Journal of Cellular Biochemistry 33:27–37 (1987)

Intracellular Signalling of Initiation of DNA Synthesis: Effect of Cell-Free Extracts From Anti-Receptor-Stimulated B Lymphocytes on Spleen Cell Nuclei

Sibghat Ullah and Milton Kern

Laboratory of Chemistry, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892

To study the relatively late intracellular signals involved in the proliferative response of B lymphocytes to antibodies specific for surface membrane immunoglobulins, extracts from antibody activated cells were mixed with *Xenopus laevis* splenic nuclei, and the incorporation of thymidine 5′-triphosphate into DNA was assessed. The slight incorporation observed with either nuclei or extract alone was markedly enhanced upon mixing the two entities when the extract was derived from cells cultured with but not without anti-receptor antibody. The appearance of active extract correlated well with the culture requirements necessary for the induction of B lymphocyte proliferation and, as revealed by time course studies, the active component arises relatively late in the activation process. Moreover, the appearance of active extracts is independent of DNA synthesis but is dependent on protein synthesis as judged from studies with metabolic inhibitors. Appropriate homogenization of activated cells yielded nuclei and cytoplasm with 85% of the activity confined to nuclei. In addition, purified active extracts exhibited DNA binding although the active component was readily distinguishable from polymerase α by chromatographic techniques. It is tentatively concluded that the active component represents either some replication protein other than polymerase or some earlier signal necessary to induce the formation or utilization of replicating proteins.

Key words: splenocyte nuclei, DNA synthesis, anti-receptor signalling, DNA binding protein

Over the last decade several laboratories have reported that DNA synthesis could be initiated by mixing cell extracts from proliferating cells with nuclei derived from *Xenopus laevis* [1–6]. These results have been interpreted to indicate that a factor from proliferating cells was involved in signalling initiation of DNA replication. However, an alternative explanation can be constructed from the available data. Thus, it has been demonstrated that DNA polymerase α activity increased markedly

Received April 29, 1986; revised and accepted September 15, 1986.

during cell growth [7]. In prior studies [1–6], the effect of mixing cell extracts from growing cells with *Xenopus* nuclei was measured on the basis of the incorporation of labeled thymidine monophosphate into DNA. Therefore, it could be reasonably argued that the marked enhancement of DNA synthesis was simply due to a cell growth induced increase in DNA polymerase α activity rather than due to a novel signal leading to initiation of DNA synthesis.

In this regard we have employed the technique of mixing B cell extracts with *Xenopus* nuclei to study the intracellular entities involved in the induction of DNA synthesis within B lymphocytes activated with anti-receptor antibody. This report characterizes some of the cell culture conditions and requirements necessary for the appearance of the active component in cell extracts. We also provide evidence that the activity of the component within cell extracts responsible for enhanced DNA synthesis is distinguishable from DNA polymerase activity. Lastly, we show that the component derived from activated B cells is localized within the nucleus and that this component exhibits DNA binding properties as well.

METHODS

Reagents

Dithiothreitol was purchased from Bethesda Research Laboratories, Inc. (Bethesda, MD). The following compounds were obtained from the Sigma Chemical Co. (St. Louis, MO): pyruvate kinase, phosphoenolpyruvate, adenosine 5′-triphosphate, dextran (2000), Brij 58, medium 199, actinomycin D, cycloheximide, and puromycin. Fluorodeoxyuridine was obtained from Calbiochem (San Diego, CA). Pharmacia, Inc. (Piscataway, NJ), supplied compounds as follows: deoxycytidine 5′-triphosphate, deoxyadenosine 5′-triphosphate, deoxyguanosine 5′-triphosphate, DNA-cellulose, and G-100 Sephadex. ^{3}H-Thymidine 5′-triphosphate was a product of New England Nuclear (Boston, MA), DEAE-cellulose (DE 52) was provided by Whatman, Maidstone (Kent, England), and fetal calf serum was purchased from Hyclone Laboratories (Logan, Utah).

