Accelerated Publications

The 68 kDa Calmodulin-Binding Protein Is Tightly Associated with the Multiprotein DNA Polymerase α−Primase Complex in HeLa Cells†

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Received November 30, 1994; Revised Manuscript Received January 23, 1995⊗

ABSTRACT: Calcium and its receptor protein calmodulin function in the regulation of proliferation of mammalian cells. A 68 kDa calmodulin-specific binding protein was shown previously to be associated with growth factor-dependent progression of a variety of mammalian cells from G1 to S phase and to stimulate DNA synthesis in permeabilized hematopoietic progenitor cells. In this report we show that the 68 kDa calmodulin-specific binding protein in HeLa cells is tightly associated with the DNA polymerase α−primase component of the 21S complex of enzymes for DNA synthesis. The 68 kDa calmodulin-binding protein and the DNA polymerase α−primase complex cofractionate during Q-Sepharose chromatography to isolate the 21S enzyme complex, native and denatured DNA−cellulose to dissociate the 21S complex, and DEAE-Bio-Gel chromatography to isolate the multiprotein DNA polymerase α−primase complex. The 68 kDa calmodulin-specific binding protein and DNA polymerase α also bind and coelute during affinity chromatography on calmodulin−agarose. They also coprecipitate with C10-agarose-linked monoclonal antibody SJK 132-20 to human DNA polymerase α. The tight association of the 68 kDa calmodulin-binding protein to the DNA polymerase α−primase complex supports a function for this protein in the regulation of DNA synthesis *in vivo*.

Transition of cells from G1 to S phase of the cell cycle is marked by the commitment of cells to undergo DNA synthesis. This is normally dependent on the interaction of specific growth factors with their receptors on the cell membrane and the availability of a suitable extracellular environment for their growth (Reddy, 1994). Although the growth factor/receptor interaction is known to be critical for G1 → S transition in cycling mammalian cells (Campisi & Pardee, 1984; Olashaw et al., 1987), the molecular event(s) stemming from such interactions on the membrane and the resulting initiation of chromosomal DNA synthesis (i.e., S phase) are not fully understood. Calcium and its receptor protein calmodulin (CaM)[1] were demonstrated through biochemical, pharmacological, and genetic studies to function in the regulation of mammalian cell proliferation (Means & Rasmussen, 1988; Whitfield et al., 1988; Lu & Means, 1993). The entry of mammalian cells into S phase is blocked by CaM antagonists, and DNA synthesis in permeabilized fibroblasts in S phase is highly sensitive to CaM-specific monoclonal antibodies (Reddy et al., 1992a). Consistent with the involvement of CaM in the cellular commitment to DNA synthesis are the observations that expression and nuclear localization of a 68 kDa calmodulin-specific binding protein (CaM-BP68)[1] are associated with growth factor-dependent progression of mammalian cells from G1 to S phase (Subramanyam et al., 1990; Reddy et al., 1992b). Calmodulin-specific binding proteins from a variety of mammalian cells were also found associated with immunopurified DNA polymerase α (Hammond et al., 1988). CaM-BP68 is associated with the replitase complex that contains DNA polymerase α and whose assembly is linked to the cell's ability to enter S phase (Reddy & Pardee, 1980; Reddy & Fager, 1993). Furthermore, purified CaM-BP68 stimulates DNA synthesis in permeabilized hematopoietic progenitor cells (Reddy et al., 1994). Taken together, these observations suggest a direct role of CaM and specific CaM-BPs in growth factor-dependent signaling pathways leading to the initiation of chromosomal DNA synthesis by virtue of their interactions with enzymes for DNA synthesis.

The advent of cell-free systems for the replication of certain DNA viruses provides a means for analysis of the DNA-synthesizing machinery in mammalian cells. Simian virus 40 (SV40)[1] has attributes that make it a model replicon for the study of the machinery for replication of chromosomal

† This work was supported by U.S. Public Health Service Grants CA-27466 and CA-15187.

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⊗ Abstract published in *Advance ACS Abstracts,* February 15, 1995.

