MOLECULAR AND CELLULAR BIOLOGY, Dec. 1987, p. 4594–4598
0270-7306/87/124594-05$02.00/0

Vol. 7, No. 12

c-*myc* Protein and DNA Replication: Separation of c-*myc* Antibodies from an Inhibitor of DNA Synthesis

CRISANTO GUTIERREZ,[1] ZONG-SHENG GUO,[1] JAMES FARRELL-TOWT,[2] GRACE JU,[2] AND MELVIN L. DEPAMPHILIS[1,*]

Department of Cell Biology, Roche Institute of Molecular Biology,[1] and Department of Molecular Genetics, Roche Research Center,[2] Hoffmann-La Roche Inc., Nutley, New Jersey 07110

Received 14 August 1987/Accepted 10 September 1987

Antibodies against human c-*myc* protein have been reported to inhibit DNA polymerase activity and endogenous DNA synthesis in isolated nuclei, suggesting a role for c-*myc* in DNA replication. Using the same antibody preparations, we observed equivalent inhibition of simian virus 40 DNA replication and DNA polymerase α and δ activities in vitro, as well as inhibition of DNA synthesis in isolated nuclei. However, the c-*myc* antibodies could be completely separated from the DNA synthesis inhibition activity. c-*myc* antibodies prepared in other laboratories also did not interfere with initiation of simian virus 40 DNA replication, DNA synthesis at replication forks, or DNA polymerase α or δ activity. Therefore, the previously reported inhibition of DNA synthesis by some antibody preparations resulted from the presence of an unidentified inhibitor of DNA polymerases α and δ and not from the action of c-*myc* antibodies.

A role for the c-*myc* protein in DNA synthesis has recently been suggested by the observation that polyclonal and monoclonal antibodies directed against c-*myc* protein inhibit DNA synthesis in nuclei isolated from human leukemic cell lines (17). Furthermore, the target for this inhibition appears to be DNA polymerase α or δ. When the substrate for these enzymes, DNase I-activated DNA, was added to nuclear preparations, DNA synthesis was inhibited to the same extent either by aphidicolin (a specific inhibitor of DNA polymerases α and δ [11]) or by the antibodies against c-*myc* protein. This is consistent with evidence that DNA replication in isolated nuclei reflects predominantly, if not exclusively, the continuation of events at replication forks that are initiated in intact cells and that replication does not involve formation of new replicons (6). Recently, it has been reported that plasmids containing the origin region of simian virus 40 (SV40) can replicate to some extent, even in the absence of SV40 large T antigen, when transfected into human HL-60 and Raji cells which are expressing the c-*myc* gene at high levels (9).

In an attempt to confirm and extend these important observations, we tested the same antibodies used by Studzinski and co-workers for the ability to inhibit replication of SV40 DNA under in vitro conditions that allowed both the initiation of new replicons and the continuation of replication at the resulting forks. SV40 replicates in the nuclei of mammalian cells as a small (5.2-kilobase-pairs) circular chromosome. The structure and assembly of SV40 chromatin and the events at SV40 DNA replication forks are essentially the same as those in mammalian cells (for reviews, see references 5, 6, and 8 and M. L. DePamphilis, *in* Y. Aloni, ed., *Molecular Aspects of the Papovaviruses*, in press). The only virally encoded protein required for replication of SV40 chromosomes is large T antigen; all other proteins, including DNA polymerases α and δ, are provided by the host cell and are apparently part of the host chromosome replication machinery. SV40 large T antigen is required to initiate SV40 DNA replication, and its helicase activity may also be utilized at replication forks (16). Therefore, if c-*myc* is involved in DNA synthesis at cellular replication forks, it is likely also to be involved at SV40 replication forks.

