TRANSCRIPTION OF DNA OF T2 BACTERIOPHAGE BY RNA POLYMERASE OF CALF THYMUS

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Received September 27, 1968

Examination of RNA synthesized at different times after T even bacteriophage infection has shown that new species appear after DNA synthesis begins. The presence of this "late" RNA can be demonstrated by hybridization competition, using RNA extracted from bacteria at several stages of the infectious cycle. RNA extracted early after infection does not exclude 40-50% of the RNA extracted at late times from combination with DNA (Hall, Nygaard, and Green, 1964). Purified E. coli RNA polymerase provided with DNA extracted from mature phage as template fails to synthesize "late" RNA (Khesin, Shemyakin, Gorlenko, Bogdanova, and Afanas'eva, 1962; Khesin, Gorlenko, Shemyakin, Bass, and Prozorov, 1963; Geiduschek, Snyder, Colvill, and Sarnat, 1966) indicating that the restriction which acts on transcription in vivo also acts in vitro and resides either in the enzyme or in the structure of the DNA. Alterations in properties of the enzyme have been reported to occur during infection (Walter, Seifert, and Zillig, 1968; Hall and Crouch, 1967) but the relationships between enzyme structure and ability to transcribe "late" RNA are not clear. The present studies, using the phylogentically disparate calf thymus RNA polymerase, indicate that this enzyme, as the E. coli enzyme, synthesizes only "early" RNA from mature T2 DNA. This appears to be the case whether RNA is synthesized in an in vitro reaction mixture containing native DNA or in a reaction mixture containing heat-denatured DNA.

Calf thymus RNA polymerase, obtained as described previously (Furth and Ho, 1965), was further purified by chromatography on phosphocellulose,

and concentrated by precipitation with ammonium sulfate. The properties of this fraction are similar to those reported previously except that heat-denatured and single stranded DNA are effective templates under the usual assay conditions. Enzymically synthesized RNA was obtained in the standard reaction mixture, but with Mg^{++} omitted (Furth and Ho, 1965). After treatment with DNAse the RNA was extracted with hot phenol and extensively dialysed to remove unincorporated nucleotides. Approximately the same amount of RNA was synthesized with native and heat-denatured T2 DNA templates; recoveries after phenol treatment and dialysis were 30% and 20% respectively. E. coli cells before and after infection with bacteriophage were obtained as described previously (Furth and Pizer, 1966). Cellular RNA was isolated using hot phenol (Scherrer and Darnell, 1962) followed by DNAse treatment. The DNAse was then inactivated by heat. After alcohol precipitation, residual phenol was extracted with ether and the ether removed with nitrogen. The RNA thus obtained contained approximately 5% deoxynucleotide (as determined by the diphenylamine reaction). RNA was determined by the orcinol reaction using yeast RNA as standard. The amount of nucleotide in the standard solutions was calculated by measuring the absorbance at 260 $m\mu$ and assuming a molar extinction coefficient of 10×10^3.

Results are reported for experiments using RNA synthesized on native T2 DNA template, heat-denatured T2 DNA template and E. coli DNA template. In the experiment illustrated in Fig. 1 RNA synthesized on native T2 DNA template was incubated with varying quantities of "early" T2 RNA, "late" T2 RNA, and E. coli RNA. While E. coli RNA has only a slight effect on hybridization, the addition of RNA synthesized after T2 infection greatly inhibits hybridization. Both RNA synthesized 0-5 minutes and 0-30 minutes after infection inhibit hybridization approximately 85%. In the range of 0-1 μ moles, native "early" RNA is a more effective competitor than "late" RNA. This difference is consistent with there being reduced amounts of "early" RNA in the sample taken at 30 minutes. The above experiments were repeated with RNA synthe-