[1] Abbreviations: AAN, aminoacetonitrile hemisulfate; BSA, bovine serum albumin; CaM, calmodulin; CaM-BP, calmodulin-binding protein; CaM-BP68, 68 kDa calmodulin-binding protein; DEAE, diethylaminoethyl; DTT, dithiothreitol; EDTA-Na_3, ethylenediaminetetraacetic acid trisodium salt, pH 7.5; EGTA-Na_3, ethylene[bis(oxyethylenenitrilo)]tetraacetic acid trisodium salt, pH 7.5; NE, 0.15 M KCl nuclear extract; PAGE, polyacrylaminde gel electrophoresis; PEG, poly(ethylene glycol); PMSF, phenylmethanesulfonyl fluoride; SDS, sodium dodecyl sulfate; SV40, simian virus 40; T-ag, simian virus 40 large tumor (T) antigen; Tris, tris(hydroxymethyl)aminomethane.

0006-2960/95/0434-3878$09.00/0

DNA in primate cells [reviewed in Challberg and Kelly (1989) and Stillman (1989)]. With the exception of the viral-encoded large T-antigen (T-ag),[1] SV40 DNA replication requires the primate host cell's DNA-synthesizing machinery. The complete replication of exogenous plasmid DNA templates containing the SV40 origin for replication can now be performed in a cell-free system supplemented with purified T-ag (Challberg & Kelly, 1989; Stillman, 1989).

Through the use of this cell-free system it was recently shown that T-ag-dependent SV40 *in vitro* replication activity in HeLa cell extracts resides exclusively with a soluble, sedimentable, 21S complex of enzymes for DNA synthesis (Malkas et al., 1990a; Li et al., 1993, 1994). The complex is disassociated into putative subassemblies during affinity chromatography on native and denatured DNA−celluloses (Li et al., 1994; Cao et al., 1994). Among the subassemblies is a 640 kDa, multiprotein (8 polypeptide), DNA polymerase α−primase complex (Vishwanatha et al., 1986). All of the 640 kDa DNA polymerase α−primase complex in HeLa cell extracts is associated with the 21S complex of enzymes for DNA synthesis (Li et al., 1993).

To better understand the function of CaM-BP68 in the initiation of DNA synthesis and its interaction with enzymes for DNA synthesis, we have investigated its association with the 21S complex in HeLa cells. This paper reports on the copurification of CaM-BP68 with the 21S enzyme complex from HeLa cells in S phase and the tight association of CaM-BP68 with the 640 kDa multiprotein DNA polymerase α−primase subassembly through several purification steps including affinity chromatography on CaM−agarose and immunoprecipitation with antibody to human DNA polymerase α.

MATERIALS AND METHODS

Subcellular Fractionation, Isolation of the 21S Complex, and Purification of the 640 kDa Multiprotein DNA Polymerase α−Primase Complex. HeLa S_3 cells in suspension culture were synchronized by the double-thymidine block technique and harvested in mid-S phase of the cell cycle as reported previously (Chiu & Baril, 1975). A 30% HeLa cell homogenate was prepared in an isotonic buffer containing 250 mM sucrose, 50 mM Tris[1]-HCl, pH 7.5, and 25 mM KCl plus 1 mM each of EDTA-Na_3,[1] EGTA-Na_3,[1] AAN,[1] and PMSF[1] using a Dounce homogenizer (pestle B). The nuclear extract (NE) and postmicrosomal supernatant (S-3) from subfractionation of the homogenate were combined and subjected to poly(ethylene glycol) (PEG)[1] precipitation by a published procedure (Malkas et al., 1990a). The 21S complex of enzymes for DNA synthesis was partially purified from the resulting supernatant by chromatography on Q-Sepharose (Li et al., 1993). Affinity chromatography on coupled columns of native and denatured DNA−cellulose dissociates the complex into putative subassemblies (Li et al., 1994; Cao et al., 1994). The effluent from the denatured DNA−cellulose column was chromatographed on a 1 × 2.5 cm DEAE[1]-Bio-Gel (Bio-Rad) column as previously described for isolation of the 640 kDa multiprotein DNA polymerase α−primase complex (Vishwanatha et al., 1986).

