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In Vivo Distribution of Ribonucleic Acid Polymerase Between Cytoplasm and Nucleoid in *Escherichia coli*

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Three forms of ribonucleic acid polymerase can be distinguished in exponentially growing *Escherichia coli* cells: (i) active, (ii) inactive, inside the nucleoid, and (iii) inactive, free in the cytoplasm.

We know that *Escherichia coli* bacteria growing at various rates contain a surplus of ribonucleic acid (RNA) polymerase. This surplus is higher at low growth rates than at high growth rates, and the number of polymerase molecules exceeds the number of RNA molecules synthesized by the enzyme two to five times (3, 7, 9, 15). If we assume a pool of dormant polymerase, the question arises whether the enzyme molecules remain bound to their deoxyribonucleic acid (DNA) templates, stay inside the *E. coli* nucleoid between termination and reinitiation of RNA synthesis, or whether RNA polymerase molecules diffuse into the cytoplasm of the cell.

It has been shown that the isolated *E. coli* nucleoid contains most of the RNA polymerase of the cell (13). However, any in vitro experiments undertaken to investigate the in vivo distribution of the enzyme would run the risk of disturbing a possible equilibrium between nucleoid-bound and cytoplasmic RNA polymerase. Therefore, we chose for our study the minicell-producing strain *E. coli* K-12 P678-54 F^- that distributes its cytoplasmic components equally between normal and minicells, but whose minicells do not contain chromosomal DNA(1). It has been shown recently that episome-free minicells may contain measurable amounts of the two large polymerase subunits, β and β' (10), and a cryptic RNA polymerase activity (11).

Cells of strain P678-54 F^- were cultured at two different growth rates, μ = 1.23 generations/h and μ = 0.32 generation/h, and labeled with [^{35}S]sulfate (cf. legend to Fig. 1). To ensure the equal distribution of cytoplasmic components between normal and minicells, the cultures were induced for β-galactosidase. Minicells were purified by differential centrifugation and sedimentation through a sucrose density gradient (12). The purity of the minicell preparation was analyzed by plating for viable cells and by microscopy examination. It was found that the viability of normal cells decreased by a factor of about 10 owing to the purification procedure. Furthermore, at the high growth rate a normal cell is approximately 12 times as big as a minicell, whereas at the low growth rate it is only 6 times as big. Therefore, the contamination of the minicell preparation by normal cells was calculated to be about 1% in cell mass for the fast- and 2% for the slow-growing culture. On the other hand, the contamination of the normal cells by minicells could be neglected. The relative amount of the two large polymerase subunits, β and β', and of β-galactosidase in total protein of the two cell types was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis of crude cell lysates (6).

Figure 1 shows gel patterns of ^{35}S-labeled polypeptides obtained from normal and minicells grown at two different rates. The β and β' bands, as well as the β-galactosidase band, were well resolved from other polypeptides. For cells not induced for β-galactosidase the appropriate band could not be detected on gels.

The quantity of label in the respective subunits was determined by subtracting a mean background that was obtained from an upper and a lower background as indicated in Fig. 1C. It should be noted that the distribution of β-galactosidase between normal and minicells as determined by radioactivity was about equal at both growth rates quoted. This result is consistent with measurements of β-galactosidase-specific activities which were equal in both cell types (data not shown). On the other hand, the relative amount of the β and β' subunits varied considerably between normal and minicells but was significant in minicells at either growth rate. Table 1 summarizes the results of three independent gel analyses.

The α_P values, i.e., the amount of core RNA polymerase relative to the amount of total protein synthesized (7), for normal cells which can be deduced from the β,β' data by extrapolating

