EVIDENCE FOR A CRYPTIC RNA POLYMERASE ACTIVITY IN DNA-FREE MINICELLS OF *Escherichia coli*

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SUMMARY: DNA-free minicells of *Escherichia coli* will not allow growth of phage T-7, nor is RNA synthesis stimulated by phage infection. Thus, these miniature cells seem not to contain *in vivo* RNA polymerase activity. However, DNA-dependent RNA polymerase activity can be unmasked in extracts with poly(dA-T) and Mn^{2+}. This activity may represent a cytoplasmic pool of inactive RNA polymerase in normal cells.

The basic DNA-dependent RNA polymerizing unit from *Escherichia coli*, or "core" RNA polymerase (E.C. 2.7.7.6) contains two subunits designated β and β', which are large enough to be detected by polyacrylamide gel electrophoresis of crude cell extracts (1). Quantitation of the β-β' subunits, or of β alone, has indicated that there are more of these subunits than can be accounted for by the apparent number of functioning RNA polymerase molecules (2-4).

Certain mutant strains of *E. coli* produce minicells, which are small anucleate bodies produced by aberrant cell divisions (5). Minicells from episome-free (but not episome-containing) strains lack DNA, are incapable of RNA and protein synthesis, and are incapable of supporting bacteriophage reproduction (6-8). Yet, electrophoresis of lysates of such minicells indicate that they contain the β-β' subunits of the RNA polymerase. Here I show that RNA polymerase activity can be unmasked in extracts of these miniature cells. This finding suggests that the excess β-β' subunits in normal cells may represent an inactive form of the RNA polymerase.

MATERIALS AND METHODS: Minicells from episome-free minicell-producing strain *E. coli* χ925 grown on appropriately supplemented A+B minimal medium (10,11) to an optical density of 1.0 at 450 nm (1.0 cm path) were purified on two glycerol gradients buffered with "BSG" (12) as previously described (11). Parental cells for

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comparison were isolated from the pellet of the first gradient. The yield of minicells was about 2% of the input cell mass. Contamination of the minicell preparations with viable cells was less than 1 per 10^5 minicells (less than 0.1% on a mass basis). Bacteriophage T-7 was utilized as described by Studier (13).

Extracts of the equivalent of 10 ml of cells at an O.D. of 0.5 were made by sonicating the concentrated cells in 0.6 ml breaking buffer (10 mM Tris-HCl pH 7.9, 50 mM KCl, 5 mM $MgCl_2$, 5 mM 2-mercaptoethanol) followed by centrifugation for 10 min at 30,000 x g at 2°C. The O.D. at 260 nm was determined from a 1:40 dilution of the extract.

RNA polymerase activity was assayed by mixing 50 µl of ice-cold extract with 100 µl of an ice-cold solution containing 40 mM Tris-HCl pH 7.9, 0.2mM 2-mercaptoethanol, 0.25 mM each ATP, CTP and GTP, 0.05 mM (50 µCi/ml) 5-[^{3}H]-UTP, 7.5 µg calf thymus DNA/ml (Sigma, highly polymerized) or 5 µg poly(dA-T)/ml (Sigma), and 10 mM $MgCl_2$ or 3 mM $MnCl_2$ and, when noted, 1.5 µg Rifampicin (14) /ml. The reaction was initiated by transfer to a 30°C bath, and terminated after 20 min by return to 0°C, immediately followed by the addition of 2 ml ice-cold 5% TCA 10 mM in $Na_4P_2O_7$. TCA-insoluble ^{3}H incorporation was determined by liquid scintillation spectrometry following filtration onto Whatman GF/C filters.

RESULTS AND DISCUSSION: Labeling of χ925 minicells with [^{14}C]-uracil as described in ref. 9 followed by extraction of the nucleic acids and electrophoretic analysis as described in ref. 15 failed to reveal any labeled RNA, or any DNA at all. Bacteriophage T-7 would form plaques with an efficiency 90% of that on strain B when plated on a lawn of strain χ925. Phage and minicells from strain χ925 were then mixed at a multiplicity of 1, and diluted 10^6-fold after 5 min. The same number of plaque-forming units (5% of the input) was found when the infected minicells were plated 10 min or 1 h after infection, even following incubation of the plates for 72 h to reveal late or unusually small bursts. Thus, these minicells were confirmed to be DNA-free, to not synthesize RNA, nor to support phage growth.

Figure 1 shows that infection of the minicells did not stimulate increased [^{3}H]-uridine uptake into TCA-insoluble form. The conversion by DNA-free minicells of uridine or uracil to a TCA-insoluble form which is not RNA has been previously documented (8,9). The conversion at 15 min represents about 3×10^{-11} moles of uridine incorporated per 10^{10} minicells, or about 5% of the input. A similar mass of normal cells under these conditions would incorporate virtually all of the input label in less than 5 min, a rate at least 50-fold greater than that seen here. A control experiment with [^{32}P]-labeled phage indicated that viral DNA was injected into the minicells. Since the early promoters of phage T-7 seem to have a high affinity for RNA

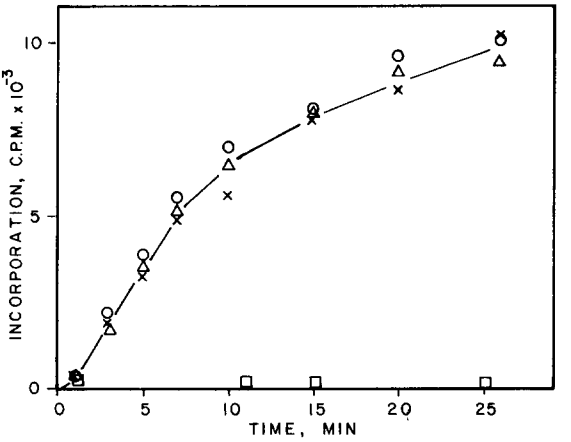

FIGURE 1. Effect of bacteriophage T-7 infection on [^{3}H]-uridine incorporation by a minicell preparation. At 0 time, samples of 10^{10} minicells which had been suspended for 0.5 h at 37°C in 2 ml complete minimal medium with aeration received 2 μg, 5 μCi [^{3}H]-uridine and: 4 x 10^{10} plaque-forming units T-7 (O); no phage (×); heat-inactivated phage (Δ). Medium alone plus label plus phage (O). Ordinate, TCA-insoluble C.P.M. per 200 μl.

polymerase (16), I concluded, in confirmation of earlier studies, that these episome-free minicells do not contain active RNA polymerase (6-8).