CaM−Agarose Affinity Purification of CaM-BP68. Active fractions of DNA polymerase α eluted by 0.15 M KCl from DEAE-Bio-Gel were dialyzed overnight at 4 °C against a 25-fold excess of buffer A containing 20 mM Tris-HCl, pH 7.4, 4 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM KCl, and 1 mM PMSF. The dialized fraction was loaded, at a flow rate of 0.75 mL/min, onto a 1 × 7 cm column of CaM−agarose (Sigma) equilibrated with buffer A. After being washed with buffer A until the absorbance of the eluate at 280 nm has reached the baseline, the column was eluted with buffer A containing 10 mM EGTA in place of $CaCl_2$. Individual fractions (5 mL) were collected and assayed for CaM-BP68 and DNA polymerase α activity as described below.

Coimmunoprecipitation of DNA Polymerase α and CaM-BP68. The procedure for immunoprecipitation of DNA polymerase α was essentially the same as that described by Cao and co-workers (1994) except that C10-agarose-linked purified mouse monoclonal antibody SJK 132-20 to human DNA polymerase α (Tanaka et al., 1982) was used.

Polyacrylamide Gel Electrophoretic and Immunoblot Analysis of Proteins. Polyacrylamide gel electrophoretic (PAGE)[1] analysis of proteins under nondenaturing and denaturing conditions was as described (Lamothe et al., 1981). The protein-stained bands were developed by Coomassie blue or sliver staining. Electroblotting of the separated proteins was performed by published procedures (Malkas et al., 1990).

CaM-BP68 Detection. CaM-BPs in individual fractions were detected essentially as described elsewhere (Reddy et al., 1994). Briefly, protein fractions from various steps of DNA polymerase α−primase purification were subjected to polyacrylamide gel electrophoresis under denaturing conditions and transferred to nitrocellulose filters. CaM-BPs on the filters were identified by using biotinylated CaM (purchased from Biomedical Technologies, Inc., Stoughton, MA) and alkaline phosphatase-conjugated avidin (purchased from Boehringer Mannheim Biochemicals, Indianapolis, IN). Prestained molecular weight markers (purchased from Bethesda Research Laboratories, Bethesda, MD) were used to identify the relative molecular weights of the CaM-BPs detected on the filters.

Other Assays. DNA polymerase α activity with activated and primed, single-stranded DNA templates (Lamothe et al., 1981) and DNA primase activity (Vishwanatha et al., 1986) were assayed according to published procedures. Protein was assayed by the procedure of Bradford (1976) using bovine serum albumin (BSA)[1] as the standard.

RESULTS

Calmodulin-binding proteins were reported to tightly complex with multiprotein forms of DNA polymerase α from bovine, hamster, and human cells (Hammond et al., 1988). The 640 kDa multiprotein form of DNA polymerase α−primase in HeLa cells was shown to exclusively associate with a 21S complex of enzymes that function in SV40 DNA replication *in vitro* (Malkas et al., 1990; Li et al., 1993). Thus, it was of interest to determine if specific calmodulin-binding proteins are tightly associated with the multiprotein DNA polymerase α complex that resides with the 21S complex of enzymes for DNA synthesis.

Cofractionation of the Multiprotein DNA Polymerase α−Primase Complex and CaM-BP68. The procedure for purification of the 21S complex of enzymes for DNA synthesis from HeLa cell homogenates involves poly(ethylene glycol) (PEG) precipitation of the combined nuclear extract (NE)/postmicrosomal supernatant (S-3) fraction fol-