Animals

Six to twelve-week-old mice (C3H/HeN) were obtained from Charles River Laboratories, Inc., (Stoneridge, NY) or the National Cancer Institute-Frederick Cancer Research Facility (Frederick, MD).

Preparation and Culturing of Cells

Frog (*Xenopus laevis*) as well as mouse (strain C3H/HeN) spleens were teased apart with wire brushes on a wire screen [8] and washed thru the screen with medium 199. *Xenopus laevis* cells were washed and stored in medium 199 preparatory to isolation of nuclei as described below. After being washed with medium 199, mouse cells were resuspended in cell culture medium composed of medium 199 supplemented with fetal calf serum to 10% and mercaptoethanol to 5×10^{-5} M. The murine B lymphocytes were activated by incubating the splenocytes, for the time intervals indicated, in cell culture medium containing 5 μg/ml of antibody with specificity for mouse IgG covalently linked to Sepharose [9]. After incubation, Sepharose-anti-mouse IgG was removed by passing the culture thru a thin layer of glass wool, and the cells were harvested by centrifugation at 500g for 5 min.

Preparation of Sub-Cellular Fractions

Cell-free extracts of mouse spleen cells were prepared by homogenizing cells in a hypotonic solution [4].

Xenopus laevis nuclei were prepared from cells using a procedure employing Brij 58 [10]. The procedure was performed at 3°C throughout. Cells (10^8) were washed in a solution containing 150 mM sucrose, 5 mM $CaCl_2$, and 25mM HEPES at pH 8.0. Following centrifugation at 500g for 5 min, the pellet was suspended in 1.0 ml of a solution containing 250 mM sucrose, 5 mM $CaCl_2$, and 25 mM HEPES at pH 8.0. After standing for 3 min in an ice bath, 1.0 ml of a solution containing 150 mM sucrose, 5 mM $CaCl_2$, 25 mM HEPES at pH 8.0, and 0.5% Brij 58 was added. The resulting mixture was agitated for 5 min in an ice bath, then diluted by adding 10 ml of a solution containing 250 mM sucrose, 5 mM $CaCl_2$, 25 mM HEPES at pH 8.0, and 2% dextran. Following centrifugation at 3,000g for 15 min, the pelleted nuclei were washed once with the last mentioned solution yielding about a 60% recovery of nuclei by microscopic count as calculated from the starting number of cells.

To study the intracellular distribution of the active component present in cell-free extracts, mouse cells were separated into cytoplasm and nuclei. Cultured cells were pelleted at 500g for 10 min, suspended in 0.25 M sucrose-0.0025 M $CaCl_2$ and immediately centrifuged (500g for 10 min). The pelleted materials were resuspended in homogenizing medium, subjected to 50 strokes in a glass-teflon homogenizer, and the nuclei and cytoplasm were separated by centrifugation at 500g for 10 min. The cytoplasm was dialyzed against a solution composed of 100 mM sucrose, 10 mM KCl, 0.5 mM $MgCl_2$, 0.5 mM dithiothreitol, and 20 mM HEPES at pH 7.8 and then clarified by centrifugation at 100,000g for 1 h. The nuclei were washed with 250 mM sucrose-2.5 mM $CaCl_2$, then washed and suspended in a hypotonic buffer composed of 20 mM HEPES at pH 7.8, 5 mM KCl, 0.5 mM $MgCl_2$, and 0.5 mM dithiothrietol. After standing in an ice bath for 10 min, the nuclei were homogenized by using 10 strokes in a loose fitting Dounce homogenizer. The homogenate was centrifuged at 3000g for 10 min, and the supernatant was adjusted to contain 100 mM sucrose and 10 mM KCl. Finally, the supernatant was centrifuged at 100,000g for 60 min to remove particulate matter.

