Transcription in Bacteriophage f1-Infected Escherichia coli: Very Large RNA Species are Synthesized on the Phage DNA

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Summary. Fractionation of pulse-labeled RNA extracted from E. coli cells infected with phage f1 and hybridization of this RNA to f1 DNA reveals that very large species are synthesized on the phage genome. Hybridization of the RNA to specific fragments of f1 DNA shows that, in the infected cell, at least one mRNA is present into which the sequences of genes III, VI, and I are all transcribed together. This result fully explains the polar effect shown by gene III mutants on the expression of genes VI and I (Pratt et al. 1966).

Introduction

F1, like M13 and fd, is a filamentous bacteriophage which infects male strains of Escherichia coli (Marvin and Hohn 1969; Denhardt 1975). These phages are so closely related that unless otherwise indicated, the results obtained in one phage are valid for the other two (for this point and for sequence analysis see: Beck and Zink 1981). The genome of the phage consists of a single-stranded, circular DNA molecule 6,407 nucleotides long (6,408 in the case of fd). This molecule is duplicated into a double-stranded form (RF1) immediately after its entry into the cell. The RF1 DNA is then transcribed by the action of the host polymerase and replicated by host enzymes with the cooperation of specific phage products (Ray 1978). Although the genome of such a small phage is quite simple, only 10 genes being located on the phage DNA, a complex pattern of transcription onto this molecule occurs inside the infected cell. Two different domains of transcription can be distinguished on the f1 DNA (La Farina and Model 1983; Smits et al. 1980 for phage M13). One of these regions is actively transcribed (region F in Fig. 1); several RNA molecules derived from this segment of the phage genome have been identified (Cashman and Webster 1979; Smits et al. 1980; La Farina and Model 1983). In contrast, the low level of transcription which occurs in the other region (region I in Fig. 1) has hampered up to now a detailed understanding of the elements that regulate the transcription of genes III, VI, I, and IV whose sequences are contained in this other segment of the phage DNA (see Fig. 1). Indirect evidence obtained in minicells infected with phage M13 has led to the identification of certain RNA molecules that could be derived from this region (Smits et al. 1978). However these data are not sufficient to explain the early finding of Pratt et al. (1966) who found that mutations in gene III have polar effects on the expression of genes VI and I.

To shed light on this point we have focused our efforts on the identification of the RNA which is derived, inside the infected cell, from this region of the f1 DNA.

We have pulse-labeled the infected cells and, after fractionation on sucrose gradients, hybridized the pulse-labeled RNA to specific segments of the f1 DNA.

Materials and Methods

Phages and Bacteria. Phage f1 and Escherichia coli K38 were from Dr. N. Zinder's collection.

Purification of RNA from Pulse-Labeled Cells. Cells grown in GCA medium (Ray and Schekman 1969) and infected
at a multiplicity of infection of 100, were pulse-labeled by exposure to [3H]Uracil (Schwartz-Mann, FRG: 10 Ci/ml; 23 Ci/mmol; final concentration in the cells: 20 μCi/ml) 5 min after the infection. One min later the cells were poured quickly onto frozen medium and RNA extracted as previously described (La Farina and Model 1978). Under these conditions, the label in the medium is not exhausted.

**DNase Digestion of the RNA Sample.** The RNA sample was made 10 mM in Na Acetate pH 5.5 and 7 mM in MgCl₂; DNase I was added at a final concentration of 20 μg/ml. After incubation at 37°C for 30 min, the sample was extracted once at room temperature with one vol water-saturated phenol, the aqueous phase was made 0.1 M in K Acetate and precipitated by 3 vol ethanol.

**Isolation of f1 Covalently Closed, Circular Double-Stranded DNA (RF₁)** was performed as described by Model and Zinder (1974).

**Restriction Endonucleases HaeIII and HindII** were a kind gift from Drs. Horiuchi and Enea.

**Sucrose Gradient Centrifugation** was performed as described by Payvar and Schimke (1979). The RNA samples were made 10 mM in methylmercuric hydroxide (Alfa, Ventron, Danvers, USA) and after 5 min they were adjusted to 1% SDS, 5 mM EDTA and 10 mM Tris pH 7.0 and loaded on 5–20% sucrose gradients containing 1% SDS, 5 mM EDTA, 10 mM Tris pH 7.0. The gradients were run at 20°C in an SW 27 rotor.

**Filter Hybridizations.** Binding of DNA to nitrocellulose filters (Schleicher and Schuell, B6) and filter hybridizations of pulse-labeled RNA to DNA were performed according to the method of Gillespie and Spiegelman (1965). Fifteen μg RF₁ DNA (linear double-stranded) f1 DNA (obtained by digesting RF₁ DNA with restriction endonuclease HindII) or molar amounts of specific fragments of it were added to each filter. By the use of fragments labeled with ³²P it was first checked that the retention of all fragments during the hybridization was complete. By hybridization with total, unfraccionated, pulse-labeled RNA from infected cells it was shown that all the hybridizations with fractionated RNA were performed in conditions of DNA excess. Before exposure to the pulse-labeled RNA, the DNA filters were preincubated for 2 h at 65°C in 10 ml standard saline citrate (0.15 M NaCl, 0.015 M Na Citrate) (SSC). The aliquots of pulse-labeled RNA were made 2 x in SSC and added to scintillation vials in the presence of a blank filter and a filter containing the desired DNA, which had been previously incubated as described above. The vials were incubated at 65°C for 24 h. The hybridizations were stopped by washing each filter twice with 2 x SSC at room temperature, incubating them at 37°C in 10 ml of 2 x SSC containing 20 μg/ml pancreatic RNase (Sigma, St Louis, USA) for 30 min and washing them twice again with 2 x SSC. After drying, the filters were counted in the presence of POPOP-PPO-toluene in an Intertechnique scintillation counter.

**Results**

Exponentially growing E. coli cells were pulse-labeled by exposure to tritiated Uracil 5 min after the infection with bacteriophage f1 (see Materials and Methods). The extracted RNA was purified from the DNA by digestion with RNase-free pancreatic DNase (Wang and Moore 1979) and fractionated on sucrose gradients. The RNA extracted from infected cells that had been similarly pulse-labeled was also fractionated in the same conditions. Every fraction of both gradients was then separately hybridized to RF₁ DNA as described in Materials and Methods. c-c, cpm hybridized in the presence of the RNA from infected cells; x-x, cpm hybridized in the presence of the RNA from uninfected cells; o-o, blanks.