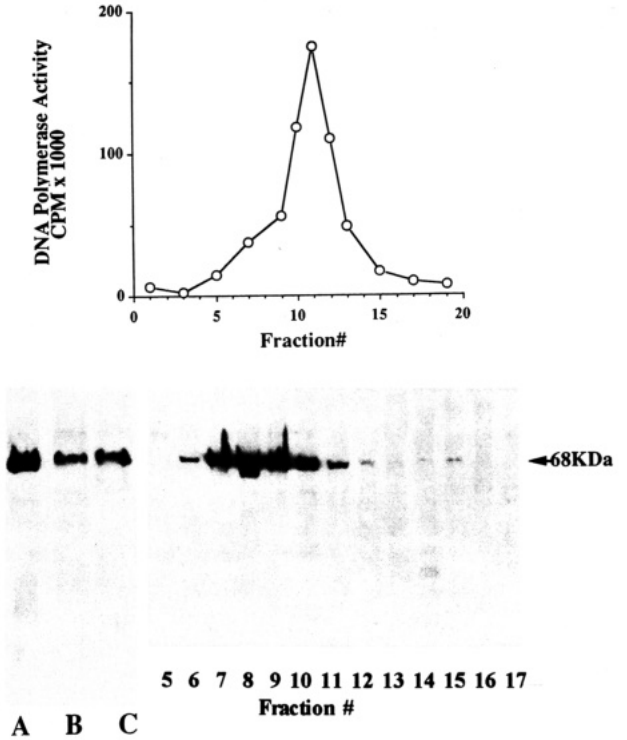

FIGURE 1: Cofractionation of DNA polymerase α and CaM-BP68 during the course of purification of the multiprotein DNA polymerase α-primase complex. (Lower panel, left) Identification of CaM-BPs in early fractions during the course of purification of the multiprotein DNA polymerase α−primase complex. Identification of CaM-BP68 in aliquots of the NE/S-3 (lane-A), PEG supernatant (lane B), and flow-through fraction from the denatured DNA−cellulose column (lane C). Elution profile of the DNA polymerase α activity (upper panel) and CaM-BPs (lower panel, right) during Q-Sepharose chromatography of the PEG supernatant for isolation of the 21S enzyme complex. The PEG supernatant (150 mg of protein) in the 50 mM Tris-HCl, pH 7.5, 1 mM EDTA-Na_3, 1 mM EGTA-Na_3, 0.05 M KC1 containing buffer was loaded onto a 1.5 × 2.5 cm column of Q-Sepharose equilibrated with the same buffer. After being washed with 10 column volumes of the equilibration buffer, the column was eluted by a gradient of increasing KC1 concentration from 0.05 to 0.5 M in the 50 mM Tris-HC1, pH 7.5, 1 mM EDTA-Na_3, 1 mM EGTA-Na_3 buffer as previously described (Li et al., 1993). Two-milliliter fractions were collected at a flow rate of 0.5 mL/min, and aliquots were assayed for DNA polymerase α activity and CaM-BP68 as described in Materials and Methods.