Fractionation of extracts on DNA-cellulose was performed as described previously [11]. DNA-cellulose columns were washed with 10 bed volumes at 3°C with a buffer containing 50 mM NaCl, 1.0 mM EDTA, 1.0 mM dithiothreitol, 10% glycerol, 100 μg/ml bovine serum albumin, and 20 mM Tris-HCl at pH 7.4. An extract sample in the same solution as used to equilibrate the column was applied to a 1.0 ml bed volume column of DNA-cellulose. The column was washed with 7–8 bed volumes of the buffer used for column equilibration at a flow rate of 2.0 ml/h. A step gradient of NaCl covering the range 0.05 to 2.0 M in the equilibrating buffer was then applied to the column to elute the bound proteins. The harvested fractions were dialyzed against 20 mM HEPES at pH 7.8, 0.5 mM $MgCl_2$, 0.5 mM dithiothreitol, 100 mM sucrose, and 10 mM KCl prior to assay. Columns were separately prepared with single-stranded and double-stranded DNA associated with cellulose. Cellulose columns free of DNA were used to assess non-specific binding of active extracts.

Measurement of DNA Synthesis

The assay for DNA synthesis using isolated nuclei and cell-free extracts of activated cells was performed as described previously [4] except that the assay was

performed in 10 × 75 mm glass tubes, and the reaction was terminated by adding an equal volume of 10% TCA and 200 μg of bovine serum albumin. The pellets were washed twice with 5% TCA and then dissolved in 200 μl of 2% sodium dodecylsulfate and the ^{3}H-thymidine 5′-triphosphate incorporated into TCA precipitable materials assessed. Polymerase α, β, and γ were determined as previously described [12] except that non-radioactive thymidine 5′-triphosphate was omitted from the reaction mixtures and 3 μCi ^{3}H-thymidine 5′-triphosphate (80 Ci/mmole) was employed.

RESULTS

Correlation of Anti-Receptor-Mediated B Cell Activation With the Appearance of Active Cell Extracts

The time course of appearance of activity in cell extracts capable of inducing stimulation of DNA synthesis upon incubation with *Xenopus laevis* nuclei is shown in Figure 1. Activity in cell extract preparations was initially detected at 36 h and was near maximal at 48 h after incubation of murine splenocytes with Sepharose-anti-mouse IgG. Cells cultured without Sepharose-anti-mouse IgG exhibited only a slight increase in extract activity over the same time course presumably as a consequence of mitogens present in the fetal calf serum with which the culture medium routinely was supplemented. Although there was a substantially greater activity detected with cells cultured with Sepharose-anti-mouse IgG in comparison to cells cultured without

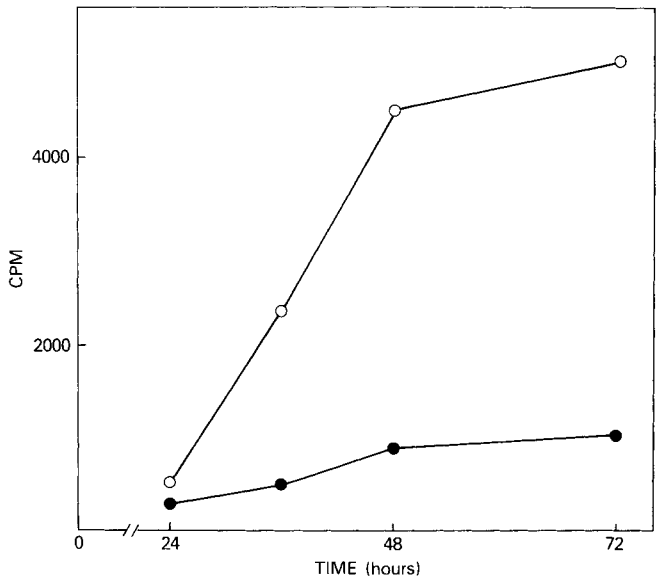

Fig. 1. Anti-immunoglobulin mediated B cell activation in relation to the time course of appearance of DNA synthesis enhancing activity. Mouse spleen cells in culture medium without and with Sepharose-anti-mouse IgG (5.0 μg/ml as antibody) were incubated for the indicated time intervals and then processed so as to prepare cell free extracts as described in Methods. Approximately 2 × 10^8 to 4 × 10^8 cells were used to obtain each time point. The extracts were mixed with *Xenopus laevis* nuclei and the incorporation of ^{3}H-thymidine 5′-triphosphate assessed. ●——●, cells cultured with Sepharose-anti-mouse IgG: ○——○, cells cultured without Sepharose-anti-mouse IgG.

this antibody, a difference in activity was not attributable to a possible differential degradation of thymidine 5′-triphosphate because the recovery of thymidine 5′-triphosphate at the end of the reaction was indistinguishable in both cases.