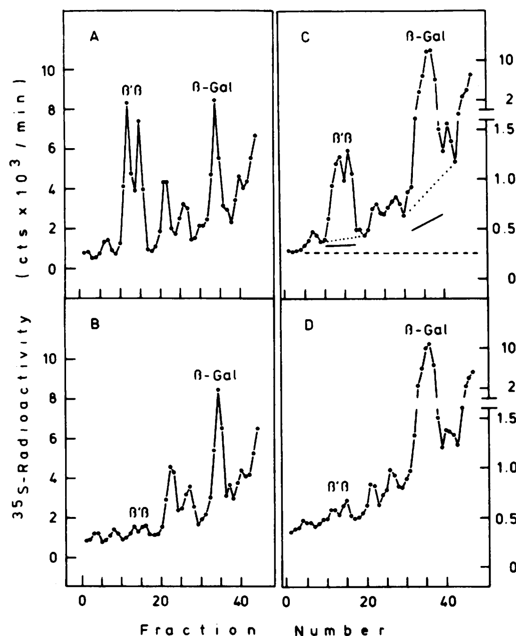

FIG. 1. *Polyacrylamide gel electrophoresis of radioactively labeled lysates from normal and minicells after growth in glucose plus amino acids medium and in acetate minimal medium. (A and B) E. coli K-12 P678-54 F$^-$ was cultured in modified M9 medium (9), containing 80 μM $K_2{}^{35}SO_4$ (63 μCi/μmol) and 1 mM $MgCl_2$, supplemented with 0.4% glucose, 0.5 μg of thiamine/ml and 100 μg of each of the following amino acids per ml: alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. β-Galactosidase was induced with 3 × 10^{-4} M isopropyl-β-thiogalactopyranoside (IPTG). The culture was grown with aeration at 37 C for several generations at a constant rate (μ = 1.23 generations/h). After centrifugation and washing with nonsupplemented M9 medium, normal and minicells were purified as described (12). The concentrated cells were dissolved in lysing buffer [0.02 M tris(hydroxymethyl)aminomethane (Tris), pH adjusted with N, N-bis(2-hydroxymethyl)glycine (bicin) to 8.6, 3% sodium dodecyl sulfate, 5% β-mercaptoethanol, and 20% glycerol] by heating for 10 min at 90 C and brief ultrasonic disruption. Samples of the clear lysates, containing 4.69 × 10^6 counts/min in ^{35}S-labeled proteins for the normal and 5.81 × 10^6 counts/min for the minicells, were applied to 7% acrylamide, 0.14% methylenebisacrylamide gels containing 0.1% sodium dodecyl sulfate. The reservoir buffer was 0.2 M Tris, pH adjusted with bicin to 8.6. The reliability of the gel system was checked with the systems used earlier (6, 7), and the gels were analyzed for the β and β' bands as well as for the β-galactosidase band as before (6, 7). (A) Lysate from normal cells; (B) lysate from minicells. Only the area around the β,β'- and β-galactosidase bands is shown. (C and D) E. coli K-12 P678-54 F$^-$ was cultured as before in modified M9 medium supplemented with 0.4 M sodium acetate, 0.5 μg of thiamine/ml and 100 μg each of leucine and threonine/ml. β-Galactosidase was induced with 3 × 10^{-4} M IPTG. The growth rate was 0.32 generation h. Normal and minicells were purified and their lysates were subjected to gel electrophoresis as before. The amount of radioactivity applied on the gels was 1.55 × 10^6 counts/min for the normal and 1.65 × 10^6 counts/min for the minicells. (C) Lysate from normal cells. The background subtraction is indicated. The mean background was obtained from a lower background determined by the radioactivity present in the upper part of the gel where no polypeptide bands could be detected, and an upper background given by a line which connects the lowest points of the respective bands. (D) Lysate from minicells. Only the area around the β,β'-, and the β-galactosidase bands is shown.*

TABLE 1. *Relative amount of β and β' subunits and β-galactosidase in total protein from normal and minicells at two different growth rates*[a]

μ (generation/h)	Cell type	β and β' subunits (%)	β-Galactosidase (%)
1.23	Normal cells	0.49	0.18
	Minicells	0.069	0.15
0.32	Normal cells	0.27	1.26
	Minicells	0.066	1.07

[a] Three independent gel electrophoretic analyses were performed for all four lysates (cf. Fig. 1). The amount of radioactivity present in the β and β' bands as well as in the β-galactosidase band was corrected for background and the three resulting values were averaged. The different sulfur contents in β,β'-, β-galactosidase, and total protein were taken into account (2, 4, 14) in calculating the relative amount of the respective polypeptides in total protein.

to the composition of core polymerase are somewhat lower than normally found for other *E. coli* strains (3, 5, 7, 9, 15). We do not know whether the lower values are characteristic for the minicell-producing strain, but they could be confirmed by incorporation of [^{3}H]leucine instead of ^{35}S.

Ywakura et al. (15) have shown that exponentially growing *E. coli* cells do not contain free β and β' subunits but all β and β' subunits are bound in RNA polymerase. Therefore we may assume that the β and β' subunits in minicells are also assembled in the enzyme. According to our earlier data, the α_P values for active core polymerase are 0.09% at μ = 0.32 generation/h and 0.34% at μ = 1.23 generations/h (7). The α_P values for total core polymerase in normal cells were found to be 0.34 and 0.61%, and in minicells 0.082 and 0.086% at these two growth rates.

The differences between the α_P values for total and for active polymerase give us the amount of total inactive polymerase, whereas the α_P values from minicells indicate the fraction of inactive enzyme that is not bound to DNA but, rather, the fraction that is released into the cytoplasm of the growing cell. This fraction is about one-third of the total inactive polymerase at the two examined growth rates.

We can distinguish, therefore, between three different forms of RNA polymerase in the cell: (i) active, engaged in RNA synthesis; (ii) inactive, bound to DNA or otherwise retained in the nucleoid; and (iii) inactive, free in the cytoplasm. The distribution of the enzyme between these three forms appears to be determined by the growth rate of the bacteria.

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