Immunochemical Detection of a Primase Activity Related Subunit of DNA Polymerase α from Human and Mouse Cells Using the Monoclonal Antibody†

Tatsuo Yagura,*,‡ Tomoko Kozu,‡ Takeshi Seno,‡ and Shigeaki Tanaka§

Department of Immunology and Virology, Saitama Cancer Center Research Institute, Inamachi, Kitaadachi-gun, Saitama-ken 362, Japan, and Basic Research Department, Pharma R & D Division, Hoechst, Japan Ltd., Kawagoe-shi, Saitama-ken 350, Japan

Received May 15, 1987; Revised Manuscript Received July 13, 1987

ABSTRACT: A hybrid cell line (HDR-854-E4) secreting monoclonal antibody (E4 antibody) against a subunit of human DNA polymerase α was established by immunizing mice with DNA replicase complex (DNA polymerase α–primase complex) prepared from HeLa cells. The E4 antibody immunoprecipitates DNA replicase complex from both human and mouse cells. The E4 antibody neutralizes the primase activity as assessed either by the direct primase assay (incorporation of [α-^{32}P]AMP) or by assay of DNA polymerase activity coupled with the primase activity using unprimed poly(dT) as a template. The E4 antibody does not neutralize DNA polymerase α activity with the activated calf thymus DNA as a template. Western immunoblotting analysis shows that the E4 antibody binds to a polypeptide of 77 kilodaltons (kDa) which is tightly associated with DNA polymerase α. The 77-kDa polypeptide was distinguished from the catalytic subunit (160 and 180 kDa) for DNA synthesis which was detected by another monoclonal antibody, HDR-863-A5. Furthermore, it is unlikely that the 77-kDa peptide is the primase, since we found that the E4 antibody also immunoprecipitates the mouse 7.3S DNA polymerase α which has no primase activity, and Western immunoblotting analysis shows that the 77-kDa polypeptide is a subunit of the 7.3S DNA polymerase α. Furthermore, after dissociation of the primase from mouse DNA replicase by chromatography on a hydroxyapatite column in the presence of dimethyl sulfoxide and ethylene glycol, the 77-kDa polypeptide is associated with DNA polymerase α, and not with the primase. These results indicate that the 77-kDa polypeptide detected with the E4 antibody is not the primase but is a subunit firmly bound to DNA polymerase α catalytic polypeptide and yet influences the activity of the associated DNA primase.

The structure of DNA polymerase α, the major replicating protein in eukaryotic cells, has been difficult to define. The structure and subunit composition of DNA polymerase α have been impeded by the relatively low amount present in tissues, the instability under conditions of fractionation and enzyme purification, and, most importantly, the exceptional sensitivity of the enzyme to proteolytic cleavage.

To compound the difficulty further, many of the proteolytically degraded fragments were found to be catalytically active. With the advent of monoclonal antibodies directed against the purified enzyme and rapid purification procedures, a uniformity in the structure of the polypeptides that constitute the DNA polymerase α core complex is beginning to emerge (Sauer & Lehman, 1982; Yagura et al., 1983a; Wang et al., 1984; Holmes et al., 1986; Loeb et al., 1986). The catalytic activity is associated with the 180- and 200–220-kilodalton (kDa)[1] polypeptides (Loeb et al., 1986). A polypeptide of 68–85 kDa, of unknown function, is also present in stoichiometric amounts (Sauer & Lehman, 1982; Wang et al., 1984; Holmes et al., 1986). DNA primase activity is found to be associated with either, or both, 55–60- and/or 48–49-kDa subunit(s) of the core complex (Tseng & Ahlem, 1982; Yagura et al., 1986; Holmes et al., 1986).

We have prepared a series of monoclonal antibodies directed against partially purified HeLa cell DNA replicase complex.

In the present work, we provide evidence that one of these antibodies binds tightly to the DNA replicase complex from both human and mouse cells and is able to inhibit DNA primase activity. Nevertheless, this antibody recognizes the 77-kDa protein subunit and not the DNA primase. The results suggest that binding of the antibody to the 77-kDa subunit interferes with the action of primase subunit.

Experimental Procedures

Reagents. The sources from which most of the biochemical reagents were obtained are the same as indicated earlier (Yagura et al., 1982b). Peroxidase-conjugated rabbit immunoglobulins to mouse immunoglobulins were from DAKO Co. (Denmark); murine hybridoma cells (SJK 287-38) that secrete the monoclonal antibody (Tanaka et al., 1982) were from Dainihon Seiyaku Co. (Osaka, Japan); the Affi-Gel protein A MAPS kit was from Bio-Rad.

DNA Polymerase α Preparations from HeLa Cells and Mouse Ehrlich Cells. All DNA polymerase α preparations used were partially purified as described previously (Yagura et al., 1982a,b), and preparations at the step of DNA–cellulose column chromatography were used for the production of monoclonal antibodies and the experiments described in this study.

