NUCLEAR LOCALIZATION OF DNA POLYMERASE α IN XENOPUS LAEVIS OOCYTES

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SUMMARY: The nuclei of Xenopus laevis stage 6 oocytes were manually isolated and separated from the cytoplasms before being subjected to acqueous extraction. DNA polymerase α is found associated with the nuclei.

DNA polymerase α represents 80-90% of the total polymerase content in growing cells (1). It has been referred to as the "cytoplasmic" DNA polymerase, since it is recovered in large amounts in the subcellular cytoplasmic fraction. This localization is rather intriguing, since DNA replication occurs in the nucleus.

It is possible that the finding of DNA polymerase α in the cytoplasm does not reflect the "in vivo" situation and is due to the fact that nuclei prepared according to conventional techniques are extracted by external solution, prior to their separation from cytoplasms (2, 3, 4).

Xenopus laevis oocytes are a unique kind of cell with which to study the problem of the localization of DNA polymerase α. Oocytes, being very large can be manually enucleated and the isolated nuclei can be subjected to aqueous extraction only after separation from the cytoplasms.

The DNA polymerase α has been defined as a large molecular weight enzyme (MW higher than 100,000); it is strongly inhibited by N-ethylmaleimide, copies activated DNA at a high rate, and cannot utilize ribopolymer templates such as poly(A)-oligo(dT) (1).

MATERIALS AND METHODS: Deozynucleoside-5-triphosphates were obtained from Schwartz Mann, Orangeburg, N. Y. [^{3}H]methyl-deoxythimidine-5-triphosphate

(15 Ci/mmol) was purchased from Amersham. Calf thymus DNA (type V, from SIGMA) was "activated" according to Loeb (5).

Stage 6 oocytes were isolated by collagenase treatment (6) from ovaries of adult female frogs not treated with hormones. After extensive rinsing in Barth's solution (7), each oocyte was punctured under a dissecting microscope with watchmaker forceps, and each nucleus gently squeezed out. The isolated nuclei were transferred with a capillary pipette into a minimum volume of Barth's solution. About one hundred nuclei were collected in a 0.6 ml nitrocellulose centrifuge tube, which was filled with TEG buffer (Tris-HCl at pH 7.5 50 mM, ethylendiaminotetraacetate 1 mM, glycerol 20% w/v). The nuclei were broken by pipetting and centrifuged 60 min at 40,000 rpm in a SW 50.1 rotor at 4°C with adaptors. The high speed supernatant was collected; it will be referred to as nuclear extract. The corresponding enucleated cytoplasms were homogenized in 1.5 ml of TEG; the extract was clarified at low speed (8,000 g, 10 min) and the resulting supernatant was centrifuged in two 0.6 ml nitrocellulose tubes as was the nuclear extract.

RESULTS AND DISCUSSION

An extract from one hundred nuclei assayed with activated DNA as previously described (8) incorporated, in a typical experiment, 480 pmoles of dTMP. An extract from the corresponding cytoplasms assayed under the same conditions incorporated 58 pmoles of dTMP. Therefore the nuclear extract contains approximately 90% of the total soluble DNA polymerase activity of the oocytes.

We have previously shown that a particulated DNA polymerase which uses poly(A)-oligo(dT) as template requires detergent treatment in order to be solubilized, and therefore is not detected when extracts are prepared as described above (8).

Figure 1 shows an inhibition curve of the polymerase activity of the nuclear extract in the presence of increasing amounts of N-ethylmaleimide. At 0.6 mM N-ethylmaleimide the polymerase activity was reduced about 90%. This

