RNA DEPENDENT DNA POLYMERASE IN CELLS OF XERODERMA PIGMENTOSUM

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SUMMARY

Cells from X.P.* skin contain an RNA dependent DNA polymerase, while in cells from normal skin this enzyme is lacking. This finding stimulates the thought that carcinogenesis in X.P. cells is due to an infection with an oncogenic RNA virus.

INTRODUCTION

X.P., a genetically determined disease is of great interest because 1) of its basic molecular defect (survey: 1) and 2) the connection between this molecular defect and carcinogenesis. The molecular defect is rather well studied. The results obtained by means of autoradiography (2,3), by CsCl density gradient methods (2,4) and investigations of the enzymes, involved in informationally directed synthesis (5,6) show in X.P. cells a failure of an early enzymatic step in the DNA repair (deoxyribonuclease).

No experimental results are available about the relationship between X.P. and carcinogenesis. This report describes experiments indicating that the RNA dependent DNA polymerase which is present in oncogenic RNA viruses (7,8,9) exists in excised parts of X.P. skin and not in skin of normal individuals.

MATERIALS

Source of the materials: Micrococcal nuclease (Worthington Biochemical Corp., Freehold, USA); poly rG and poly dC (Biopolymers Lab., Dover, USA). The hybrid poly rG·dC was made according to Spiegelman et al. (10). Nonidet NP 40 (Deutsche Shell Chemie Ges., Hamburg, Germany).

*Abbreviation: X.P., Xeroderma pigmentosum

METHODS

X.P. skin was taken from apparently unaffected regions of the back with a dermatome. Control skin came under similar conditions from the mamma region of healthy individuals. For preparation of the crude extract, the skin was pulverized in a mortar together with dry ice, which ultimately was evaporated. For further treatment a modified method of Scolnick et al. (11) was applied. 0.5 g of disrupted skin were suspended in 0.5 ml saturated $(NH_4)_2SO_4$ containing 50 mM Tris-HCl, pH 7.8 and 0.1 mM EDTA and homogenized with 1 g glass beads (diameter : 0.45 - 0.55 mm) in a cell homogenizer (type MSK, B. Braun, Melsungen, Germany) for 2 min. This preparation was centrifuged at 15000 x g for 1 hour at 2°C. The pellet was resuspended in 0.5 ml buffer 1: 50 mM Tris-HCl, pH 7.8, 50 mM KCl and 1 mM dithiothreitol. A diluted solution of "Triton X-100" was added by drops to a final concentration of 0.3 % (v/v). This preparation was incubated at 37°C for 15 min and centrifuged at 15000 x g for 1 hour at 2°C. The supernatant was dialyzed for 12 hours at 2°C against 500 ml buffer 1. The dialyzed extract was then assayed for enzyme activity.

RMLV* was obtained from spleens of infected NMRI mice and purified as described before (12).

Protein determinations were performed according to Lowry et al. (13). The virus preparation contained 0.3 mg of protein per ml, the X.P. extract 0.13 mg/ml and the extract from normal skin 1.2 mg/ml.

In all experiments DNA and RNA, resident in the enzyme preparations (extracted from RMLV and skin) were destroyed by a preincubation with micrococcal nuclease 150 µg/ml in the presence of Ca^{2+} (2 mM) at 20°C for 30 min. The nuclease reaction was terminated by addition of ethyleneglycol-bis-(aminoethyl ether) tetraacetic acid (4 mM).

For observing full activity of the RNA dependent DNA polymerase the purified virus preparation was preincubated for 10 min at 0°C with 0.2 % "Nonidet NP-40" and 0.1 M dithiothreitol.

The DNA polymerase assay mixture contained the following components: 40 mM Tris-HCl (pH 7.8), 60 mM KCl, 2 mM $(CH_3COO)_2Mg$, 2 mM dithiothreitol, 0.1 mM each of dATP, dCTP, dTTP, ^{3}H-dGTP (5000 cpm/pmole; The Radiochemical Centre, Amersham), and 0.4 A_{260} of poly dC·rG or 0.4 A_{260} of native herring DNA (12)

*Abbreviation: RMLV, Rauscher murine leukaemia virus.

per 1 ml assay mixture. For determination of the enzyme activity 90 µl of the assay mixture werecombined with 10 µl enzyme preparation and incubated at 37°C. The acid-precipitable radioactivity was collected and counted as previously described (12).

RESULTS

In Table 1 the ability of DNA and of synthetic RNA-DNA hybrid poly rG·dC to serve as template for RMLV DNA polymerase is compared. It is obvious that poly rG·dC acts as a more effective template than native DNA. Therefore this synthetic template seems to be a good tool for detecting RNA dependent DNA polymerase activities in cells.

Table 1.
Template specificity of DNA polymerase of RMLV. The reaction mixture was incubated at 37°C for 30 min. Procedure and assay are as described under methods.

Template	Incorporation rate $\frac{10^{-10}\text{moles dGTP incorporated}}{30\text{min} \cdot \text{mg protein}}$
None	0.02
DNA	1.83
rG · dC	9.36

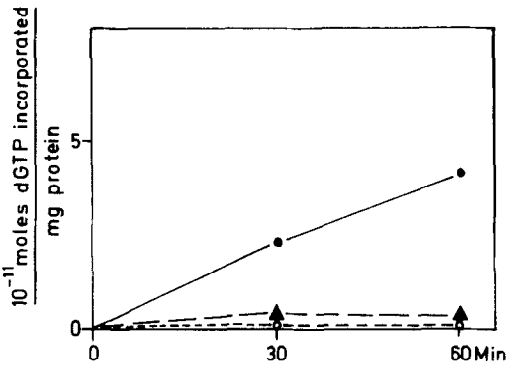

Fig. 1: Template specificity of DNA polymerase of normal human skin. Kinetics of incorporation of ^{3}H-dGTP into acid precipitable materials by the polymerase preparation extracted from normal human skin. The assay system was the same as described under methods. Enzyme activity was determined in the assays without template (o) with added native DNA (•) and with poly rG·dC (▲).