Preparation of Hybridomas. Two female (BALB/c × C57BL/6) F_1 mice were immunized by four weekly intraperitoneal injections with DNA–cellulose fractions of HeLa DNA replicase complex (DNA polymerase α–primase complex) (Yagura et al., 1982b) (80 units per injection) precipitated in alumina gels with killed *Bordatella pertussis* (10-mg

† This work was supported in part by Grants-in-Aid for Cancer Research from the Ministry of Education, Science and Culture of Japan.

‡ Saitama Cancer Center Research Institute.

§ Hoechst, Japan Ltd.

[1] Abbreviations: EDTA, disodium ethylenediaminetetraacetic acid; kDa, kilodalton(s); Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; SDS, sodium dodecyl sulfate.

0006-2960/87/0426-7749$01.50/0

alumina gels and 2×10^9 killed organisms per mouse). The mice received a booster intraperitoneal injection with the DNA replicase preparations in phosphate-buffered saline and were sacrificed 3 days later. Spleen cells of the mouse were fused with P3X63Ag8-653 mouse myeloma cells as described by Tanaka et al. (1982), with minor modifications.

Purification of Monoclonal Antibodies. Monoclonal IgG molecules were purified from either hybridoma culture supernatants or ascites fluids from mice inoculated with hybridoma cells by chromatography on columns of Affi-Gel protein A according to the manufacturer's direction (Bio-Rad). After concentration against 50% saturated ammonium sulfate (0 °C), IgG molecules were thoroughly dialyzed against phosphate-buffered saline and stored at −80 °C until use. The IgG subclasses of the monoclonal antibodies used were IgG_{2b} (HDR-854-E4) and IgG_1 (HDR-863-A5).

Binding and Neutralization Assays with Monoclonal Antibodies. Binding and neutralization assays were performed as described previously (Yagura et al., 1983a; Tanaka et al., 1982) with slight modifications. DNA replicase complex or 7.3S DNA polymerase α was incubated with the antibody for 1 h in an ice bath. For neutralization assays, the enzyme was incubated for 1 h and then directly tested for enzyme activity. For immunoadsorption assays, sheep anti-mouse IgG antibody-bound *Staphylococcus aureus* suspension was added to the mixture after the initial incubation, and the mixture was incubated for an additional 1 h at 0 °C. The supernatant fraction obtained by centrifugation of the mixture was tested for enzyme activity.

Assays for DNA Polymerase α and Primase Activities. The procedures for assays were as described previously (Yagura et al., 1982a,b).

The reaction mixture for the activity of primase-dependent DNA synthesis (total volume 50 μL each) contained 25 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) (pH 7.6)/1 mM dithiothreitol/2 mM $MgCl_2$/40 mM KCl/0.125 mM each of dTTP, dCTP, and dGTP/0.01 mM [^{3}H]dATP (0.4–1.6 Ci/mmol)/2 mM ATP/1 μg of poly(dT)/5 μg of bovine serum albumin/0.2 μg of mouse stimulating factor. The activity of primase in DNA replicase complex was assayed in the same reaction mixture except that the concentration of ATP was lowered to 100 μM and [^{32}P]ATP (4 μCi/50-μL assay) was added. The activity of the primase free from DNA polymerase α was assayed in the reaction mixture supplemented with the purified 7.3S mouse DNA polymerase α (a subspecies which has no primase activity) (Yagura et al., 1983b, 1986).

Characterization of Monoclonal Antibody Defined Antigens by Western Immunoblots. Enzyme preparations were solubilized in sodium lauroylsarcosine (1.5%) and 10 mM dithiothreitol in a boiling water bath for 5 min and resolved by sodium dodecyl sulfate–polyacrylamide gel (8%) electrophoresis as described by Laemmli (1970) and then transferred to nitrocellulose paper. Antigenic components reactive with monoclonal antibodies were detected by the methods described by Yagura et al. (1986) and Senshu et al. (1985). The sheets were treated with 5% (w/v) nonfat dry milk in 10 mM Tris-HCl (pH 7.6)/0.15 M NaCl/1 mM EDTA/0.1% Triton X100 for 1 h at room temperature and then with the monoclonal antibody (1–2 μg/mL) in 1% nonfat dry milk/10 mM Tris-HCl (pH 7.6)/0.15 M NaCl/3% bovine serum albumin/10% calf serum, either at 4 °C overnight or for 5 h at room temperature. The sheets were next treated with horseradish peroxidase conjugated rabbit anti-mouse IgG, which was diluted 3000-fold, for 40 min at room temperature and then treated with 3,3′-diaminobenzidine tetrahydrochloride and hydrogen peroxide. Protein markers used for calculation of molecular mass were phosphorylase *b* (94 kDa), albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa) (Pharmacia Fine Chemicals), and *Escherichia coli* RNA polymerase (β, 165 kDa; β′, 155 kDa; α, 39 kDa) (Boehringer Mannheim Biochemicals).