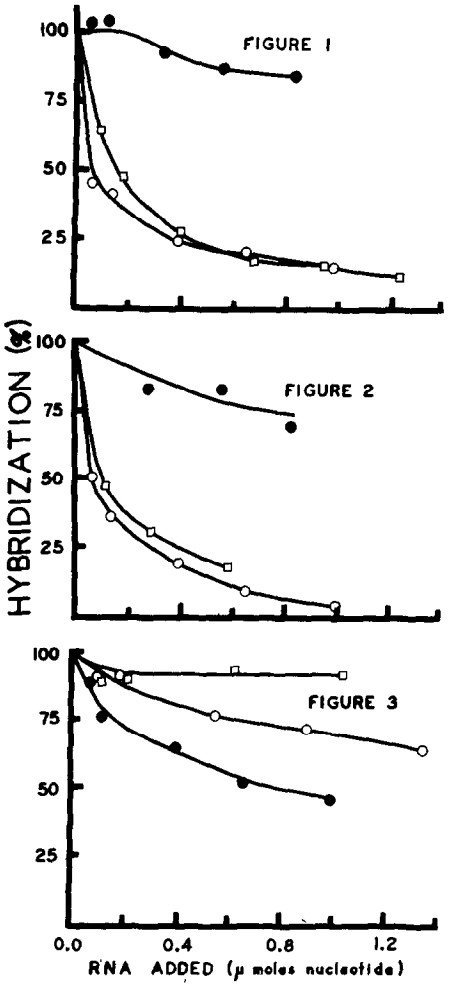

Figure. Hybridization competition with RNA synthesized by calf thymus RNA polymerase on native T2 DNA template, Figure 1; heat-denatured T2 DNA template, Figure 2; and E. coli DNA template, Figure 3.

Vessels contained (in 0.5 ml) 12 mμ moles of DNA nucleotide, 0.30 M NaCl, 0.03 M Na Citrate, approximately 0.1 mμ mole (as nucleotide) of radioactively labeled (^{32}pU) RNA, and incubated at 67° for 12 hours. The extent of hybridization was determined as described by Nygaard and Hall (1963), but with RNAse treatment of the DNA-RNA hybrid. Radioactivity content of the filters was determined in a windowless Geiger-Muller counter. In the experiment reported in Fig. 1, 12 cpm were attached to the filter when incubation at 67° was omitted. This value has been subtracted and 100% represents 320 net cpm, 29% of the input RNA. Zero time values for the experiments reported in Figs. 2 and 3 were 34 and 7 cpm respectively; 100% represented 177 net cpm (30%) in Fig. 2 and 182 cpm (16%) in Fig. 3. ●---●---●, E. coli RNA; O---O---O, "early" T2 RNA; □---□---□, "late" T2 RNA.

sized with heat-denatured T2 DNA as template. (Fig. 2). The results are similar; E. coli has only a small effect on hybridization, and "early" and "late" T2 RNA inhibit hybridization 80% or more.

To exclude the possibility that RNA isolated from T2 infected cells was competing in a non-specific fashion, RNA synthesized on E. coli DNA template was incubated at 67° with varying amounts of the several RNA species (Fig. 3). While E. coli RNA is an effective competitor, "late" T2 RNA is ineffective. Significant competition by "early" T2 RNA indicates that some E. coli messenger RNA remains 5 minutes after infection.

The similarities of the competition observed with "early" and "late" T2 RNA indicates that RNA made in vitro by calf thymus RNA polymerase lacks appreciable quantities of "late" messenger. Whatever the restrictions are which prevent transcription of "late" messenger by E. coli polymerase, these restrictions also appear to operate on RNA polymerase obtained from a very different source, suggesting that regulation of transcription relates to modifications of the structure of DNA rather than to modifications of RNA polymerase. This modification of DNA may not involve changes in the helical configuration of the DNA template, as similar competition curves are obtained with RNA synthesized by calf thymus enzyme when denatured T2 DNA is the template. These results with heat-denatured T2 DNA as template are in contrast with results obtained with E. coli RNA polymerase. The latter enzyme when given heat-denatured DNA synthesizes RNA which is different from RNA isolated from infected cells (Green, 1964). Whether part of the RNA made by the E. coli enzyme is "late" RNA has not been established.

While the extent of the competition is consistent with the hypothesis that little RNA is made in vitro that is not also made in vivo, we cannot rule out the synthesis of RNA complimentary to the RNA made in vivo. Such an RNA could form an RNA-RNA hybrid with the RNA isolated from infected cells, and thereby be unable to hybridize with DNA. RNAse resistant material was formed on incubation of synthetic messenger with both "early" and

"late" T2 RNA, but not E. coli RNA (Furth and Pizer, unpublished observations). However, interpretation of these results is not possible due to a small amount of DNA contaminating the RNA preparations. It should also be noted that we are unable at the present time to synthesize enough RNA enzymically to compete pulse labeled in vivo RNA with in vitro RNA.

ACKNOWLEDGEMENTS

This investigation was supported by a grant from the National Institutes of Health, United States Public Health Service GM 10390. One of us (J. J. F.) is a Research Career Development Awardee (GMK3-12888) of the National Institutes of Health, United States Public Health Service.

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