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Nucleoprotein Complexes Released From Lymphoma Nuclei That Contain the *abl* Oncogene and RNA and DNA Polymerase and RNA Primase Activities

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Abstract We report on the discovery and isolation of DNA- and RNA-containing macromolecular nuclear complexes whose purified major DNA possessed electrophoretic mobilities of ~ 90 and ~ 25 kbp. The deoxyribonucleoprotein-ribonucleoprotein complexes contain RNA and DNA polymerase and primase activities and were isolated from nuclei of murine RAW117 large-cell lymphoma cells by restriction digestion with *Msp*-I, gentle extraction with solutions containing $MgCl_2$, but without chelating agents, and low ionic strength gel electrophoresis. Two-dimensional (isoelectric focusing/M_r) gel electrophoresis and silver staining of the proteins of the complexes after treatment with DNase I indicated the presence of ~ 30 protein components. In vitro DNA and RNA polymerase/primase assays showed that the DNP/RNP complexes had very high enzyme specific activities. Using the DNP/RNP complexes a discrete DNA polymerase α product of ~ 85 kbp was synthesized that was not synthesized in the presence of the DNA polymerase α inhibitor aphidicolin. RNA polymerase assays in the presence of excess α-amanitin indicated that the complexes possessed significant RNA polymerase I activity. Preparing the complexes at various times after the release of cells from a double thymidine block showed the complexes as well as the complex-associated enzyme activities to be cell-cycle dependent. The DNA and RNA polymerase-related activities were highest in late S phase, 7 and 9 h, respectively, after release from the double thymidine block. The complexes synthesized a specific in vitro DNA polymerase product using endogenous substrate and nucleotide precursors. Hybridization studies showed that the complexes contained the *abl* oncogene which is expressed in RAW117 cells, but not the β-casein gene which is not expressed in this cell system.

Key words: cell division, DNA replication, transcription, multienzyme complex

Although a great deal of information has accumulated on gene regulation at the level of DNA primary sequence organization and its requirement for key protein factors [Engelke et al., 1980; Manley et al., 1980; Waslyk et al., 1980; Learned and Tjian, 1982; McKnight et al., 1984; Haltiner et al., 1986], the mechanisms involved in the in vivo regulation of eukaryotic gene expression in relation to the cytological organization of chromatin remain elusive. DNA-protein complexes and interactions associated with these that arise when chelated, cell-free extracts are added to cloned DNAs have added to our understanding of the role of DNA primary sequence in gene regulatory processes, particularly transcription [Weintraub and Groudine, 1976; Simpson, 1982; Dignam et al., 1983; Dynan and Tjian, 1983; Coppola and Luse, 1984; Gronostajski et al., 1984; Miller et al., 1985]. Such whole cell-free extracts, however, are usually the result of using relatively harsh salt-, chelator-, and chaotrope-containing extraction and chromatographic solutions, and the purified factors from such extracts may not associate with the same DNA-protein structures that exist in vivo.

Elegant studies have shown that purified gene regulatory factors, such as those involved in SV40 DNA transcription, can regulate RNA polymerase (pol) activity in vitro [Dynan and Tjian, 1983]. Eukaryotic replication has been studied using particular factors purified by a variety of chromatographic techniques, and these have been analyzed for their abilities to promote DNA synthesis using synthetic oligonuleotide substrates [Gronostajski et al., 1984]. In the eukaryote, however, few of these studies have used naturally occurring endogenous oligonucleotide substrates.

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Endogenous DNA has been studied by restriction digestion of mouse nuclei with specific enzymes, such as *Msp*-I [Weintraub and Groudine, 1976; Nickols and Felsenfeld, 1983]. This has lead to the identification of specific hypersensitive DNA sites that are associated with the framing 3'-sequences of mouse globin genes. Other restriction enzymes failed to release similar complexes.

Deoxyribonucleoprotein/ribonucleoprotein (DNP/RNP) complexes containing ribosomal DNA and RNA pol I activity have been released by restriction digestion of purified nucleoli with *Msp*-I, whereas other restriction enzymes failed to do so [Rosenberg, 1986, 1987]. Such restriction sites may play a role in delineating active chromatin regions [Weintraub and Groudine, 1976; Nickols and Felsenfeld, 1983]. We thus used *Msp*-I restriction digestion of nuclei from murine RAW117 cells, a well-characterized murine metastatic large-cell lymphoma cell line [Brunson and Nicolson, 1978], to release DNP/RNP complexes with high specific in vitro DNA and RNA pol and primase activities.

