RNA-DEPENDENT DNA POLYMERASE: PRESENCE IN NORMAL HUMAN CELLS*

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Summary: RNA-dependent DNA polymerase has been reported in oncogenic RNA viruses, as well as in human leukemia cells, suggesting a close relationship between this activity and malignancy. However, we have detected an RNA-dependent DNA polymerase activity in normal human lymphocytes stimulated with phytohemagglutinin, indicating either that this enzyme is not unique to RNA-viruses, or that a viral genome is present in non-malignant human cells. Therefore, the use of this activity as an indication of malignancy or a target for the control of cancer should be approached with caution.

The presence of the enzyme, RNA-dependent DNA polymerase, in all oncogenic RNA viruses thus far examined (1-3), provides a possible mechanism for integrating their genetic information into that of the host. Attention has been focused on the relationship of this enzyme to malignancy, a relationship which is supported by the presence of an RNA-dependent DNA polymerase activity in lymphocytes from patients with acute lymphatic leukemia and its apparent absence in stimulated lymphocytes from normal donors (4). This discovery raises the

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possibility of using this enzyme both as an indicator of malignancy, as well as a site for the control of leukemia. However, our results indicate that the enzyme, RNA-dependent DNA polymerase, is not unique to cancer cells; we find it to be present in stimulated lymphocytes from normal donors. This finding has broad implications concerning the flow of genetic information in normal cells.

METHODS

The lymphocytes used in these studies were isolated from human peripheral blood by the method of Cooper (5), cultured as previously described in the presence of phytohemagglutinin for 3 days (6). The cells were harvested by centrifugation, washed with 0.15 M potassium chloride, and suspended at a concentration of 10^7 cells/ml in 20% glycerol, 0.02 M potassium phosphate, pH 7.4, 0.001 M potassium EDTA, and 0.004 M reduced glutathione. The suspension was sonicated at 0° for 80 seconds at a setting of 4 on a Branson Sonifier model S-75, equipped with a microtip. The disrupted cell preparations were frozen and thawed three times and then assayed for enzyme activity.

Electrofocusing in a pH gradient was carried out at 0° on 110 ml electrofocusing columns (LKB Produkter, Stockholm). A 2 ml sample of a 100,000 x g supernatant from disrupted cell suspensions was applied to the middle of a pre-established pH gradient containing 1% "Ampholine" carrier ampholytes pH 3-6 (LKB) in a 0-45% sucrose gradient. The pH gradient was established at 600 v for at least 24 hours before application of the sample, then a further 16-18 hours after the sample was added. Fractions were then collected and assayed for DNA-dependent DNA polymerase by the method of Loeb (7) and RNA-dependent DNA polymerase by the method of Gallo et al. (4).

