IDENTIFICATION OF THE PRODUCT OF PHAGE 434 *cro* GENE

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1. Introduction

During the lytic development of lambdoid phages, an important regulatory role is played by the gene *cro* (*tof*) whose product represses the transcription of early genes [1]. Recently, the Cro protein of phage λ was identified and characterized [2,3]. To compare regulatory proteins of different temporary phages we made an attempt to find a similar protein of phage 434, among proteins that are formed in a coupled transcription–translation system in vitro. We used as template fragment G which is the smallest product formed after the phage λimm434 DNA is split by *Eco* RI restriction endonuclease. After the complete primary structure of the G fragment had been established [4], it became clear that its greater, right part was composed of λ-DNA sequences whereas its smaller part, 361 base pairs long, corresponded to the immunity region of the phage 434 chromosome (fig.1). If the functional arrangements of immunity regions in phages λ and 434 are similar, the left part of the G fragment may be expected to contain the phage 434 *cro* gene and its promoter–operator sequence whose presence can be shown by electron microscopic study of complexes of the fragment with RNA polymerase.

In this study, the sites of tight binding of *E. coli* RNA polymerase on the G fragment were located by electron microscopy and the product of phage 434 *cro* gene formed in a DNA-dependent protein synthesis system containing the G fragment was identified.

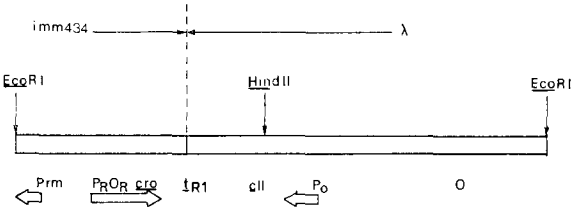

Fig.1. Physical map of the G fragment of phage λimm434 DNA containing the right part of the immunity region of phage 434. Arrow indicates the point of cleavage by *Hin*dII restriction endonuclease. CII, O and *cro* are genes: P_O, promoter initiating oop RNA transcription; P_R, promoter of right transcription of phage 434; P_{rm}, promoter determining phage 434 repressor maintenance; O_R, operator; t_{R1}, terminator. Broad arrows indicate the beginning and direction of transcription. The boundary of the immunity region from phage 434 is indicated with vertical dashed lines.

2. Materials and methods

The G fragment of bacteriophage λimm434 DNA was isolated as in [4]. *E. coli* RNA polymerase was purified according to [5]. The RNA polymerase binding to the DNA fragment and the fixation of the complex by formaldehyde were done as in [6], except that fixation was done by dialysis against a rising (from 0.05–0.4%) CH_2O concentration. Specimens for electron microscopy were prepared by the method in [7]. Cell-free protein synthesis was carried out using a slightly modified method of [8]. S30 extracts were prepared from two isogenic *E. coli* strains:

W3110 and its ρ mutant *psu*4, both kindly supplied by C. Yanofsky [9]. Electrophoresis of the synthesized protein was performed as in [10] in a Laemmli system [11]. ρ factor was a gift from E. L. Kapitza.

