Synthesis of phage-specific transfer RNA molecules by vibriophage ϕ 149

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^{32}P-Labelled tRNA was isolated from uninfected and phage ϕ 149-infected *Vibrio cholerae* cells. These tRNA preparations were then hybridised with DNA isolated from phage ϕ 149. Significant hybridisation was observed only with tRNA from phage ϕ 149-infected cells. This strongly suggests that infection of classical vibrio with phage ϕ 149 results in the synthesis of phage-specific tRNA molecules.

Phage-specific tRNA Vibrio cholerae

1. INTRODUCTION

Phages capable of infecting *Vibrio cholerae* and its different biotypes can be classified into 4 groups depending on their serological [1] and morphological properties [2]. The classical and EItor biotypes of *Vibrio cholerae*, which have been responsible for all recent epidemics [1] can be differentiated on the basis of their sensitivity to infection with the group IV phages; group IV phages infect only the classical biotypes of *V. cholerae*. Phage ϕ 149 is a group IV phage with a linear double-stranded DNA genome having M_r 36×10^6 [3]. The biochemical functions encoded by this molecule have not been identified. In this communication we report that infection of classical vibrios with phage ϕ 149 results in the synthesis of phage-specific tRNA molecules. T_5 [4] and T-even phages [5–7] are the only other systems in which the synthesis of phage-specific tRNA has been reported.

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2. MATERIALS AND METHODS

2.1. *Cells and phage*

Vibrio cholerae Ogawa 154 strain was used to propagate the cholerabacteriophage ϕ 149 and as an indicator host.

Cells were grown in low phosphate nutrient broth [8] at 37°C with shaking (180 rev./min) to mid log phase (about 5×10^8 c.f.u./ml). The cells were infected with phage ϕ 149 at m.o.i. = 0.2, then incubated until complete lysis had occurred (4–5 h). Chloroform (0.5%, v/v) was added to the cell lysate and cell debris removed by centrifugation at $6000 \times g$ at 4°C. Phage particles present in the supernatant were pelleted by centrifugation at 37000 rev./min for 90 min at 5°C (Sorvall Dupont OTD50 ultracentrifuge, A841 rotor), and then resuspended in 0.5 ml 10 mM Tris–HCl (pH 8.0), 10 mM $MgSO_4$. The phage was subsequently purified by banding in CsCl step gradient (1.3–1.6 g/ml) by centrifugation at 30000 rev./min for 1 h (Sorvall Dupont OTD50 ultracentrifuge AH650 rotor). The recovered phage band was dialysed overnight at 4°C against 10 mM Tris–HCl (pH 8.0), 1 mM EDTA and then stored at 4°C in the presence of small amounts of chloroform.

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2.2. *Isolation of phage DNA*

Phage preparations were extracted 3 times with redistilled phenol (equilibrated with 0.15 M NaCl, 0.015 M sodium citrate, pH 6.0). The phage DNA present in the aqueous phase was dialysed extensively against 15 mM NaCl, 1.5 mM sodium citrate (pH 6.0) and then stored at 4°C at 100–150 μg/ml.

2.3. *Isolation of [^{32}P]tRNA*

Cells were infected with ϕ 149 at mid-log phase at m.o.i. = 5 p.f.u./cell; 1 mCi ^{32}P (carrier-free orthophosphate) was added 2 min after infection and the cells incubated at 37°C for a further 25 min. The cells were then rapidly chilled and 0.01 vol. 1 M NaN_3 added. The infected cells were collected by centrifugation at 10000 × *g* for 5 min and tRNA isolated by phenol extraction and isopropanol fractionation as in [9]. [^{32}P]tRNA was further purified by gel filtration on Sephadex G-100 columns [10] pre-calibrated with authentic 5 S RNA and tRNA. Isolation of [^{32}P]tRNA from uninfected cells was done using identical conditions.

2.4. *Hybridisation*

Hybridisation of [^{32}P]tRNA with denatured DNA from phage ϕ 149 was done as in [11]; 30 μg DNA was immobilized on each filter and was hybridised with varying amounts of [^{32}P]tRNA.

3. RESULTS AND DISCUSSION

^{32}P-Labelled tRNA isolated from both uninfected and phage ϕ 149-infected cells by phenol extraction and isopropanol extraction (as in section 2) was contaminated with small amounts of 5 S RNA. This contaminant was removed by gel filtration on Sephadex G-100 (fig.1); purified tRNA was recovered from the appropriate pooled fractions by ethanol precipitation. Purified [^{32}P]tRNA was used directly for hybridisation with phage ϕ 149 DNA immobilised on cellulose nitrate membranes. A significant proportion (about 2%) of the tRNA from ϕ 149-infected cells hybridised with purified ϕ 149 DNA (fig.2). tRNA from uninfected cells failed to show any appreciable hybridisation under identical conditions. These results strongly suggest that the tRNA from ϕ 149-infected cells hybridising with ϕ 149 was not of host origin and was transcribed from the phage DNA after infection. Thus, infection of classical *Vibrio* with phage ϕ 149 results in the synthesis of phage-specific tRNA molecules.