RESULTS

In initial experiments on crude homogenates of phytohemagglutinin-

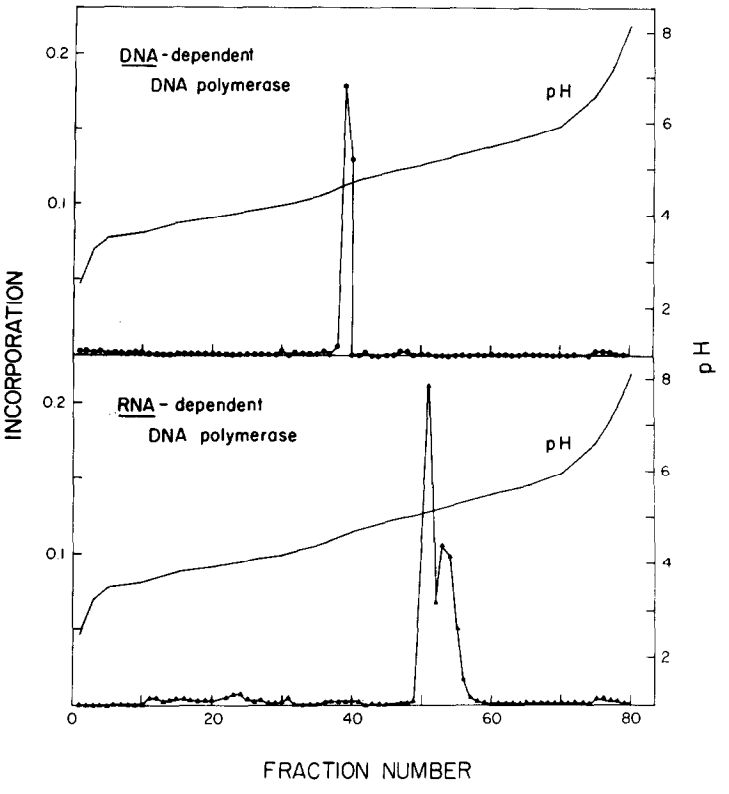

Fig. 1. Profile of DNA polymerases after electrofocusing extracts of phytohemagglutinin-stimulated lymphocytes from a normal donor. The fractions from the column (1.1 ml) were neutralized with Tris-maleate buffer, pH 8.2, immediately after the pH measurements were made and assayed directly, or after storage at -70°. DNA-dependent DNA polymerase was assayed with "activated" calf thymus DNA as a primer (7). The reaction mixture in a total of 0.3 ml contained 25 µmoles Tris-maleate, pH 7.8, 1.8 µmoles magnesium chloride, 0.3 µmole β-mercaptoethanol, 10 mµmoles each dATP, dCTP, dGTP, 10 mµmoles [α-^{32}P]-dTTP (about 6 x 10^5 dpm), 177 mµmoles of "maximally activated" calf thymus DNA (7) and 100 µl of the enzyme fraction. RNA-dependent DNA polymerase was assayed with yeast RNA as a primer in a reaction mixture similar to the one used for DNA-dependent DNA polymerase except that it contained in a total volume of 0.25 ml, 10 µmoles Tris-chloride, pH 8.2, 1.5 µmoles magnesium chloride, 5.0 µmoles dithiothreitol, 0.1 µmole each dATP, dCTP, dGTP, 10 mµmoles [α-^{32}P]-dTTP (about 6 x 10^5 dpm), 2.5 µg yeast RNA and 50 or 100 µl of the enzyme fraction. Incubation was for 2 hours at 37°. The reaction was terminated with 300 mµmoles of calf thymus DNA as carrier and 0.5 ml of 1N perchloric acid, 0.01 M potassium pyrophosphate. The precipitate was washed by repeated centrifugation and incorporation is given in mµmoles/hr/100 µl enzyme. Values for the incorporation of radioactivity with no enzyme present (0.002 mµmoles) have been subtracted from all assays.

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stimulated lymphocytes from 9 normal volunteers we were unable to detect RNA-dependent DNA polymerase activity. Similar results were

reported independently by Gallo et al. (4). However, after purification by electrofocusing this activity was readily apparent (Fig. 1). RNA-dependent DNA polymerase activity was completely separated from DNA-dependent DNA polymerase activity. The DNA-dependent activity appears as a single peak with an isoelectric point of 4.7, while the RNA-dependent activity is heterogeneous and mainly localized at pH 5.3.

So far, without exception, RNA-dependent DNA polymerase activities have been detected by this method in phytohemagglutinin-stimulated lymphocytes from 6 normal donors. In some of these experiments (as seen in Fig. 2) the total activity was considerably less than that found in the experiment seen in Figure 1, and the heterogeneity of the enzyme was more apparent; the peak at pH 5.3 was smaller and the minor peaks more prominent. Figure 1 and Figure 2 represent the extreme examples of data obtained with cell populations from 6 normal donors. The multiplicity of enzyme peaks might have resulted from the isolation and fractionation procedures themselves, possibly through association of the enzyme with oligonucleotides.

The primer requirement for the RNA-dependent DNA polymerase from phytohemagglutinin-stimulated lymphocytes can be met by either yeast RNA or by poly rA·poly rU, which, in the presence of 0.5 mM Mn^{2+}, is about twice as effective as the yeast RNA. A similar preference for the homopolymer has been reported for the enzyme from acute lymphatic leukemia cells (4). As seen in Figure 1 and Figure 2, maximally activated calf thymus DNA (7) does not serve as a template for the RNA-dependent activity even though it is an excellent primer for all known DNA polymerases. The data presented here suggest that DNA is the product of the RNA-dependent activity since deoxynucleoside triphosphates labeled with either ^{3}H- or α-^{32}P are incorporated into an acid-precipitable form. However, an alternate explanation, such as terminal transfer of dTM^{32}P to RNA, has not yet been ruled out. Such

