THE PRESENCE OF A COMMON ACTIVE SUBUNIT IN LOW AND HIGH MOLECULAR WEIGHT MURINE DNA POLYMERASES.

Norman B. Hecht and Donna Davidson

Department of Biology
Tufts University
Medford, Massachusetts 02155

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SUMMARY:

An enzymatically active subunit was isolated from the high molecular weight (6-8S) DNA polymerase. This activity had an identical sedimentation coefficient and the same elution profile from Sephadex G-100 as the low molecular weight (3.3S) DNA polymerase.

INTRODUCTION:

Distinguishable DNA polymerases have been isolated from the nuclear, cytoplasmic, and mitochondrial fractions of eucaryotic cells (1,2,3,4,5,6). In general, the low molecular weight (3.3S-3.5S) enzyme is the predominant nuclear activity while the majority of the high molecular weight (6-8S) DNA polymerase is isolated from the cytoplasm. Evidence suggesting a common peptide in the two DNA polymerases has recently been presented by Chang and Bollum (7). They have found one antibody population that reacts with both the low and high molecular weight DNA polymerases isolated from several different eucaryotes. In this communication we present evidence demonstrating the existence of an active subunit derived from the high molecular weight DNA polymerase. This activity appears identical to the low molecular weight nuclear activity when analyzed by sucrose gradient centrifugation and gel filtration. Details of the characterization and properties

of the nuclear and cytoplasmic DNA polymerases isolated from mouse testis have been presented elsewhere (5,6,8).

MATERIALS AND METHODS:

Nuclei and soluble cytoplasm were prepared from 8-12 week old B6AF1/J mouse testes by the 2.2M sucrose method described by Muramatsu (9). The two subcellular fractions were sonicated in extraction buffer (0.02M Tris pH 8.1, 0.05M NaCl, 0.001 M DTT, 0.001M EDTA) at a setting of 4 with a Branson sonicator (Model W-140 C) for 6 ten second bursts interspersed with ten second cooling intervals. $MgCl_2$ and DNase were added to final concentrations of 0.01M and 40 ug/ml; the preparations were incubated 30 minutes at 25°C and then centrifuged for 1 hour at 100,000 Xg. The supernatants were removed and dialyzed overnight against extraction buffer.

RESULTS AND DISCUSSION:

When aliquots of the nuclear and cytoplasmic protein extracts were analyzed for DNA polymerase activity, sedimentation coefficients of 3.5S and 6-8S, respectively, were obtained as previously reported (6). Figure 1 shows the results obtained when a final concentration of 0.125M $(NH_4)_2SO_4$ was added at 4°C to the nuclear and cytoplasmic extracts. No changes are seen in the sedimentation coefficient of the nuclear activity. In contrast, most of the cytoplasmic activity was converted to a form sedimenting at 3.5S. Although the majority of the 6-8S activity shifted its position in the gradient, a small but reproducible fraction was not converted. This is consistent with the existence of more than one DNA polymerase in the cytoplasmic fraction.