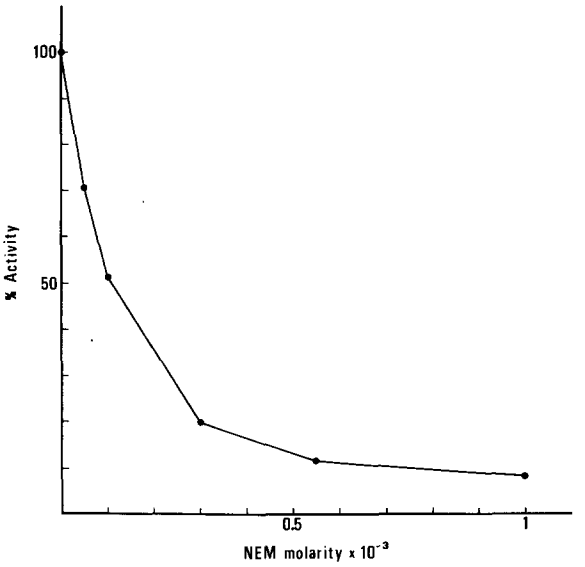

Fig. 1 - Inhibition curve of the DNA polymerase activity of nuclear extract by N-ethylmaleimide. Each assay contained 10 µl of nuclear extract mixed with 50 µl of a mixture containing 0.2 mg/ml of activated calf thymus DNA, 8 mM Mg acetate, 0.1 mM each of dATP, dGTP, dCTP, 1 µ M [^{3}H]TTP, 0.02 M potassium phosphate pH 7.5 and the indicated increasing concentrations of N-ethylmaleimide. The protein concentrations of the nuclear extract was 250 µg/ml. Values are expressed as percent of activity in the absence of N-ethylmaleimide.

result suggests that the major polymerase activity contained in this extract is due to the polymerase α.

The high speed nuclear supernatant was applied to a 10-34% glycerol linear gradient and centrifuged in a SW 41 rotor for 15 hours at 38,000 rpm at 4°C (Fig. 2). Fractions were assayed with activated DNA, in the presence of either 1 mM 2-mercaptoethanol or of 1 mM N-ethylmaleimide at 37°C for 30 min (after appropriate dilution, since polymerase α is very sensitive to salt). The major activity sediments faster than the 4S marker, is completely inhibited by N-ethylmaleimide and does not use poly(A)-oligo(dT). This activity represents

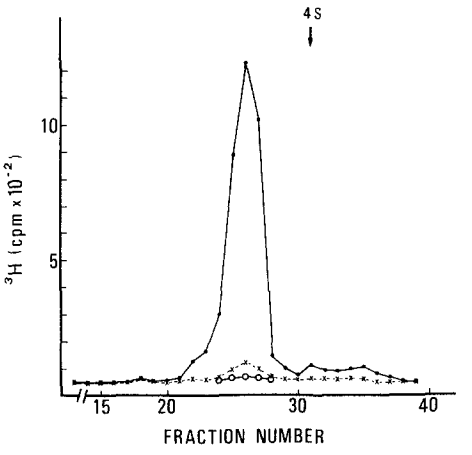

Fig. 2 - Analysis on glycerol gradient of DNA polymerase activity of the nuclear extract. 300 µl of nuclear extract were layered on a linear 10-34% w/v glycerol gradient (300 mM KCl, 50 mM Tris-HCl pH 7.5) and centrifuged in a SW 41 rotor at 38,000 rpm for 15 hours at 4°C. 10 µl of each fraction were assayed with 50 µl of a mixture containing 0.2 mg/ml of activated calf thymus DNA, 8 mM µg acetate, phosphate buffer pH 7.5, 0.1 mM each of ATP, dGTP, dCTP, 1 µ M [^{3}H]TTP and 3 µλ of stock aqueous solution of either 2-mercaptoethanol to give a final 1 mM concentration (—●—) or of N-ethylmaleimide to give a final 1 mM concentration. (-X-) The peak fractions were also assayed with poly A, oligo dT as template (8) (—o—). Assays were carried out at 37°C for 30 min. 4S RNA marker was run in a separate gradient.

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therefore the DNA polymerase α of Xenopus laevis oocytes.

When the gradient was assayed using poly(dA-dT) as template (data not shown) a peak of activity sedimenting more slowly than the 4S marker was detected. The amount of such activity varies considerably from preparation to preparation and is probably due to DNA polymerase β, which requires different procedures in order to be efficiently solubilized (e.g. high salt, sonication, detergent).

We would like to stress that the different procedures result in different

quantitative and qualitative yields of enzymes (9, 10). For example, we reported previously that using a different extraction procedure, an analysis in glycerol gradient of a nuclear extract shows mainly DNA polymerase β (8). It must be added that in that experiment we assayed for DNA polymerase activity in high salt. It is known that DNA polymerase α is much more sensitive to salt than DNA polymerase β (1).

These and previous data indicate that Xenopus laevis oocytes contain three types of major DNA polymerases. Two of them, polymerase α and polymerase β, are localized in the germinal vesicle. DNA polymerase α is efficiently released by the mild extraction procedure which we used, while DNA polymerase β and γ require, as they do in mammals, more drastic procedures in order to be solubilized (1).

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