Initiation as well as continuation of DNA replication can be achieved by using a soluble extract of SV40-infected monkey CV-1 cells supplemented with deoxyribonucleoside triphosphates and polyethylene glycol (3, 4). Incubation in this system of SV40 covalently closed, superhelical DNA, SV40 chromosomes, or circular, covalently closed, superhelical plasmid DNA containing the SV40 *ori* region (pSVori) results in bidirectional replication from the genetically defined origin of replication sequence (*ori*). Replication involves RNA-primed DNA synthesis (J. Taljanidisz, R. S. Decker, Z.-S. Guo, M. L. DePamphilis, and N. Sarkar, Nucleic Acids Res., in press) and depends on a functional SV40 large T antigen, a functional SV40 *ori*, and an extract of cells that permit SV40 DNA replication (3, 4, 20). Thus, the properties of DNA replication in this in vitro system are consistent with the properties of SV40 chromosome replication in vivo (5; DePamphilis, in press).

Effect of c-*myc* antibodies on SV40 DNA replication in vitro. We obtained one polyclonal (RW [19]) and two monoclonal (F5 and C3 [18]) antibodies against human c-*myc* protein (Table 1). These antibodies had been prepared against recombinant human c-*myc* protein, purified by affinity chromatography on *Staphylococcus aureus* protein A-Sepharose, and shown to inhibit cellular DNA synthesis (17). In vitro SV40 DNA replication was measured as described previously (3, 4). Briefly, extracts of SV40-infected CV-1 cells (20 μl of cytosol [5 to 9 mg of protein per ml; 1] mixed with 10 μl of high-salt nuclear extract [4 to 8 mg of protein per ml; 1]) were preincubated with each of the antibodies on ice for 30 min before the addition of a buffered salt solution containing nucleoside triphosphates, deoxynucleoside triphosphates, DNA substrates, and polyethylene glycol (3, 4). Replication reactions (50 μl) containing 30 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), pH 7.8, 0.5 mM dithiothreitol, 7 mM magnesium acetate, 1 mM EGTA [ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N'*,*N'*-tetraacetic acid], 200 μM each of CTP, UTP, and GTP, 4 mM ATP, 100 μM each of dATP and dGTP, 40 μM each of

* Corresponding author.

TABLE 1. Antibodies tested for inhibition of SV40 DNA replication and DNA synthesis on activated DNA

Antibody	Source (reference)	Immunogen	c-*myc* reactivity[a]	DNA synthesis (%) on[b]:		
				pSVori	SV40 chromosomes	Activated DNA
Preimmune rabbit serum	G. Studzinski (17)	None	−	100	100	100
Polyclonal RW	G. Studzinski (19)	c-*myc* (*E. coli*)	+	33	8	14
Polyclonal RC	R. Chizzonite (13)	c-*myc* peptides 269 to 283, 305 to 317, 336 to 348	+	97		96
Polyclonal JFT	J. Farrell-Towt	c-*myc* (insect)	+	89		100
Monoclonal C3	G. Studzinski (18)	c-*myc* (*E. coli*)	+	36	5	8
Monoclonal IF7	R. Chizzonite (13)	c-*myc* peptide 305 to 317	+	97		77
Monoclonal B3(RW)	G. Studzinski (17)	c-*myc* (*E. coli*)	+			3[c]
Monoclonal B3(JFT)	J. Farrell-Towt	c-*myc* (*E. coli*)	+			100
Monoclonal F5	G. Studzinski (17, 18)	c-*myc* (*E. coli*)	−[d]	22	8	15
Monoclonal IL-2	R. Chizzonite (15)	Interleukin-2	−	92		94
Monoclonal H1	G. Studzinski (17)	Histone H1	−	73[c]	80[c]	93[c]
Monoclonal SJK 132	Pharmacia, Inc.	DNA polymerase α	−			28[c]
Polyclonal c-*fos*	T. Curran (2)	c-*fos* peptide 127 to 152	−	93[c]	93[c]	95[c]