MATERIALS AND METHODS

Isolation of DNP/RNP Complexes

DNP/RNP complexes were isolated by *Msp*-I restriction of nuclei isolated from the highly metastatic murine RAW117-H10 cells [Brunson and Nicolson, 1978] avoiding the use of strong detergents and chaotropic or chelating agents [Rosenberg, 1986, 1987]. The electrophoretic system we used was a modification [Rosenberg, 1986] of the low ionic strength system originally developed for the fractionation of nucleosomes and nucleosomal oligosomes [Varshavsky et al., 1976]. Nuclei were prepared as follows: Cells (~ 4–5 g) were washed once by low speed centrifugation at 800*g* for 10 min in ice-cold 0.13 M NaCl, 0.005 M KCl, 0.008 M $MgCl_2$, 0.88 M sucrose. The resultant pellet was resuspended in ice-cold 0.010 M NaCl, 0.015 M $MgCl_2$, 0.010 M Tris/HCl, pH 7.4, and allowed to swell on ice for 20 min. Following this incubation, the pellet was centrifuged at 1,085*g* for 10 min and washed in the same buffer by repeating the centrifugation step. The pellet was then homogenized in 10 vol buffer in the presence of 0.005% NP-40. At this phase of the preparation, two more washes in buffer were performed with centrifugation, as before. The following protease inhibitors were included in all buffers and solutions: 5 mM phenylmethyl sulfonyl fluoride and 50 mg/ml aprotinin. Prior to restriction digestion with *Msp*-I, the nuclei were washed once in the following buffer: 0.060 M KCl, 0.015 M $MgCl_2$, 10^{-5} M $CaCl_2$, and 0.015 M Tris/HCl, pH 7.5 (K buffer) [Rosenberg, 1987]. The morphological integrity of the nuclei preparation was monitored by phase microscopy.

Nuclei (1 mg/ml) were digested with *Msp*-I (1600 units; Bethesda Research Laboratories) in K buffer in 500 μl Eppendorf tubes for 2.5 h at 37°C. We found that the DNP/RNP complex yields were better by performing 6–8 minidigestions with ~ 180–200 μl sample volumes. After the first treatment with *Msp*-I, digests were microfuged at maximum speed for 30 sec, and the supernatant was decanted and retained for further analyses. The remaining pellet was then extracted with 500 μl of 0.010 M $MgCl_2$, 0.100M Tris/HCl, pH 7.3. This was accomplished by resuspending the pellet by gentle vortexing and aspiration, incubating the mixture for 10–20 min at room temperature, and microfuging as above. Unlike the isolation procedure for the nucleolar transcripton [Rosenberg, 1986], a second digestion with *Msp*-I was required. The redigestion was performed as before, and the pellet was again extracted with the $MgCl_2$ solution and microfuged. The resultant pellet containing the DNP/RNP complexes was then resuspended in 0.010 M Tris/HCl, 0.010 M boric acid, pH 7.8 (Tris-borate buffer), for low ionic strength electrophoresis. Chelating agents were omitted.

Components of the DNP/RNP Complexes

Cells were grown in the presence of [α^{32}P]TTP in plastic roller bottles (Corning: 850 cm^2) containing Hepes-buffered high glucose Dulbecco-modified Eagle's medium (DME) supplemented with 5% fetal bovine serum to a concentration of 3×10^5 cells/ml. [α^{32}P]TTP or [^{3}H]uridine (ICN) was added (1 mCi/400 ml cell suspension) and incubation continued for 18 h. The cells were harvested by centrifugation, washed twice in phosphate-buffered saline, and processed immediately to obtain purified nuclei. The yield of cells from 400 ml of medium was ~ 1 g, and the complexes were then prepared as described above. To show that the DNP/RNP complexes contained DNA the labelled preparations were fractionated using low ionic strength gel electrophoresis. Samples were suspended in Tris-

borate buffer containing 0.001% sucrose. Gel electrophoresis was performed in a standard flat-bed apparatus (BRL). The gel mixture consisted of 0.8% agarose (ultrapure; BRL) in Tris-borate buffer. Electrophoresis was performed for 5 h at 60 V/cm. The gel was dried and autoradiography performed in the presence of an intensifying screen at −70°C. Samples radiolabeled with [α^{32}P]TTP or [^{3}H]uridine were also fractionated by native protein electrophoresis [Cavanaugh and Nicolson, 1989] and autoradiography was performed as described above. Alternatively, equivalent unlabeled preparations were analyzed by two-dimensional (isoelectric focusing/reducing SDS-PAGE) gel electrophoresis. Some of the preparations were pretreated with DNase I (2 μg/ml) for 1 h at 37°C prior to gel electrophoresis and silver staining. DNA was purified by phenol extraction, ethanol precipitation, and washing, and analyzed by DNA electophoresis as described [Maniatis et al., 1982]. DNA pol products from in vitro reactions labeled with [α^{32}P]dCTP (2 μCi/50 μl sample) were purified and analyzed [Maniatis et al., 1982].

