THE EFFECT OF THE TERMINATION RHO FACTOR AND RIBONUCLEASE III ON THE TRANSCRIPTION OF BACTERIOPHAGE ϕ X174 DNA IN VITRO

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1. Introduction

The transcription of bacteriophage ϕX174 has been actively investigated in recent years. In phage infected *E. coli* cells Hayashi and Hayashi [1,2] have identified 10 discrete ϕX174 specific RNA components with mol. wts. ranging from 3.0×10^6 to about 0.2×10^6. Recent studies on size and number of ϕX174 RNA conducted by Clements and Sinsheimer [3] have demonstrated the presence of 18 distinct RNA species within the same range of molecular weight. In contrast to these observations RNA synthesized in vitro using *E. coli* RNA polymerase and ϕX174 RF DNA as a template had a different size distribution [4]. Each of the in vitro RNAs was larger than the complete transcript of the ϕX174 genome (1.7×10^6 dalton). Addition of the termination factor ρ to the RNA-synthesizing system resulted in a decrease of the RNA's size to that of in vivo RNA. However, even in the presence of ρ factor several low mol. wt. RNAs identified in vivo were missing in vitro. We suggested that these RNAs might originate as a result of a cleavage of the initial RNA transcripts by the special enzyme, RNAase III. The involvement of the RNAase III in the processing of T7-early RNA and ribosomal RNA precursor was observed recently [5–7]. In this paper we describe the effect of the ρ factor and RNAase III on the transcription of ϕX174 RFI DNA in vitro. It is shown that in the RNA polymerase system containing ϕX174 RFI DNA, ρ factor, and RNAase III a set of RNAs is synthesized that is very similar in size distribution to the ϕX174 RNAs from infected cells.

Abbreviations: RF, double-stranded circular replicative form DNA of ϕX174; RFI, supercoiled RF DNA with both strands closed; EDTA, ethylenediamine tetraacetic acid; SDS, sodium dodecyl sulphate; ATP, GTP, UTP and CTP, abbreviations for ribonucleoside-5'-triphosphates.

2. Methods

2.1. *Enzymes*

RNA polymerase holoenzyme was purified from *E. coli* MRE 600 cells by the method of Burgess [8] with slight modifications, ρ factor and RNAase III were purified according to the method of Darlix [9] including the glycerol gradient sedimentation.

2.2. *DNA of bacteriophage ϕX174*

RFI DNA was isolated from *E. coli* C infected with wild type ϕ X174 by the procedure developed in our laboratory. This involved gentle lysozyme-SDS lysis followed by selective precipitation of high mol. wt. host DNA with 1.2 M NaCl, phenol treatment, gel filtration on a Sepharose 2B column, chromatography on a methylated serum albumin–kieselguhr column and glycerol gradient sedimentation.

2.3. *RNA synthesis in vitro*

^{14}C-Labelled ϕX RNA was synthesized in the standard reaction mixture containing (per ml): 40 μmol Tris·HCl pH 7.9, 20 μmol $MgCl_2$, 150 μmol NaCl, 0.1 μmol EDTA, 0.1 μmol dithiothreitol, 0.2 μmol (each) ATP, GTP and CTP, 0.1 μmole [^{14}C] UTP (20 μCi/μmole), 12 μg ϕX174 RFI DNA and 24 μg RNA polymerase (1500 units/mg [8]). ρ Factor

and/or RNAase III were added as indicated in figure legends. The reaction mixtures were incubated for 15 min at 37°C and then 20 μg of RNAase-free DNAase (Worthington) was added. After a further 10 min. incubation at 0°C SDS was added to 0.5%. RNA was extracted by phenol treatment according to Kirby [10].

2.4. *Gel electrophoresis of [^{14}C]RNA*

Gel electrophoresis was performed in composite gels (2.4% acrylamide, 0.5% agarose, 0.1% SDS) as described by Bishop [11]. In vitro synthesized RNA was dissolved in 30% glycerol–1% SDS and 5 μl samples were placed on gels. After the run (8 mA/gel, 80 min) gels were frozen and sliced to 2 mm discs. Radioactivity of the discs was measured in toluene–absolute ethanol scintillation fluid with a Mark II liquid scintillation counter.

