EVIDENCE FOR THE POLYCISTRONIC TRANSCRIPTION OF RIBOSOMAL RNA IN *ESCHERICHIA COLI*

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Summary: Using rifampicin to inhibit the initiation of transcription, the time that an RNA polymerase molecule can continue transcribing ribosomal RNA (rRNA) has been measured. The time found is too long for the synthesis of single rRNA chains at previously measured rates of RNA chain growth, but is consistent with the continuous transcription of a 16S, a 23S and a 5S cistron after a single initiation event. It is proposed that the clustered genes for rRNA are transcribed as operons containing one cistron for each species of rRNA.

The chromosome of *E. coli* contains a single cluster of six genes for 16S ribosomal RNA (rRNA) and six genes for 23S rRNA (1). Measurements of the specific radioactivities of the 16S, 23S and 5S rRNA species after inhibition of chain initiation with rifampicin (RIF) have implied that three cistrons are transcribed in the order 16S-23S-5S in a single transcriptional event (2).

In the present study, measurements of the incorporation of labeled uracil into total RNA and into stable (ribosomal and transfer) RNA after propagation to completion in the presence of RIF have been analyzed to give the length of time an RNA polymerase molecule can continue transcribing rRNA in the absence of further initiation. The measured transcription time is too long for the synthesis of single rRNA chains at previously measured rates of RNA chain growth (3-6), suggesting that rRNA is synthesized by transcription across several cistrons.

Materials and Methods

Our laboratory strain of *E. coli* K12(λ) was grown as described

previously (7), with 10 mM glucose or 0.1% casamino acids added to basal medium as indicated. Growth rate (μ) was determined turbidimetrically (7).

The incorporation of uracil-^{14}C into RNA was measured by adding 0.5 ml of culture to an equal volume of cold 10% CCl_3COOH with two drops of a 1% solution of bovine serum albumin as carrier. The precipitate was collected on a glass fiber filter (Reeve-Angel) and washed with 5 ml of cold 5% CCl_3COOH. The filters were dried, placed in vials with 15 ml of toluene-based scintillation medium and counted in a Packard model 3380 spectrometer. Rifampicin (lot no. U-2995) and uracil-2-^{14}C (55 mCi/mmole) were from Schwarz/Mann, Orangeburg, N.Y.

Results and Discussion

Representative measurements of the incorporation of uracil-^{14}C into RNA in growing cells are shown in Fig. 1. At each point, R_t is the radioactivity in total RNA (mostly stable RNA) and R_∞ is the final level of radioactivity attained when RNA chains growing at time t are propagated to completion while chain initiation is blocked by RIF. The additional time allowed to reach R_∞ is long enough for complete decay of labeled messenger RNA (mRNA), such that the radioactivity at R_∞ should be found only in stable RNA. This has been confirmed by sucrose gradient sedimentation of the purified RNA. Experiments like that of Fig. 1 have been performed with cells growing in different media and at different temperatures and in all cases the R_t and R_∞ curves are approximately parallel.

The time-lag (x) between the R_∞ and R_t curves is given by the increase in radioactivity at infinite time ($R_\infty - R_t = S_\infty$) divided by the slope of the R_∞ curve (see Fig. 1). That is,

$$x = \frac{S_\infty}{dR_\infty/dt} \qquad (1)$$

Assuming that the contribution of labeled mRNA to R_t and of tRNA to R_∞ are both negligible, the quantity S_∞, the amount of radioactivity incorp-

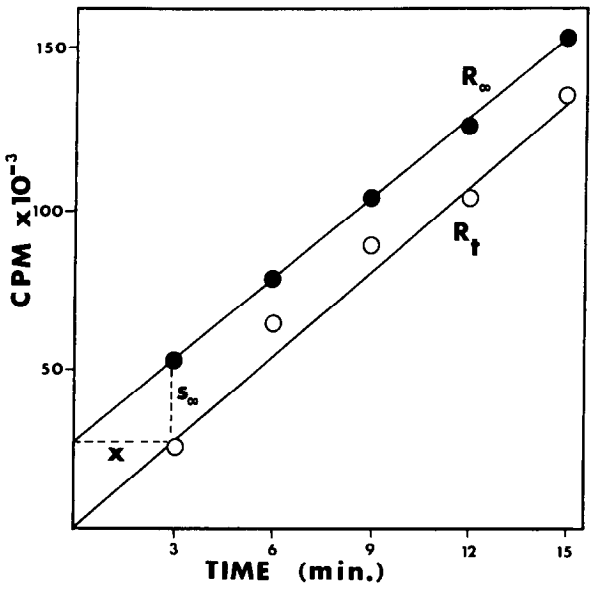

Figure 1. Measurement of R_t and R_∞ in exponentially growing cells. *E. coli* K12(λ) growing at 30°C in basal medium + 10 mM glucose (μ = 0.5 doublings/hr) was labeled with 0.31 μCi/ml of uracil-^{14}C. At intervals, 0.5 ml samples were precipitated with CCl_3COOH to measure R_t and duplicate 0.5 ml samples were added to 0.5 ml of basal medium containing 10 mM glucose and 400 μgm/ml RIF. After 30 min. of incubation at 37°C, the latter samples were precipitated with CCl_3COOH to measure R_∞.

