SHUT-OFF OF EARLY MESSENGER RNA SYNTHESIS IN E. COLI INFECTED WITH PHAGE T2

Akio Matsukage* and Teiichi Minagawa

Department of Botany, Faculty of Science

Kyoto University, Kyoto, Japan

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The phage specific proteins synthesized in E. coli infected with T-even phages are classified into early and late proteins according to time sequence of their appearance. The synthesis of early proteins starts immediately after infection. Among them early enzymes stop to be synthesized between 15 or 20 min after infection (Flaks et al., 1959; Kornberg et al., 1959), while the internal protein does not until cell lysis (Murakami et al., 1959; Minagawa, 1961a). The synthesis of late proteins (tail fiber, coat protein, and lysozyme) initiates later than 7 min and continues till cell lysis (De Mars, 1955; Koch and Hershey, 1959; Hershey et al., 1960). From those facts, it is more reasonable to categorize phage induced proteins into three groups instead of two (Minagawa, 1961b).

There is little knowledge of the mRNA specific for each group. If this time sequential synthesis of proteins is regulated at the level of transcription, there may be three groups of mRNA's which behave in parallel with the protein groups. It has been shown that there is a species of mRNA (late RNA) at the stage different from mRNA at the early stage (Hall et al., 1964), and this species of mRNA is assumed to be specific for late proteins.

In this short communication we shall describe results of experiments investigating the hypothesis that synthesis of a part of the early mRNA may

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* Present address; Department of Molecular Biology, Cancer Research Institute, Kanazawa University, Kanazawa, Japan

stop at a certain period after infection.

MATERIALS AND METHODS

Overnight grown cells of E. coli strain H were inoculated in TG(Hershey and Melechen, 1959) at 37° C to get $5x10^8$ cells /ml. Bacteria were collected by centrifugation and suspended in a half volume of fresh TG with low phosphate concentration (0.1 mM instead of 0.7 mM) and aerated for 30 min to lower the phosphate concentration in the cells. Then, bacteria were infected at time 0 with 10 particles of T2H per cell, and labeled by addition of ^{32}P-orthophosphate, to prepare ^{32}P-phage RNA. Synthesis of RNA and other macromolecules was stopped by pouring the culture onto crushed ice made of a solution containing 0.02 M $MgCl_2$, 0.06 M NaN_3, and 0.01 M Tris-HCl buffer at pH 7.4. Bacteria were collected and resuspended in a small volume of the above solution. After cell lysis by repeated freeze and thaw in the presence of lysozyme (200 µg/ml) and DNase (5 µg/ml), RNA was extracted and purified by the SDS-phenol method and ethanol precipitation. Finally, purified RNA was dissolved in 0.01 M Tris-HCl buffer at pH 7.4 containing 0.5 M KCl, filtered through Milipore filter (HA) and stocked in a deep freeze. Unlabeled RNA's were prepared by the same method as ^{32}P-RNA except that filtration at the final step was omitted.

T2 DNA was prepared from purified phage by the method of Mandell and Hershey (1960) and denatured by heating at 100°C for 15 min, followed by rapid chilling in an ice bath.

Hybridization between DNA and RNA was performed by the membrane filter method of Nygaard and Hall (1963) with digestion by RNase (10 µg/ml for 20 min at 37°C).

RESULTS AND DISCUSSION

Presence of heterologous RNA can be examined by hybridization of DNA and isotope-labeled RNA in the presence of large amount of unlabeled known or standard RNA. If RNA labeled with ^{32}P at the late period of T2 infection is mixed with phage DNA in the presence of an excess amount of unlabeled RNA made at the early period, ^{32}P-RNA specific for late period is hybridized with DNA

and remains on Milipore filter as RNase-resistant complex, since it does not compete for the DNA binding sites with unlabeled early RNA. Two kinds of late ^{32}P-RNA preparations were examined. One was labeled between 0.5 and 15 min , and the other was labeled between 0.5 and 19 min after infection, respectively.

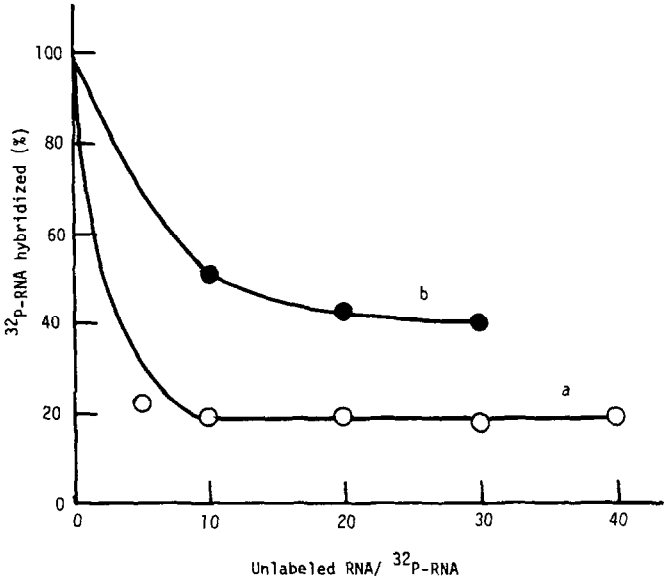

Fig. 1. Competition between ^{32}P-RNA and unlabeled early RNA for the DNA binding sites. Reaction mixture contains 1 µg/ml of denatured T2 DNA, 50 µg/ml of ^{32}P-RNA and indicated amount of unlabeled RNA. Incubation conditions; at 67°C for 2 hrs in 0.01 M Tris-HCl buffer at pH 7.4 containing 0.5 M KCl. After incubation, free RNA was digested by pancreatic RNase (10 µg/ml) for 20 min at 37°C, DNA-RNA complex was collected on Milipore filter (HA), and dried to measure radioactivity by G. M. counter.
a; ^{32}P-RNA labeled between 0.5 and 15 min (3670 cpm/µg) versus unlabeled RNA prepared at 4 min after infection.
b; ^{32}P-RNA labeled between 0.5 and 19 min (5300 cpm/µg) versus unlabeled RNA prepared at 4 min after infection.

