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ATP-dependent association of nuclear proteins with isolated rat liver nuclei

(nucleoplasmin/nonhistone chromosomal high mobility group protein)

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ABSTRACT ***In vitro* association of *Xenopus* nucleoplasmin and mammalian nonhistone chromosomal high mobility group 1 (HMG_1) protein with nuclei isolated from rat liver was examined. Efficient association of nuclear proteins with isolated nuclei requires ATP, HCO_3^-, and Ca^{2+}. Association occurred at 33°C but not at 4°C. ATP could be replaced by adenosine 5′-[α,β-methylene]triphosphate (pp[CH_2]pA), a nonhydrolyzable ATP analog. pp[CH_2]pA associated with nuclei at 33°C and nucleoplasmin and HMG_1 rapidly associated with the pp[CH_2]pA-bound nuclei at 4°C. Competition studies showed that these associations at both 33°C and 4°C were specific. More than 80% of the bindings of nuclear proteins to the nuclear surface were blocked by wheat germ agglutinin.**

The nucleus, which is surrounded by a double nuclear membrane composed of proteins and lipids, possesses unique functions related to genetic activities. It contains many nuclear proteins that are accumulated selectively from the large number of proteins synthesized in the cytoplasm (1–3). To elucidate the mechanism underlying the selective transport of proteins into the nucleus, determination of both structural features of the nuclear proteins transported and the nature of the nuclear components involved in the nuclear transport is necessary. Evidence is accumulating for the existence of specific signals to direct nuclear proteins into the nucleus. Studies using nucleoplasmin, a 165-kDa pentameric karyophilic protein of *Xenopus* oocytes, and limited pepsin digestion products of this protein showed that the C-terminal "tail" portion contains information for selective nuclear uptake (4). Furthermore, a study using simian virus 40 large tumor antigen showed that the amino acid sequence of residues 127–133, Lys-Lys-Lys-Arg-Lys-Val-Glu, acts as a signal for nuclear accumulation (5–7). Recently, chemical conjugation of a synthetic peptide containing this sequence with nonnuclear proteins was found to endow them with the ability to migrate into the nucleus (8–10).

Little is known about the nature of the nuclear envelope component that recognizes the protein signals and participates in the subsequent transport of proteins into the nucleus. An electron microscopic study showed nucleoplasmin coated with colloidal gold accumulated in the nuclear pore region when injected into the cytoplasm of *Xenopus* oocytes (11). Thus, nuclear proteins are believed to migrate into the nucleus through a nuclear pore.

Recently, a cell-free system for nuclear transport of nucleoplasmin was developed with synthetic nuclei in a *Xenopus* oocyte extract (12). The *in vitro* study showed ATP is required for accumulation of nucleoplasmin in nuclei.

In this paper, we describe kinetic studies of the association of nucleoplasmin and nonhistone chromosomal high mobility group 1 protein (HMG_1) with nuclei by using an *in vitro* system free of cytoplasmic proteins.

MATERIALS AND METHODS

Purification of Nucleoplasmin, HMG_1, Phycoerythrin, and Rat Liver Nuclei. Nucleoplasmin was purified from unfertilized eggs of *Xenopus laevis* as described (4). HMG_1 was purified from calf thymus as described (13). Phycoerythrin was purified as described (14). Nuclei were prepared from adult rat liver as described (1).

Labeling of Proteins. Nuclear proteins labeled with Na ^{125}I using lactoperoxidase (15) were nucleoplasmin (^{125}I-nucleoplasmin; specific activity, 1.6×10^6 cpm per μg of protein) and HMG_1 (^{125}I-HMG_1; specific activity, 1.1×10^6 cpm per μg of protein).

Conjugation of Phycoerythrin with Nucleoplasmin. Phycoerythrin and nucleoplasmin were conjugated with *N*-succinimidyl 3(2-pyridyldithio)propionate (SPDP; Pharmacia) and succinimidyl 4-(*P*-maleimidophenyl)butyrate (SMPB; Pierce) as described (16). The fraction corresponding to a molecular mass of 350–400 kDa was rechromatographed by Sepharose CL6B gel filtration. The main peak corresponding to 350–400 kDa was used for this experiment.

To make larger conjugates, excess SPDP and SMPB were used. The fraction corresponding to a molecular mass of ≈2000 kDa was similarly rechromatographed by Sepharose CL4B and the main peak corresponding to ≈2000 kDa was used.