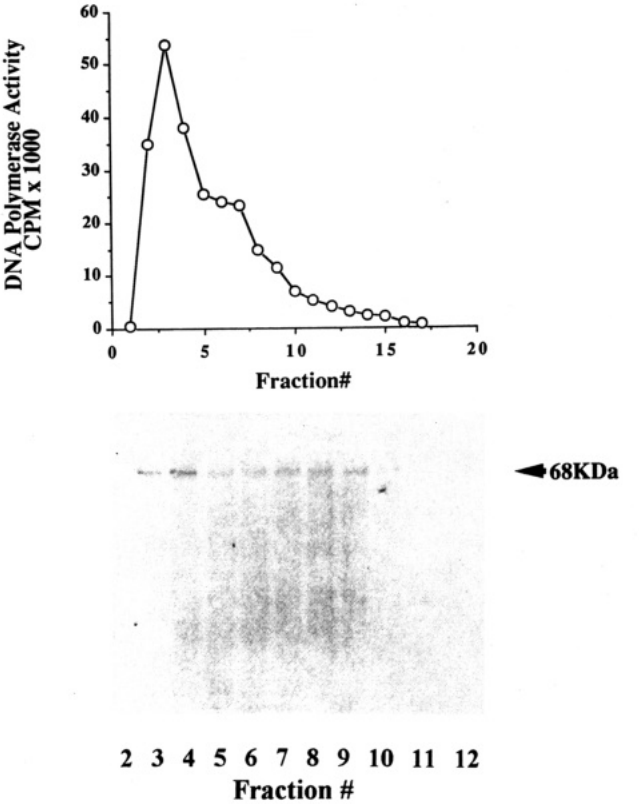

FIGURE 2: Coelution of DNA polymerase α and CaM-BP68 during DEAE-Bio-Gel chromatography of the flow-through fraction from denatured DNA−cellulose. Elution profile of the DNA polymerase α activity (upper panel) and CaM-BP68 (lower panel) in the eluted fractions from the DEAE-Bio-Gel column. The flow-through fraction from the denatured DNA−cellulose column in chromatography of the 21S enzyme complex on coupled native and denatured DNA−cellulose columns was equilibrated with buffer containing 50 mM Tris-HC1, pH 7.5, 1 mM EDTA, 1 mM EGTA, and 50 mM KC1 by dialysis and loaded onto a 1 × 2.5 cm column of DEAE-Bio-Gel equilibrated with the dialysis buffer. After being washed with 10 column volumes of the equilibration buffer, the column was eluted by a stepwise gradient of increasing KC1 concentrations of 0.1 M (data not shown) followed by 0.15 M KCl (shown in upper panel) by a published procedure (Lamothe et al., 1981). Fractions of 1.5-mL volume were collected, and aliquots were assayed for DNA polymerase α activity and CaM-BP68 as described in Materials and Methods. As shown previously, all of the DNA polymerase α−primase activity in the denatured DNA−cellulose column effluent is recovered in the 0.15 M KC1 eluate during DEAE-Bio-Gel chromatography (Lamothe et al., 1981; Vishwanatha et al., 1986).

lowed by Q-Sepharose chromatography of the resulting supernatant (Malkas et al., 1990a). The 21S complex is totally recovered in the supernatant (PEG supernatant) following PEG precipitation of NE/S-3 and binds to Q-Sepharose requiring 0.15−0.3 M KC1 for its elution. The 640 kDa multiprotein DNA polymerase α−primase complex is exclusively associated with the 21S complex (Malkas et al., 1990a; Li et al., 1993) that coelutes from Q-Sepharose at the same KC1 concentration (Malkas et al., 1990a; Li et al., 1993).

As shown in Figure 1 (lower left panel, lanes A−C), CaM-BP68 is also present in the starting NE/S-3 fraction (lane A) and cofractionates with the 21S complex of enzymes for DNA synthesis during PEG precipitation (lane B) and denatured DNA−cellulose chromatography (lane C). CaM-BP68 in the PEG supernatant also binds to Q-Sepharose and coelutes with the peak of DNA polymerase α activity (fractions 4−16 in upper panel and fractions 6−15 in lower panel of Figure 1) that is associated with the 21S complex (Malkas et al., 1990a; Li et al., 1993).

Chromatography of the 21S enzyme complex on coupled columns of native and denatured DNA−cellulose dissociates the complex into putative subassemblies (Li et al., 1993, 1994; Cao et al., 1994). The 640 kDa multiprotein DNA polymerase α−primase component of the 21S complex does not bind to the DNA−celluloses under these conditions (Vishwanatha et al., 1986) and appears in the flow-through fraction from the denatured DNA−cellulose column. The 68 kDa CaM-BP also does not bind to native or denatured DNA−cellulose (Figure 1, lower left panel, lane C) and appears with the multiprotein DNA polymerase α−primase complex in the flow-through fraction from the denatured DNA−cellulose column (data not shown). As shown in Figure 2, the DNA polymerase α activity (upper panel) and CaM-BP68 (lower panel) in the denatured DNA−cellulose flow-through fraction cofractionate during chromatography on DEAE-Bio-Gel. DNA polymerase α (Figure 2, upper

