ASYMMETRIC TRANSCRIPTION OF B. SUBTILIS PHAGE SPP1 DNA IN VITRO

by

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SUMMARY

B. subtilis phage SPP1 DNA has separable strands and binds ribosomal RNA only to the heavy pyrimidine-rich strands. The RNA synthesized in vitro with the heterologous E. coli RNA polymerase hybridizes only with the heavy strand.

INTRODUCTION

A considerable amount of evidence, mainly accumulated by Szybalski and coworkers, indicates that the binding of ribosomal RNA and of some synthetic polynucleotides (like poly IG and poly G) to DNA is due to the presence of pyrimidine-rich clusters on the latter (Kubinski et al.,1966; Szybalski et al., 1966). The same authors have proposed a possible role of the pyrimidine-clusters as "recognition" sites for RNA polymerase of the DNA template in connection with the initiation or the termination of the RNA synthesis. Their hypothesis has received strong support from in vivo work on phage λ (Taylor et al., 1967) and on phage T_7 (Summers and Szybalski, 1968).

The DNA of the B. subtilis phage SPP1 exhibits the property of having separable strands and binding ribosomal RNA only to the heavy pyrimidine-rich strand (Riva et al., 1968). This paper reports experiments which show that practically all the RNA synthesized in vitro on the SPP1 DNA template with E. coli RNA polymerase (that is, in an heterologous system) hybridizes with the pyrimidine-rich heavy DNA strand.

MATERIALS AND METHODS

E. coli RNA polymerase, purified according to Chamberlin and Berg (1962) was the generous gift of Dr. O. Grau.

SPP1 DNA and its separated strands were prepared as described elsewhere (Riva et al., 1968). This method yields DNA preparations consisting of whole molecules as judged by the sedimentation velocity and by the high recovery of infectious activity after denaturation and renaturation. After collecting and pooling fractions corresponding to the light (L) and heavy (H) strands, ribosomal RNA was eliminated by alkali digestion (0.2 M KOH, 5 hrs at 37°C, followed by neutralization with KH_2PO_4). The denatured DNA template for RNA synthesis was obtained by heating 10 min at 100°C in 0.01 M Tris-chloride, pH 7.5, and rapid cooling.

In vitro RNA synthesis and RNA isolation followed the method described by Geiduschek et al. (1964). The reaction mixture contained per ml: 100 µmoles Tris-chloride, pH 7.5, 10 µmoles $MgCl_2$, 1 µmole each of ATP, UTP, CTP and 0.1 µmole of ^{3}H-GTP, 1 µmole Spermidine, 50 µg of native SPP1 DNA (or 20 µg of denatured SPP1 DNA) and 50 units of E. coli RNA polymerase. The reaction was carried out for 45 min at 30°C. The asymmetry of the synthesized RNA was measured by determining the conversion to RNase resistant complementary RNA duplexes upon annealing (Colvill et al., 1965).

RNA-DNA hybridization was carried out according to Nygaard and Hall (1964). Labelled RNA and the separated strands of SPP1 DNA were hybridized for 6 hrs at 60°C in 2 x SSC, digested with RNase (12.5 μg/ml pancreatic RNase + 1.25 μg/ml T_1 RNase) and then filtered through nitrocellulose filters (Schleicher and Schuell, B6, 24 mm dia.) with 0.5 M KCl-0.01 M Tris-chloride, pH 7.5.

Before annealing, RNA was heated for 10 min at 100°C in 0.001 M Tris-chloride, pH 7.5, and cooled in ice in order to eliminate any RNase-resistant aggregates that might be present.