The correction for DNA synthesis by cell extract incubated alone was somewhat variable from experiment to experiment. However, as can be seen in Table I, batch treatment of the crude extract with DEAE-cellulose essentially eliminated this blank. In addition, the incorporation of ^{3}H-thymidine 5′-triphosphate, after applying the correction, was indistinguishable whether crude extract or DEAE-cellulose treated extract was used.

It has been demonstrated that cells, briefly treated with Pronase prior to cell culture, exhibited enhanced proliferative responsiveness to anti-immunoglobulins [9,13,14]. Furthermore, although murine lymphocytes respond readily to anti-mouse IgM they are essentially nonresponsive to anti-mouse IgG but acquire responsiveness subsequent to cell treatment with Pronase. The data of Table II establish that only Pronase-treated cells cultured with anti-mouse IgG yielded extracts which induced incorporation of ^{3}H-thymidine 5′-triphosphate in the presence of nuclei. Moreover,

TABLE I. Effect of DEAE-Cellulose Batch Treatment on Extract Activity*

Components added	^{3}H-Thymidine 5′-triphosphate incorporation (cpm)	
	Uncorrected	Corrected[a]
Extract (crude)	5,790	—
Extract (DEAE)	270	—
Nuclei	1,420	—
Nuclei + Extract (crude)	19,540	12,280
Nuclei + Extract (DEAE)	14,100	12,360

*The procedure for determining ^{3}H-thymidine 5′-triphosphate incorporation into DNA is described in the legend to Figure 1 and Methods. The crude extract was dialyzed to equilibrium with 0.4 M phosphate, pH 7.5. For every 5.0 mg of extract protein the equivalent of 0.3 ml of DEAE-cellulose as bed volume in 0.4 M phosphate, pH 7.5 was added. The mixture was centrifuged to recover DEAE-treated extract.
[a]Corrected for the sum of cpm of nuclei alone and extract (crude) or extract (DEAE) alone.

TABLE II. Effects of Prior Treatment of Cells with Pronase on the Appearance of Activity in Extracts after Cell Culture with Goat Antimouse IgG*

Initial cell treatment	^{3}H-Thymidine 5′-triphosphate incorporation in the presence of nuclei (cpm)[a]	^{3}H-Thymidine incorporation into cells (cpm)[b]
None	0[c]	0[c]
Pronase	5,020	13,560

*Four hundred million splenocytes in 40 ml of medium 199 incubated for 1.0 h at 37°C without or with 60 mg of crude Pronase. The cells were washed twice with medium 199 supplemented with fetal bovin10% and cultured with anti-mouse IgG. The cultured cells were used to prepare either cell extracts or to determine ^{3}H-thymidine incorporation into DNA of cells. The cell culture procedures and assay conditions are described in Methods.
[a]These values have been corrected both for the incorporation by nuclei alone plus cell extract alone (1,080).
[b]These values have been corrected for cpm incorporated by cells incubated without anti-mouse IgG (3,740 cpm).
[c]Indistinguishable from controls.

assessment of the incorporation of ^{3}H-thymidine into cells showed that only the Pronase-treated cells exhibited a proliferative response.

Characteristics of the Cell Extract

The intracellular site at which the active component accumulates was determined for cells previously cultured with Sepharose-anti-mouse IgG since mouse lymphocytes proliferate in response to insolubilized but not to soluble anti-mouse IgG. After homogenization, the nuclei and cytoplasm were separated, the nuclei were lysed, and the resultant nuclear lysate as well as the cytoplasmic fraction were tested for their ability to stimulate DNA synthesis in the presence of *Xenopus laevis* nuclei. The data of Table III show that the active component derived from murine B lymphocytes was largely confined to the nucleus of such cells. It should be noted that in other experiments the activation observed upon addition of cytoplasmic and nuclear extract in combination was additive. For example, in a representative experiment, the thymidine 5′-triphosphate incorporated was 8,200 cpm for the combination and 8,570 cpm for the sum of the two fractions added separately.

