When circular, ψ6-A74 replicative DNA (RF-DNA) is used as a template for RNA synthesis in vitro by a DNA-dependent RNA polymerase (RF 2,3,7) purified from E. coli K-12, the synthesized RNA has heterogeneous sedimentation constants ranging from 50S to 16S (ref. 1). As synthesize proceeds, progressively heavier molecules of RNA are obtained. Pulse and chase experiments have shown that some of the label initially incorporated into RNA with low S values is transferred to more rapidly sedimenting fractions, indicating the growth of the polynucleotide chains in this system. Using this system it is possible to prepare RNA labeled with a different radiolabel at each end of the molecule. The differentially labeled RNA can be used to determine the direction of polynucleotide chain growth. This communication will present evidence which indicates that RNA synthesis by DNA-dependent RNA polymerase proceeds from the 5′ to the 3′ end of the molecule.

Further: E. coli C-222 DNA-dependent RNA polymerase and ψ6 RF-DNA were prepared as described previously. To synchronize the initiation of the reaction, the reaction mixture (see legend of Fig. 1) was preincubated at 25° for 30 min. The reaction was initiated by adding a mixture of four nucleoside 3′-triphosphates to a final concentration of: 300 ATP (0.1 M), Schwartz Bioresearch, Inc. 250 CTP, 280 CTP, 280 GTP, 280 GTP, 2 mol

The incubation mixture was sampled prior to cluing at (7 min); the remainder was incubated for an additional 45 min. The reaction mixture was chilled at 0° to 4° in an ice-water bath purified by the method described previously. The two samples were subjected to sucrose gradient analysis. A profile of the sample taken at 7 min is shown in Fig. 2A. It has been shown that the heavier peak coinciding with RF-DNA is a hybrid complex composed of double-stranded RF-DNA and newly synthesized RNA. It was concluded that this hybrid complex is an intermediate in genetic transcription. It has also been shown that the RNA isolated in this complex can be freed from the template RF-DNA by treatment with formamide or heat. As illustrated in Fig. 2A, the hybridized RNA is completely released from RF-DNA upon incubation with 70% formamide for 20 min at 37°. This released RNA now sediments with the originally free RNA of 8 to 1S. It is evident, from Fig. 2A and Fig. 2B (formamide-treated 35S-35S) that more than 20% of the 8 to 1S RF-DNA synthesized during the first 7 min of incubation is transferred to RNA species with S values of more than 20 during the additional 45 min incubation with 70% GTP and 100% excess of unlabeled ATP and UTP. While the 7 min sample gives a profile with a homogeneous S distribution, subelute

References: 4, 11, 13, 18, 20, 21.
Fig. 2. Saturating density gradients profiles of the reaction products. DNA was purified by gel chromatography from the 27th and 35th min samples of Fig. 1. After dialysis overnight against 0.01 M Tris-HCl buffer (pH 7.3) containing 0.1 M NaCl, half of the sample was layered onto a 2.5 to 15% sucrose gradient in the same buffer, and spun in a SW 25.1 rotor for 18 h at 4°C. The other half of the sample was treated with 25 μg/ml bromphenol for 30 min at 37°C, dialyzed against the above-mentioned buffer, and processed for sedimentation as above. Radioactivity was determined as in Fig. 1. (A) 27 min sample, before (--) and after (---) formamide treatment. (B) 35 min sample after formamide treatment. (C) (---) DNA used as a marker was prepared from a lymphoid cell line by a method similar to that of Karvinen and Hasegawa. (---) incubated DNA was used as a marker was prepared from a lymphoid cell line by a method similar to that of Karvinen and Hasegawa. The profile of the DNA was determined from a sedimentation run in a separate tube. 1 M NaCl was used as a marker. The ordinate is the fraction of radioactivity in each gradient fraction calculated from the ratio of radioactivity in each fraction to the total radioactivity in the sample. The profiles of the DNA were determined from a sedimentation run in a separate tube. 1 M NaCl was used as a marker. The ordinate is the fraction of radioactivity in each gradient fraction calculated from the ratio of radioactivity in each fraction to the total radioactivity in the sample.
RNA chain growth yields a heterogeneous size distribution with 5 to 6 methylthymine peaks. Fig. 2B is a plot of the ratio of 32P/35P (in counts/min) of each fraction as calculated from Fig. 2B. The ratio is highest for the largest RNA species and decreases steeply as the size of the RNA decreases. This steep decrease in the 32P/35P ratio implies that the initially synthesized polymerization stopped, or decreased in rate, at distinct sites on the template DNA.

The heaviest peaks (specified by arrows in Fig. 2B) with a constant 32P/35P ratio were collected. After concentrating 20-fold by pressure filtration, the sample was run on a sucrose gradient for further purification. The mean relative length of the peak 32P-labeled, most recently synthesized region and the tail 35P-labeled region, verehesed during the first 5 min of these polymerase--DNA reaction were calculated by assuming that the base compositions of both regions of the chain were identical to that of the DNA-7 DNA (32P/35P = 25/32; 33/39). The peak is 83%.

Fig. 3. Deposition of differentially labeled RNA by venom phosphodiesterase. The reaction mixture consisted of 0.3 mg/ml Tris-arcade (pH 8.1) 0.2 mg/ml magnesium nitrate, 1/114 mg/ml venom phosphodiesterase (Worthington Biochemical Corp.) and an appropriate amount of labeled DNA. The reaction was stopped by adding 0.5 ml of 0.1 M sodium acetate as a buffer, and 0.2 ml of 30% trichloroacetic acid. After centrifugation at 40,000 rpm for 7 min, trichloroacetic acid in the supernatant was collected with ether. 5 ml of 2 ml of 0.2 M sodium acetate was added to 20 ml of 0.4 M sodium periodate. The supernatant was washed several times in 95% ethanol, and the tail is 13% of the molecule. If the tail has the 3' terminus and the head the 5' terminus, there should be a differential release of H3P and H3P into the acid-soluble fraction upon treatment with venom phosphodiesterase (EC 3.1.4.2). H3P would appear without delay, whereas H3P would appear only after an initial lag. On the other hand, if the tail has the 3' terminus, the situation would be reversed. Fig. 3 shows a 10 min lag in the release of H3P into the acid-soluble fraction in contrast to the linear release of H3P. Some 5% of the input H3P had been released into the acid-soluble fraction before the appearance of appreciable amounts of H3P (0 min). H3P is eliminated somewhat earlier than expected from the ratio of H3P to H3P in the sample.

This may be due to degradation present in the enzyme treatment or a control RNA, prepared similarly but labeled oppositely (i.e., H3P in the tail and H3P in the head), gave the opposite result.

Considering the specificity of venom phospholipases, these data prove that RNA is synthesized from the 3' to the 5' end. This conclusion is consistent with the data recently published by KREMER et al. and MAURA and HIRNITZ.

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