rRNA Transcription Rate in *Escherichia coli*

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The rate of in vivo transcription elongation for *Escherichia coli* rRNA operons was determined by electron microscopy following addition of rifampin to log-phase cultures. Direct observation of RNA polymerase positions along rRNA operons 30, 40, and 70 s after inhibition of transcription initiation yielded a transcription elongation rate of 42 nucleotides per s.

Gene expression originates with the transcription of DNA into RNA and can be influenced not only by the frequency with which RNA polymerases initiate transcription but also by the rate at which the nascent RNA chains are elongated. Biochemical estimates of the rate of rRNA chain elongation in *Escherichia coli* range from 12.5 to 108 nucleotides per s at 37°C (2, 3, 5, 6, 15, 18, 19, 22, 23). To determine more directly the rate of transcript elongation in vivo, we have visualized the process of rRNA transcription by electron microscopy. After addition of rifampin, which inhibits initiation of transcription while allowing elongation in progress to continue unchanged (24), the positions of RNA polymerases along templates coding for rRNA were measured as a function of time.

Log-phase cultures of *E. coli* W3110 grown at 37°C in LB medium (16) ($\mu = 2.4$ doublings per h) were exposed to rifampin (200 $\mu\text{g/ml}$) for 0, 30, 40, or 70 s and then prepared for electron microscopy by the Miller chromatin-spreading technique (10, 12). Bacterial cell contents were dispersed in pH 9 water without EDTA. Samples were viewed in a JEOL 100C transmission electron microscope. Measurements from micrographs were made with a Numonics 2200 digitizing tablet and Jandel SigmaScan software.

The Miller spreading technique relies on low ionic strength to gently disperse chromatin. RNA polymerases engaged in transcription at the time of cell lysis remain attached to their DNA templates and are visualized on the DNA as electron-dense particles at the base of nascent transcripts. Transcriptionally active genes are visualized as an array of increasing nascent fibril lengths emanating from a central chromatin fiber. In chromatin spreads, *E. coli* rRNA operons can be specifically identified by their dense packing with RNA polymerases and their characteristic double "Christmas tree" morphology (Fig. 1A). RNase III cleavage of nascent transcripts between the 16S and 23S cistrons gives rise to the two gradients of rRNA fibril lengths observed (8, 12).

To determine the rate of rRNA chain elongation in *E. coli*, initiation of transcription was inhibited with rifampin and the progression of previously initiated RNA polymerases was observed along the rRNA operons. As the time following rifampin addition increased, the length of the operons densely packed with RNA polymerases decreased in a 5'-to-3' direction (Fig. 1). The seven rRNA operons in *E. coli* vary somewhat in length because of differences in their component 5S and tRNA genes. Five of the seven operons can be distinguished in electron micrographs by patterns of

upstream and downstream transcription (8). In the presence of rifampin, however, identification of specific operons becomes more difficult. As initiation of transcription is inhibited, surrounding transcriptional markers used to identify the rRNA operons are also lost. Because the identity of each rRNA operon visualized could not be determined, measurements were based on an average rRNA operon length of 5.5 kb derived from DNA sequence (4, 14) and 5S and tRNA composition (1) data. The transcribed portions of the rRNA operons averaged 4.5 ± 0.5 kb ($n = 11$), 3.8 ± 0.5 kb ($n = 48$), and 2.6 ± 0.5 kb ($n = 36$) in length following 30, 40, and 70 s of exposure to rifampin, respectively.

Linear regression analysis of the length of rDNA template densely packed with RNA polymerases versus time following rifampin addition (Fig. 2) yielded a transcription elongation rate of 42 nucleotides per s (standard error of slope = ± 2 nucleotides per s, $r^2 = 0.82$). Elimination of the 0-s data from regression analysis yielded a transcription elongation rate of 43 ± 3 nucleotides per s ($r^2 = 0.66$), indicating that inhibition of transcription initiation occurred rapidly.

Inhibition of transcription initiation was, however, not complete. Often one or more RNA polymerases were observed between the promoter and the cluster of polymerases completing transcription (Fig. 1C). From differences in RNA polymerase density between the actively transcribed and the infrequently transcribed portions of the operons, we estimate that ~6% of RNA polymerases escaped inhibition by rifampin. At all times of inhibition there were 12 ± 4 polymerases per kb on the actively transcribed portions of rRNA operons, the same density as observed previously in the absence of rifampin (8), while the density of RNA polymerases between the promoter and downstream cluster averaged 0.8 polymerase per kb.