The results of the gel permeation profiles of cytoplasmic

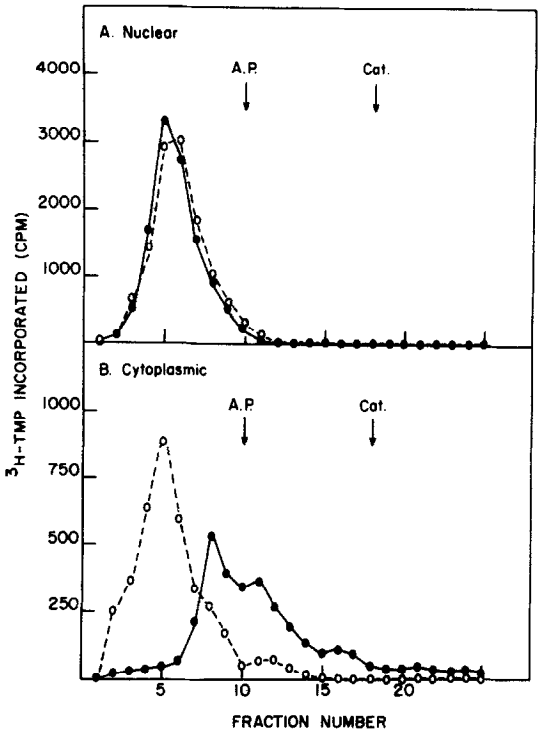


FIGURE 1: Sucrose density gradient centrifugation of nuclear and cytoplasmic derived enzyme extracts. Enzyme extracts were prepared as previously described (6). 0.2 ml aliquots were layered on 5-20% sucrose gradients (sucrose dissolved in extraction buffer) and centrifuged 13 hours at 40,000 rpm in a SW 50.1 rotor in a Beckman L2-65 centrifuge. Sedimentation was to the right. For the salt treated samples, aliquots (0.01 ml) of a freshly prepared ammonium sulfate solution (2.5M) were added at 4°C to 0.2 ml of enzyme extract. Control gradients were run as balance tubes for the .125M $(NH_4)_2SO_4$ treated samples and the two gradients are plotted on one coordinate. 0.1 ml aliquots were assayed for DNA polymerase as previously described (8). Sedimentation coefficients were determined by the method of Martin and Ames using E. Coli alkaline phosphatase and catalase as markers. The arrow marked A.P. indicates the position of the alkaline phosphatase ($S_{20,w}$=6.3) and the arrow marked Cat. indicates the position of catalase ($S_{20,w}$=11.3).

Closed circles (●———●) represent the control.
Open circles (o-----o) represent the salt treated extracts.

(6-8S), nuclear (3.5S), and 0.125M $(NH_4)_2SO_4$ treated cytoplasmic activities are seen in Figure 2. The elution pattern of the cytoplasmic and nuclear activities (Fig. 2A and Fig. 2B) is the same as the profiles of similar activities isolated from rabbit bone

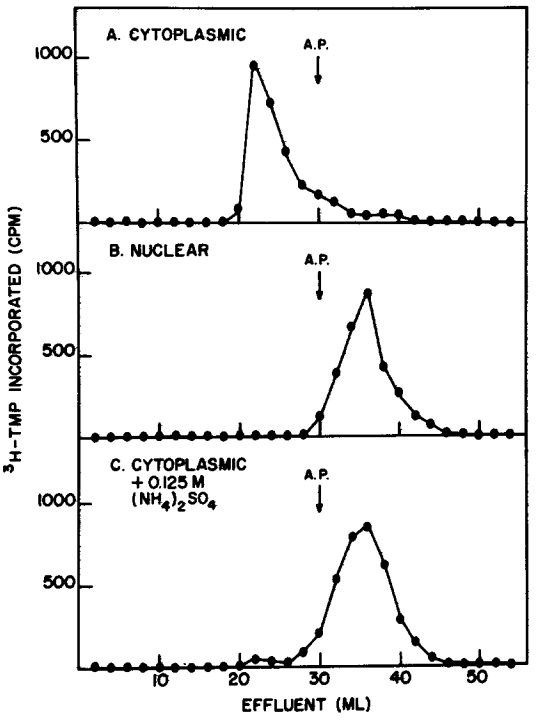
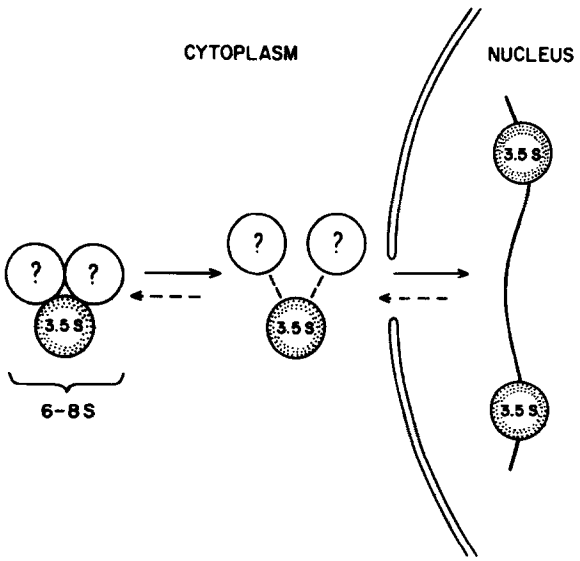