The effect of culturing cells in the presence of metabolic inhibitors on the appearance of the active component in cell extracts was assessed (Table IV). Fluorodeoxyuridine, a potent inhibitor of DNA synthesis in such cells [15] was without

TABLE III. Intracellular Distribution of the Extract Component Stimulating DNA Synthesis*

Source of the cell extract component	^{3}H-Thymidine 5′-triphosphate incorporation in the presence of *Xenopus* splenic nuclei[a] (cpm)
Cytoplasm	1,430
Nucleus	7,900

*Murine splenocytes were cultured for 72 hr with anti-mouse IgG covalently linked to Sepharose, the harvested cells were homogenized, and cytoplasm and nuclei were isolated as described in Methods.
[a]These values have been corrected for the sum of the incorporation by nuclei lone plus that of cell extract component alone. This correction was 610 and 700 cmp in the case where cytoplasm-derived and nucleus-derived extract was used, respectively.

TABLE IV. Effect of Culturing Cells in the Presence of Metabolic Inhibitors on the Appearance of the Active Component in Extracts*

Metabolic inhibitor added	^{3}H-Thymidine 5′-triphosphate incorporation in the presence of *Xenopus* splenic nuclei[a] (cpm)
None	6,350
Fluorodeoxyuridine	6,570
Actinomycin D	960
Cycloheximide	1,390
Puromycin	2,060

*Cells (4×10^6/ml) were cultured in the presence of Sepharose-anti-mouse IgG (5.0 μg/ml as antibody) for 24 hr and, where indicated, the inhibitors were added to achieve a final concentration of 20 μg/ml fluorodeoxyuridine, 10^{-4} M puromycin, 10^{-3} M cycloheximide and 5.0 μg/ml actinomycin D. At 60 hr, the Sepharose-anti-mouse IgG was removed, the cells were washed, cell-free extracts were prepared as described in Figure 1 and Methods.
[a]The values shown have been corrected for the sum of the cpm incorporated by nuclei plus that of cell extract alone. This correction averaged 500 cpm and ranged between 450 and 620 cpm.

effect, whereas puromycin, cycloheximide and actinomycin D markedly inhibited the appearance of the cell extract factor.

Inasmuch as synchronized cells exhibit increases in polymerase α activity prior to cell division [7], we determined the rate of appearance of polymerase α in cell extracts subsequent to culturing B cells with Sepharose-anti-mouse IgG. The polymerase α activity assessed with activated DNA, was compared to DNA synthesis activity measured in the presence of *Xenopus laevis* nuclei (Fig. 2). The two activities followed the same time course. By contrast, polymerase β and γ were essentially unchanged over the time course studied (data not shown).

To determine whether the polymerase α activity in cell-free extracts accounted for the initiation of DNA synthesis when the *Xenopus* nuclei assay was used; we subjected the mouse cell-free extracts to partial purification and then separately assessed the polymerase α activity and the DNA synthetic activity in the presence of nuclei. The ^{3}H-thymidine 5′-triphosphate incorporation determined in the presence of activated DNA at pH 7.2 was taken as a measure of polymerase α, since it was essentially completely inhibited by the presence of N-ethylmaleimide.

The mouse cell-free extract was separated on Sephadex G-100 (Fig. 3) into four arbitrary fractions and analyzed for activity on activated DNA and on *Xenopus* nuclei (Table V). It is clear that 95% of the polymerase α activity (measured with activated DNA) was in fraction I while $< 11\%$ of the activity observed with nuclei was found in this fraction. On the other hand, 83% of the activity observed with nuclei was found in fraction III, while this fraction exhibited $< 5\%$ of the polymerase α activity.