Fig. 1 shows the kinetics of DNA synthesis by a crude enzyme preparation, extracted from normal human skin with DNA- and poly rG·dC templates. In the polymerase assay double-stranded DNA is clearly the best template. Only very small activities are detected using poly rG·dC template.

Fig. 2 shows the response of the crude extract from X.P. skin to native DNA and poly rG·dC. The preference of the enzyme preparation for poly rG·dC is remarkable. However in spite of the striking preference for poly rG·dC, double-stranded DNA stimulates DNA synthesis in the cell extract from X.P. skin to the same extent as with extract of normal human skin.

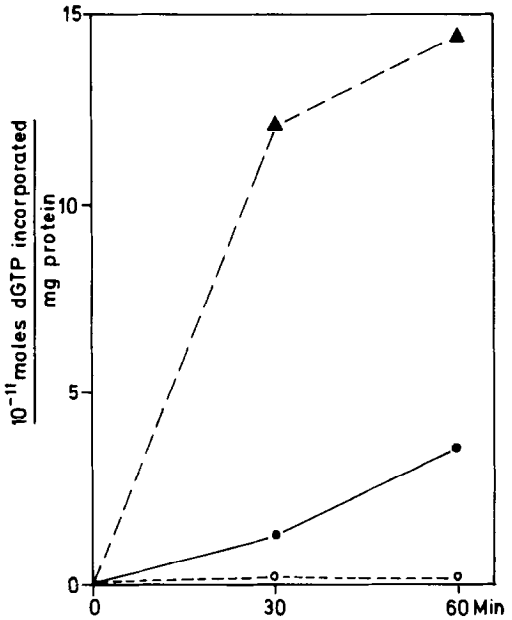

Fig. 2: Template specificity of DNA polymerase of X.P. skin. Kinetics of incorporation of ^{3}H-dGTP by the polymerase preparation from X.P. skin. The assay system was the same as described under methods. Enzyme activity was determined in the assays without template (o), with added native DNA (•) and with poly rG·dC (▲).

DISCUSSION

RNA dependent DNA polymerase of oncogenic RNA viruses can be detected by its capacity to copy synthetic RNA-DNA hybrids (10). Because of the fact, that certain synthetic RNA-DNA hybrids are better templates than DNA this enzyme has also been detected in lymphoblasts of leukaemic patients (14). Recently Scolnick et al. (11) and Penner et al. (15) have found RNA dependent DNA polymerases in uninfected normal human and murine cells, too. These discoveries indicate no clear re-

lationship of the viral enzyme to malignancy.

X.P. is a disease which is characterized by multiple basal and squamous cell carcinomas (4). The basic defect has been found in the genetically determined failure of the initial enzymatic step in DNA repair (1). UV-irradiated cells of X.P. patients do not excise pyrimidine dimers. The connection between the defective DNA repair and carcinogenesis in X.P. could not be proven experimentally (1). Our results presented here, demonstrate that cells of X.P. skin contain the RNA dependent DNA polymerase, while in normal cells this enzyme is lacking. By these findings we suggest that transformation of X.P. cells may be caused by oncogenic RNA viruses. It may be assumed that the ability of X.P. cells to be transformed is due to the defective DNA repair and the resulting physiological state of proneness to virus infection. Such an explanation of carcinogenesis in X.P. tissue would be contradictory to the somatic mutation theory (16).

On the other hand the activity of DNA dependent DNA polymerase extracted from cells of normal skin and from X.P. cells is nearly the same. This is in accordance with the observed fact that X.P. cells exhibit normal activity of cellular DNA replication (3).

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REFERENCES

1. Cleaver, J.E., J. Invest. Derm. 54: 181 (1970).
2. Cleaver, J.E., Nature 218: 652 (1968).
3. Jung, E.G. and Schnyder, U.W., Schweiz. med. Wschr. 100: 1718 (1970).
4. Setlow, R.B. , Regan, J.D., German, J. and Carrier, W.L., Proc. Natl. Acad. Sci., U.S. 64: 1035 (1969).
5. Müller, W.E.G., Zahn, R.K., Brehm, G. and Korting, G., Arch. Derm. Forsch. 240: 334 (1971).
6. Beck, J., Müller, W.E.G., Maidhof, A., Zahn, R.K., Brehm, G. and Korting, G., in preparation.
7. Baltimore, D., Nature 226: 1209 (1970).
8. Temin, H.M. and Mizutani, S., Nature 226: 1211 (1970).
9. Spiegelman, S., Burny, A., Das, M.R., Keydar, J., Schlom, J., Travnicek, M. and Watson, K., Nature 227: 563 (1970).
10. Spiegelman, S., Burny, A., Das, M.R., Keydar, J., Schlom, J., Travnicek, M. and Watson, K., Nature 228: 430 (1970).
11. Scolnick, E.M., Aaronson, S.A., Todaro, G.J. and Parks, W.P., Nature 229: 318 (1971).
12. Müller, W.E.G., Zahn, R.K. and Seidel, H.J., Nature , submitted for publication.

13. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.S., J. Biol. Chem. 193: 265 (1951).
14. Gallo, R.C. Yang, S.S. and Ting, R.C., Nature 228: 927 (1970).
15. Penner, P.E., Cohen, L.H. and Loeb, L.A., Biochem. Biophys. Res. Commun. 42: 1228 (1971).
16. Szilard, L., Proc. Natl. Acad. Sci., U.S. 45: 20 (1959).