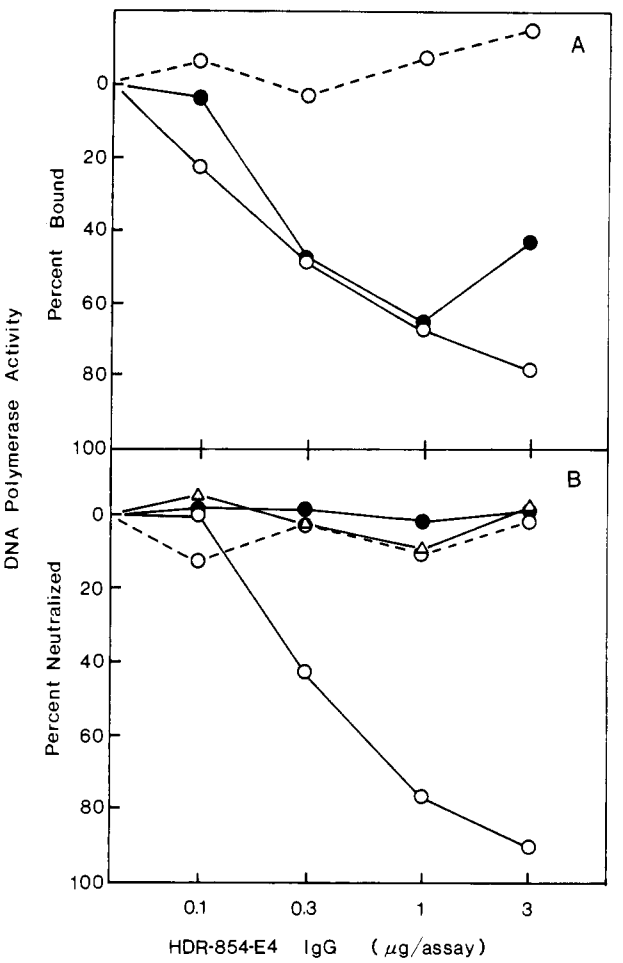

FIGURE 1: Effect of monoclonal antibody HDR-854-E4 on HeLa DNA replicase complex activity with different templates. Immunoadsorption (A) and neutralization (B) assays were performed as described under Experimental Procedures. The DNA polymerase α activity with the activated calf thymus DNA or poly(dT)·oligo(rA) as tempate was detected by measuring the incorporation of [^{3}H]dATP in the presence of the other three nonradioactive dNTP substrates. The activity on unprimed poly(dT) was detected with [^{3}H]dATP in the presence of 2 mM ATP as a substrate for primase activity. This activity was totally dependent on the presence of ATP (Yagura et al., 1982b). As a control of nonspecific immunoreactivity, we measured the effect of mouse antibody secreted by unhybridized myeloma cells (P3-X63-Ag8) using unprimed poly(dT) (O---O). (A) Immunoadsorption of DNA replicase complex by the E4 antibody. The templates used and the values for 100% activity were as follows: (O—O) 8 pmol with poly(dT); (●—●) 17 pmol with the activated DNA. (B) Neutralization of DNA replicase complex activity by the E4 antibody. The template used and values for 100% activity were as follows: (O—O) 76 pmol with poly(dT); (●—●) 60 pmol with the activated DNA; (Δ—Δ) 220 pmol with poly(dT)·oligo(rA).

Centrifugation in Glycerol Gradient. The fraction of DNA replicase was layered onto a 5-mL linear gradient of 10–30% glycerol in 50 mM Tris-HCl (pH 7.6)/0.5 mM ethylenediaminetetraacetic acid (EDTA)/1 mM dithiothreitol/0.3 M KCl and centrifuged in a Hitachi RPS 65T rotor for 3 h at 60 000 rpm at 0 °C. In a case where the linear gradient was prepared with the buffer containing 20% dimethyl sulfoxide and 10% ethylene glycol, the centrifugation was for 16 h at 60 000 rpm at 0 °C.