The two activities could also be separated to some extent upon DEAE-cellulose chromatography (Table VI). For example, while only 7% of the recovered polymerase

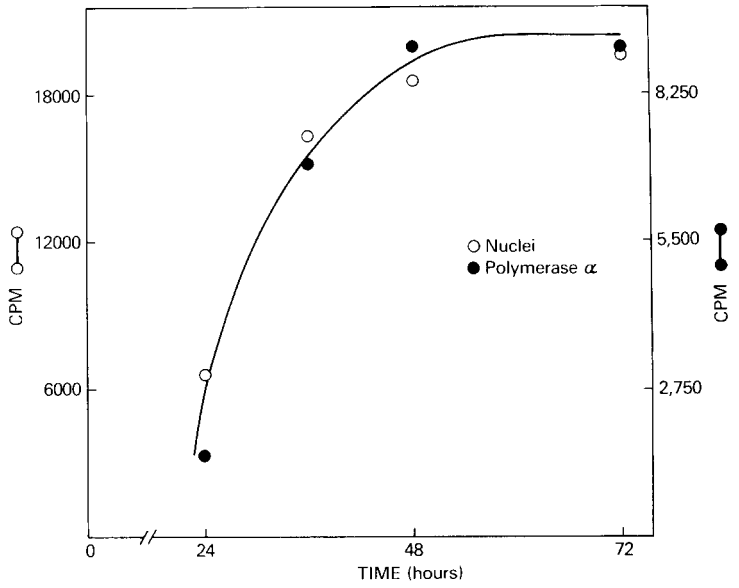

Fig. 2. Correlation between nuclei mediated enhanced DNA synthesis and polymerase activity of activated mouse B cell-free extracts. Mouse spleen cells were cultured and cell-free extracts prepared at the indicated time intervals. Polymerase activity and ^{3}H-thymidine 5′-triphosphate incorporation in the presence of nuclei was then determined. The procedures are described in the legend to Figure 1 and in Methods.

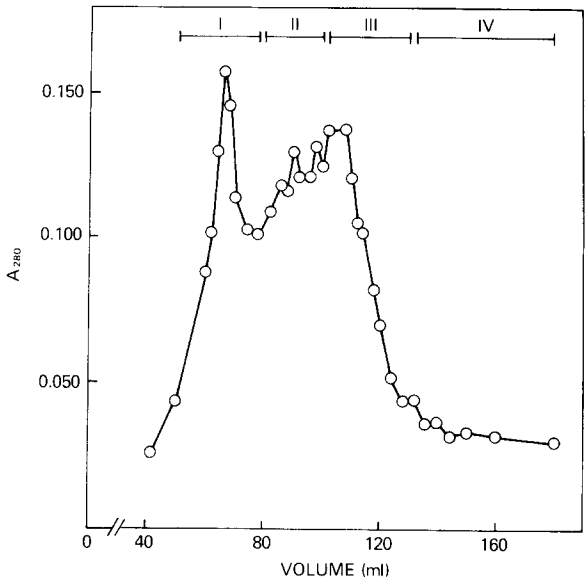

Fig. 3. Sephadex G-100 chromatography of crude cell extracts. Cells were cultured for 72 h with Sepharose-anti-mouse IgG, and the cells were lysed to prepare crude extracts. The extract was equilibrated with 0.4 M phosphate buffer, pH 7.5, and passed thru a DEAE-cellulose column equilibrated with the same buffer. The protein solution passing thru the column was concentrated in vacuo using a collodian sac and the dialyzed against buffered saline (0.02 M phosphate buffer, pH 7.5–0.15 M NaCl) and applied to a G-100 column (93 × 1.5 cm) equilibrated with buffered saline. The indicated column fractions were concentrated in vacuo as described above and then dialyzed against the buffer used to prepare cell extracts.

TABLE V. Activity of Sephadex G-100 Fractions*

Column fraction	^{3}H-Thymidine 5′-triphosphate incorporation (cpm) in the presence of	
	Nuclei	Activated DNA
I	2,910	33,020
II	1,560	0[a]
III	22,600	1,540
IV	180	350

*The column fractions, concentrated by vacuum dialysis, were separately assayed for thymidine 5′-triphosphate incorporation into DNA in the presence of nuclei and in the presence of activated DNA as described in Methods. The values shown are corrected for the contribution by nuclei alone plus column fraction alone.