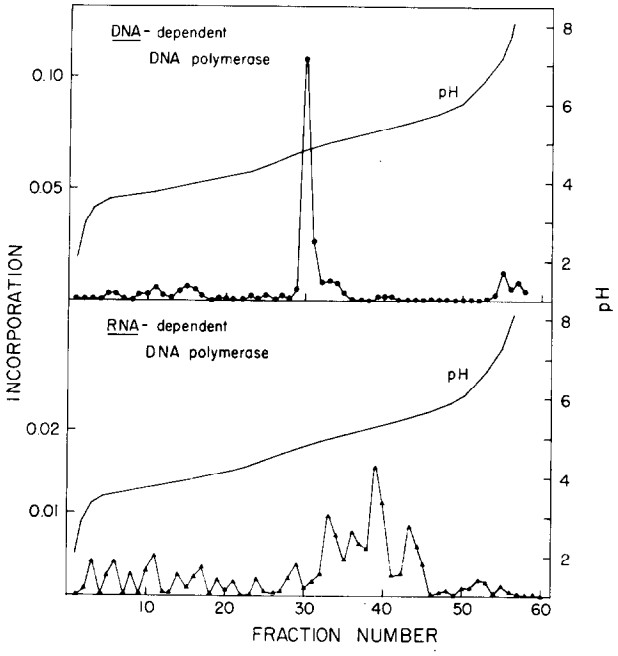

Fig. 2. Profile of DNA polymerases after electrofocusing extracts of stimulated lymphocytes from a different normal donor. Procedure and assays are as detailed in the legend to Fig. 1, except that the fraction size is 1.5 ml and numbers 21 to 38 are triplicate assays. Values for the incorporation of radioactivity with no enzyme present (0.007 mμmoles) have been subtracted from all assays.

characterization studies have been hampered by extreme lability of the enzyme after electrofocusing.

We have confirmed the results reported by Gallo et al. (4) in that RNA-dependent DNA polymerase is detectable in homogenates of lymphocytes from patients with acute lymphatic leukemia (unpublished results). We also find this activity to be present in lymphocytes from three patients with chronic lymphatic leukemia (unpublished results). After resolution by electrofocusing, multiple peaks of activity are present.

DISCUSSION

We report for the first time the presence of RNA-dependent DNA polymerase in non-malignant animal cells. Our ability to detect this activity in purified preparations from normal cells stimulated with

phytohemagglutinin in spite of its apparent absence in crude homogenates suggests the possible presence of inhibitors in the cell extracts which may have been removed by the purification procedure. Alternately, degradative enzymes such as DNAse found in the cell extract might preferentially destroy the product of the RNA-dependent enzyme, but not that of the DNA-dependent DNA polymerase. Also, the DNA product could be protected by the large amount of DNA primer added to the reaction mixture. So far we have only shown that RNA or poly rA·poly rU evokes the incorporation of deoxynucleotides into acid-insoluble material. Proof that the product is complementary to the RNA template is still required for both normal and leukemic lymphocytes.

The presence of RNA-dependent DNA polymerase in oncogenic tumor viruses, as well as in human leukemia cells, has suggested a close relationship between this activity and maliganancy (3). Our finding of an RNA-dependent DNA polymerase in cells from normal donors does not support this suggestion. Nevertheless, it does not exclude an oncogenic function for these activities. In fact, the presence of such viral genomes in normal cells is compatible with a provirus (8) or oncogene hypothesis (9).

Quite apart from its possible role in malignancy, a mechanism for the "reverse flow" of information from RNA to DNA is also attractive for normal functions such as DNA replication, gene amplification, and might explain the hitherto unknown function for heterogeneous nuclear RNA. Its present in lymphocytes might reflect the immunologic functions of these cells and be particularly relevant to theories requiring RNA transfer between cells for antibody production. Hence, the enzyme found in the phytohemagglutinin-stimulated lymphocytes used in this study might not be indicative of a latent virus, but actually represent a normal component of human cells. In fact, after electrofocusing,

the RNA-dependent activity was in some cases of the same order of magnitude as the DNA-dependent activity (see Fig. 1), perhaps indicating a major role for this enzyme in normal lymphocyte function.

Regardless of the role of RNA-dependent DNA polymerase, the activities present in both normal and neoplastic cells need to be further characterized if this enzyme is to serve as an indicator of malignancy or if chemotherapeutic agents based on the inhibition of this activity are to be developed.

Note Added in Proof: Since submission of this manuscript, a report by E. M. Scolnick, S. A. Aaronson, G. J. Todaro and W. P. Parks has appeared in Nature 229:318 (1971), also reporting the presence of an RNA-dependent DNA polymerase activity in non-malignant cells. These authors point out that their results could have resulted from anomalous replication by DNA-dependent DNA polymerase. In the present investigation the RNA-dependent and DNA-dependent activities were completely separated, showing that the former activity was the function of a distinctly separate enzyme in normal cells.

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