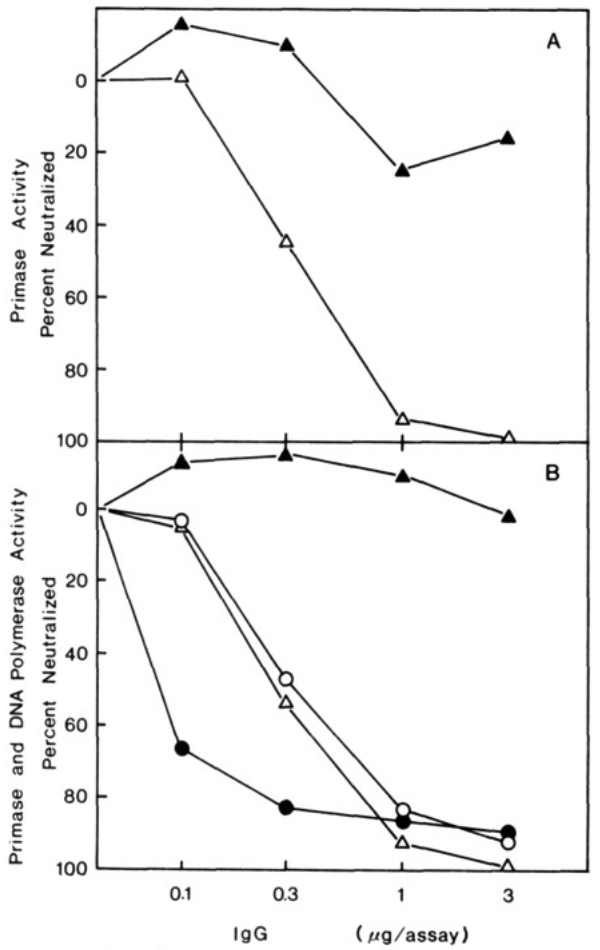

FIGURE 2: Effect of HDR-854-E4 antibody on DNA primase activity. Neutralization of the primase activity of HeLa DNA replicase complex using poly(dT) was determined by adding graded amounts of the E4 antibody, either in the absence (primase reaction) (A) or in the presence (coupled reaction of DNA and initiator RNA syntheses) (B) of dATP as described under Experimental Procedures. The SJK 287-38 monoclonal antibody that specifically neutralizes DNA polymerase α of KB cells was used as a control [(▲) and (●) in figures]. The values for 100% primase activity (AMP incorporation) and DNA polymerase activities (dAMP incorporation) were as follows: (A) 3.8 pmol of dAMP in assay of the effect of the E4 antibody (△) and SJK 287-38 antibody (▲). (B) 2.4 pmol of AMP for the E4 antibody (△) and SJK 287-38 antibody (▲) and 9.8 pmol of dAMP for the E4 antibody (○) and SJK 287-38 antibody (●).

RESULTS

Specificity of Monoclonal Antibody HDR-854-E4. Hybridomas prepared against partially purified HeLa DNA replicase complex (DNA polymerase α–primase complex) (Yagura et al., 1982b; Kozu et al., 1982) were screened for the production of active antibodies by both immunoadsorption and neutralization assays. In order to measure both DNA polymerase α and DNA primase activities in the same sample, unprimed poly(dT) was used as a template in the presence of ATP and dATP which was radioactively labeled. The incorporation of dAMP into acid-insoluble material is dependent on both DNA primase and DNA polymerase activity. As shown in Figure 1, one hybridoma, HDR-854-E4, secreted monoclonal antibody (E4 antibody) which exhibited both immunoprecipitating (Figure 1A) and neutralizing (Figure 1B) activity. The E4 antibody does not react directly with the catalytic site of DNA polymerase α activity when assayed as a separate entity using either activated DNA or poly(dT)·oligo(rA) as tempate-primers (Figure 1B). In another experiment, it was shown that the E4 antibody does not alter the size of either initiator RNA or DNA synthesized on single-stranded fd phage DNA (results not shown).

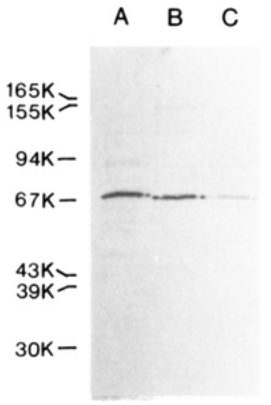

FIGURE 3: Western immunoblotting analysis of peptide recognized by HDR-854-E4 antibody. Lanes A and B contain DNA replicase complexes from HeLa cells and mouse Ehrich cells, respectively. Lane C contains mouse 7.3S DNA polymerase α. These enzymes were partially purified by successive chromatography on DEAE-cellulose, phosphocellulose, hydroxyapatite, and DNA–cellulose columns (Yagura et al., 1982a,b). Western immunoblotting analysis using the E4 antibody is as described under Experimental Procedures. The amount of the enzymes applied onto the sodium dodecyl sulfate–polyacrylamide gel is as follows: HeLa DNA replicase, 71.2 units; mouse DNA replicase, 68 units; mouse 7.3S DNA polymerase α, 21.4 units.

Another hybridoma we obtained (HDR-863-A5) secreted monoclonal antibody (A5 antibody) which exhibited only immunoprecipitating activity (results not shown). As will be shown in a later section, the A5 antibody binds to 180- and 160-kDa polypeptides which corresponded in size to the catalytic polypeptide of DNA polymerase α from KB cells reported by Wang et al. (1984).

The experiments in Figure 2 demonstrate that the E4 antibody specifically decreases DNA primase activity. In Figure 2A, primase activity was determined by measuring the incorporation of AMP into acid-insoluble materials. In this experiment, the direct effect of the E4 antibody on initiator RNA synthesis could be examined because the substrate for DNA synthesis, dATP, was omitted from the reaction mixture. In contrast, antibody prepared specifically against DNA polymerase α, SJK 287-38 (Tanaka et al., 1982), caused only a limited inhibition of DNA primase activity. In the coupled assay in which both dATP and ATP were added and the incorporation of dAMP and AMP into acid-insoluble materials was determined, the E4 antibody inhibited both DNA primase and DNA polymerase activities (Figure 2B). Thus, the E4 antibody reacts either directly or indirectly with the primase subunit but has little effect on the catalytic subunit for DNA polymerase α.