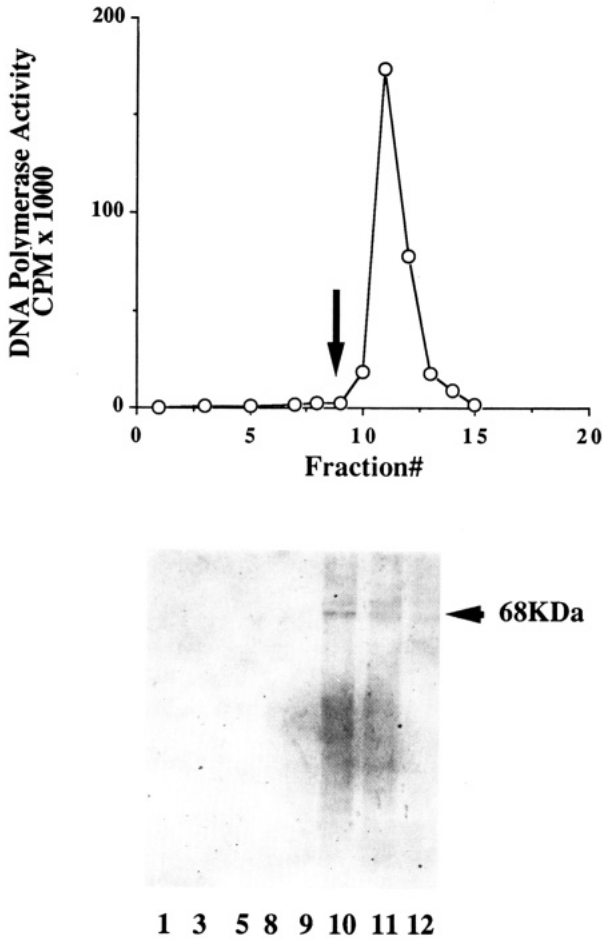

FIGURE 3: Coelution of the DNA polymerase activity and the CaM-BP68 during CaM−agarose affinity chromatography. Elution profile of the DNA polymerase α activity (upper panel) and CaM-BPs (lower panel) during CaM−agarose affinity chromatography. The arrow indicates the switch from elution with the 2 mM calcium-containing buffer to buffer containing 10 mM EGTA in place of calcium. The assay and chromatographic procedures are as described in Materials and Methods.

panel) and CaM-BP68 (Figure 2, lower panel) both bind to DEAE-Bio-Gel and coelute at a KCl concentration of 0.15 M.

CaM-BP68 and DNA Polymerase α Remain Associated during CaM−Agarose Affinity Chromatography. In the overall purification scheme of CaM-BP68 from nuclear lysates of mammalian cells, the CaM−agarose affinity chromatographic step enriches recovery of CaM-BP68 about 270-fold (Reddy et al., 1994). Therefore, as an alternative approach to critically test the specific association between CaM-BP68 and DNA polymerase α, we subjected the 640 kDa multiprotein DNA polymerase α−primase complex recovered from DEAE-Bio-Gel to CaM−agarose affinity chromatorgraphy. As shown in Figure 3, both the DNA polymerase α activity and CaM-BP68 were retained on the CaM−agarose column in the presence of 2 mM $CaC1_2$ and coeluted as a sharp peak following the substitution of the calcium-containing buffer with the buffer containing 10 mM EGTA in place of calcium. Since DNA polymerase α alone does not bind to CaM−agarose (data not shown), the binding of the DNA polymerase α activity in the 640 kDa multi-protein complex to CaM−agarose in the presence of calcium, but not in the presence of EGTA, is consistent with its tight association with CaM-BP68.