3. Results and discussion

3.1. *Electron microscopy of RNA polymerase complexes with the G fragment*

Figure 2 is an electron micrograph which shows

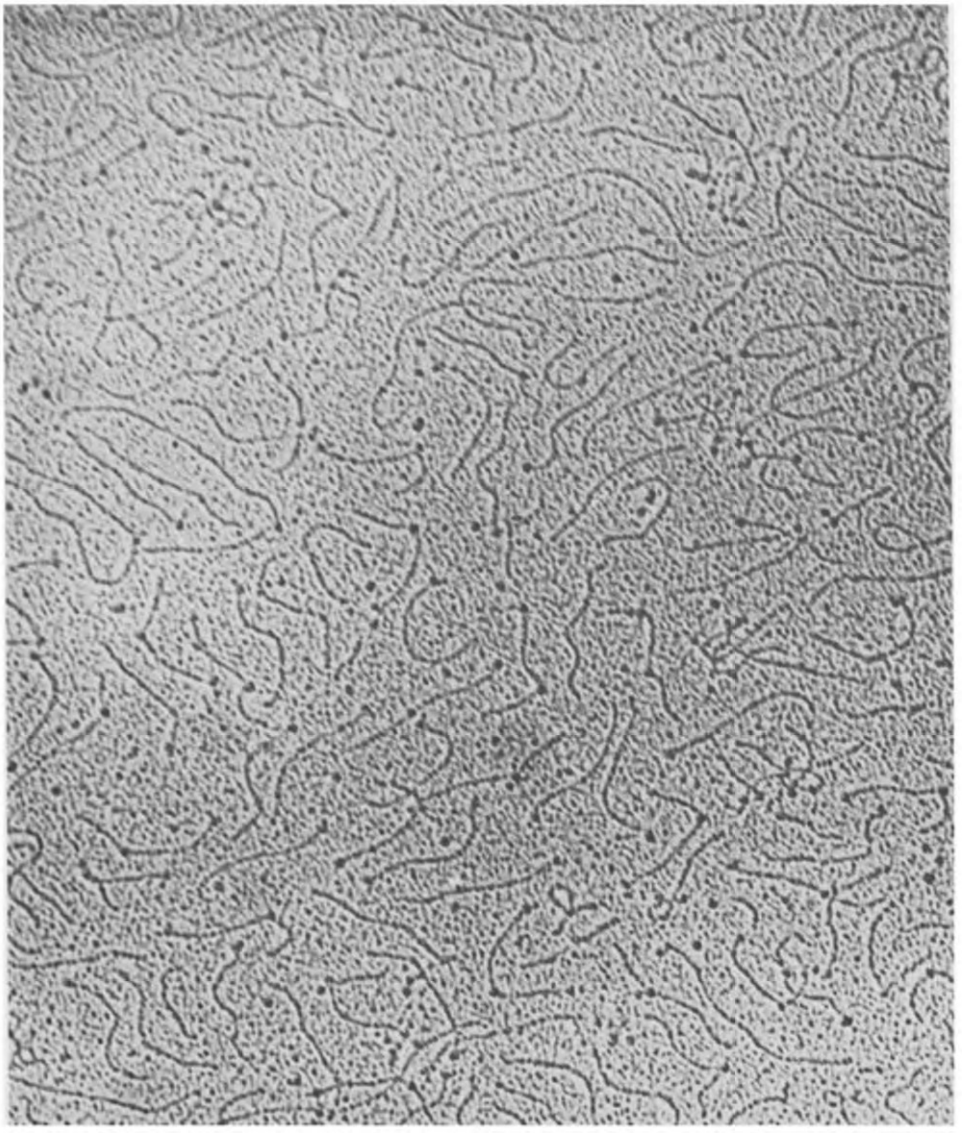

Fig.2. Electron micrograph of RNA polymerase complexes with the G fragment.

that 3 types of RNA polymerase complexes exist with the G fragment:

(i) Complexes of one RNA polymerase molecule with the terminal part of the fragment;
(ii) Complexes of one RNA polymerase molecule with the inner part of the fragment located at ~1/3rd of the distance from its end;
(iii) Complexes of two RNA polymerase molecules, one of which is at the end of the fragment and the other in its inner part.

A histogram showing the distribution of RNA polymerase molecules complexed with the G fragment is presented in fig.3. The fragment thus has two points of specific RNA polymerase binding, one of which is located near the fragment end and the other at a distance of ~2/3rds of the fragment length from the first. It is clear from primary structure analysis [4] that the P_0 promoter is located in the fragment at a distance of ~2/3rds of its length from the immunity region end of the fragment. It is therefore likely that the inner point of RNA polymerase binding corresponds to the P_0 promoter of phage λ whereas the other point corresponds to promoters in the 434 immunity region. The frequency of RNA polymerase binding to the end of the fragment is significantly higher than that of RNA polymerase binding to its inner part (fig.3). Possibly, this is related to the fact that the primary region contains two closely spaced promoters, P_R and P_{rm}, which increases the likelihood of RNA polymerase being bound in this region.