When these conjugates were injected into living cells, the 350- to 400-kDa conjugate entered the nucleus but the 2000-kDa conjugate did not.

Incubation of Isolated Nuclei with Nuclear Proteins. Freshly prepared nuclei were used in all experiments. Isolated nuclei ($4–5 \times 10^7$ nuclei per ml) were preincubated in complete reaction medium (see Table 1) without ATP for 2 hr at 33°C. Preincubation shortened the lag for association of nucleoplasmin with nuclei. After 2 hr, 45 μl of nuclear suspension was mixed with 2 μl of 10 mM ATP unless otherwise mentioned and 0.05 μg of iodinated protein in a final volume of 50 μl, and it was incubated at 33°C with gentle shaking. After incubation, samples were centrifuged at 3000 × *g* for 2.5 min to separate the nuclei. The nuclei were then washed twice with complete reaction medium without ATP and counted in a γ-counter.

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Abbreviations: HMG_1, nonhistone chromosomal high mobility group 1 protein; ^{125}I-HMG_1, ^{125}I-labeled HMG_1; ^{125}I-nucleoplasmin, ^{125}I-labeled nucleoplasmin; WGA, wheat germ agglutinin; pp[CH_2] pA, adenosine 5′-[α,β-methylene]trisphophate.

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In experiments on the association of ^{125}I-nucleoplasmin with pretreated nuclei, 2×10^6 nuclei were incubated at 33°C in complete reaction medium but with various amounts of ATP or adenosine 5′-[α,β-methylene]triphosphate (pp[CH_2]-pA) for 2–4 hr (pretreatment). They were then collected by centrifugation at 1500 $\times g$ for 5 min, suspended in 50 μl of buffer (10 mM Hepes, pH 7.8/60 mM KCl/2 mM $CaCl_2$/1 mg of bovine serum albumin per ml) containing 0.05 μg of ^{125}I-nucleoplasmin or HMG_1 and incubated on ice for 20 min. Radioactivity associated with the nuclei was counted as described above.

The association of fluorescent-labeled nucleoplasmin with nuclei was examined by fluorescence microscopy without any fixation procedures.

Detection of Association of Nucleotide with the Nuclei. A sample of 8×10^6 nuclei was incubated with 0.4 mM pp[CH_2]pA in 600 μl of buffer in complete reaction medium. After incubation, the nuclei were collected by centrifugation (1500 $\times g$; 5 min) and washed twice with the same buffer. Nucleotides were extracted from the nuclei with 0.5 M perchloric acid, neutralized, and analyzed on a Toyo Soda HPLC DEAE 2SW IEX-540 column (25 cm × 4.6 mm) as described by Kamiike *et al.* (17).

Digestion of Iodinated Nucleoplasmin with Pepsin. ^{125}I-Nucleoplasmin was digested with pepsin as described (4).

RESULTS

Association of Nucleoplasmin and HMG_1 with Isolated Rat Liver Nuclei. Nucleoplasmin, a major karyophilic protein in *X. laevis* oocytes, is a pentamer with a monomer molecular mass of ≈33 kDa that induces nucleosome assembly *in vitro* (18–20). This protein rapidly enters the nucleus when injected into the cytoplasm of *Xenopus* oocytes (4) or mammalian cultured cells (16). HMG_1 isolated from calf thymus also migrates into the nucleus when injected into the cytoplasm of cultured mammalian cells (2, 21).

We conjugated phycoerythrin, a fluorescent protein (≈200 kDa), with nucleoplasmin. Fluorescence of the conjugated protein (350–400 kDa) in nuclei was detected after incubation of the nuclei at 33°C in the presence, but not in the absence, of ATP (Fig. 1 *A–D*). Moreover, when nuclei were incubated with a larger protein conjugate of nucleoplasmin and phycoerythrin (≈2000 kDa), the fluorescence was detected only on the surface of nuclei if ATP was present (Fig. 1 *E* and *F*). This suggests that the nuclear envelope plays a primary role in the ATP-dependent association of nucleoplasmin with the isolated nuclei.

