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Synthesis of DNA Complements of Natural RNAs: A General Approach

(avian myeloblastosis virus/avian reverse transcriptase/Moloney sarcoma virus/Qβ bacteriophage)

S. SPIEGELMAN, K. F. WATSON, AND D. L. KACIAN

Institute of Cancer Research, College of Physicians and Surgeons, Columbia University, New York, N.Y. 10032

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ABSTRACT **The availability of a purified RNA-instructed DNA polymerase (reverse transcriptase) from avian myeloblastosis virus provided the opportunity to explore whether this enzyme could be used as a general tool for synthesizing DNA complements of a wide variety of natural RNAs. The results described show that this potentially useful situation is in fact realized. The avian viral transcriptase can mediate the synthesis of DNA complementary to RNAs of such widely divergent origins as Qβ bacteriophage and Moloney sarcoma virus. These findings open up novel pathways for the experimental resolution of several interesting problems. Thus, given a purified RNA message, one should be able to synthesize the corresponding DNA genetic material. If suitably labeled, the synthetic DNA has various obvious uses, including its use via molecular hybridization as an analytical probe for the corresponding gene on the chromosomes or for its message in a complex mixture of RNA molecules.**

Of immediate practical interest is the import of these findings for viral oncology. They imply that for many purposes we will not be compelled to isolate or use the "reverse transcriptase" from each oncogenic RNA virus in order to synthesize its complementary DNA. The ability of one enzyme to accept a variety of oncogenic RNAs will obviate many of the logistical difficulties that arise, particularly in attempts to illuminate the etiology of human cancer.

The RNA tumor or oncornaviruses (1) exhibit DNA polymerase activities (2–8) that respond to a wide variety of nucleic acids as template. The endogenous viral RNA, DNAs of various origins (9–13), and synthetic homopolymer duplexes (11, 14) all stimulate synthesis. The product DNA has been shown to be complementary to the endogenous RNA or to the added DNA (4, 9, 10, 15, 16).

Recently, we reported (17) the purification of the reverse transcriptase from avian myeloblastosis virus. The purified enzyme preparation was shown to contain two subunits in equimolar amounts, one of 110,000, and the other of 69,000, molecular weight. Throughout the various stages of purification on different columns and in glycerol gradients, the responses to homologous/viral RNA, native DNA, and the synthetic homopolymer duplexes seemed to be mediated by a single protein entity. The final preparation, which was better than 97% pure, retained the ability to recognize the complete spectrum of templates.

It was of obvious interest to see whether this flexibility extended to heterologous, natural single-stranded RNAs. Were this the case, the comparatively easily obtained avian virus reverse transcriptase could be used to generate DNA complements of oncogenic RNAs in instances in which it is difficult to obtain virus in amounts adequate for active enzyme preparations.

It is the purpose of this paper to describe experiments that demonstrate that this eminently useful situation does in fact obtain. We show, by a number of detailed examples, that the purified avian viral reverse transcriptase will accept genetically unrelated RNA molecules as templates, which leads to the formation of DNA that will hybridize to the RNA used to instruct the synthesis.

MATERIALS AND METHODS

Compounds and Enzymes. Unlabeled nucleoside triphosphates and dithiothrietol were obtained from P-L Biochemicals. Tritiated triphosphates came from Schwarz BioResearch and Amersham-Searle. Ribonuclease A (EC 2.7.7.16) was purchased from Worthington. Optical grade cesium sulfate was supplied by Gallard-Schlesinger.

Purification of RNA-Dependent DNA Polymerase from Avian Myeloblastosis Virus. The purification of avian myeloblastosis virus and isolation of the DNA polymerase were performed as described (17). Enzyme purified through the carboxymethyl-Sephadex or phosphocellulose chromatography steps was used for the work reported here. Procedures for enzyme assay have been detailed elsewhere (17).

Isolation of Viral RNAs. Qβ virus and viral RNA were prepared by the procedures of Pace *et al.* (18). The isolation of avian virus RNA has been described (17).

Hybridization of DNA Product to RNA. DNA product labeled with tritium was synthesized in suitable multiples of the standard assay mixture. After incubation at 37°C for 60 min, sodium dodecyl sulfate was added to 0.1% and the reaction mixture was extracted with an equal volume of phenol–cresol solution. The product was then separated from residual substrate on a Sephadex G-50 (coarse grade) column equilibrated with 0.01 M Tris·HCl (pH 7.4)–0.1 M NaCl–3 mM EDTA–0.1% sodium dodecyl sulfate, and the excluded material was recovered by ethanol precipitation. The sample was then treated with alkali to hydrolyze the RNA and tested for complementarity with various RNAs (4).