3. Results and discussion

RNA synthesized in vitro using highly purified *E. coli* RNA polymerase and φX174 RFI DNA as a

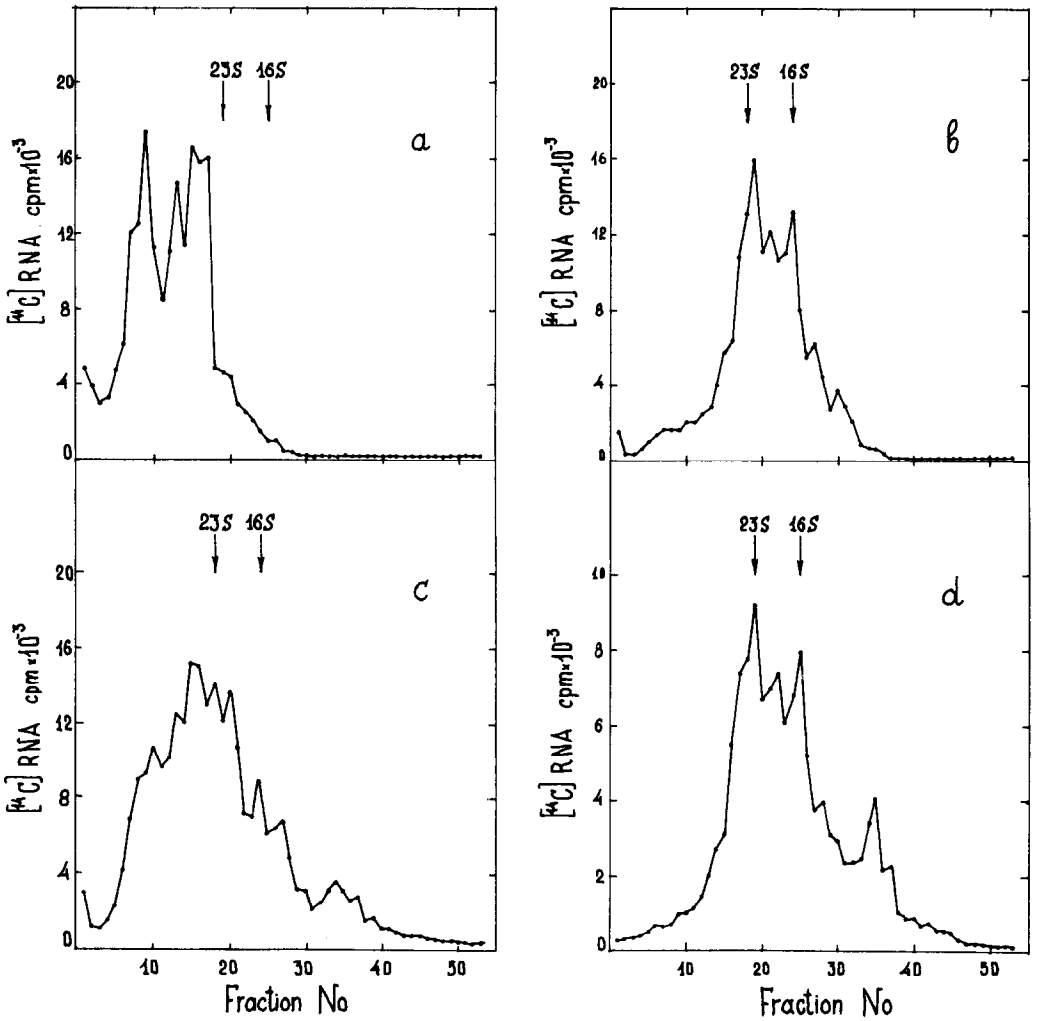

Fig. 1. Size distributions of φX174 RNA synthesized in vitro. The conditions of RNA synthesis, isolation and gel electrophoresis were as described in Methods. Arrows show the position of 23S and 16S ribosomal RNA markers. RNA synthesized in vitro (a) without factors, (b) with 1 μg ρ factor, (c) with 20 μg RNAase III and (d) with 1 μg ρ factor and 20 μg RNAase III.

template was analysed in SDS–polyacrylamide–agarose gel electrophoresis (fig.1a). High mol. wt. RNAs synthesized in this system had a characteristic discrete size distribution; at least five species of RNA were usually observed. The largest of them had a mol. wt. of approximately 3.0×10^6, i.e. it was a product of about two rounds of ϕX174 genome transcription, and the smallest one was of 1.6×10^6 dalton, i.e. corresponded to the product of about one round of transcription. In our RNA preparations we were failed to detect any considerable amounts of material with a size larger than 3.0×10^6 dalton, as was observed by Hayashi et al. [4] in a similar system. This discrepancy was probably a result of a longer incubation time in Hayashi's experiments.