Detection of the Polypeptide Recognized by the E4 Antibody by Western Immunoblotting Procedure. The results so far presented suggest that the E4 antibody affects the DNA primase activity, which is known to be a component of the DNA replicase complex.

In order to analyze the specificity of interaction of the E4 antibody with the different components present in the DNA replicase complex, we carried out Western blots. The partially purified fraction of HeLa DNA replicase complex was subjected to SDS–polyacrylamide gel electrophoresis, electrotransferred to nitrocellulose paper, and incubated with the E4 antibody. The E4 antibody singularly detected a polypeptide of 77 kDa (Figure 3, lane A). To further ascertain that the 77-kDa polypeptide is a component of the HeLa DNA replicase complex and not an artifact resulting from nonspecific binding of the antibody, the partially purified DNA replicase

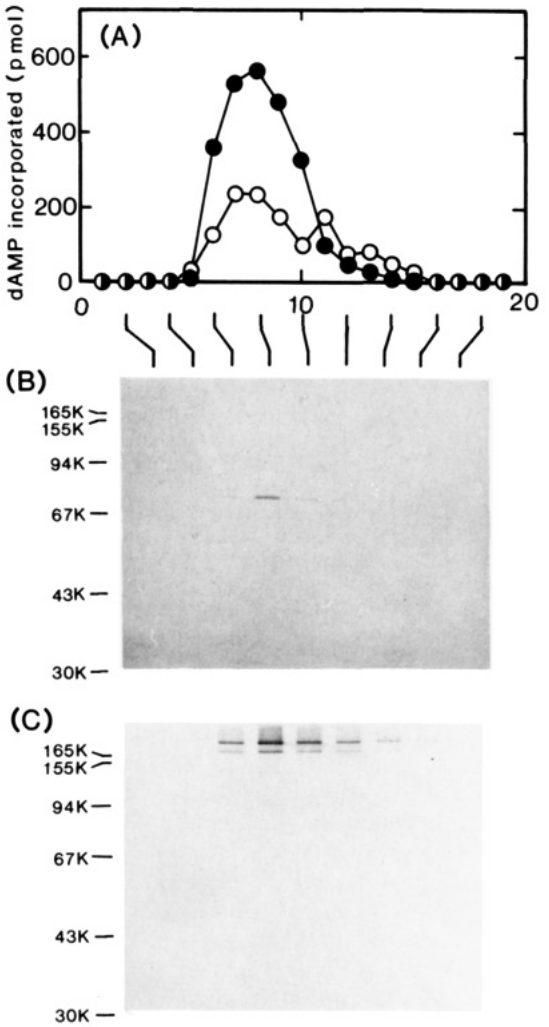

FIGURE 4: Cosedimentation of the 77-kDa polypeptide with the 180- and 160-kDa catalytic polypeptides of the DNA replicase complex. The partially purified HeLa DNA replicase was fractionated through the glycerol gradient followed by the analysis of each fraction by Western immunoblotting as described under Experimental Procedures. Fractions were collected from the bottom of the gradient. (A) DNA polymerase activity was assayed with the activated DNA (○) and poly(dT) in the presence of ATP (●). Incorporation was determined by using 20 μL of each fraction. The sedimentation coefficient of DNA replicase was 7.6–8 S. (B) Western immunoblotting analysis using the E4 antibody and (C) Western immunoblotting analysis using the A5 antibody. Each fraction of the gradient was analyzed as described under Experimental Procedures.

complex was fractionated by centrifugation through a glycerol gradient, and each fraction was subjected to Western immunoblot (Figure 4). Figure 4B shows that the 77-kDa polypeptide sediments coincident with the DNA replicase complex. Figure 4C shows that the monoclonal antibody directed against the catalytic subunits of DNA polymerase α (180- and 160-kDa polypeptide), the A5 antibody, does not recognize the 77-kDa polypeptide and, conversely, the E4 antibody does not recognize the catalytic polypeptides (see Figure 4B). The molecular mass of the polypeptides recognized with the E4 and A5 antibodies was determined by 4–20% gradient polyacrylamide gel electrophoresis. Evidence indicates that the 77-kDa polypeptide is not a portion of the DNA polymerase α catalytic subunit that is released by limited proteolysis during purification. This conclusion is supported by peptide mapping studies of Wong et al. (1986).

The molecular weights of these polypeptides are consistent with those reported for KB cell DNA polymerase α by Wong et al. (1986), and the 160-kDa polypeptide may be a partial