[a] Immunoblotting was done by using recombinant c-*myc* (insect) protein as previously described (14).
[b] DNA replication on pSVori and SV40 chromosomes was carried out as described in the legend to Fig. 1. DNA synthesis on activated DNA was measured as previously described (21). Cell extracts were preincubated on ice with 20 μg of IgG per ml, unless otherwise stated, for 30 min before deoxynucleoside triphosphates were added. Final concentrations in the assay (100 μl) were 50 mM Tris hydrochloride, pH 7.4, 1 mM dithiothreitol, 80 μg of activated DNA per ml, 100 μM each dATP, dCTP, and dGTP, 20 μM [^{3}H]dTTP (200 cpm/pmol), and 400 μg of bovine serum albumin per ml.
[c] Monoclonal B3(RW) was used at 2 μg/ml, monoclonal H1 at 80 μg/ml, monoclonal SJK 132 at 120 μg/ml and polyclonal c-*fos* at 100 μg/ml.
[d] Previously reported to be positive with c-*myc* (*E. coli*) protein (18).

[^{32}P]dCTP and [^{32}P]dTTP, 10 mM phosphoenolpyruvate, 3 μg (0.2 U/μg) of pyruvate kinase (Boehringer Mannheim Biochemicals), and 3.2% polyethylene glycol were incubated at 37°C for 2.5 h. Replication products were purified as previously described (4), digested either with *Eco*RI and *Dpn*I (in the case of pSVori used as the template) or with *Eco*RI (in the case of SV40 chromosomes or DNA used as the template), and fractionated by electrophoresis in 0.8% agarose in Tris borate-EDTA buffer (12). The gel was dried and exposed to Kodak X-Omat AR film with a Cronex-Plus intensifying screen (Du Pont Co.) at −70°C. To quantitate the replication efficiency, the portions of the gel containing the products of interest were excised and the radioactivity was measured in the presence of scintillation liquid.

Addition of polyclonal RW, monoclonal F5, or monoclonal C3 to the in vitro SV40 DNA replication system inhibited pSVori replication in a dose-dependent manner (Fig. 1A). We did not observe significant inhibition of SV40 DNA replication with equivalent amounts of immunoglobulin G (IgG) from preimmune rabbit serum or with a monoclonal antibody against histone H1 that does not inhibit cellular DNA synthesis. Similar results were obtained when SV40 DNA was substituted for the pSVori template (data not shown). The extent of inhibition was somewhat greater when isolated SV40 chromosomes were added to the system in place of pSVori (Fig. 1B) or when they were present as endogenous viral chromatin in extracts of SV40-infected CV-1 cells (Fig. 1). These results are summarized in Table 1. The antibody concentrations required for inhibition of viral DNA replication were the same as those reported by Studzinski et al. (17) for DNA synthesis in isolated nuclei. We also observed inhibition of DNA synthesis in nuclei isolated from uninfected CV-1 cells preincubated with these antibody samples (data not shown). These results suggest that c-*myc* protein is involved in both SV40 and cellular DNA replication.

Several attempts were made to demonstrate that purified c-*myc* protein would block inhibition of DNA synthesis by c-*myc* antibodies as reported previously (17). However, samples of c-*myc* protein, kindly provided by G. Studzinski,