Cell Synchronization

RAW117-H10 cells were synchronized by the double thymidine block method [Stein and Borun, 1972]. The cells were grown in suspension in roller bottles in Hepes-buffered high glucose DME supplemented with 5% fetal calf serum to a concentration of 3×10^5 cells/ml. Thymidine was added to a final concentration of 2 mM, and the cells were incubated for 16 h. To determine the S phase peak 200 ml cell aliquots (6×10^4 cells) were removed hourly and incubated with 10 mCi of [^{3}H]thymidine at 37°C for 20 min. After incubation, 5 ml of ice-cold 10% trichloroacetic acid (TCA) was added, and the cells were collected on a Whatman GF/C filter, washed twice with 10 ml 10% TCA and once with 10 ml ice-cold ethanol, air dried, and counted in a liquid scintillation counter. After incubation, the cells were collected by low speed centrifugation, washed once in complete medium, resuspended in prewarmed medium, and incubated for 9 h. [^{3}H]thymidine was again added, and the cells were incubated for an additional 16 h. After this incubation, the cell collecting, washing, and resuspending was repeated, and aliquots (200 ml) of the suspension were collected at 3, 5, 7, 9, 11, and 13 h. The cells were then concentrated by centrifugation and immediately processed to obtain isolated nuclei. The DNP/RNP complexes were prepared as described above at each time point.

DNA and RNA Polymerase and RNA Primase Assays

The DNA pol assays were performed as follows: DNP/RNP complexes (0.01–0.05 μg) from unsynchronized or synchronized preparations were removed from low ionic strength or native electrophoresis gels by electroelution and analyzed for in vitro enzyme activity. To assess for the presence of DNA pol α, aphidicolin, which inhibits DNA pol α and distinguishes between DNA pols β and α but not δ, was included in parallel assays at a 20-times excess (w/w). The DNA pol activity associated with the complexes was determined by a 1 h incorporation of [α^{32}P]dCTP into exogenous or endogenous DNA. Alternatively, DNP/RNP complex preparations from unsynchronized cells were assayed in the presence or absence of actinomycin D at 5-times excess (w/w) to determine the presence of primase activity in the RNA pol assay. Primase activity is inhibitable by actinomycin D, and enzyme activity that persists in the presence of actinomycin D can be attributable to poly A tailing. Samples were analyzed with or without RNase A (boiled) pretreatment. Exogenous DNA used in these assays was obtained from BRL (Bethesda, MD). RNA pol assays were performed with an in vitro transcription kit (BRL); however, we substituted the DNP/RNP complexes for substrate in the assays, and [α^{32}P]UTP (10 μCi) was added to the reaction. RNA pol I assays were performed in the presence of 200-times (w/w) excess α-amanitin. When it was appropriate, assays were performed in the presence and/or absence of RNase A to aid in distinguishing nascent RNA and DNA pol products. Controls were performed for both DNA and RNA pol assays in which DNase was included to determine if portions of the DNP/RNP's DNA were protected and to further discern nascent DNA from RNA pol products. All in vitro assays were conducted in a volume of 100 μl in 0.008 M $MgCl_2$, 1 μg/μl bovine serum albumin, 0.030 M Tris/HCl, pH 7.5. Activities were then assessed for both DNA and RNA pol or primase by performing a standard filter-binding TCA precipitable procedure using GF/C filters (Whatmann). The filters were washed extensively and treated

with Scintifluor before radioscintillation counting.

Hybridization Studies

DNA was purified from DNP/RNP complexes derived from synchronized RAW117 cells 3 and 7 h after release from a double thymidine block. In addition, total cellular DNA was isolated from the RAW117 cells and purified for comparative purposes. DNAs in both cases were purified as described and resolved by standard DNA electrophoresis [Maniatis et al., 1982]. The DNA was then transferred to Nytran (Schleicher and Schuell, Keene, NH) by the method of Southern, and hybridization was performed at moderate stringency (67°C, 0.5× SSC, 48 h). The purified DNP/RNP complex DNA was probed for the v-*abl* oncogene (probe provided by Dr. M. Tainsky), a gene known to be expressed in RAW117 cells [Rotter et al., 1985], and β-casein (probe provided by Dr. J. Rosen), an unexpressed gene in RAW117 cells. Probes were labeled by the random prime method using the Boehringer Mannheim Random Primed DNA Labeling Kit (BMB, Indianapolis, IN) and hybridization was performed as described previously [Rotter et al., 1985].

RESULTS

DNP/RNP Complexes From RAW117 Cell Nuclei

Treatment of RAW117-H10 cell nuclei with *Msp*-I dislodged and released very large DNP/RNP complexes that when analyzed by sucrose density ultracentrifugation migrated rapidly as complexes of approximately ~ 10,000 s. When analyzed by standard DNA electrophoresis, the major DNAs isolated from such complexes migrated discretely with mobilities equivalent to ~ 25 and ~ 90 kbp (Fig. 1, lane 2). The electrophoretic mobilities of the radiolabeled DNP/RNPs themselves are slightly slower in the low ionic strength gel system compared to the complexes' purified DNAs, the two major species migrating with relative mobilities estimated to be > 50 and > 150 kbp DNA, respectively (Fig. 1, lane 1). Native protein electrophoresis [Cavanaugh and Nicolson, 1989] of the radiolabelled complexes did not resolve the two species as clearly as in the low ionic strength gel system (Fig. 1, lanes 7, 8).