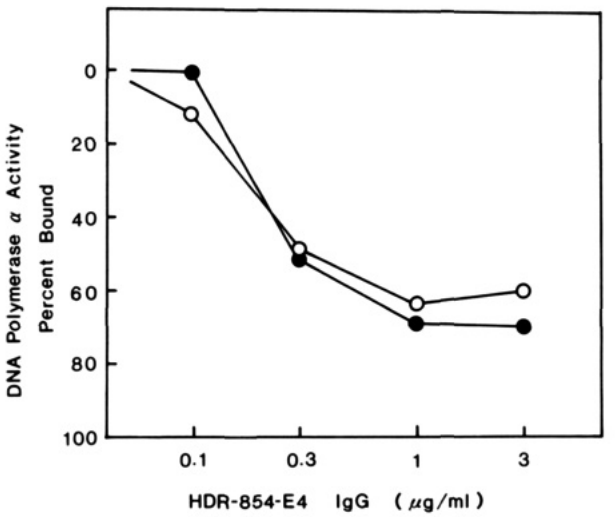

FIGURE 5: Interaction of HDR-854-E4 antibody with mouse DNA replicase and 7.3S DNA polymerase α. Immunoadsorption assay was performed as described under Experimental Procedures. Mouse DNA replicase complex (○) and 7.3S DNA polymerase α (●) were assayed with poly(dT) in the presence of ATP and the activated DNA, respectively, as template. The values for 100% polymerase activity were as follows: 21 pmol for DNA replicase and 18 pmol for 7.3S DNA polymerase α.

degradation product of the 180-kDa polypeptide as suggested by these authors.

Lack of Association of the 77-kDa Polypeptide with DNA Primase Activity. Results obtained so far indicated the possibility that the 77-kDa polypeptide is DNA primase. However, in other purified DNA replicase complexes, DNA primase is invariably associated with a polypeptide smaller than 60 kDa (Sauer & Lehman, 1982; Wang et al., 1984; Yagura et al., 1986; Holmes et al., 1986). In order to study this association more precisely, we examined the DNA replicase complex obtained from mouse Ehrlich cells, where the separation of DNA polymerase from primase is more easily obtained than that from HeLa cells (Yagura et al., 1986).

The antibody which was initially raised against the HeLa cell DNA replicase complex also interacts with mouse enzymes. As shown in Figure 5, a 50% reduction in DNA polymerase activity exhibited by the mouse DNA replicase complex (immunoadsorption assay) is obtained at an antibody concentration of 0.3 μg/mL, while the neutralization effect of the E4 antibody against the mouse DNA replicase complex was weaker than that against the HeLa DNA replicase complex [10–20% reduction in the primase-dependent DNA polymerase activity was obtained at an antibody concentration of 0.3 μg/mL (data not shown)]. A similar reduction in activity is also obtained using a purified fraction containing DNA polymerase α, that is devoid of DNA primase activity and sediments at 7.3 S (7.3S DNA polymerase α) (Yagura et al., 1983b, 1986; Kozu et al., 1986). The Western immunoblotting analysis showed that the E4 antibody interacts with the 77-kDa polypeptide from mouse DNA replicase (Figure 3, lane B). Moreover, 7.3S DNA polymerase α also contains a polypeptide that interacts with the E4 antibody (Figure 3, lane C). Quantitative analysis of the ratio of the Western immunoblotting staining density of the 77-kDa polypeptide to the amount of polymerase activity exhibited by the DNA replicase complex and the 7.3S DNA polymerase α shown in Figure 3 indicates that the amount of the 77-kDa polypeptide of 7.3S DNA polymerase α is almost the same as in mouse DNA replicase (data not shown).

The results indicate that the E4 antibody reacts with the 77-kDa polypeptide in the absence of DNA primase activity.