Table 1 shows the complete composition of our reaction medium. When isolated nuclei were incubated with ^{125}I-nucleoplasmin or ^{125}I-HMG_1 at 33°C in complete reaction medium, efficient association of iodinated proteins with nuclei was detected, but at 4°C association did not occur. Maximal levels of association were reached within 2–3 hr of incubation. When ATP, $KHCO_3$, or $CaCl_2$ was omitted from the reaction mixture no association was observed. Association of nuclear proteins with nuclei was similar when ATP was replaced by GTP or UTP. With CTP, it was ≈70%; with ADP, it was about half that with ATP. No association was seen when ATP was replaced by AMP. HPLC analysis of nucleotides in the reaction mixture showed that ADP was converted to ATP, suggesting that nuclear myokinase activity was required for ADP to support this reaction. It was also found that $NaHCO_3$ could replace $KHCO_3$ but $MgCl_2$ could not replace $CaCl_2$. We confirmed that nuclear-associated radioactivity results from the presence of intact nucleoplasmin or HMG_1 and not of degradation products by NaDodSO$_4$/PAGE analysis of nuclear proteins and autoradiography. Nonnuclear proteins such as bovine serum albumin, IgG, and polypeptide chain elongation factor 2 did not associate with nuclei even in the complete reaction mixture.

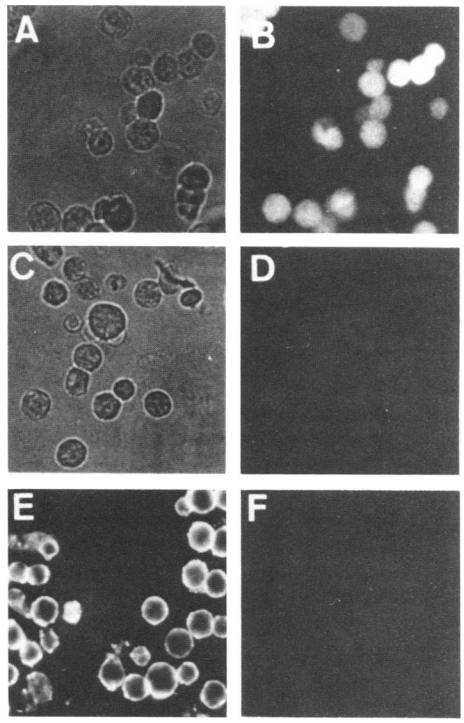

FIG. 1. Association of nucleoplasmin phycoerythrin with nuclei. Nucleoplasmin conjugated with phycoerythrin (350–400 kDa) was incubated with nuclei in the presence (*A* and *B*) or absence (*C* and *D*) of 0.4 mM ATP. The larger conjugate (2000 kDa) was incubated with nuclei in the presence (*E*) or absence (*F*) of 0.4 mM ATP. After incubation for 2 hr at 33°C, the reaction mixture was directly mounted on a glass slide and observed by phase-contrast (*A* and *C*) and fluorescence (*B*, *D–F*) microscopy.

To eliminate the apprehension that ATP-dependent association of nucleoplasmin with isolated nuclei occurs by diffusion through holes in the nuclear envelope followed by binding to intranuclear components, we examined the uptake of nucleoplasmin into nuclei treated with nonionic detergent. This treatment removes all lipids and thus destroys the outer membrane and part of the inner nuclear membrane (22). As shown in Fig. 2*A*, nuclei that had been treated with 1% Triton X-100 did not associate nucleoplasmin.

Role of Nucleotides. To obtain further information on the role of ATP, we examined the effects of pp[CH_2]pA and p[CH_2] pA, nonhydrolyzable analogs of ATP and ADP. On incubation at 33°C for 2 hr, pp[CH_2]pA had nearly the same effect as ATP on association of nucleoplasmin with nuclei,

Table 1. Effects of ATP, $KHCO_3$, and $CaCl_2$ on association of nucleoplasmin with nuclei

Reaction		Radioactivity, cpm			
		^{125}I-Nucleoplasmin		^{125}I-HMG_1	
Medium	Temperature, °C	Nuclei	Supernatant	Nuclei	Supernatant
Complete	33	71,000	28,700	45,000	50,000
Complete	4	3,400	92,300	6,000	89,000
Minus ATP	33	2,300	90,700	5,400	90,000
Minus $KHCO_3$	33	3,700	87,800	6,100	89,500
Minus $CaCl_2$, plus $MgCl_2$	33	1,200	88,000	4,500	92,000
Plus $MgCl_2$	33	76,300	14,700	44,000	55,000

Complete reaction medium is 10 mM Hepes, pH 7.8/60 mM KCl/2 mM $CaCl_2$/3 mM $KHCO_3$/0.4 mM ATP/1 mg of bovine serum albumin per ml.