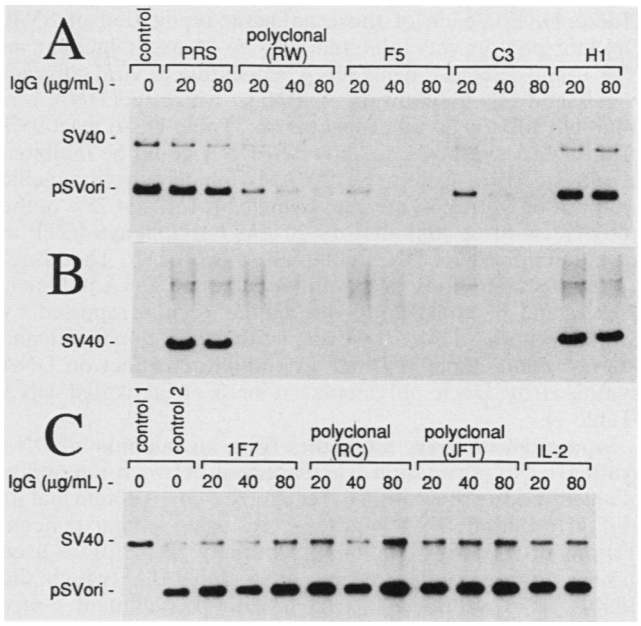

FIG. 1. Effect of c-*myc* antibodies on in vitro replication of SV40 templates. Extracts of infected CV-1 cells were prepared as described in the text. After preincubation of the extracts with various concentrations of purified IgG from the c-*myc* antisera, plasmid pSVori, containing the SV40 origin of replication (A and C), or purified SV40 chromosomes (B) were added. Autoradiograms are shown of the replication products after digestion either with *Eco*RI and *Dpn*I (A and C) or with *Eco*RI only (B). (A and B) Control, No antibody added (A) or no antibody or SV40 chromatin added (B); PRS, preimmune rabbit serum; polyclonal RW, polyclonal anti-c-*myc* serum (provided by G. Studzinski and R. Watt); F5 and C3, monoclonal anti-c-*myc* sera; H1, monoclonal anti-histone H1 serum. (C) Control 1, No antibody and no pSVori added; control 2, no antibody added; IF7, monoclonal anti-c-*myc* serum; polyclonal (RC) and (JFT), polyclonal anti-c-*myc* sera; IL-2, monoclonal anti-interleukin-2 serum.

had no effect on SV40 DNA replication, either when preincubated with c-*myc* antibodies or when added alone. Therefore, an effort was then made to confirm the preceding observations by using antibodies that were prepared independently. We tested three different sera (Table 1): (i) polyclonal JFT serum prepared against recombinant human c-*myc* protein expressed in insect cells (14); (ii) a polyclonal serum raised against synthetic peptides of human c-*myc* protein, designated polyclonal RC (13); and (iii) the monoclonal antibody IF7 prepared against a c-*myc* synthetic peptide (13). By using the same in vitro SV40 DNA replication system described above, there was no replication inhibition of pSVori DNA (Fig. 1C and Table 1), endogenous SV40 chromatin (Fig. 1C and Table 1), or purified SV40 chromatin (data not shown) by any of these three antibodies.

Effect of c-*myc* antibodies on DNA polymerase α and δ activities. To determine whether the inhibition of in vitro DNA replication observed with some c-*myc* antibody preparations was due to inhibition of DNA polymerase activity, cellular DNA that was activated as a primer template for DNA polymerase by limited digestion with pancreatic DNase I was added to the permissive cell extract in place of the intact SV40 DNA substrates described above. Preincubation of permissive cell extracts with any one of the c-*myc* antibodies that inhibited in vitro SV40 DNA replication (polyclonal RW and monoclonal F5 and C3) also inhibited DNA synthesis on activated DNA to the same extent as that observed for DNA replication in SV40 chromosomes (Table 1). For each of these antisera, replication of SV40 DNA or pSVori was inhibited 90% by 80 μg of IgG per ml (Fig. 1 and Table 1), while DNA replication in viral chromosomes and DNA synthesis on DNase I-digested DNA was inhibited 90% by 20 μg of IgG per ml (Table 1). At least 95% of the DNA synthesis on activated DNA could be inhibited in our in vitro system by 20 μM aphidicolin (a specific inhibitor of DNA polymerases α and δ [4, 11], and 75% of the activity could be inhibited by 20 μM butylphenyl-dGTP (a specific inhibitor of DNA polymerase α [3, 11]). Therefore, these c-*myc* antibody preparations inhibited DNA polymerases α and δ, consistent with similar results reported by Studzinski et al. (17). However, neither polyclonal JFT and RC nor monoclonal IF7 had any inhibitory effect on DNA synthesis by DNA polymerase α or δ on activated DNA (Table 1).