The incorporation of [α^{32}P]TTP into the complexes indicated that DNA synthesis and the production of nascent DNA are associated with

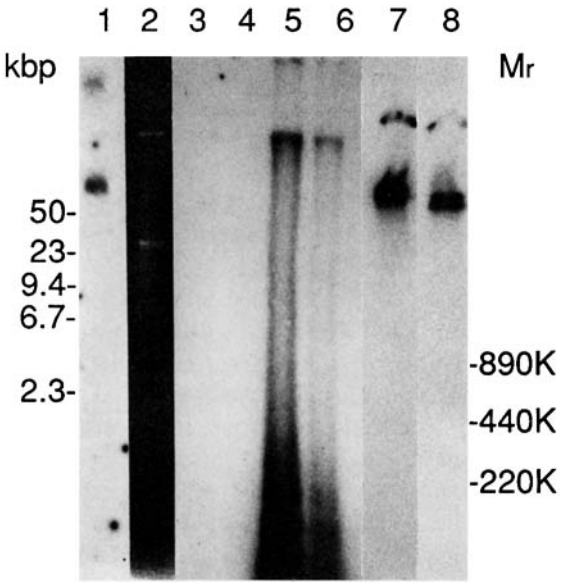

Fig. 1. Gel electrophoresis of the DNP/RNP complex, complex DNA, and the DNP/RNP complex DNA pol synthesis products. **Lane 1:** DNP/RNP complexes were labelled with [α^{32}P]TTP and separated by low ionic strength electrophoresis. **Lane 2:** DNA from the DNP/RNP complexes was purified and analyzed by standard DNA electrophoresis as described in Materials and Methods. **Lanes 3–6:** In vitro DNA synthesis reactions were preformed with the DNP/RNP complexes labeled with [α^{32}P]dCTP in the presence (lanes 3, 4) or absence (lanes 5, 6) of aphidicolin for 7 h (lanes 3, 5) or 3 h (lanes 4, 6) after release of a double thymidine block, and the subsequent products were resolved by low ionic strength electrophoresis. **Lanes 7, 8:** Native gel electrophoresis of [α^{32}P]TTP-labeled (lane 7) or [^{3}H]uridine-labeled (lane 8) DNP/RNP complexes. DNA standards for low ionic strength and DNA gel electrophoresis are shown to the left of the figure; protein standards for native gel electrophoresis are shown to the right of the figure.

the DNP/RNP complexes (Fig. 1, lane 7). RNA synthesis was also associated with these complexes, as indicated by the incorporation of [^{3}H]uridine into the DNP/RNP complexes (Fig. 1, lane 8). The in vitro products from the DNA pol reaction were purified by routine phenol extraction/ethanol washing and precipitation and analyzed by standard DNA electrophoresis (Fig. 1, lanes 3–6). In samples derived from synchronized cell populations 3 and 7 h after release from a double thymidine block, a DNA pol product ~ 85 kbp was synthesized along with several smaller products (< 400 bp) that we speculate could be origins of replication. Both of the major DNP/RNP complexes were necessary to synthesize the products, and it was noteworthy that the products were synthesized from the DNP/RNP endogenous substrate without the addition of synthetic oligonucleotides. When aphidicolin, a potent inhibitor of DNA pol α, was

included in the assay, the product was not seen. Compared to previous studies with chromatographic-purified DNA pol/primase and synthetic oligonucleotides as substrate [Gronostajski et al., 1984], the DNP/RNP complexes synthesized DNA pol products from endogenous substrate that were obviously more discrete. The in vitro RNA pol assays showed that the DNP/RNP complex synthesized a heterogeneous array of products ranging in size from ~ 2,000 to ~ 200 bp (data not shown). Electroelution of unlabelled DNP/RNP complexes from native protein or low ionic strength gel systems and performing enzyme assays indicated that both DNA and RNA pol activities were associated with the eluted complexes (Table I, F–K, M–O).

To further determine the complexity of the DNP/RNP complexes' proteins, we performed two-dimensional (isoelectrofocusing/SDS-PAGE) gel electrophoresis (Fig. 2). The DNP/RNP components from unsynchronized H10 cells were resolved without (Fig. 2A) or with (Fig. 2B) pretreatment of the DNP/RNP complex with DNase I. This experiment demonstrated that the DNP/RNP complexes consisted of at least 30 proteins. In the DNase I–treated sample at least 8 proteins were susceptible to the enzyme, as indicated by their apparent change in mobility, suggesting that these might be DNA-binding proteins. Two of the proteins were partially susceptible to DNase I treatment, as indicated by a change in their pI. Other proteins observed in the DNase I–treated sample were attribut-

TABLE I. DNA and RNA Polymerase and Primase Activities of DNP/RNP Complexes Purified From RAW117 Large-Cell Lymphoma Nuclei

