second precursor is simply the time integral of the specific radioactivity of the first, it will rise in proportion to τ^2, as long as the specific radioactivity of the first does not approach saturation. Similarly the amount of radioactivity in the third object is the time integral of the specific radioactivity of the second and will rise to proportion to τ^3.

C. DISCUSSION

The function ϕ which we have called the newly synthesized fraction of the RNA (in the special case of RNA synthesis) has turned out to be of great practical use. It can be expressed:

$$\phi_X = \frac{\mu_X}{\mu}\frac{X}{T} = \frac{X^*}{\mu T} \tag{9}$$

It is measured by the radioactivity (X^*) of a given fraction, and can be computed readily from the experimental data. At early times the total radioactivity of a precursor may be measured accurately even when the specific radioactivity is uncertain as a result of dilution with unlabeled material. T is, of course, the total RNA corresponding to the sample size on which X^* was determined.

μ is readily determined from the specific radioactivity of the total RNA using the relations:

$$\mu_T = \frac{T^*}{T} = \mu\phi_T = \mu(1 - e^{-\tau}) \tag{10}$$

where τ has been determined from the growth curve, $Q = Q_0e^{\tau}$. Since T is used both in calculation of μ and ϕ_X an absolute measure of RNA is unnecessary. In fact a number proportional to the equivalent cell mass in the sample is sufficient.

In using equation (10) it is, of course, necessary to know that T^* is in fact the total radioactivity in the sequence of reactions being considered. Further if T^*/T is

not proportional to $1 - e^{-\tau}$ a failure of restrictive condition *c* or *d* is indicated and the significance of the errors resulting must be considered. Examples of the method of calculation of ϕ and its use in a complex case are given in Paper III.

In the interpretation it is convenient to plot log ϕ against log τ. This procedure expands both scales at early times. During the usefully long period when ϕ is proportional to τ, τ^2, or τ^3 a straight line of slope 1, 2, or 3 results. Thus precursor-product relationships can be readily recognized. The relative quantity of each fraction is given by the final value of ϕ after the specific activity of that fraction has been saturated.

In a simple case such as that of a single precursor it is easy to test the precursor-product relationship quantitatively without further calculation. In this case ϕ for the precursor is given by equation (2). On the log-log plot the constants in equation (2) affect the position but not the shape of the curve. Thus a curve of $1 - e^{-\tau}$ on tracing paper may be translated over the experimental plot until the best fit is achieved. The experimental points, at late times, should level off at $\phi = M/T$. $\tau = 1$ on the $1 - e^{-\tau}$ tracing should lie over $\tau = M/T$ on the experimental graph.

In more complex cases, or if there is a failure of a sequential relationship, the nature of the relationships can be deduced from the slopes, positions, and curvatures of the various fractions. The use of τ defined by the growth curve simplifies all of these operations and allows the comparison of experiments carried out at different growth rates.

While ϕ is adequately defined by equation (9) the following general definition may perhaps be useful. ϕ is the ratio of the number of labeled atoms at a given time in a given class of molecules to the number of atoms in the whole cell which would be labeled after a long period of growth during which the labeling conditions were precisely constant. ϕ is thus a measure of the number of newly synthesized molecules which occur in a given class, and has been named the "newly synthesized fraction." The term newly synthesized is used here to refer to those molecules which have actually been assembled from low molecular weight precusors (including the tracer) after the tracer has been added. If over a significant period of time there is a rise in the specific radioactivity of a pool of low molecular weight presursors, the general definition above is still valid but the meaning of the term newly synthesized must be carefully considered.

APPENDIX

A SPECIAL METHOD USING SPECIFIC RADIOACTIVITIES

In certain circumstances the analytical methods permit the measurement of the specific radioactivity of the fractions, but not their total radioactivity. In such cases the equations given here are still useful. However, in the absence of independent measures of the time constant and the size of a fraction the test of precusor-product relationships is not as effective. The purpose of this Appendix is not to examine such procedures but to

bring attention to the peculiar properties of the function μ_N/μ_M, the ratio of the specific radioactivity of the product to that of the precursor in the simple case examined in section 3. From equations (2) and (3) we can write:

$$\frac{\mu_N}{\mu_M} = \frac{M}{N}\left(\frac{T}{M}\cdot\frac{1-e^{-\tau}}{1-e^{-(T/M)\tau}} - 1\right) \tag{11}$$

At early times this ratio has, of course, very small values, and at late times it approaches unity. However, at any given time it does not vary by more than a factor of two for any possible relative quantities of precursor and product. For example, when $\tau = 0.01$, $\mu_N/\mu_M = 0.01$ for the case of an infinitely small precursor. For the other extreme case when the precursor is large and the product infinitely small $\mu_N/\mu_M = 0.005$ when $\tau = 0.01$.

This surprising result is not evident by inspection of the equation. It should be useful in a variety of special circumstances. For example, incomplete purification of the precursor will cause the experimental curve to lie entirely outside of the region permitted by this function. This can easily be recognized and does not require knowledge of the relative size of the pool of precursor. The existence of two sequential precursors or the failure of restrictive condition (*e*) will yield values of μ_N/μ_M much smaller than those predicted by equation (11).

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