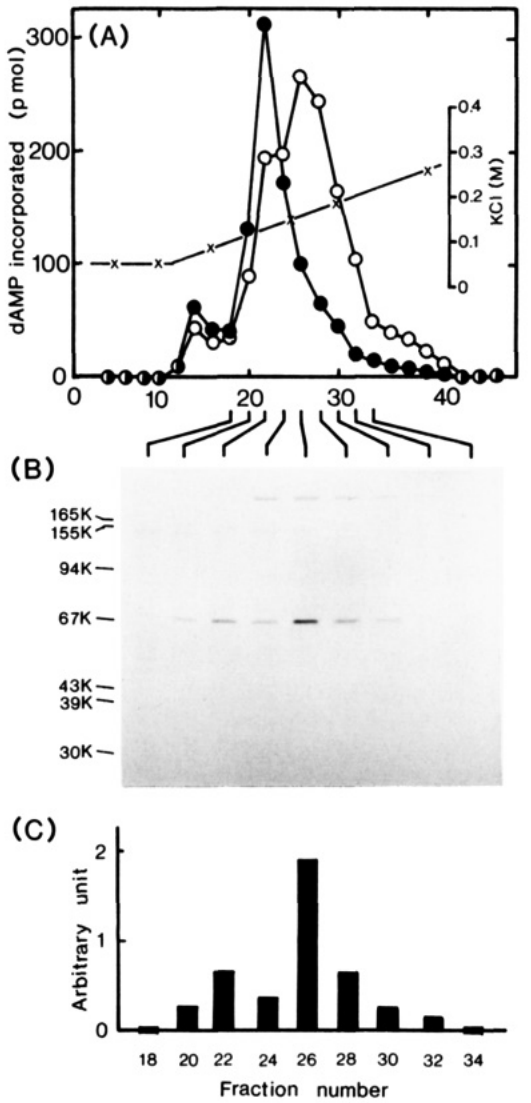

FIGURE 6: Copurification of the 77-kDa polypeptide with both mouse DNA replicase and 7.3S DNA polymerase α. The crude extract prepared from cultured mouse Ehrlich cells were subjected to chromatography on DEAE-cellulose as described earlier (Yagura et al., 1982a). Each fraction eluted from the column was assayed for the primase-dependent DNA synthesis activity on poly(dT) (●) and the DNA polymerase α activity on activated DNA (○) (A). The primase-dependent DNA synthesis activity was eluted at 0.13 M KCl, and the DNA polymerase α activity was in two peaks at 0.13 M KCl and 0.18 M KCl. The fractions around the polymerase activity were subjected to Western immunoblotting analysis using the E4 antibody (B). Diagram C shows the amounts of staining density of each band on nitrocellulose paper measured by using a Bio-Rad Model 1650 scanning densitometer (15-nm range) with a reflectance mode and recorded at 50-mV range (1 unit = 10%).

Furthermore, that the 77-kDa polypeptide is not associated with DNA primase is shown in experiments in which the DNA replicase complex is separated into two DNA polymerase fractions by DEAE-cellulose chromatography, only one of which has DNA primase activity. Extracts prepared from mouse Ehrich culture cells were fractionated by DEAE-cellulose chromatography (Figure 6A). The DNA replicase complex containing primase activity was detected with unprimed poly(dT). A second peak containing DNA polymerase activity devoid of primase activity was obtained (7.3S DNA polymerase α). Western blot analysis of each column fraction showed that both species contained the 77-kDa polypeptide (Figure 6B). The amount of 77-kDa polypeptide was roughly proportional to the amount of DNA polymerase α activity in each peak (Figure 6C). Since the 7.3S DNA polymerase α

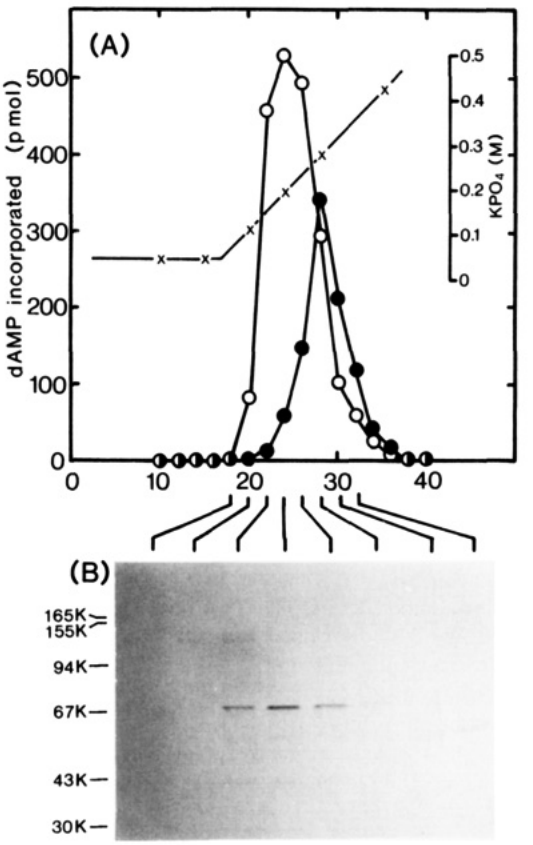

FIGURE 7: Lack of association of the 77-kDa polypeptide with DNA primase. The mouse DNA replicase complex was subjected to hydroxyapatite column chromatography in the presence of dimethyl sulfoxide and ethylene glycol. The partially purified mouse DNA replicase, essentially free from 7.3S DNA polymerase α by repeated column chromatographies (see Figure 3 legend), was used. Hydroxyapatite column chromatography in the presence of 20% dimethyl sulfoxide and 10% ethylene glycol was performed as described earlier (Yagura et al., 1986). In (A), DNA polymerase α activity was assayed with the activated DNA (○). The activity of primase detached from DNA replicase complex was detected with the addition of 7.3S DNA polymerase α to the reaction mixture containing poly(dT), ATP, and [^{3}H]dATP (●) as described earlier (Yagura et al., 1986). (B) Detection of the 77-kDa polypeptide with Western immunoblotting using the E4 antibody. Fractions around the activities shown in (A) were examined for the presence of 77-kDa polypeptide with Western immunoblotting as described under Experimental Procedures.

is free from primase activity, these results again that the 77-kDa polypeptide is not a component of DNA primase.

However, these results did not provide conclusive proof that the 77-kDa polypeptide is not the primase because we cannot show at present that the mouse 7.3S DNA polymerase α is really devoid of the primase subunit. Therefore, we next performed the following experiments.

As shown previously (Yagura et al., 1986), separation of DNA primase activity from the mouse DNA replicase complex was obtained by using hydroxyapatite column chromatography in the presence of dimethyl sulfoxide and ethylene glycol (Figure 7A). Figure 7B shows that the 77-kDa polypeptide elutes in fractions coincident with DNA polymerase α and not in fractions containing DNA primase. These results indicate that the 77-kDa polypeptide is not primase but affects catalysis by DNA primase in the initiation of RNA synthesis.