Experiment/complex(s)	TCA-precipitable radioactivity[a] (Mean CPM ± SEM[b] × 10^{-3})	
	RNA pol/primase	DNA pol
A. Untreated DNP/RNP complexes	1,627 ± 235	1,365 ± 235
B. RNase A–treated DNP/RNP complexes	1,329 ± 156	1,555 ± 88
C. α-amanitin–treated[c] DNP/RNP complexes	318 ± 65	ND[d]
D. Aphidicolin-treated[e] DNP/RNP complexes	ND	292 ± 94
E. Actinomycin D–treated[e] DNP/RNP complexes	264 ± 78	ND
F. ~ 90 kbp DNA DNP/RNP complex eluent (low ionic strength gel)[f]	1,574 ± 215	869 ± 47
G. ~ 90 kbp DNA DNP/RNP complex eluent plus equivalent exogenous DNA (low ionic strength gel)[f]	ND	86 ± 2
H. ~ 25 kbp DNA DNP/RNP complex eluent (low ionic strength gel)[f]	1,058 ± 215	330 ± 32
I. RNase A–treated ~ 90 kbp DNA DNP/RNP complex eluent (low ionic strength gel)[f]	499 ± 95	1,380 ± 30
J. RNase A–treated ~ 25 kbp DNA DNP/RNP complex eluent (low ionic strength gel)[f]	442 ± 47	279 ± 29
K. RNase A–treated ~ 25 kbp DNA DNP/RNP complex eluent plus equivalent exogenous DNA (low ionic strength gel)[f]	ND	37 ± 5
L. RNase A–treated DNP/RNP complexes plus equivalent exogenous DNA	421 ± 97	527 ± 47
M. RNase A– and DNase I–treated DNP/RNP complexes eluted from native protein gel	2,062 ± 663	497 ± 57
N. RNase A– DNase I–treated DNP/RNP complexes eluted from native from native protein gel plus equivalent exogenous DNA[g]	18 ± 2	5 ± 0.5
O. RNase A– and DNAse I–treated DNP/RNP complexes eluted from native protein gel[g] plus α-amanitin[g]	180 ± 17	ND
P. Control (reaction buffer)	0.23 ± 0.02	0.24 ± 0.002

[a]Activities for 3 independent experiments (n = 9)— measured according to M M during a 1 h incubation at 37°C using ~ 3 μg protein (~ 0.09 μg DNA) of DNP/RNP complex mixture or DNP/RNP complex eluted after low ionic strength or native gel electrophoresis.

[b]SEM, overall standard error of the mean for the 3 independent experiments (n = 9).

[c]200-times (w/w) excess.

[d]ND, not determined.

[e]20-times (w/w) excess.

[f]DNP/RNP complex was electroeluted from a specific portion of the low ionic strength gel (~ 0.05 μg protein, ~ 0.002 μg DNA).

[g]DNP/RNP complexes were electroeluted from a specific portion of the native gel (~ 1 μg protein, ~ 0.03 μg DNA).