As shown in Fig. 1, in the case of the former, the level undiluted by excess unlabeled early RNA was about 20 %, while it was about 40 % in the latter. The latter result is almost the same as that obtained with RNA pulse-labeled between 15 and 19 min (Hall et al., 1964). For this difference the followein explanations are possible.

i) The synthesis of a new species of late RNA which is not synthesized as late as 15 min, starts between 15 and 19 min after infection, and it can not

be excluded by unlabeled early RNA. However this may be unlikely, since the maturation starts near 12 min.

ii) The synthesis of all of early RNA's continues till cell lysis, though the ratio of early RNA to late RNA changes after 15 min.

iii) Some of early RNA's stop to be synthesized at a certain time. Under this hypothesis three types of RNA's should be synthesized in the cells after infection.

a) eI-RNA: the synthesis starts immediately after infection and stops at a certain time,

b) eII-RNA: the synthesis starts at the early period and continues till cell lysis, and

c) l-RNA: the synthesis starts after a certain while and continues till cell lysis.

Presence of eII-RNA is understandable by the synthesis of internal protein and that of l-RNA is evident from the above experiment.

The following experiments were carried out to prove the presence of eI-RNA. Four kinds of RNA's, that is, unlabeled early (prepared at 4 min) and late (prepared at 40 min as described below) RNA's, and ^{32}P-labeled early (labeled between 0.5 and 4 min) and late (labeled between 15 and 19 min) RNA's were prepared. As a large amount of unlabeled RNA is employed in competitive hybridization, it is necessary to obtain the clear result that unlabeled late RNA should not contain eI-RNA. Assuming eI-RNA synthesis stops at 15 min and is degradated with a half life of 2 min, its remaining amount decrease to less than 1/5000 ($= 2^{-\frac{40-15}{2}}$) in RNA prepared at 40 min after infection. Under this assumption unlabeled late RNA was prepared at 40 min from cells superinfected at 10 min after primary infection to ensure lysis inhibition (Doermann, 1948).

Fig. 2 shows the competitive hybridization between early ^{32}P-RNA and unlabeled late RNA (a), late ^{32}P-RNA and unlabeled late RNA (b) and early ^{32}P-RNA and unlabeled early RNA (c). The curve a in Fig.2 indicates that the competi-

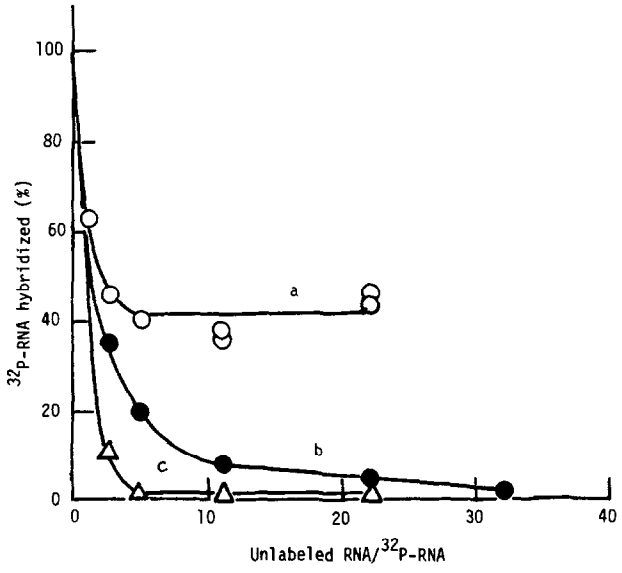

Fig. 2. Competition between ^{32}P-RNA and unlabeled late RNA for DNA binding sites. Procedures and conditions were the same as those in Fig. 1.
a; ^{32}P-RNA labeled between 0.5 and 4 min (5400 cpm/μg) versus unlabeled late RNA prepared at 40 min after infection,
b; ^{32}P-RNA labeled between 15 and 19 min (13800 cpm/μg) versus unlabeled late RNA prepared at 40 min after infection, and
c; ^{32}P-RNA labeled between 0.5 and 4 min versus unlabeled early RNA prepared at 4 min after infection (control).

tion is partial, and a part of the early RNA is no more present or very scarce in the late RNA preparation. But the unlabeled late RNA can perfectly exclude late ^{32}P-RNA labeled between 15 and 19 min (curve b), these being homologous. We conclude from these facts that early RNA, synthesized immediately after T2 infection, involves two types, the synthesis of one (eII-RNA) continues till cell lysis and that of the other (eI-RNA) stops before 15 min after infection. This excludes the hypothesis ii).

We are interested in the correlation between eI-RNA and early protein. Experiments with UV irradiated T2 phage (Dirksen et al., 1960) or a kind of amber mutant (Nishimoto et al., unpublished), which is known to synthesize continuously an early enzyme, dCTPase, may suggestive results. It is shown that the protein synthesized at an early period, is necessary for the late RNA

synthesis (Matsukage and Minagawa, in preparation). Whether there is some protein, which acts as a repressor of eI-RNA synthesis, is under investigation.

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