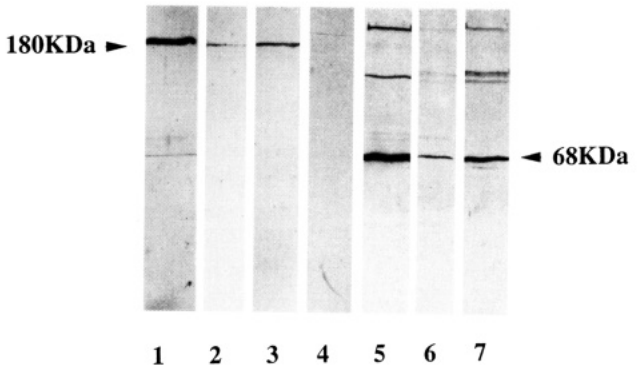

FIGURE 4: Coimmunoprecipitation of DNA polymerase α and the CaM-BP68 from various chromatographic fractions during the purification. Aliquots of the peak of DNA polymerase α activity, containing 10 μg of protein each in duplicate, eluted from the Q-Sepharose, DEAE-Bio-Gel, and CaM−agarose columns were immunoprecipitated using a vast excess of C10-agarose-linked monoclonal antibody to human DNA polymerase α by a published procedure (Cao et al., 1994). The solubilized immunoprecipitate was assayed for the 180 kDa DNA polymerase α catalytic polypeptide by immunoblot analysis and CaM-BPs by biotinylated CaM binding assay as described in Materials and Methods. Lanes 1−3: DNA polymerase α 183 kDa polypeptide in the solubilized immunoprecipitate of the eluted fraction from the Q-Sepharose (lane 1), DEAE-Bio-Gel (lane 2), and CaM−agarose (lane 3) columns, respectively. Lanes 5−7: CaM-BPs in the solubilized immunoprecipitate of the eluted fraction from the Q-Sepharose (lane 5), DEAE-Bio-Gel (lane 6), and CaM−agarose (lane 7) columns, respectively. Lane 4: A representative control immunoblot for DNA polymerase α was performed on the solubilized immunoprecipitate by incubation of an aliquot of the Q-Sepharose-eluted fraction with C10-agarose-linked goat anti-mouse IgG under the same conditions as for the incubation of C10-agarose-linked SJK 132-20 antibody. The same control incubations were performed for the DEAE-Bio-Gel and CaM−agarose fraction of DNA polymerase α with the same results (data not shown). The procedures for immunoprecipitation and CaM-BP analysis were as described under Materials and Methods. Immunoblot analysis of DNA polymerase α was performed according to a published procedure using a 1:200 dilution of rabbit polyclonal antibody (purified IgG) specific for the 183 kDa catalytic subunit of human DNA polymerase α (Vishwanatha et al., 1986), followed by incubation with peroxidase-conjugated sheep anti-rabbit IgG.

Coimmunoprecipitation of CaM-BP68 and DNA Polymerase α−Primase with Agarose-Linked Antibody to Human DNA Polymerase α. Although CaM-BP68 appears to cofractionate with the DNA polymerase α−primase subassembly of the 21S enzyme complex through several chromatographic steps, it was important to demonstrate a specific association of these proteins. As shown in Figure 4, C10-agarose-linked monoclonal antibody SJK 132-20 to human DNA polymerase α immunoprecipitated both the 183 kDa DNA polymerase α subunit (lanes 1−3) and CaM-BP68 (lanes 5−7) from three different chromatographic fractions during the course of purification of the DNA polymerase α−primase complex. SDS−polyacrylamide gel analysis showed that the 70 kDa polypeptide and the 40 plus 50 kDa primase subunits of the DNA polymerase α−primase complex also coprecipitated under these conditions (data not shown). CaM-BP68 and DNA polymerase activities were completely precipitated under these conditions since no DNA polymerase α or CaM-BP68 was recovered in the supernatant following centrifugation of the C10-agarose-linked SJK 132-20 antibody, DNA polymerase α−CaM-BP68 complex. The precipitation also appears specific for the antibody/DNA polymerase interaction since incubation with control mouse

IgG did not result in precipitation of DNA polymerase α (Figure 4, lane 4) or CaM-BP68 (data not shown).

DISCUSSION

The onset of DNA synthesis in mammalian cells during the cell cycle is governed by extracellular stimuli and growth factor/receptor interaction. The mechanism(s) involved in the transduction of the signal generated by growth factor/receptor interaction on the membrane into the nucleus for organization and activation of the DNA synthesizing machinery is (are) not yet understood.

The level of CaM increases during the G1 phase of hepatocytes stimulated to proliferate and is translocated to the nucleus to associate with CaM-BPs in the nuclear matrix (Serratosa et al., 1988; Pujol et al., 1989). The nuclear localization of a 68 kDa calmodulin-specific binding protein (CaM-BP68) was previously shown to be associated with growth factor-dependent transition of cells from G1 to S phase of the cell cycle (Subramanyam et al., 1990; Reddy et al., 1992b). CaM-BP68 was also shown to stimulate DNA synthesis in permeabilized hematopoietic progenitor cells (Reddy et al., 1994) and to be associated with the replitase complex that contains DNA polymerase α (Subramanyam et al., 1990). CaM-BPs were also reported to be associated with immunopurified DNA polymerase α from a variety of mammalian cells (Hammond et al., 1988).