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orated by the completion of chains which were growing at time t, is

$$S_\infty = n_g \cdot s \cdot f \cdot (\ell_c - \overline{\ell_t}) \qquad (2)$$

where n_g is the number of growing chains, s is the radioactivity per uracil residue entering the growing chains, f is the base fraction of uracil residues, ℓ_c is the length in nucleotides of a complete chain and $\overline{\ell_t}$ is the average length of a growing chain at time t. If RNA polymerase molecules are distributed randomly on the DNA, then $\overline{\ell_t} = \ell_c/2$. Thus,

$$S_\infty = n_g \cdot s \cdot f \cdot \ell_c/2 \qquad (3)$$

Note that S_∞ remains constant with time (Fig. 1), implying that a steady-state exists in growing cells such that n_g is constant.

The rate of change of R_∞ is given by

$$\frac{dR_\infty}{dt} = s \cdot f \cdot \ell_c \left[\frac{dn_c}{dt} + \frac{dn_g}{dt}\right] \qquad (4)$$

where n_c is the number of completed chains. At steady-state n_g is constant so

$$\frac{dR_\infty}{dt} = s \cdot f \cdot \ell_c \cdot \frac{dn_c}{dt} \qquad (5)$$

Substituting equations (3) and (5) into equation (1) we get

$$x = \frac{n_g}{2dn_c/dt} \qquad (6)$$

Imagine now an RNA polymerase molecule P_1 which has just initiated transcription of a stable RNA cistron. The time taken by this molecule to traverse a transcriptional unit is t_c, operationally defined as the length of time such a molecule can continue transcribing in the presence of RIF. The number of chains which are completed during the time (t_c) the molecule P_1 takes to traverse the transcriptional unit is the same as the number of chains n_g which were in process of transcribing ahead of P_1 on the same transcriptional unit. Expressed mathematically this is

$$\frac{dn_c}{dt} \cdot t_c = n_g \qquad (7)$$

Substituting equation (7) into equation (6) we get

$$x = t_c/2 \qquad (8)$$

That is, the time-lag x between the curves in Fig. 1 is one-half the time a single polymerase molecule takes to transcribe the distance between RIF-sensitive positions on the stable RNA cistrons.

Data such as those in Fig. 1 have been obtained for cultures at a number of growth rates. Some representative values of t_c are shown in Table I. In general, t_c is inversely proportional to the bacterial growth rate μ. The values of t_c, however, are always greater than is predicted for the transcription of a single rRNA chain at the RNA chain growth rates which others have measured. At $\mu = 0.33$, for example, an RNA chain growth rate of 15 nucleotides/sec. has been found (4). At this rate, the transcription of the 3200 nucleotides of 23S rRNA should take 213 sec., yet we find $t_c = 480$ sec. Similar discrepancies are found at other bacterial growth rates (Table I).

TABLE I

Comparison of Measured and Calculated RNA Chain Growth Rates

Medium	μ doublings/hr.	t_c (sec.)	Chain Growth Rate (nucleotides/sec)	Reference
Glucose	1.33	-	55	3
Glucose + CAA	1.2	96	56*	
Glucose + CAA	1.07	-	26	4
Glucose + CAA	1.07	144	37*	
Glucose	0.5	-	13	5
Glucose	0.49	-	24	6
Glucose	0.5	396	14*	
Proline	0.33	-	15	4
Glucose	0.32	480	11*	

* Calculated from the completion time t_c for a unit of 5400 nucleotides.

It should be noted that estimates of the RNA chain growth rate by different methods often do not agree closely. For example, two estimates (5,6) at μ near 0.5 doublings/hr. have given values of 13 and 24 nucleotides/sec., respectively. It is therefore difficult to judge the exact degree of discrepancy between direct estimates of RNA chain growth rate and estimates from the measured values of t_c.

The values of t_c can be most easily reconciled with the chain growth rates if we assume that an RNA polymerase molecule which has initiated transcription on the rRNA cistrons can then proceed for about 5400 nucleotides in the presence of RIF. A transcriptional unit of 5400 was chosen to represent one cistron each of the 16S and 23S rRNA species, allowing for the fact that each of these RNA species is transcribed in a form about 10% larger than the corresponding chains found in ribosomes (8,9). A 5S cistron about 120 nucleotides long has also been included to conform with the suggestion of Pato & von Meyenburg (2). The omission of this additional 120 nucleotides, however, would make little difference in our calculations and there is no direct evidence to support its inclusion.

Using 5400 nucleotides as the length of a transcriptional unit, the values of t_c yield RNA chain growth rates which are in reasonable agreement with those determined by others (Table I). Since the technique used here allows only stable RNA to contribute to the measured value of R_∞, the inclusion of unstable RNA or a "spacer" in the transcription of rRNA would not produce the observed result. These measurements therefore support the conclusion (2) that rRNA is transcribed in the form of polycistronic operons.

The organization of genes whose products are functionally related into a single transcriptional unit or operon has been recognized as a means of coordinating the synthesis of the several gene products. In the case of ribosomal RNA, one molecule each of the 16S, 23S and 5S species are present in a functional 70S ribosome. A mechanism to insure their coordinated synthesis might thus be biologically advantageous.

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