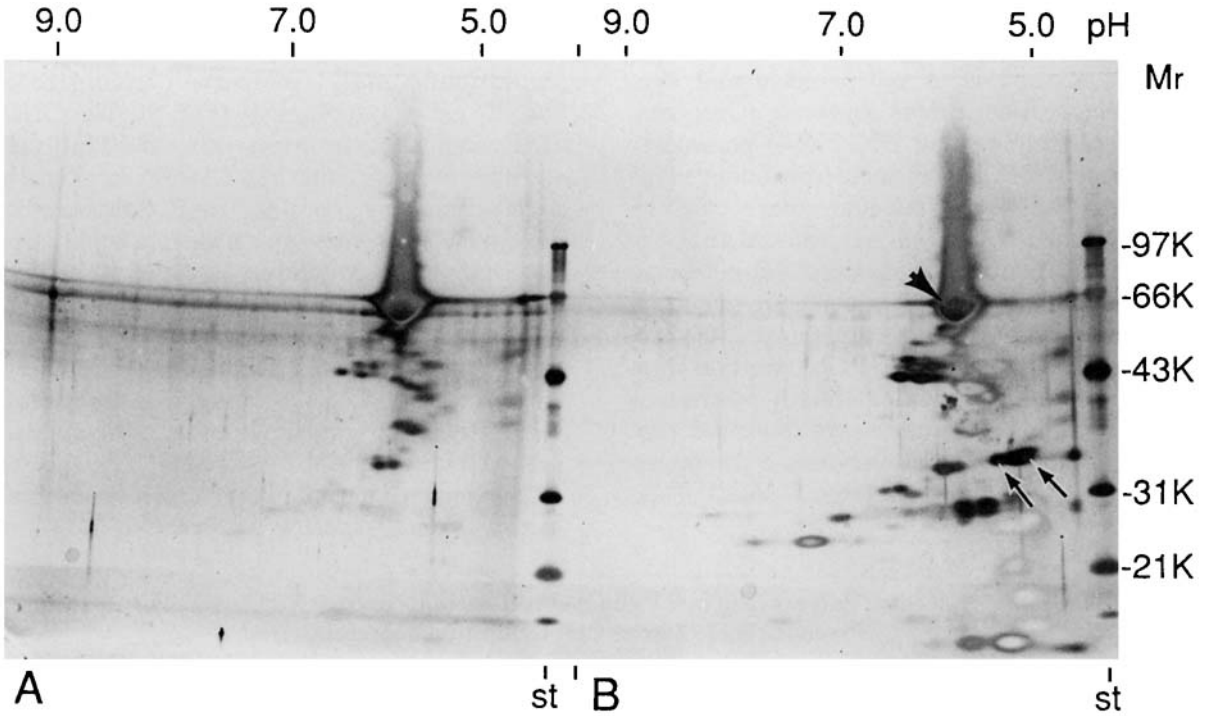

Fig. 2. Two-dimensional (isoelectric focusing/reducing SDS-PAGE) gel electrophoresis of the DNP/RNP complex proteins. **A:** DNP/RNP complexes were analyzed without pretreatment with DNase I. **B:** DNP/RNP complexes were analyzed after pretreatment with DNase I (1 μg/ml) for 1 h at 37°C. Arrows indicate DNase I proteins; arrowhead indicates bovine serum albumin from the purification buffer; st, M_r standards (the M_r values are indicated at the right of the figure); pH in the gels is indicated at the top of each panel.

able to DNase I itself; however, some DNP/RNP components may comigrate with the spots that we have assigned to DNase I (Fig. 2B).

DNA and RNA Pol and RNA Primase

A series of in vitro DNA and RNA pol and RNA primase assays were performed on the isolated DNP/RNP complexes and electroeluents of the low ionic strength gel-electrophoresis-separated complexes that contained discrete DNA of ~ 25 or ~ 90 kbp DNA (Table I). Similar assays were also performed on electroeluents of the DNP/RNP complexes purified by native protein gel electrophoresis. This allowed us to examine whether the enzyme activities were from possible contaminating or co-eluting factors in our preparations. High RNA pol activities were associated with either the mixture (Table I, A,B,M) or the separated DNP/RNP complexes containing ~ 25 kbp (Table I, H) or ~ 90 kbp DNA (Table I, F). To determine if RNA pol 1 activity was present in the DNP/RNP complexes, an assay was performed in the presence of 200-times excess (w/w) α-amanitin, an inhibitor of nuclear RNA pols II and III but not pol I. We found that the average RNA pol activities as assessed by incorporation of [α^{32}P]UTP in our filter-binding studies was about 10^7 cpm; of this, about 20–30% was inhibitable by α-amanitin and probably represented other RNA pol activities (Table I, C,O). To make sure that we were, indeed, assaying for RNA pol activities, equivalent samples were treated with RNase A (boiled). The RNA pol activity persisted and was not affected by this treatment (Table I, B,I,J). We have also assessed the in vitro pol activities using other ribonucleotides, such as [α^{32}P]GTP and [α^{32}P]CTP, to control for poly A tailing activity. Potential transcription activity was noted for both of the separated DNP/RNP complexes containing ~ 25 or ~ 90 kbp DNA (Table I, F,H). Analyses of the in vitro products from these assays by glyoxyl gel electrophoresis indicated that a series of RNAs with sizes ranging from ~ 2,000 to 100 bp were formed (data not shown). Because we have not yet sequenced or performed S1 nuclease protection assays on these RNAs, they have not been called transcripts.

We also found that both the unseparated and separated DNP/RNP complexes had DNA pol activity (Table I, A,F,H). The DNP/RNP complex containing ~ 25 kbp DNA consistently had

about 20% of the DNA pol activity of the complex containing ~ 90 kbp DNA (Table I, cf. F,H). Bio-rad protein and diphenylamine DNA assays of the electroeluted DNP/RNP complexes indicated that this observation was not due to failure to quantitatively elute the complexes from the gel, because ~ 0.05 μg of complex electroeluent protein (0.002 μg DNA) was used in all of the assays. The addition of an equivalent amount (w/w) of exogenous DNA (containing SV-40 promoter) to either DNA or RNA pol reactions caused significant enzyme inhibition. Using the boiled RNase A–treated DNP/RNP complexes together resulted in approximately 70% enzyme inhibition by the exogenous DNA (Table I, cf. B,L), and using the electroeluted (boiled) RNase A–treated DNP/RNP complexes with DNase I treatment had little effect on the assay (Table I, M). Thus, particular DNA and/or RNA sequences necessary for RNA and DNA pol activities in the complexes appear to be protected. The addition of exogenous DNA to these samples resulted in approximately 99% enzyme inhibition (Table I, N).

To determine if the DNP/RNP complexes contained DNA pol α activities, in vitro assays were performed on the DNP/RNP complexes in the presence of 20-times excess (w/w) aphidicolin, an inhibitor of DNA pol α but not β or δ (Table I, D). In vitro studies to determine the presence of RNA primase were performed in the absence or presence of 30-times excess (w/w) actinomycin D, an inhibitor of primase activity (Table I, E). Sizeable RNA primase activities were detected.