RESULTS AND DISCUSSION

Fig. 1 shows the result of a preparative separation of the

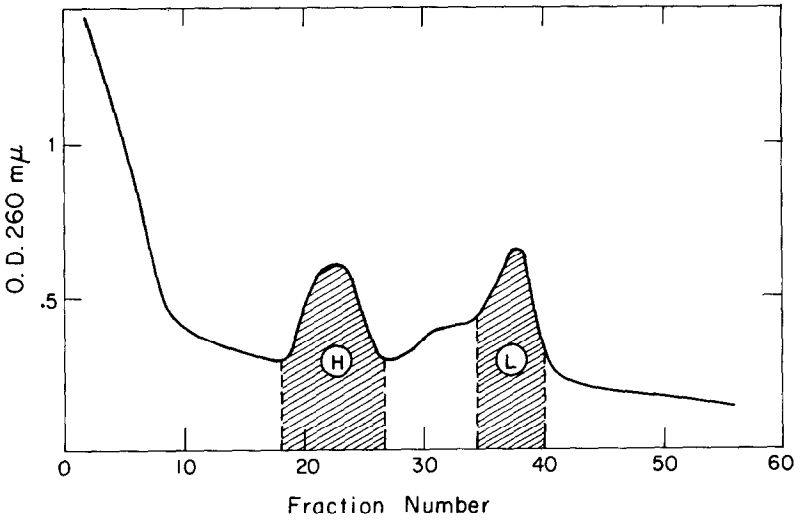

Fig. 1 - Preparative CsCl-gradient separation of the complementary strands of SPP1 DNA. 3 ml of solution containing 40μg of DNA and 120 μg of ribosomal RNA in 0.03 M NaCl-0.003 M NaCitrate were heated 10 min at 100°C and chilled. CsCl and water were added to give a final volume of 13 ml and a density of 1.700 g cm^{-3}. The mixture was then run in a No. 50 Spinco angle rotor 38 hrs at 38,000 RPM and 20°C. 0.25 ml fractions were collected from the bottom and their absorbance at 260 mμ was read.

two strands of SPP1 DNA. It is known from previous work (Riva et al.,1968) that this technique provides complete separation of the two viral DNA strands as judged by reannealing-infectivity tests on the separate and mixed "bands." It has also been shown that ribosomal RNA binds only to the H strand of SPP1 DNA, increasing its buoyant density in CsCl by as much as 20 mg/ml; any binding to the L strand, if it exists, is not sufficiently extensive or stable to change its density in a neutral CsCl gradient.

^{3}H-RNA was synthesized in vitro on native and denatured SPP1 DNA templates. The time course of RNA synthesis was determined by taking 10 µl samples at various times and measuring the amount of ^{3}H-GTP incorporated into trichloroacetic acid-precipitable material. The initial rate of incorporation of ^{3}H-GTP was 1.2 mµmoles/ml/min for native DNA template and 0.12 mµmoles/ml/min for denatured DNA template. The final weight ratio of RNA synthesized to DNA template was about 0.75 for native DNA and 0.19 for denatured DNA template.

The purified SPP1 RNA was assayed for the presence of self-complementary polynucleotide sequences; the concentration-dependent conversion to ordered RNase resistant RNA duplexes is shown in Fig. 2. When transcription is on native DNA template, the product is highly asymmetric, whereas the product of transcription on a denatured DNA template shows a considerable degree of symmetry. That the asymmetry of the in vitro synthesis is strongly dependent on the DNA secondary structure, has been reported in several instances (Hayashi et al., 1963; Geiduschek et al., 1964). It is commonly surmised that the ordering of DNA secondary structure is essential in order for a specific DNA-enzyme interaction, restricted to a limited number of specific

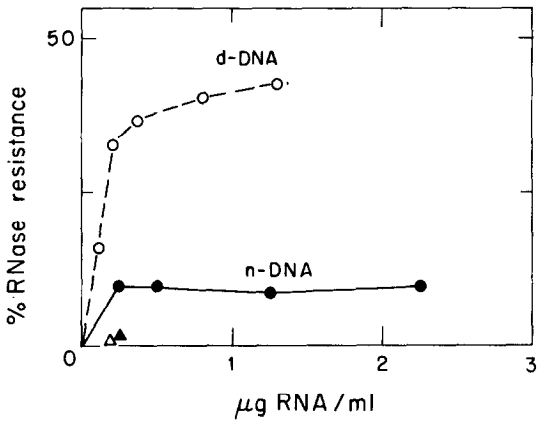

Fig. 2 - Self-complementarity of RNA synthesized on native SPP1 DNA template (●—●) and on denatured SPP1 DNA template(o—o). Control points (▲, Δ) were obtained by heating and quenching unannealed RNA, treating with RNase and measuring the percent of RNase-resistance.