[a]Indistinguishable from the radioactivity incorporated by nuclei alone plus column fraction alone.

α activity was eluted with 0.15 M KCl some 45% of the activity observed with the nuclei assay was found in this fraction.

Fraction III derived from G-100 chromatography was tested for its capacity to bind to both native and to denatured DNA. It can be calculated from the recovered activity that 70–75% was adherent to either native or to denatured DNA-cellulose. About 25% of the column adherent materials could be eluted with 0.2 M NaCl, and the remainder with 2.0 M NaCl. In other experiments (not shown) some activity was

TABLE VI. Partial Separation of Nuclei-Dependent and Activated DNA-Dependent Extract Activity by DEAE-Cellulose Chromatography*

Column fraction	^{3}H-Thymidine 5′-triphosphate incorporation (cpm) in the presence of	
	Nuclei	Activated DNA
Applied sample	97,140	33,800
0.02 M PO_4	0[a]	0[a]
0.02 M PO_4-0.15 M KCl	45,830	1,950
0.02 M PO_4-0.4 M KCl	56,210	25,050

*A cell extract was adjusted to 0.4 M phosphate, pH 7.5 and passed thru a DEAE-cellulose column as described in Figure 3. After concentration and then dialysis against 0.02 M phosphate (PO_4) pH 7.5 the column was washed with two bed volumes of the indicated solutions. These solutions were then concentrated and dialyzed against the assay buffer. Fifty μl samples in triplicate were assayed in the presence of either nuclei or activated DNA.
[a]Indistinguishable from appropriate controls.

TABLE VII. Binding of the active component to DNA-cellulose columns*

Column fraction	Activity of fractions (cpm) derived from cellulose columns containing		
	Native DNA	Denatured DNA	No DNA
Not absorbed	4,470	5,720	12,900
0.2 M NaCl eluted	3,010	3,820	1,670
2.0 M NaCl eluted	10,690	10,060	1,610

*Fraction III (8.0 mg protein) described in Figure 2 and Table V was applied to a 1.0-ml bed volume of either native DNA-cellulose, denatured DNA-cellulose, or cellulose without DNA, and the indicated fractions were collected. Each fraction (2.0 ml) was concentrated in vacuo to approximately 0.5 ml and then tested for the incorporation of ^{3}H-thymidine 5′-triphosphate in the presence of *Xenopus laevis* nuclei.

eluted at 0.6, 1.0, and 2.0 M NaCl, thereby exhibiting a similarity to the elution patterns for DNA binding proteins reported for other cells [11].

In the presence of mouse cell-free extracts, nuclei derived from mouse spleen cells cultured for 72–96 h exhibited essentially the same results as nuclei derived from *Xenopus* except that the enhancement of DNA synthesis was only about one-fourth to one-third as great. Moreover, nuclei derived from mouse spleen cells cultured with Sepharose-anti-mouse IgG or with concanavalin A showed a 2–3 fold greater incorporation of ^{3}H-thymidine 5′-triphosphate than cells cultured without either agent.

DISCUSSION

These results show that extracts derived from B cells cultured with anti-mouse immunoglobulin contained a component or components which in the presence of *Xenopus laevis* nuclei yielded markedly enhanced incorporation of ^{3}H-thymidine 5′-triphosphate into DNA. There was a close correlation between the culture conditions required for the proliferation of cells responding to Sepharose-anti-mouse IgG and the culture conditions necessary for the appearance of active extracts upon lysis of such cells. Thus, as shown herein, Pronase-treated cells cultured with anti-mouse IgG exhibited proliferation and yielded active extracts while untreated cells cultured under

these conditions did not result in proliferation nor yield active extracts. In experiments not shown, it was observed that premature removal of Sepharose-linked anti-mouse IgG resulted in inactive extracts. It should be noted that premature removal of antibody has also been shown to inhibit proliferation [14]. Furthermore, in the presence of excess mouse immunoglobulins added to selectively block the combining sites on anti-mouse immunoglobulins there was neither proliferation observed [14] nor the appearance of active extracts. These findings are compatible with the findings of others [1–6] in which there was an association between the proliferative ability of cells and the detection of active extracts.