Cell Cycle Analysis

Cell cycle analysis of RAW117-H10 cells indicated that this cell line undergoes mitosis in ~ 10 h. In a separate experiment to determine the effectiveness of the double thymidine block for cell synchrony, [^{3}H]thymidine incorporation was monitored. We found that RAW117 cells possessed two peaks of thymidine incorporation corresponding to early and late DNA synthesis (~ 3 and ~ 8 h after release from the block). By 11 h after release, RAW117 cells had undergone division. The DNP/RNP complexes' DNA pol activity followed the cell cycle with activity peaking in late S, 7 h after release from the thymidine block, just prior to mitosis (Table II). We also noted that the synthesis of the DNP/RNP complexes' major DNA pol product reflected this general trend (Fig. 1, lanes 5, 6), with the products from the 7 h sample showing an autoradiographic signal ~ 5 times as intense as that of the 3 h sample.

When the two major DNP/RNP complexes were monitored together (as in Table II), RNA pol activity was analogous to the known changes in RNA pol I activity during the cell cycle. RNA pol activity peaked in late S phase, 9 h after release from the double thymidine block (Table II). Other RNA Pol activities (RNA pol II and possibly III) were detected throughout the cell cycle. This was expected, since transcription occurs at all phases of the cell cycle with basal levels reached during the resting periods. By 13 h we noted that the RAW117-H10 cells had begun to lose cell cycle synchrony.

Hybridization Studies

Hybridization studies were performed on the DNP/RNP complexes' purified DNAs from synchronized cell populations (3 and 7 h after release from a double thymidine block) and compared to the total cellular DNA for the presence of the *abl* oncogene which is expressed in

TABLE II. Cell-Cycle Dependence of DNA and RNA Pol/Primase Using DNP/RNP Complexes From RAW117 Large-Cell Lymphoma Nuclei

Release from double thymidine block (h)	TCA-precipitable radioactivity[a] (mean CPM ± SEM[b] × 10^{-3})		
	[α^{32}P]UTP	[α^{32}P]UTP + α-amanitin[c]	[α^{32}P]dCTP
3	228,220 ± 3.9	1,670 ± 0.1	83,730 ± 1.6
5	215,350 ± 5.8	11,480 ± 0.1	127,300 ± 4.6
7	395,990 ± 2.8	45,090 ± 0.1	282,370 ± 4.8
9	426,270 ± 7.1	377,390 ± 0.1	70,190 ± 2.0
11	990,020 ± 4.1	58,070 ± 0.1	16,180 ± 0.7
13	438,770 ± 3.4	11,150 ± 0.1	586,520 ± 31

[a]See footnote in Table I.
[b]See footnote in Table I.
[c]See footnote in Table I.

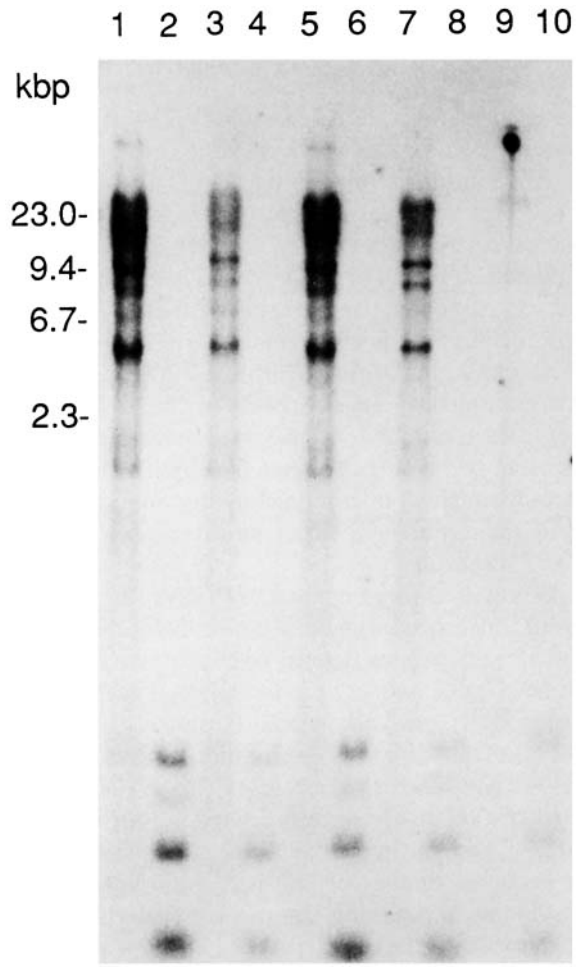

Fig. 3. Analysis of the DNP/RNP complexes' purified DNA or total cellular DNA for the presence of the *abl* oncogene known to be expressed in RAW117 cells by hyridization at moderate stringency. **Lanes 1–4:** Purified DNA from DNP/RNP complexes (isolated 3 h after release from double thymidine block). Lanes 2, 4 are the same as in lanes 1, 3, except that the purified DNA from the DNP/RNP complexes has been redigested with *Msp*-I. Note that the *abl* probe recognizes DNA fragments that are less than 400 bp after redigestion of the isolated DNA with *Msp*-I. Lanes 1, 2 show DNA samples prior to washing with 10 mM Tris, 10 mM $MgCl_2$, pH 7.8, whereas lanes 3, 4 show the same samples after washing with buffer. **Lanes 5–8:** Purified DNA from DNP/RNP complexes (isolated 7 h after release from double thymidine block). Lanes 6, 8 are the same as in lanes 5, 7, except that the purified DNA from the DNP/RNP complexes has been redigested with *Msp*-I. Lanes 5, 6 show DNA samples prior to washing with 10 mM Tris, 10 mM $MgCl_2$, pH 7.8, whereas lanes 7, 8 show the same samples after washing with buffer. **Lanes 9, 10:** Total cellular DNA was purified from RAW117 cells and not digested (lane 9) or digested (lane 10) with *Msp*-I. DNA standards are indicated at the left of the figure.