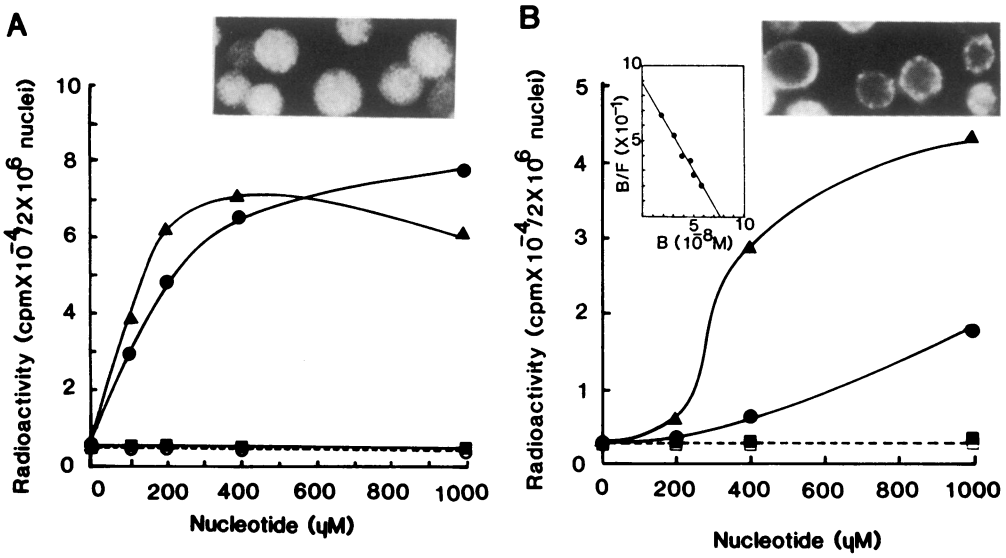

FIG. 2. Effects of pp[CH_2]pA and p[CH_2]pA on association of iodinated nucleoplasmin with nuclei. (*A*) Isolated nuclei were incubated with ^{125}I-nucleoplasmin at 33°C for 2 hr in the complete reaction medium but with various amounts of ATP (●), pp[CH_2]pA (▲), or p[CH_2]pA (■). As a control, nuclei treated with buffer containing 10 mM Hepes (pH 7.8), 60 mM KCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$, and 1% Triton X-100 were similarly incubated with ^{125}I-nucleoplasmin in the complete reaction medium with various amounts of ATP (○). (*B*) ^{125}I-Nucleoplasmin was incubated at 4°C for 20 min with nuclei pretreated with various amounts of ATP (●) or pp[CH_2]pA (▲) in the presence of $KHCO_3$ and $CaCl_2$. As a control, ^{125}I-nucleoplasmin was incubated with nuclei pretreated with pp[CH_2]pA in the absence of $KHCO_3$ (■) or $CaCl_2$ (□). Photomicrographs: (*A*) Nuclei incubated with nucleoplasmin phycoerythrin (350–400 kDa) at 33°C for 2 hr in the complete reaction medium; (*B*) pretreated nuclei incubated with the same conjugate at 4°C for 20 min. (*Inset*) Scatchard plots of 4°C binding of nucleoplasmin and nuclei. Various concentrations (0.3–2.4 μg) of ^{125}I-nucleoplasmin were added to the reaction mixture (45 μl) containing 2×10^6 pretreated nuclei. After incubation for 1 hr at 4°C, the specific counts associated with nuclei were measured.

whereas p[CH_2]pA did not support association (Fig. 2*A*). These results indicate that NTP is required for association of nucleoplasmin with nuclei, but that the primary reaction is not hydrolysis of the triphosphate portion.