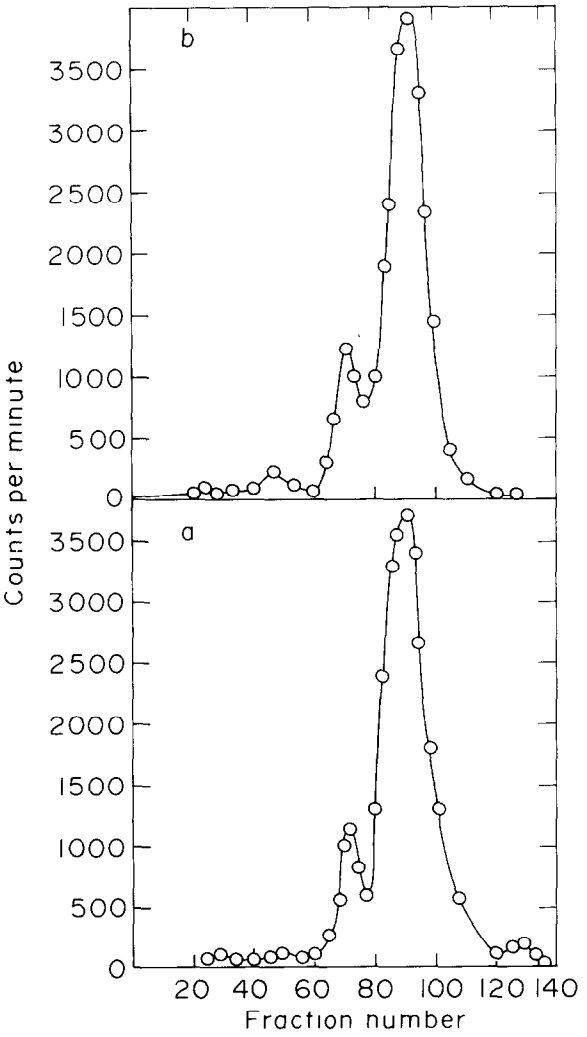

Fig.1. Gel filtration of crude ^{32}P-labelled tRNA preparations on a Sephadex G-100 column (2 cm × 150 cm); 0.5 ml crude tRNA preparation was applied to the column and eluted with 1 M NaCl [12] at 10 ml/h; 3.5 ml fractions were collected. The radioactivity contained in 10 μl aliquots of each fraction was monitored by liquid scintillation counting. The positions of the major and minor peaks correspond to authentic tRNA and 5 S RNA, respectively. The void volume was reached at fraction 42. (A) tRNA from ϕ 149-infected cells; (B) tRNA from uninfected cells.

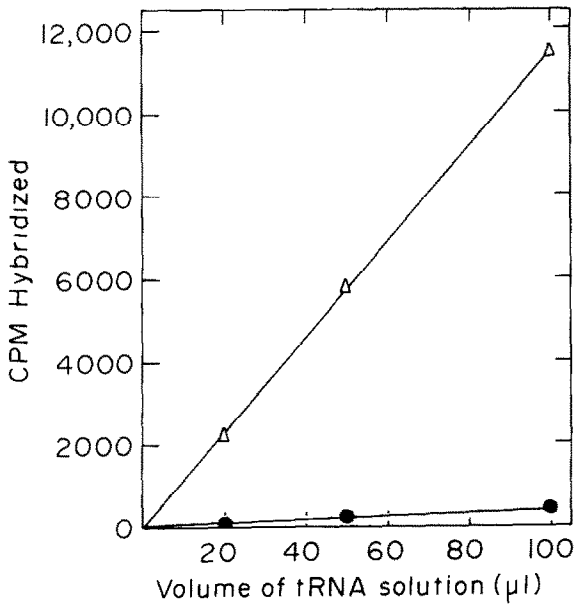

Fig.2. Hybridisation of ^{32}P-labelled tRNA (6000 cpm/μl) from ϕ 149-infected cells (△) and uninfected cells (●) with ϕ 149 DNA; 30 μg ϕ 149 DNA was immobilised to each filter and hybridised with varying amounts of [^{32}P]tRNA as in [11].

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