It is now apparent that in mammalian cells, as in lower organisms, DNA replication is facilitated by the assembly of enzymes for DNA synthesis into multienzyme complexes [reviewed in Malkas et al. (1990b) and Reddy and Fager (1993)]. A 21S complex of enzymes for DNA synthesis that functions in SV40 DNA replication *in vitro* was isolated from HeLa cell extracts (Malkas et al., 1990a; Li et al., 1993). The complex was purified and dissociated into putative subassemblies (Li et al., 1994; Cao et al., 1994). Among the subassemblies is a 640 kDa, multiprotein DNA polymerase α−primase complex (Vishwanatha et al., 1986).

In this report we show that CaM-BP68 cofractionates with the 21S complex of enzymes for DNA synthesis in HeLa cells and is tightly associated with the DNA polymerase α−primase component of the complex. CaM-BP68 remains associated with the DNA polymerase α−primase subassembly of the complex through several purification steps including affinity chromatography on CaM−agarose. Although the CaM-BP68 and DNA polymerase α activity peaks are not completely coincidental during Q-Sepharose chromatography of the 21S enzyme complex (Figure 1), they are completely coincidental during chromatography of the DNA polymerase α−primase complex on DEAE-Bio-Gel (Figure 2) and CaM−agarose (Figure 3). The lack of complete coincidence of the polymerase α and CaM-BP68 activities with the complex during Q-Sepharose chromatography may not be surprising, considering the size of the loosely associated 21S complex (i.e., 35 polypeptides) and the possible presence of components within the complex that interfere with the assays. This is the case for the assay of DNA ligase I that is associated with the 21S complex (Li et al., 1993). It is also intriguing to note that the CaM-BP68 interaction with DNA polymerase α−primase remained unaffected by EGTA treatment that chelates Ca^{2+} during CaM affinity chromatography. Ca^{2+} and CaM play pivotal roles during transition of cells from G1 to S (Reddy, 1994). This may imply that Ca^{2+} and CaM may play a role in nuclear localization of CaM-BP68 following growth factor/receptor interactions, but once inside the nuclei CaM-BP68 interaction with the DNA−primase complex may be independent of Ca^{2+} and CaM. Thus CaM-BP68 may function in the transduction of calcium signals to the replication complex during growth factor stimulation. In any event, the fact that CaM-BP68 and DNA polymerase α cofractionate during affinity chromatography on CaM−agarose and are coprecipitated by monoclonal antibody to human DNA polymerase α supports a tight association of these proteins.

The results from this study are in agreement with previous findings of the association of CaM-BP68 with the replitase complex and the possible involvement of this protein in chromosomal DNA replication in mammalian cells (Reddy et al., 1992b, 1994). These results are also in agreement with the previous report of the cofractionation of CaM-BPs with DNA polymerase α during its immunopurification from a variety of mammalian cells (Hammond et al., 1988). It is of interest that a number of proteins (i.e., 6−8 polypeptides) in addition to primase and the 70 kDa polypeptide were found to associate with the 183 kDa DNA polymerase α catalytic subunit during affinity chromatography of human (Rogge & Wang, 1992) and yeast (Miles & Formosa, 1992) cell extracts on a matrix containing the agarose-linked DNA polymerase α catalytic subunit. Although no activities or function(s) for these additional proteins were demonstrated in those studies, some of the additional proteins were in the same size range (i.e., 65−70 kDa) as CaM-BP68 (Rogge et al., 1992).

The finding of a tight association of CaM-BP68 with the DNA polymerase α−primase component of the 21S complex of enzymes for DNA synthesis should now help to define the role of CaM-BP68 in DNA replication by use of the assay for SV40 DNA replication *in vitro*.

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BI9427678