FIGURE 2: Gel filtration of nuclear and cytoplasmic derived enzyme extracts. Nuclear (1mg) and cytoplasmic (8 mg) extracts were applied to Sephadex G-100 (1cm x 40cm) equilibrated with extraction buffer (Fig. 2A and 2B) or extraction buffer containing .125 M $(NH_4)_2SO_4$ (Fig. 2C). V_o is at 20.5 ml. 2 ml. fractions were collected and 0.1 ml aliquots were assayed for DNA polymerase. The columns were calibrated with blue dextran, E. coli alkaline phosphatase, bovine serum albumin, ovalbumin, and horse heart cytochrome *c*.

marrow extacts (4). Figure 2C shows the elution pattern of a cytoplasmic extract that has been treated with 0.125M $(NH_4)_2SO_4$. Most of the activity eluted at the same position as the nuclear 3.5S activity. A small but reproducible fraction of the 6-8S activity was not converted and eluted at the same position as untreated 6-8S. This further supports the idea that more than one form of cytoplasmic DNA polymerase exists. Nuclear enzyme preparations showed no change in molecular weight when analyzed on Sephadex G-100 in extraction buffer or in extraction buffer

containing 0.125M $(NH_4)_2SO_4$. A cytoplasmic preparation that had been made 0.125M $(NH_4)_2SO_4$ and analyzed on a column that was equilibrated only with extraction buffer (no $(NH_4)_2SO_4$) eluted in an identical fashion to an untreated cytoplasmic preparation suggesting a reversible association. A similar reversible conversion occurs when a salt treated 6-8S fraction is dialyzed against extraction buffer (6).

Molecular weights of the activities have been determined by reference to protein standards. The 6-8S activity eluting near the exclusion volume has a molecular weight of approximately 100,000 daltons. The nuclear and converted cytoplasmic activities have an estimated molecular weight of 60,000 daltons by this technique. These estimates are in agreement with those for rat liver nuclear DNA polymerase reported by Haines et al (10). They postulate that since the estimation of molecular weight by Sephadex G-100 approaches twice the molecular weight estimation obtained by dodecyl sulphate-polyacrylamide gel electrophoresis, the enzyme isolated on Sephadex may exist as a dimer. Chang and



Bollum have reported a molecular weight of 40-50,000 for a similar activity from rabbit bone marrow (11). Although this communication deals with enzymes found in mouse testis, we feel the observations are of general interest since we have obtained similar results with mouse brain and liver. We suggest that the 3.5S subunit serves as a replicative deoxynucleotidyl transferase since it is found bound to DNA in nuclei and can catalyze a single round of complementary synthesis (11). In the cytoplasm the 3.5S moiety is found associated with other molecules producing the characteristic 6-8S cytoplasmic activity. A model concerning the conversion is seen in Figure 3. We postulate that the 3.5S moiety could be shuttled back and forth between the nucleus and cytoplasm depending upon the physiological state of the cell. Peptides of unknown properties can associate with the 3.5S monomer to produce a 6-8S fraction (N.B.H. unpublished observations). Since the latter has a molecular weight of approximately 100,000 we postulate two subunits may attach to the 3.5S molecule. The possibility that one form of the 6-8S activity consists of an aggregate of 3.5S monomers is not excluded. The physiological function of this reversible dissociation remains to be elucidated.

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