Separation of c-*myc* antibodies from an inhibitor of DNA synthesis. No correlation was observed between the ability of an antibody preparation to recognize c-*myc* protein and its ability to inhibit DNA synthesis. By using similar concentrations of IgG, all of the c-*myc* antibody preparations used in the present study, except monoclonal F5 (which did inhibit DNA synthesis), reacted with recombinant c-*myc* protein expressed in insect cells as determined by immunoblotting (Table 1). Furthermore, polyclonal JFT (which did not inhibit DNA synthesis) and polyclonal RW (which did inhibit DNA synthesis) had equivalent titers against c-*myc* protein as measured in a radioimmunoassay with ^{125}I-labeled-c-*myc* protein (described below). This discrepancy between the reactivity of each antibody toward c-*myc* protein and the ability of the antibody to inhibit DNA synthesis raised the question of whether the antibody and inhibition activities were one and the same.

To determine whether the DNA polymerase inhibition activity present in polyclonal RW copurifies with the antibody against c-*myc*, polyclonal RW was repurified in two ways. First, it was incubated with a 10-fold excess of immobilized *S. aureus* protein A (Pansorbin; Calbiochem-Behring) extensively washed with 50 mM Tris hydrochloride, pH 7.4. The protein A-antibody complex was then removed by centrifugation, and the supernatant was assayed for its ability to inhibit DNA polymerase activity on activated DNA (as described in Table 1) and to detect c-*myc* protein as determined by a radioimmunoassay. Briefly, human c-*myc* protein was extracted from the nuclei of infected insect cells (14) and purified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, followed by elution of the c-*myc* band and concentration by spin filtration (Amicon Corp.). The protein was labeled by iodination with ^{125}INa (Amersham Corp.) by using Iodobeads (Pharmacia, Inc.) to a specific activity of 4×10^3 to 6×10^3 cpm/pmol. A standard curve for c-*myc* antibody was determined by incubating serial dilutions of a known concentration of polyclonal JFT with a uniform amount of ^{125}I-labeled c-*myc* protein in RIPA buffer (14) overnight at 4°C. The percent precipitable counts were determined after the addition of a 10-fold excess of Pansorbin. The amount of c-*myc* antibody in polyclonal RW was obtained by incubating different dilutions of the serum with ^{125}I-labeled c-*myc* protein and comparing the percent precipitable counts with the standard curve. Surprisingly, the supernatant fraction obtained after protein A precipitation contained all of the DNA polymerase inhibitor, but as expected, none of the c-*myc* antibody was present (Fig. 2).

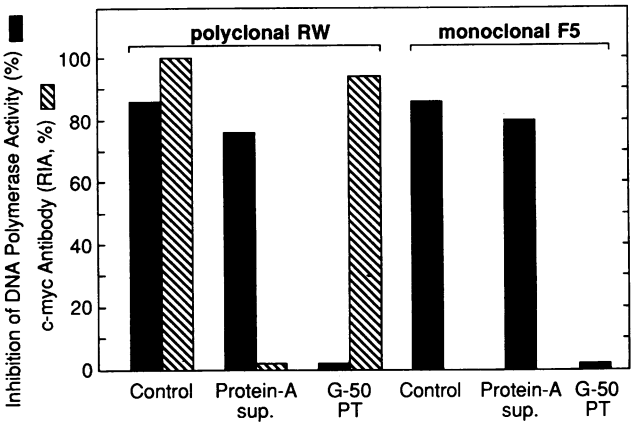

FIG. 2. Separation of DNA polymerase inhibitor from c-*myc* antibodies. Polyclonal RW and monoclonal F5 were treated either with immobilized protein A or by gel filtration through Sephadex G-50 as described in the text. Samples were analyzed for the ability to inhibit DNA polymerase activity on activated DNA (as described in Table 1, footnote *b*) and for the amount of c-*myc* antibody by radioimmunoassay. Control, No treatment; Protein-A sup., fraction not bound to immobilized protein A; G-50 PT, pass-through of Sephadex G-50 column. No c-*myc* reactivity could be detected in the preparation of monoclonal F5 antibody.