Discussion

We have purified a monoclonal antibody, HDR-854-E4, that specifically neutralizes DNA primase activity found associated with human and mouse DNA replicase complex. We have used the term DNA replicase complex to define the complex

formed with DNA polymerase α and DNA primase. Surprisingly, the E4 antibody did not react with the primase polypeptide but instead reacted with a 77-kDa polypeptide. It is probable that the E4 antibody recognizes the primary structure of a portion of the 77-kDa polypeptide since the E4 antibody can bind to the 77-kDa polypeptide after electrophoresis under denaturing conditions. Judging from its putative function and molecular mass, the 77-kDa polypeptide, which we have detected in HeLa cells and mouse cells, is equivalent to the 77-kDa polypeptide detected in KB cell DNA polymerase α as reported by Wang et al. (1984) and to the yeast 74-kDa polypeptide (Plevani et al., 1985) as well as the calf thymus 68-kDa polypeptide (Holmes et al., 1986).

The fact that the E4 antibody also inhibits primase activity in the DNA replicase complex suggests that the 77-kDa polypeptide is intimately associated with the DNA primase subunits and serves to initiate RNA synthesis in the DNA replicating complex. In fact, Holmes et al. (1986) reported that a calf thymus primase isolated from the DNA polymerase α–primase complex was associated with a part of the 68-kDa polypeptide, suggesting the initimate association of both subunits in the DNA replicase complex. At present, we have no evidence for the tight association of the 77-kDa polypeptide with our preparation of isolated primase, since our most purified fractions have only a 58-kDa polypeptide (Yagura et al., 1986). Furthermore, peptide mapping analysis of subunit polypeptides purified from KB cells suggested that the species of 77, 55, and 49 kDa are not structurally related to one another (Wong et al., 1986). Nevertheless, the model we proposed is consistent with that of Holmes et al. (1986), in which switching between the initiator RNA synthesis and subsequent DNA synthesis may be regulated by the 77-kDa polypeptide.

In the current study, we have used monoclonal antibodies to define the function of the different components of the DNA replicase complex. Our results indicate that each of these components may be intimately associated and may affect the catalytic activity of one another. However, it is also possible that antibody binding to one subunit polypeptide merely results in the inhibition of function of another subunit by stearic hindrance. Thus, in order to determine the mechanism for initiation of RNA synthesis by the DNA replicase complex, it might be necessary at the same time to define the three-dimensional relationships among the different polypeptides that constitute the DNA replicating complex.

Acknowledgments

We thank Drs. L. A. Loeb and F. Perrino of the University of Washington for critical reading of the manuscript.

Registry No. EC 2.7.7.7, 9012-90-2; DNA primase, 64885-96-7.

References

Holmes, A. M., Cheriathundam, E., Bollum, F. J., & Chang, L. M. S. (1986) *J. Biol. Chem. 261*, 11924–11930.

Kozu, T., Yagura, T., & Seno, T. (1982) *Nature (London) 298*, 180–182.

Kozu, T., Seno, T., & Yagura, T. (1986) *Eur. J. Biochem. 157*, 251–259.

Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.

Loeb, L. A., Liu, P. K., & Fry, M. (1986) *Prog. Nucleic Acid Res. Mol. Biol. 33*, 57–110.

Plevani, P., Foiani, M., Valsasnini, P., & Chang, L. M. S. (1985) *J. Biol. Chem. 260*, 7102–7107.

Sauer, P., & Lehman, I. R. (1982) *J. Biol. Chem. 257*, 12394–12398.

Senshu, T., Akiyama, K., Ohsawa, T., & Takahashi, K. (1985) *Eur. J. Biochem. 146*, 261–266.

Tanaka, S., Hu, S.-Z., Wang, T. S.-F., & Korn, D. (1982) *J. Biol. Chem. 257*, 8386–8390.

Tseng, Y., & Ahlem, C. N. (1982) *J. Biol. Chem. 257*, 7280–7283.

Wang, T. S.-F., Hu, Z.-Z., & Korn, D. (1984) *J. Biol. Chem. 259*, 1854–1865.

Wong, S. W., Paborsky, L. R., Fisher, P. A., Wang, T. S.-F., & Korn, D. (1986) *J. Biol. Chem. 261*, 7958–7968.

Yagura, T., Kozu, T., & Seno, T. (1982a) *J. Biochem. (Tokyo) 91*, 607–618.

Yagura, T., Kozu, T., & Seno, T. (1982b) *J. Biol. Chem. 257*, 11121–11127.

Yagura, T., Tanaka, S., Kozu, T., Seno, T., & Korn, D. (1983a) *J. Biol. Chem. 258*, 6698–6700.

Yagura, T., Kozu, T., Seno, T., Saneyoshi, M., Hiraga, S., & Nagano, H. (1983b) *J. Biol. Chem. 258*, 13070–13075.

Yagura, T., Kozu, T., & Seno, T. (1986) *Biochim. Biophys. Acta 870*, 1–11.