RESULTS

Response to heterologous single-stranded RNA

The response of the purified reverse transcriptase to a number of viral RNA templates is shown in Fig. 1. Each RNA stimulates the incorporation of [^{3}H]dTTP into acid-insoluble product. It would appear that Moloney sarcoma virus RNA is superior to the homologous avian RNA. However, inter-

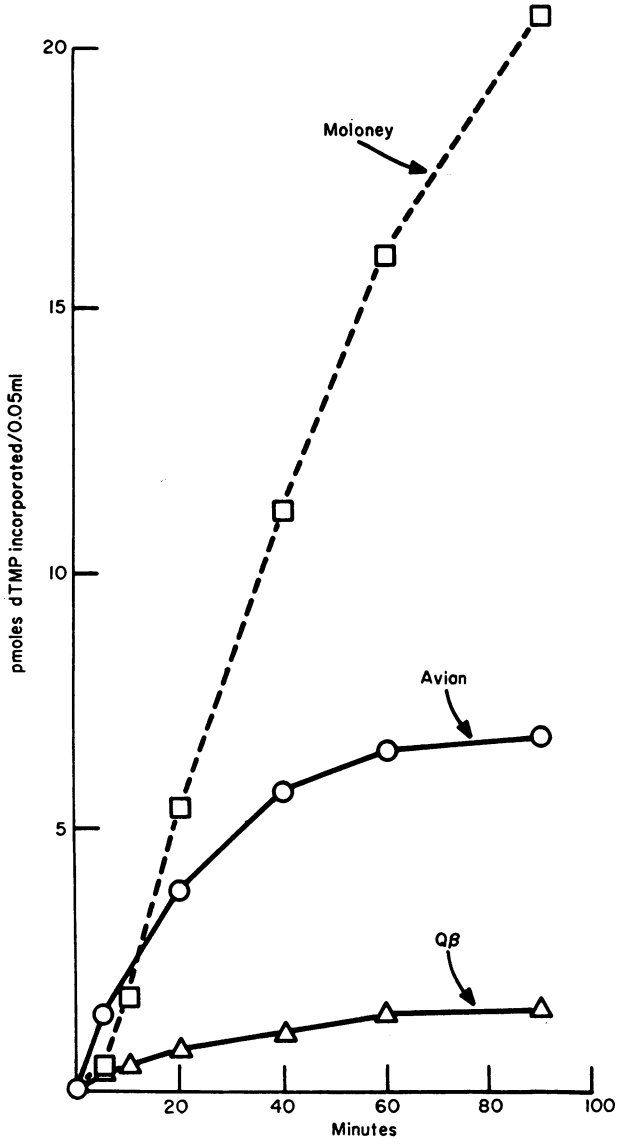

FIG. 1. Kinetics of the avian DNA polymerase reaction with natural RNA templates. Moloney, represents Moloney sarcoma virus; Avian, represents avian myeloblastosis virus.

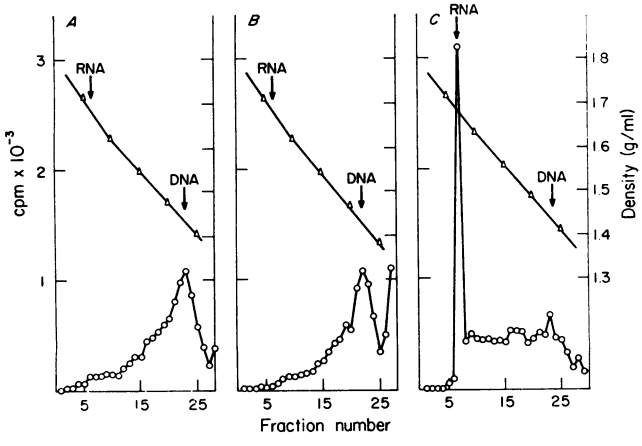

FIG. 2. Complementarity between avian RNA template and product. (*A*) Product DNA self-hybridized. (*B*) Product DNA hybridized to Qβ RNA. (*C*) Product DNA hybridized to avian viral RNA.

TABLE 1. *Effect of RNase treatment on template function of natural RNAs*

Viral RNA	pmol/0.1 ml reaction −RNase	pmol/0.1 ml reaction +RNase	Activity remaining (%)
Avian myeloblastosis*	24.2	0.4	1.7
Moloney sarcoma*	12.4	0.0	0.0
Qβ RNA	1.9	0.2	10.5

2 μg of each RNA, in 0.04 ml of 0.01 M Tris·HCl (pH 7.4)–3 mM EDTA, was incubated with or without the addition of 1 μg of RNase A (heated at 100°C for 10 min to destroy contaminating DNase) for 15 min at 37°C. The samples were chilled to 0°C, the remaining assay components were added, and a zero-time aliquot was removed. The reactions were incubated at 37°C for 20 min, and the net acid-precipitable radioactivity was determined. [³H]dTTP had a specific activity of 350 cpm/pmol for the reactions with avian and Moloney sarcoma viral RNAs and 3000 cpm/pmol for the reactions with Qβ RNA.