A second sample of polyclonal RW was fractionated by gel filtration through Sephadex G-50 equilibrated in 50 mM Tris hydrochloride, pH 7.4. The pass-through was recovered by centrifugation of the column. Again, the c-*myc*-reactive material, which was recovered in the pass-through, was separated from the DNA polymerase inhibitor, which remained in the included volume (Fig. 2). Analogous results were obtained with monoclonal antibody F5, except that the level of c-*myc* antibody was too low to quantitate. We also attempted to precipitate the DNA polymerase inhibition activity by treatment of polyclonal RW with antirabbit IgG and monoclonal F5 with antimouse IgG. Neither treatment diminished the level of inhibitor remaining in the supernatant

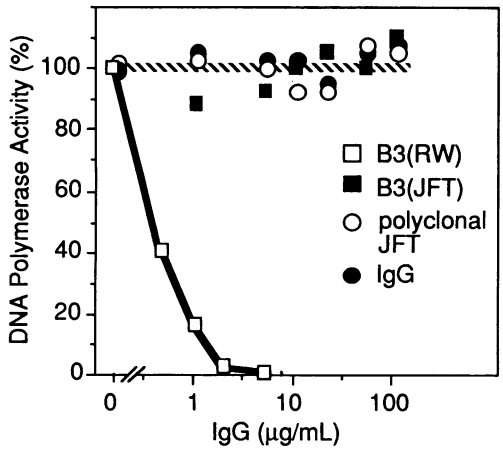

FIG. 3. Effect of c-*myc* antibody preparations after purification on DNA polymerase activity. Extracts of CV-1 cells preincubated with different concentrations of purified IgG were tested for DNA synthesis on activated DNA as described in Table 1. All antibody preparations [except monoclonal B3(RW)] were extensively dialyzed against Tris hydrochloride, pH 7.4, and protein concentrations of the dialyzed samples were determined by the Bradford procedure (1). B3(RW), Monoclonal anti-c-*myc* (provided by G. Studzinski); B3(JFT), monoclonal B3 purified independently (by J. Farrell-Towt); polyclonal JFT, as described in Table 1; IgG, control monoclonal anti-interleukin-2 antibody as described in Table 1.

fraction (data not shown). In comparison, antimouse IgG precipitated monoclonal antibody SJK 132 directed against human DNA polymerase α under the same conditions and thus removed this DNA polymerase inhibitor from solution. These data demonstrate that DNA polymerase inhibition activity and c-*myc* antibodies could be readily separated.

All of the c-*myc* antibodies reported to inhibit DNA synthesis (17) had been purified by affinity chromatography through the same protein A-Sepharose column (R. Watt, personal communication). Therefore, polyclonal RW and monoclonal F5 and C3 may have been inadvertedly contaminated during their purification by an unidentified inhibitor of DNA polymerases α and δ. To test this hypothesis, a sample of another c-*myc* monoclonal antibody, B3, that was exceptionally inhibitory in DNA synthesis assays (17) was kindly provided by G. Studzinski [B3(RW)] and the original B3 hybridoma medium was kindly provided by R. Watt. As expected, B3(RW) was more effective at inhibiting DNA polymerase activity on activated DNA (50% inhibitory concentration < 1 μg/ml; Fig. 3) than were the other antibodies that we tested (Fig. 1A and Table 1). This monoclonal antibody was purified from its hybridoma medium in the same way that polyclonal JFT was purified. Briefly, the IgG was purified by protein-A Sepharose affinity chromatography by using MAPS II buffers (Bio-Rad Laboratories) and concentrated by precipitation in 50% ammonium sulfate, followed by resolubilization in phosphate-buffered saline and extensive dialysis against 10 mM Tris hydrochloride, pH 7.4. After this purification protocol, monoclonal B3(JFT) no longer inhibited DNA polymerase activity (Fig. 3), SV40 DNA replication (data not shown), or DNA synthesis in isolated nuclei from CV-1 or HL-60 cells (data not shown). Monoclonal B3(RW), monoclonal B3(JFT), and polyclonal JFT were equally effective at recognizing human c-*myc* protein (Table 1).