The active component appears to arise prior to the onset of S phase of the cell cycle as judged from the fact that fluorodeoxyuridine totally inhibited DNA synthesis but was without effect on the formation of active extract. That the active component is a protein or associated with a protein is suggested by the inhibition of the appearance of active extract when puromycin, cycloheximide, or actinomycin D were present during cell culture. This interpretation is consistent with the reports of others showing that the active component is inactivated by trypsin treatment [1–4].

The active component is ordinarily released from cells by homogenization subsequent to exposure of cells to low salt concentrations (See Methods). Indeed, in experiments not shown, 1.0 mM ethylenediaminetetraacetate at pH 7.2 was used with equal success. Therefore, it is highly unlikely that the localization of the active component principally in the nucleus of cell homogenates is somehow the result of the artifactual inactivation of an active component in the cytoplasmic fraction as a consequence of exposure to the low salt sucrose solution used for nuclei/cytoplasm preparation.

In this context, it should be noted that polymerase α was originally considered to be a cytoplasmic entity until improved methods which prevented leakage of enzymes from the isolated nuclei provided evidence that polymerase α was of nuclear origin [16] and that the presence of polymerase in the cytoplasmic fraction was due to extraction from nuclei by buffer and inorganic salts even at low ionic strength [17]. In view of the experience with polymerase, it is necessary to reserve a decision as to whether in the present study the active component found in the cytoplasm reflects leakage from the nuclei or reflects a truly cytoplasmic entity.

The findings presented here are compatible with the view that the active component represents a signal in the pathway leading to DNA synthesis. Thus, the active component was confined principally to the nucleus, exhibited DNA binding properties, and was readily distinguishable from polymerase by chromatographic techniques. Since, as pointed out above, the elaboration of the active component precedes the onset of S phase; the time of its appearance is compatible with a possible role in signalling DNA synthesis. While the precise nature and function of the putative signal is not known, the findings are consistent with the view that the product in active extracts reflects either some component in the DNA replication system other than polymerase or reflects some entity which signals the elaboration or utilization of the replicating complex, ie, the active component is possibly the ultimate or perhaps the penultimate signal leading to the onset of DNA synthesis.

REFERENCES

1. Benbow RM, Ford CC: Proc Natl Acad Sci USA 72:2437, 1975.
2. Jazwinski SM, Wang JL, Edelman GM: Proc Natl Acad Sci USA 73:2231, 1976.
3. Das M: Proc Natl Acad Sci USA 77:112,1980
4. Gutowski JK, Cohen S: Cell Immunol 75:300, 1983
5. Gutowski JK, Innes J, Weksler ME, Cohen S: J Immunol 132:559, 1984.
6. Cohen S, Gutowski JK: In Ford RJ, Maizel AL, (eds): "Mediators in Cell Growth and Differentiation." New York: Raven Press, 1985, p. 11.
7. Chiu RW, Baril EF: J Biol Chem 250:7951, 1975.
8. Swenson RM, Kern M: Proc Natl Acad Sci USA 57:417, 1967.
9. Kern M: J Immunol 134:2260, 1985.
10. Benz WC, Strominger JL: Proc Natl Acad Sci USA 72:2413, 1975.
11. Alberts B, Herrick G: Meth Enzymol 21D:198, 1971.
12. Hubscher U, Kuenzle CC, Spadari S: Nucleic Acid Res 4:2917, 1977.
13. Gollapudi SVS, Ramanadham M , Kern M: Biochem Biophys Res Comm 119:1, 1984.
14. Ramanadham M, Gollapudi SVS, Kern M: J Immunol 134:4, 1985.
15. Zimmerman DH, Kern M: J Immunol 111:761, 1973.
16. Kornberg A: "DNA Replication." New York: WH Freeman and Company, 1980, p 203.
17. Lynch, WE, Surrey S, Lieberman I: J Biol Chem 250:8179, 1975.