* 70S RNA.

pretation of such differences in response must await further investigation. We have observed similar disparities with different preparations of the same RNA, all of which exhibit virtually identical sedimentation profiles.

To show that the RNA added as template is required for activity, each of the RNAs was incubated with RNase A before addition to the reaction mixture. In each case, RNase treatment severely reduces the observed incorporation to a small percentage of that obtained with control RNA (Table 1).

Hybridizability of the product DNA to the RNA template

The experiments described above, although suggestive, do not constitute a rigorous demonstration that product complementary to the proffered RNA is being synthesized. It was therefore necessary to show that the material synthesized in the reaction hybridizes to the added RNA, but not to heterologous RNA.

When the relevant experiments were performed, it was found in each case the product hybridized to the RNA that was added as template, and not to heterologous RNA (Figs. 2–4). We conclude that the avian DNA polymerase is able to

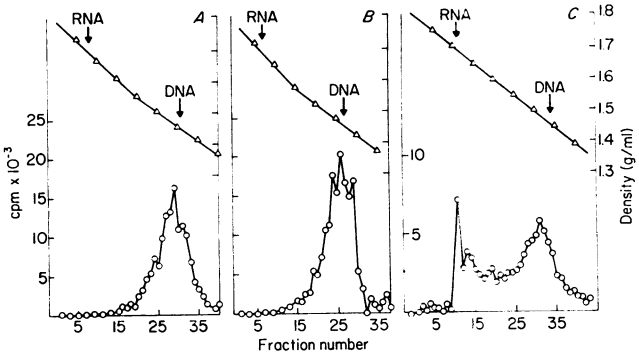

FIG. 3. Complementarity between Moloney RNA template and product. (*A*) Product DNA self-hybridized. (*B*) Product DNA hybridized to Qβ RNA. (*C*) Product DNA hybridized to Moloney-viral RNA. Moloney, represents Moloney sarcoma virus.

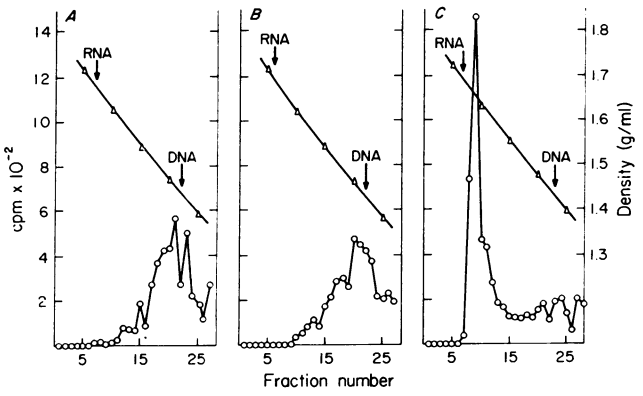

FIG. 4. Complementarity between Qβ RNA template and product. (*A*) Product DNA self-hybridized. (*B*) Product DNA hybridized to avian myeloblastosis viral RNA. (*C*) Product DNA hybridized to Qβ RNA.

use various natural RNA templates to synthesize complementary DNA.

DISCUSSION

The results described show that purified avian virus reverse transcriptase will, when presented with heterologous RNA, synthesize DNA sufficiently complementary to form stable hybrids with the RNA templates. It should be noted that although 70S avian myeloblastosis and Moloney sarcoma viral RNAs were used in the present study, it has been reported (19, 20) that such molecules possess breaks that are absent when extreme care is exercised in harvesting and processing the RNA. These breaks clearly do not destroy template function, but they may adversely affect chain initiation or elongation and help to explain the small size of the DNA product usually observed. Such questions may be more amenable to experimental analysis by the use of templates that are more readily obtainable as intact biologically competent molecules (e.g., Qβ RNA). It is also necessary to compare the completeness of the copying of homologous and heterologous RNA templates.

In any event, it is clear that the situation found with the avian reverse transcriptase is strikingly different from that observed with the RNA bacteriophage replicases. Qβ replicase exhibits a rigid template requirement, restricted to Qβ RNA (21, 22) and variant molecules derived from it (23).

Consequently, Qβ replicase could not be used as a general tool for the production of RNA copies. The fact that the avian reverse transcriptase does not show this restriction makes it a very valuable device, which we have already exploited to synthesize useful amounts of DNA complementary to such diverse oncogenic RNA agents as the Moloney sarcoma virus, the mouse mammary-tumor virus, and the Mason–Pfizer monkey virus, which is associated with a spontaneous mammary tumor in a rhesus monkey (24–26).

Needless to say, the reverse transcriptase method for the synthesis of DNA copies of RNA cannot replace the elegant chemical approach of Khorana and his colleagues (27). Reverse transcriptase could, however, provide a convenient and rapid supplement in instances in which detailed knowledge of sequence is not pertinent.

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