RAW117 cells (Fig. 3). As a control, DNP/RNP complex DNA from RAW117 cells released 3 and 7 h from a double thymidine block were also analyzed for the β-casein gene, which is not expressed in RAW117 cells, and compared to the total cellular DNA. These studies indicated that the DNP/RNP complex possessed large *Msp*-I restriction fragments ranging in size from roughly 9–1 kbp containing the *abl* oncogene. An aliquot of the complexes' DNA, which theoretically possesses ~ 400 potential *Msp*-I cleavage sites in the *abl* oncogene if all sites were accessible to the enzyme, was redigested with *Msp*-I and analyzed. The results indicated that at least some of the *abl* oncogene's restriction sites recognized by *Msp*-I are protected from enzymatic attack in the DNP/RNP complexes. In contrast, the smaller DNA fragments (~ 400 bp) indicate the enzyme's ability to cut within the gene of DNA isolated from the complexes. The β-casein gene, although present in the total cellular DNA, was not contained in the DNP/RNP complexes, since only a background pattern emerged after reprobing under the same conditions used for the *abl* hybridization study. That the β-casein probe was functional was shown in control hybridization experiments with total cellular DNA using a variety of restriction enzymes (data not shown).

DISCUSSION

It has been suggested that DNA in eukaryotic cells is synthesized by macromolecular complexes rather than soluble enzymes [Martin et al., 1974; Seki and Mueller, 1976; Vishwanatha et al., 1986; Newport and Forbes, 1987]. Reddy and Pardee [1983] found that enzymes involved in DNA synthesis were part of a macromolecular complex that they termed a "replitase." Wold and Kelley [1988] have purified and partially characterized a replication complex required for in vitro replication of SV-40 DNA that is composed of four tightly bound protein components.

The nuclear DNP/RNP complexes reported here are probably representative of suborganellular structures that could be part of the functionally active genetic machinery. Since both in vitro DNA and RNA pol and RNA primase activities were cell-cycle-dependent and found in the DNP/RNP complexes, we propose that such structures be called *repliscriptons* for multifunctional structures that are equipped with at least some of the machinery involved in DNA replication and/or transcription processes. We have found a polycistronic messenger RNA of ~ 1,700 bp that contains *abl* oncogene, immunoglobulin μ-chain, and p53 oncogene messages associated with discrete nucleoprotein components of the

DNP/RNP complexes [manuscript submitted]. In addition, the synthesis in vitro of a large, discrete, cell-cycle-dependent DNA pol α product formed from endogenous DNP/RNP substrate also lends credence to this notion.

The role of the DNP/RNP complexes during the cell cycle remains to be determined. It is known that DNA polymerases cannot directly initiate replication and that small pieces of RNA primers are required [Watson, 1981]. The initiation points for DNA replication, the DNA primase reaction, may therefore be started by a form of RNA, not DNA pol or perhaps by novel subclasses of these pols. The DNP/RNP complexes we have isolated may contain a unique RNA pol, since RNA and DNA pol activities were associated with the complexes and discrete in vitro DNA pol α products were synthesized by the DNP/RNP complexes. In addition, other studies indicate that eukaryotic transcription most likely occurs in vivo on a DNA hybrid duplex and that transcription and replication are coupled. Therefore, it is not surprising that the DNP/RNP complexes contain DNA and RNA and DNA pol, RNA pol, and RNA primase.

Analysis of RAW117 cell DNP/RNP complexes suggests that its proteins are associated with DNA and probably RNA, with some of the proteins partially protected and not readily accessible to digestion with proteinase K (unpublished data). The majority of the proteins that comprise these DNP/RNP complexes have a slow turnover rate as determined from [^{35}S]methionine labeling studies [unpublished observations], which is consistent with the behavior of RNA and DNA pols in vivo. Two-dimensional gel electrophoresis studies indicate that the DNP/RNP complexes are composed of ~ 30 proteins. The mobilities of at least 11 of the abundant proteins are sensitive to DNase I, and two of the proteins are partially accessible to DNase I, as shown by an apparent change in their pI after enzyme treatment. These observations confirm that the complexes' DNA is in intimate association with at least some of the DNP/RNP complex proteins. Our observations suggest that because of the DNP/RNP complexes' size, cell-cycle-dependence, presence of DNA pol and RNA pol and primase activities, ability to synthesize cell-cycle-dependent DNA pol products in vitro, presence of an actively expressed oncogene, and enzymatic release from nuclei by *Msp*-I restriction digestion, they may comprise a new class of functional nuclear suborganellular elements.

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