I then attempted to elicit RNA polymerase activity in extracts of the minicells. I assayed extracts of both the minicells and the parental cells using two different DNA templates (calf thymus DNA or poly[dA-T]) and two different cations (Mg^{2+} or Mn^{2+}). Calf thymus DNA was chosen because transcription on that template is relativly insensitive to lack of the initiation factor σ (17,18), and lack of σ might explain the lack of *in vivo* RNA polymerase activity in the minicells. Poly(dA-T) was utilized because two reports have attributed unusual properties to the template activity of that polynucleotide (19,20), especially in the presence of Mn^{2+} (20). RNA polymerase activity was defined as that activity in the crude extracts sensitive to inhibition by Rifampicin (14).

Table 1 shows the results of these assays. The template-cation combination poly(dA-T) plus Mn^{2+} caused a dramatic

TABLE 1. RNA Polymerase Activity in Extracts of Minicells and Normal Cells.

Template	Extract from (cell)	Rif[a]	Incorporation with[b]		Relative Activity[c]	
			Mg^{2+}	Mn^{2+}	Mg^{2+}	Mn^{2+}
Calf Thymus DNA	Normal	-	5668	10364		
		+	759	2850		
			(4909)	(7514)	0.65	1.00
	Mini	-	437	349		
		+	150	318		
			(287)	(31)	9.26	1.00
Poly(dA-T)	Normal	-	7222	36097		
		+	1097	6972		
			(6125)	(29125)	0.82	3.88
	Mini	-	927	2372		
		+	457	461		
			(470)	(1911)	15.16	61.65
Minus Template	Normal	-	3272	9178		
	Mini	-	130	315		

[a] Symbols indicate presence (+) or absence (-) of Rifampicin.

[b] C.P.M. incorporated per 150 µl assay. Numbers in parentheses indicate difference between assays with and without Rifampicin.

[c] Rifamicin-sensitive activity relative to activity with Calf Thymus DNA plus Mn^{2+} for each extract. Normal cell extract contained 39 O.D. 260 units/ml; the minicell extract contained 19.

stimulation of a Rifampicin-inhibitable, DNA-dependent RNA polymerase activity in the minicell extract. This combination was unique among those tested in clearly allowing the conclusion that RNA polymerase activity could be elicited in that extract. The same combination caused stimulation of the polymerase activity in the normal cell extract, but the maximum range of stimulation was about 10-fold less than the apparent stimulation in the minicell extract. The mechanism of the activation of RNA polymerase by Mn^{2+} and poly(dA-T) is obscure, but it is not

difficult to imagine that conditions which artificially activate a presumed cryptic polymerase also stimulate normal polymerase activity. The activities in the normal cell extract were not completely DNA-dependent, probably due to the presence of exogenous DNA in that extract.

It is difficult to ascribe the activity in the minicell extract to the presence in the minicell preparations of "normal" RNA polymerase. The activity cannot be accounted for by parental cell contamination, as the specific activity in the minicell extract should then be on the order of 1/1000 of that in the normal cell extract. Based on estimation of protein concentration from optical density measurements (there was insufficient material for precise protein estimation) and data from assays with poly-(dA-T) and Mn^{2+}, the specific activity in the minicell extract seems to be about 1/7 of that in the normal cell extract. Moreover, other minicell preparations with varying (but low) parental cell contamination gave similar results. The lack of stimulation of [^{3}H]-uridine uptake by the minicells following the introduction of phage DNA argues against the presence of active polymerase in the minicells themselves. The existence of polymerase of 1/7 of normal cell activity should have been detected in these experiments. The activity in the minicells is not unprimed synthesis because it requires template (table 1) and proceeds without delay (data not shown).

The lack of *in vivo* RNA polymerase activity in these minicells, the presence of the β-β' subunits of the polymerase (9), and the presence of RNA polymerase activity in extracts under unusual conditions of assay suggest that DNA-free minicells contain a cryptic, normally inactive form of the RNA polymerase. It is impossible from this data to tell if the *in vitro* activity represents slugish activation of all of the polymerase suggested by the presence of the β-β' subunits, whether it represents the complete activation of a portion of the putative polymerase, or, indeed, if all of the β-β' subunits represent RNA polymerase.

About half of the minicells in a culture are products of the last cell generation. Thus, the "average" cytoplasm in a minicell population has not aged for an extensive length of time. Unless RNA polymerase activity decays very rapidly, there is no reason to expect that the formation of a minicell will influence RNA polymerase activity. The cryptic RNA polymerase activity of

minicells may therefore be related to a cryptic RNA polymerase which may exist in the cytoplasm of normal E. coli cells.

There are currently reports of at least four different forms of RNA polymerase from normal, uninfected cells of E. coli (1,20, 22,23). Unfortunately, differences in experimental conditions (especially assay conditions) preclude meaningful comparisons of all of their various properties. Further studies on systems from both normal cells and minicells should help to clarify the relations between, and the regulation of, the various forms of the bacterial RNA polymerase.

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