The frequency with which transcription is initiated at rRNA promoters can be calculated from the observed RNA polymerase densities and the transcription rate. At 37°C in LB medium with $\mu = 2.4$ doublings per h, an RNA polymerase initiates transcription every 2 s at each of the rRNA operon promoters and takes 2.2 min to complete transcription of an entire operon. During the 70-s interval following rifampin treatment, the frequency of transcription initiation dropped to one initiation every 30 s. Initiation frequency will also change under different growth conditions because rRNA promoters are subject to growth rate control (9, 21).

Full-length rRNA operons with normal morphology are occasionally observed in chromatin spreads following rifampin treatment. The unperturbed operons are most often seen in dense clumps of chromatin. These operons were excluded from analysis because we speculate that they were

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FIG. 1. Electron micrographs of representative *E. coli* rRNA operons before and after exposure to rifampin. (A) Prior to rifampin exposure. RNase III cleavage of nascent transcripts between the 16S and 23S cistrons (arrow) gives rRNA operons their characteristic double Christmas tree morphology. Direction of transcription is from left to right. (B) After 40-s rifampin exposure. Rifampin prevents initiation of transcription without inhibiting elongation (24). Because no new transcripts can be initiated, RNA polymerases are not observed on promoter-proximal regions of transcription units; most of the 16S cistron is now devoid of RNA polymerases. The arrow marks the RNase III cleavage site. (C) After 70-s rifampin exposure. Previously initiated RNA polymerases have continued transcription elongation along the rDNA template. Except for a small percentage which have apparently escaped inhibition by rifampin (arrows), RNA polymerases are now observed only along the promoter-distal portion of the 23S cistron. Bar = 1 kbp.

contained within a clump of cells which may have shielded them from the immediate action of rifampin.

Previous measurements of rRNA chain elongation rates ranging from 12.5 to 105 nucleotides per s have been indirect. In most studies, total RNA was isolated and size fractionated before the specific RNA species under investigation was measured. Considering problems of RNA instability and contamination from overlapping RNA populations, it is not surprising that values reported for rRNA transcription rate vary widely. Transcription rates were

underestimated initially (15) because the rRNA operon structure was not yet known. Recent estimates of transcription rates may be too high because of the mistaken assumption that most tRNAs cotranscribed with rRNA are located at the distal ends of rRNA operons (22), when in fact only three of the seven rRNA operons in *E. coli* have tRNA genes downstream of their 23S cistrons. A more accurate rate would have been reported by Bremer and colleagues (2, 22, 23) if measurements had been based on individual tRNA species at known distances from their respective promoters.

FIG. 2. *E. coli* rRNA transcription elongation rate. The lengths of rRNA operons densely packed with RNA polymerases are plotted as a function of time following addition of rifampin to the growth medium. Measurements were based on an average rRNA operon length of 5.5 kb derived from the DNA sequence for *rnnB* (4) and information on the cotranscribed 5S and tRNA genes of the other 6 rRNA operons (1, 14). The average was weighted by the relative gene dosage of each operon (5, 7, 11) because of the fact that the rRNA operons are distributed from 5 to 90 min on the *E. coli* chromosome and hence are replicated at different times. A least-squares regression line yields a rate for transcription elongation of 42 nucleotides per s (standard error of slope = ± 2 nucleotides per s, $r^2 = 0.82$).

Such measurements by Morgan et al. (20) are consistent with the 42-nucleotides-per-s rate of rRNA transcription reported here. Biochemical estimates of mRNA elongation rate are also within this range, but they are fraught with the same difficulties discussed above. We are currently measuring transcription rates along the *S10* and α ribosomal protein operons to determine whether rRNA and mRNA transcription rates are indeed the same.

Although most previous estimates indicated that rRNA chain elongation rates were invariant under nutritional conditions leading to differences in growth rate, in some cases a variation in transcription rate with growth rate was reported (6, 18). Differences in elongation rates in different media were, however, not as large as the variation reported by different investigators using the same medium. Current estimates for the variation in transcription rate with growth rate range from 0 to 30% (13) between the extremes of different growth rates. Such differences are not sufficient to account for the wide range of previously reported rate estimates.

The ability to visualize individual genes in action is a unique opportunity afforded by electron microscopy. Operons which have been affected by drug or other treatments can be distinguished from those which have not. Regions of the same gene which have and have not been perturbed by treatment can be identified (17). Additionally, transcription rates can be measured by monitoring the actual movement of RNA polymerases along DNA templates rather than by extrapolation from purified transcripts.

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