Thus size distribution of the in vitro RNAs (fig.1a) was very different from the distribution of virus-induced RNAs [1–3], as most of the in vivo RNA species had molecular weights less than 1.6×10^6.

It seems that the formation of ϕX174 RNA in infected cells is under the control of some additional factors for instance of termination ρ factor. The addition of ρ factor to RNA-synthesizing system in vitro resulted in a drastic alteration of RNA size (fig.1b). In this case RNA species with molecular weights higher than 1.6×10^6 disappeared completely and only smaller molecules were present. A similar decrease of RNA size in the presence of ρ factor was previously reported by Hayashi [4]. In both cases the sizes of RNA produced in the system containing ρ factor were within the size range of in vivo RNA. However several species observed in the preparations of in vivo RNA (mainly with a low mol. wt.) were missing from this pattern.

The formation of low mol. wt RNA in the infected cells might be a result of the cleavage of preformed RNA by a special enzyme. It was demonstrated [5–7] that early T7 RNA and ribosomal RNA precursor were cleaved by an enzyme, RNAase III into discrete RNA species. If RNAase III has a role in sizing ϕX174 RNAs in vivo it may be possible to mimic this situation in vitro. Assuming that all possible initiation and termination events take place in the cell-free RNA-synthesizing system with ρ factor, we can try to obtain all in vivo RNA species if both termination ρ factor and RNAase III are present. The size distribution of in vitro RNAs synthesized in the presence of both ρ factor and RNAase III shown in fig.1d. It may

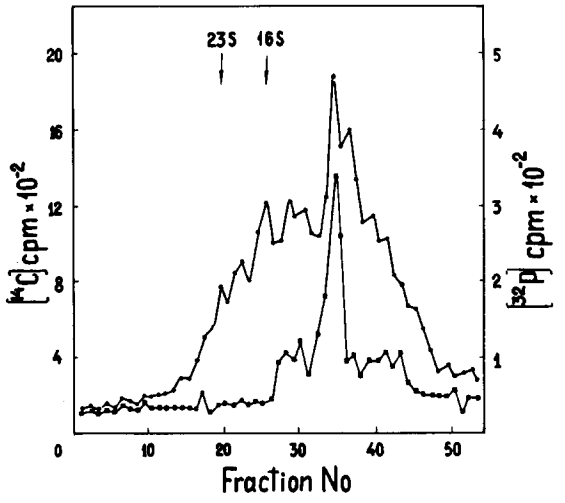

Fig.2. Size distribution of double-labelled ϕX174 RNA synthesized in vitro. All conditions were as in fig.1 except that the concentrations of RFI DNA, RNA polymerase, ribonucleoside triphosphates, ρ factor and RNAase III were two times higher and 200 μCi [γ-^{32}P]CTP were also added. Symbols: (–●—●–) [^{14}C]RNA counts, (–■—■–) [^{32}P]RNA counts.

be seen that the relative amount of RNA with a mol. wt. less than 0.6×10^6 increased and at least two new species of RNA appeared in the region of fractions 33–38. These effects became even more pronounced if the concentrations of DNA, RNA polymerase, ρ factor and RNAase III were elevated (fig.2).

The size distribution of RNA shown in fig.2 is in good agreement with that reported by Hayashi for the in vivo system [2]. The same distribution was obtained not only as a result of simultaneous action of ρ factor and RNAase III during the synthesis, but when RNA was synthesized with ρ factor alone, isolated and then treated with RNAase III (data not shown). If only RNAase III was present in the RNA-synthesizing system the gel pattern of the RNA product (fig.1) differed from those shown in fig.1d and fig.2, though some material was present in fractions 34–39.

Our results suggest that RNAase III participates in processing of ϕX174 RNA in vitro. It is reasonable to assume that RNAase III also participates in the formation of ϕX174 specific RNAs in vivo. Stronger support for this assumption may well be found in experiments with ϕX174 infected *E. coli* strains deficient in RNA-ase III.

It is not clear at present which RNA species are the targets of RNAase III action. The analysis of 5′-termini of individual RNAs may be useful in the solution of this problem. We confirmed the observation [12] that not only [γ-^{32}P]ATP and [γ-^{32}P]GTP but also [γ-^{32}P]CTP is incorporated into ϕX174 RNA synthesized in vitro, and showed that in the system containing ρ factor and RNAase III virtually all incorporated [γ-^{32}P]CTP is found in a single RNA species (fig.2). This RNA component is a product of RNAase III action.

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