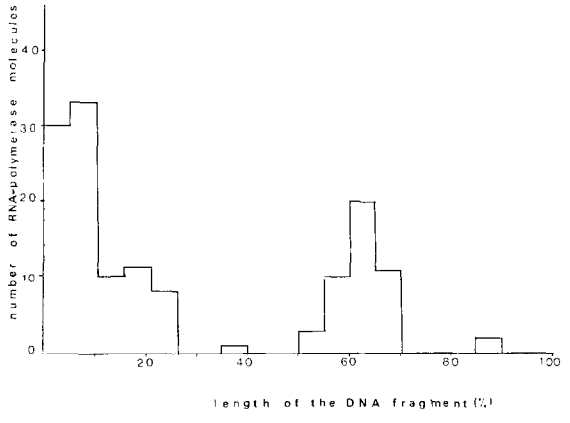

Fig.3. Distribution of sites of tight RNA polymerase binding on the G fragment.

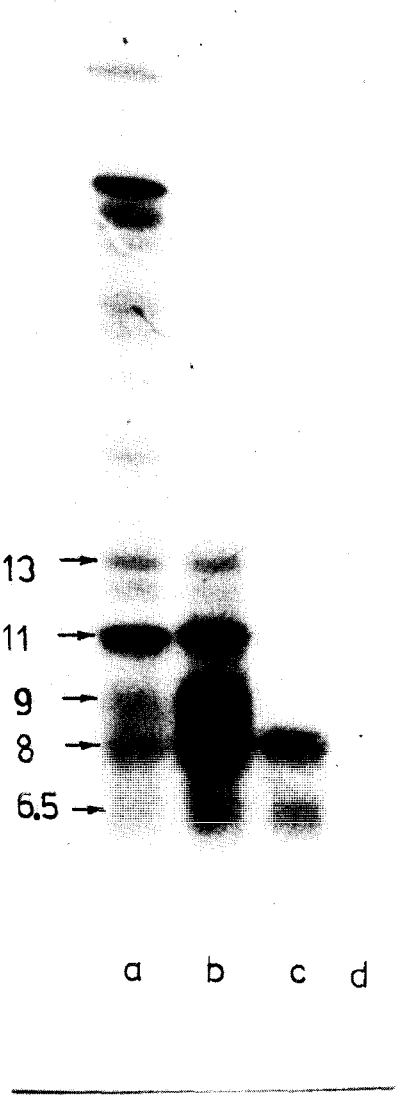

Fig.4. Electrophoresis of proteins formed in a S30 system on different templates. The synthesis of proteins, their electrophoretic separation in 15% polyacrylamide gel, and autoradiography were carried out as in [8,10,11]. S30 extracts were prepared from *E. coli* W3110 cells. Used as templates were native λimm434 DNA (a), G fragment (b), and *Hin*dII endonuclease-treated G fragment (c). No template was added to sample (d). Arrows indicate the polypeptides discussed in the text; their molecular weights are given in 10^3. The molecular weights of proteins were determined by use of the *E. coli* RNA polymerase α-subunit (40 000), chymotrypsinogen (28 000), cytochrome *c* (12 500), and BSA (67 000) as reference standards.