Our observations are consistent with those of Kaczmarek et al. (10) that microinjection of another monoclonal antibody directed against c-*myc* protein (expressed in *Escherichia coli*) into the nuclei of cultured fibroblasts does not prevent entry of cells into the S phase, while injection of antibodies against DNA polymerase α does inhibit. In addition, we found that high concentrations of a polyclonal sera against c-*fos* protein, another nuclear proto-oncogene, also did not interfere with initiation or continuation of SV40 DNA replication (Table 1). These observations suggest that neither c-*myc* nor c-*fos* proteins are required for DNA synthesis at SV40 or cellular replication forks and that neither c-*myc* nor c-*fos* proteins are required for initiation of SV40 DNA replication. However, the question of whether these proteins are required for initiation of cellular DNA replication needs further investigation.

In summary, the results presented here demonstrate that the previously reported inhibition of DNA synthesis by some antibodies directed against c-*myc* (17) resulted from the presence of an unidentified inhibitor of DNA polymerases α and δ and not from the action of c-*myc* antibodies. The c-*myc* antibodies could be separated from the DNA synthesis inhibitor either by precipitating the antibodies with protein A or by size exclusion chromatography through Sephadex G-50. Furthermore, two polyclonal sera and one monoclonal antibody prepared independently did not inhibit SV40 DNA replication in vitro or DNA synthesis on activated DNA, although they did recognize c-*myc* protein as effectively as those used by Studzinski et al. (17). Finally, when the c-*myc* antibody preparation that was most effective in blocking DNA synthesis, monoclonal B3(RW), was purified from hybridoma medium in our laboratory, it failed to inhibit DNA synthesis. Our results emphasize that caution must be exercised in the preparation of antibodies that are to be tested as inhibitors of DNA replication. Although we have not identified the DNA synthesis inhibitor present in some c-*myc* antibody preparations, DNA synthesis by DNA polymerases α and δ, enzymes strongly implicated in cellular and viral DNA replication and repair, is very sensitive to Cl^-, divalent metal chelators, and sulfhydryl-reactive agents as well as a variety of other chemicals (7).

We wish to thank George Studzinski and Rosemary Watt for their gracious cooperation and insightful comments during the course of this study and Paul Wassarman for suggesting the gel filtration experiment.

This work was supported by Hoffmann-La Roche, Inc.

ADDENDUM IN PROOF

While this paper was in press, a role for the c-*myc* product in the promotion of cellular DNA replication was suggested by Iguchi-Ariga et al. (S. M. M. Iguchi-Ariga, T. Itani, Y. Kiji, and H. Ariga, EMBO J. 6:2365–2371, 1987).

LITERATURE CITED

1. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. 72:248–254.
2. **Curran, T., C. Van Beveren, N. Ling, and I. M. Verma.** 1985. Viral and cellular *fos* proteins are complexed with a 39,000-dalton cellular protein. Mol. Cell. Biol. 5:167–172.
3. **Decker, R. S., M. Yamaguchi, R. Possenti, M. K. Bradley, and M. L. DePamphilis.** 1987. In vitro initiation of DNA replication in simian virus 40 chromosomes. J. Biol. Chem. 262:10863–10872.
4. **Decker, R. S., M. Yamaguchi, R. Possenti, and M. L. DePamphilis.** 1986. Initiation of simian virus 40 DNA replication in vitro: aphidicolin causes accumulation of early-replicating intermediates and allows determination of the initial direction of