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initiation sites, to take place. Asymmetry can arise either when only one strand is transcribed unidirectionally or when small regions of both strands are transcribed with a switch in the direction of transcription. In order to show that in our system one strand of helical SPP1 DNA is preferentially transcribed in vitro, asymmetric ^{3}H-RNA was annealed with the two separated strands of SPP1 DNA (Fig. 3a); the ^{3}H-RNA hybridizes almost exclusively with the H strand. As a control, to prove that the L strand is intrinsically capable of forming DNA-RNA hybrids, symmetric ^{3}H-RNA (synthesized on the denatured template) was shown to hybridize equally well with both DNA strands (Fig. 3b). The low efficiency of hybridization of symmetric RNA to the DNA strands is probably due to the low molecular weight of the RNA; in fact, the RNA-RNA hybrid formation, at the RNA concentration used, cannot fully account for it (see Fig. 2).

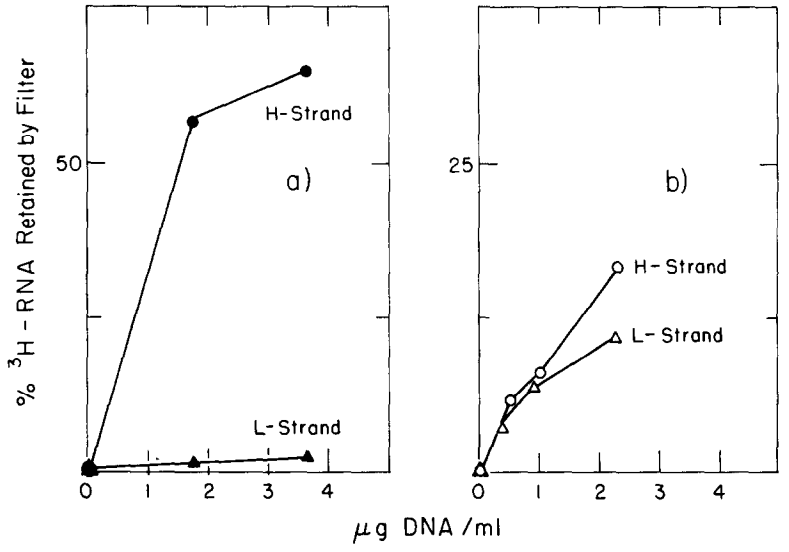

Fig. 3a- Hybridization of in vitro RNA synthesized on native template with separated strands of phage SPP1 DNA. In the annealing mixture ^{3}H-RNA is present at a concentration of 0.15 μg/ml. Hybridization with the heavy (H) strand: (●—●). Hybridization with the light (L) strand: (▲—▲).

Fig. 3b- Hybridization of in vitro RNA synthesized on denatured template with the two separated strands of SPP1 DNA. The concentration of ^{3}H-RNA in the annealing mixture is 0.1 μg/ml. Hybridization with the heavy strand (○—○). Hybridization with the light strand (Δ—Δ).

These results indicate that the H strand of SPP1 DNA is preferentially (if not exclusively) transcribed by E. coli RNA polymerase. The small proportion of RNA forming RNase resistant material upon self-annealing could be either due to a small degree of symmetric transcription or to a slight contamination of the primer with denatured DNA.

These results on this in vitro heterologous system, together with the previous observations on the in vitro asymmetric transcription of phage α (Tocchini-Valentini et al., 1963), of phage λ (Taylor et al., 1967) and of phage T_7 (Summers and Szybalski, 1968) demonstrate that both in vivo and in vitro

the pyrimidine-rich clusters are located on the same DNA strand as the signals for the initiation (and possibly the termination) of transcription. No evidence exists, at present, that the pyrimidine clusters are the signals for the initiation of transcription. However, experiments of M. Chamberlin, quoted by Summers and Szybalski (1968), show that both RNA initiation and termination are very frequent when poly dG.dC or poly dA.dT are transcribed in vitro.

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