3.2. *Identification of the product of phage 434* cro *gene*

An electron microscopic analysis has confirmed that the G fragment does contain the right promoter–operator site of the phage 434 immunity region. For this reason the fragment may be expected to cause synthesis of Cro protein in a coupled transcription–translation system. We made a comparative analysis of the products synthesized in a S30 system containing an intact DNA of λimm434, the G fragment, and a G fragment cleaved by *Hin*dII restriction endonuclease (fig.4). The G fragment directed the synthesis of the 4 main polypeptides with mol. wt 11 000, 9000, 8000 and 6500, respectively; each of these polypeptides also forms in a system containing intact DNA. After the fragment was split by *Hin*dII restriction endonuclease, there occurred synthesis of the last two polypeptides only. Since *Hin*dII cleaves the G fragment at one site only, i.e., within the *c*II gene, and leaves the *cro* gene intact, one of the two remaining peptides may be taken to be the Cro protein. We attempted to identify this peptide as follows.

The synthesis of mRNA which begins on the P_R promoter is known to be terminated by the ρ-dependent terminator t_{RI} (fig.1) located to the right of the *cro* gene. One could therefore expect that the presence of ρ factor in a S30 system would not affect the synthesis of Cro protein, but should decrease the synthesis of other polypeptides. We compared protein syntheses in the G fragment containing S30 systems prepared from two isogenic *E. coli* strains: W3110 (parental ρ^+) and *psu*4 (ρ-defective mutant). It can be seen from fig.5 that there was synthesized a relatively greater amount of protein with mol. wt ~8000 in the wild-type system than in the mutant one. A more reliable result emerged when an exogenous ρ factor was added to the system: there occurred a considerable decrease in the synthesis of all polypeptides with the exception of the one with mol. wt ~8000.

As regards the remaining 3 polypeptides synthesized in the cell-free system containing the G fragment, the 2 most slowly moving ones appear to have been products of the *c*II gene and of the proximal part (40–45%) of the phage λ O gene. On the other hand, it was not possible to relate the fastest moving polypeptide to any of the known genes present in the G fragment.

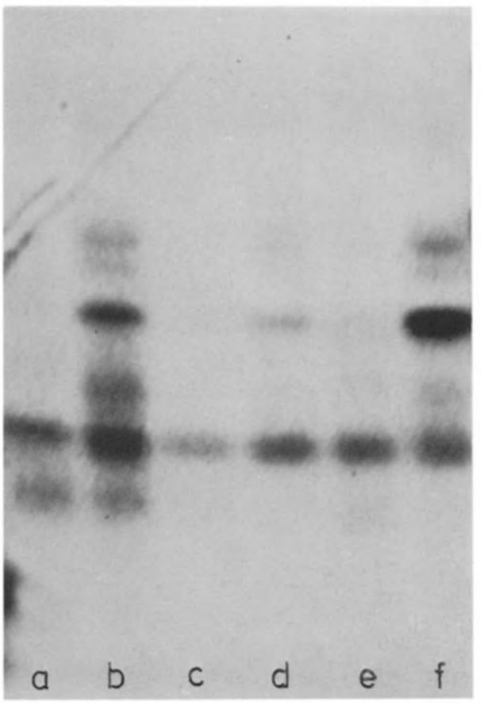

Fig.5. Effect of ρ factor on protein synthesis directed by different templates in a S30 system. Protein synthesis, electrophoresis, and autoradiography were carried out as indicated in the legend to fig.4. S30 extracts were obtained from *E. coli* W3110 cells (a,b) or *psu*4 (c–f). Templates were the G fragment (b,d,f) or the G fragment treated with *Hin*dII endonuclease (a,c,e). Before incubation, purified ρ factor was added to samples (c) and (d) to 6 μg/ml final conc.

A paper has appeared [12] where the Cro protein of phage 434 is ascribed mol. wt 11 000, which must correspond to a polypeptide chainlength of some 100 amino acids. We believe that this is a considerable overestimation and that our result is closer to reality also for the following additional reasons.

1. The molecular weight of Cro protein as determined by us, agrees well with the molecular weight estimate derived from an analysis of the G fragment primary structure, which gave mol. wt 8138.6 and a polypeptide chainlength of 71 amino acids [4];
2. The size of 434 Cro protein determined by us, is close to that of the Cro protein of the related phage λ which consists of 66 amino acid residues [2].

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