RNA DIRECTED DNA SYNTHESIS: IDENTIFICATION IN L5178Y MOUSE LEUKEMIC CELLS AND DISTRIBUTION OF THE POLYMERASE IN A SYNCHRONIZED L5178Y CELL POPULATION*

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1. Introduction

The recent identification of RNA dependent DNA polymerase in acute leukemic cells from humans [1] leads to the question of whether the polymerase is present in other mammalian leukemic cells, particularly those available in large quantities from tissue culture. The initial finding of an RNA dependent DNA polymerase in oncogenic RNA viruses [2–6] and the presence of the polymerase in human leukemic cells provide a basis for gene amplification and leukemogenesis. The present study reports on the characteristics of an RNA dependent DNA polymerase from L5178Y leukemic mouse cells. In addition the activity of the enzyme in a synchronous population was studied to determine whether the enzyme is active during the normal DNA synthesis (S) period or at other times in the cell cycle.

2. Materials and methods

Cells were cultured in Fischer's medium [7] plus 10% horse serum as previously described [8]. Cells were synchronized by the method of Doida and Okada [9] as described previously. Details of synchrony and cell parameters are given in previous publications [10–13].

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Table 1
Characteristics of L5178Y RNA dependent DNA polymerase.

System	cpm/assay
Complete	2492
0° (incubate)	361
0 time (immediate termination)	0
minus 'enzyme'	0
minus 'enzyme' plus boiled 'enzyme'	29
minus 'template'	0
minus 'template' plus RNase treated 'template'	116
minus 'template' plus DNase treated 'template'	2362
Product: DNase treated	311
Product: RNase treated	2396
Product: 0.5 N NaOH	2118
minus dATP, dCTP, dGTP	416
minus $MgCl_2$	304
minus 'template' plus yeast RNA	393
incubate 1 hr 37°	1119
minus TTP-^{3}H plus 5 μCi TTP-^{3}H	1546

The complete system contained in a final volume of 1 ml the following: 0.50 mg protein from the 'nucleic acid free' extract ('enzyme'); 1.0 μmole each of dATP, sCTP, and dGTP; 50 μmoles tris-HCl buffer pH 8.4; 5.0 μmoles $MgCl_2$; 10 μCi ^{3}H-methyl TTP (7 Ci/mmole, New England Nuclear); 20 μmoles dithiothreitol; 50 μmoles NaCl; and 36 μg of rat liver RNA ('template'), Assays were incubated at 37° for 2 hr, after which 1 mg of yeast RNA was added and the assay was made 10% in trichloroacetic acid. The resultant precipitates were washed 3 times with 10% trichloroacetic acid, dissolved in 1 N NaOH at 100°, plated on a glass fiber filter and counted in a liquid scintillation counter. Rat liver RNA ('template') was purchased from General Biochemicals and treated with DNase and the enzyme removed by phenol extraction. DNase and RNase were present at 4 mg per ml and were purchased from Worthington. All data are given with endogenous activity (about 10%) determined with water substituted for the 'template' subtracted. Yeast RNA was purchased from Worthington and was present at 36 mg per assay.

A 'nucleic acid free extract' was prepared essentially as described by Gallo et al. [1]. Exponential L5178Y cells or synchronized L5178Y cells taken at one half hour intervals from a synchronous population were vigorously homogenized for 30 strokes in a Potter Elvehjem homogenizer at 0°. The homogenizing solution was 5 volumes of 25 mM tris-HCl buffer pH 8.3, 1 mM $MgCl_2$, 6 mM NaCl, 5 mM dithiothreitol and 0.15 mM EDTA. The samples were centrifuged at 27,000 *g* and the pellet discarded. Nucleic acids were removed from the supernatant by successive precipitations with $MnCl_2$ and protamine sulfate. This crude preparation was utilized as the enzyme source in this report. Details of the enzyme assay are given in the legend to table 1. A crude 0.1% Triton X-100 extract of the L5178Y cells also exhibited RNA dependent DNA polymerase activity but the endogenous activity due to DNA template in the extract was prohibitively high.

All biochemicals not specifically mentioned were obtained in the purest available form from usual sources. Degree of synchrony was measured utilizing ^{3}H-thymidine and cell number counts determined with a Coulter Counter as previously described [10–13].

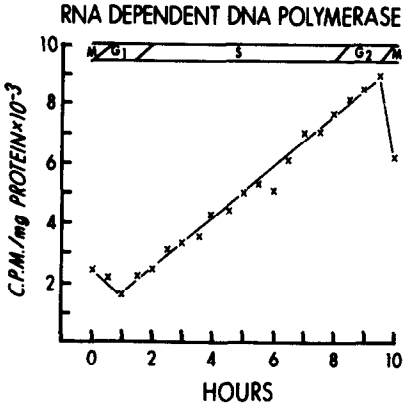

Fig. 1. RNA dependent DNA polymerase activity in a synchronized L5178Y cell population. At 0 hr the cells were released from the mitotic block. At each successive one-half hour the cells were assayed for RNA dependent DNA polymerase. At each one-half hour a 'nucleic acid free extract' was prepared as given in Materials and methods. The assay was exactly as given in the legend to table 1. Above the graph is a representation of the cell cycle. Each point is the mean of three observations.

3. Results and discussion

The data in table 1 indicate that L5178Y mouse leukemic cells possess an RNA dependent DNA polymerase. The enzyme is dependent on RNA as a template as evidenced by the low activity found with RNase treated template and the high activity found with DNase treated template. That the product is DNA, is evidenced by the lack of degradation by RNase, degradation by DNase, trichloroacetic acid insolubility, and alkali resistance (table 1). The reaction is dependent on Mg^{2+} and dATP, dCTP and dGTP. Unlike the human cell polymerase [1] the enzyme seems relatively specific for mammalian RNA since yeast RNA was relatively ineffective as a template.

The data in fig. 1 indicate that the RNA dependent DNA polymerase is a continuous enzyme in the synchronous L5178Y cell population as opposed to a step or peak enzyme [14]. DNA dependent DNA polymerase has been described as a peak enzyme in *Sacharomyces cerevisiae* [15], a continuous [16] or a peak enzyme [17] in mouse L cells, and a peak enzyme [18] in HeLa cells. The RNA dependent DNA polymerase is active throughout the cell cycle and not active only in the S period; indeed the highest activity occurs at 9.5 hr post mitosis in the early M period.

The results indicate that the RNA dependent DNA polymerase is active throughout the cell cycle and therefore would be present for information transfer from RNA viruses or for gene amplification. The presence of the polymerase in mouse leukemic cells does not indicate a direct relationship to viral leukemogenesis but is consistent with a viral etiology. The fact that the enzyme is present in mouse leukemic cells and is active throughout the cell cycle points to the fact that it may be coded for by the host cell DNA. The occurrence of the polymerase in tissue culture cells provides a convenient source for purification and study of the enzyme and its implications.

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