DNA synthesis. Mol. Cell. Biol. **6**:3815–3825.
5. **DePamphilis, M. L., and M. K. Bradley.** 1986. Replication of SV40 and polyoma virus chromosomes, p. 99–246. *In* N. P. Salzman (ed.), The *Papovaviridae*, vol. 1. Plenum Publishing Corp., New York.
6. **DePamphilis, M. L., and P. M. Wassarman.** 1980. Replication of eukaryotic chromosomes: a close-up of the replication fork. Annu. Rev. Biochem. **49**:627–666.
7. **Fry, M., and L. A. Loeb.** 1986. Animal cell DNA polymerases. CRC Press, Inc., Boca Raton, Fla.
8. **Hay, R. T., E. A. Hendrickson, and M. L. DePamphilis.** 1984. Sequence specificity for the initiation of RNA-primed simian virus 40 DNA replication *in vivo*. J. Mol. Biol. **175**:131–157.
9. **Iguchi-Ariga, S. M. M., T. Itani, M. Yamaguchi, and H. Ariga.** 1987. c-*myc* protein can be substituted for SV40 T antigen in SV40 DNA replication. Nucleic Acids Res. **15**:4889–4899.
10. **Kaczmarek, L., M. R. Miller, R. A. Hammond, and W. E. Mercer.** 1986. A microinjected monoclonal antibody against human DNA polymerase-α inhibits DNA replication in human, hamster, and mouse cell lines. J. Biol. Chem. **261**:10802–10807.
11. **Lee, M. Y. W. T., N. L. Toomey, and G. E. Wright.** 1985. Differential inhibition of human placental DNA polymerases δ and α by BuPdGTP and BuPdATP. Nucleic Acids Res. **13**: 8623–8630.
12. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
13. **Miyamoto, C., R. Chizzonite, R. Crowl, K. Rupprecht, R. Kramer, M. Schaber, G. Kumar, M. Poonian, and G. Ju.** 1985. Molecular cloning and regulated expression of the human c-myc gene in *Escherichia coli* and *Saccharomyces cerevisiae*: comparison of the protein products. Proc. Natl. Acad. Sci. USA **82**: 7232–7236.
14. **Miyamoto, C., G. E. Smith, J. Farrell-Towt, R. Chizzonite, M. D. Summers, and G. Ju.** 1985. Production of human c-*myc* protein in insect cells infected with a baculovirus expression vector. Mol. Cell. Biol. **5**:2860–2865.
15. **Smith, G. E., G. Ju, B. L. Ericson, J. Moschera, H.-W. Lahm, R. Chizzonite, and M. D. Summers.** 1985. Modification and secretion of human interleukin-2 produced in insect cells by a baculovirus expression vector. Proc. Natl. Acad. Sci. USA **82**: 8404–8408.
16. **Stahl, H., P. Droge, and R. Knippers.** 1986. DNA helicase activity of SV40 large tumor antigen. EMBO J. **5**:1939–1944.
17. **Studzinski, G. P., Z. S. Brelvi, S. C. Feldman, and R. A. Watt.** 1986. Participation of c-myc protein in DNA synthesis of human cells. Science **234**:467–470.
18. **Sullivan, N., C. Green, M. Pasdar, and R. Watt.** 1986. Characterization and nuclear localization of the v- and c-myc proteins. Curr. Top. Microbiol. Immunol. **132**:355–361.
19. **Watt, R. A., A. R. Shatzman, and M. Rosenberg.** 1985. Expression and characterization of the human c-*myc* DNA-binding protein. Mol. Cell. Biol. **5**:448–456.
20. **Yamaguchi, M., and M. L. DePamphilis.** 1986. DNA binding site for a factor(s) required to initiate simian virus 40 DNA replication. Proc. Natl. Acad. Sci. USA **83**:1646–1650.
21. **Yamaguchi, M., E. A. Hendrickson, and M. L. DePamphilis.** 1985. DNA-primase-DNA polymerase α from simian cells: modulation of RNA primer synthesis by ribonucleotide triphosphates. J. Biol. Chem. **260**:6254–6263.