Next, we incubated nuclei with pp[CH_2]pA or ATP without nuclear proteins (pretreatment) and then collected these pretreated nuclei and tested their ability to take up ^{125}I-nucleoplasmin. If nuclei were pretreated with nucleotide at 33°C in the presence of HCO_3^- and Ca^{2+}, ^{125}I-nucleoplasmin would become associated with the nuclei. The association reached a plateau within 20 min even at 4°C. The association of ^{125}I-nucleoplasmin with pretreated nuclei increased with increased concentrations of nucleotide in the pretreatment, and nuclei that had not been treated with nucleotides did not associate with nucleoplasmin (Fig. 2*B*). Fig. 2*B* (*Inset*) presents a Scatchard plot analysis of 4°C binding of nucleoplasmin to pretreated nuclei, which determined K_a as 1×10^7 (1/M) and maximum binding sites as 1×10^6 molecules per nucleus. In a parallel experiment, nucleotides extracted from nuclei after pretreatment were analyzed by HPLC, which revealed that pp[CH_2]pA becomes associated with nuclei during pretreatment. Association of ATP with nuclei was also detected in a similar manner but the amount was much less than that of pp[CH_2]pA. It is likely that ATP is hydrolyzed much more rapidly than pp[CH_2]pA. As shown in Fig. 2*B*, nucleoplasmin did not associate with nuclei pretreated at 33°C in the absence of HCO_3^- or Ca^{2+} or with all of these factors at 4°C. Association of pp[CH_2]pA was low or undetectable in these conditions, showing that the association of nucleoplasmin and nucleotides is coincidental. The association of nucleoplasmin with pretreated nuclei did not depend on the presence of additional nucleotide or HCO_3^- or temperature, but it did depend on the presence of Ca^{2+}. Using pepsin-treated nucleoplasmin, we confirmed that only a tail fragment or pentamers possessing one or more tails associated with nuclei in our *in vitro* system (Fig. 3). This is consistent with the *in vivo* experiment previously reported (4).

When nuclei and nucleoplasmin conjugated with phycoerythrin (350–400 kDa) were incubated at 33°C, the fluorescence was observed throughout the nucleus (Fig. 2*A*), while

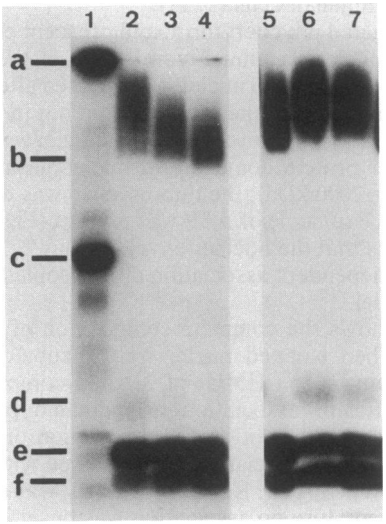

FIG. 3. Association of pepsin-digested products of nucleoplasmin with nuclei. Results with intact ^{125}I-nucleoplasmin (lane 1) and products formed by its digestion with pepsin for 10 (lane 2), 40 (lane 3), and 90 min (lane 4) are shown. Nuclear association of products formed by digestion with pepsin for 40 min was examined. Lane 5, products remaining in the supernatant after incubation; lane 6, products associated with nuclei after incubation in the complete reaction medium at 33°C for 2 hr; lane 7, products associated with pretreated nuclei after incubation at 4°C for 20 min. The positions of intact pentamer (a), core pentamer (b), intact monomer (c), 16-kDa tail (d), 14-kDa core (e), and 12-kDa tail (f) are indicated. Preferential association of 16-kDa and 12-kDa tail fragments with the nuclei is observed. The 14-kDa core monomer associated with nuclei was derived from the pentameric form of nucleoplasmin that retained one or more tail fragments. A core pentamer that had lost all its tail fragments did not associate with the nuclei (upper regions of lanes 6 and 7).

when pretreated nuclei and conjugated protein were incubated at 4°C, the fluorescence localized on the surface of the nuclei in a dotted profile (Fig. 2*B*). These observations suggest that the mode of association of nucleoplasmin with nuclei is different in these two reactions: in one case the protein enters the nucleus; in the other case the protein remains bound to the nuclear surface.

To investigate this possibility, we incubated nuclei that had been preincubated with ^{125}I-nucleoplasmin with excess unlabeled nucleoplasmin without ATP at 4°C overnight. When nuclei were incubated together with pp[CH_2]pA and ^{125}I-nucleoplasmin at 33°C, further incubation with unlabeled nucleoplasmin reduced the radioactivity of the nuclei to ≈75% of the initial level. When nuclei pretreated with nucleotide were incubated with ^{125}I-nucleoplasmin at 4°C, subsequent incubation with unlabeled nucleoplasmin reduced the radioactivity associated with the nuclei to 14% of the initial value. These results suggest that >70% of the nucleoplasmin associated with the nuclei at 33°C seemed to localize in the nuclei, whereas ≈85% of that associated with pretreated nuclei at 4°C localizes on the nuclear surface. When nuclei that had been incubated with ^{125}I-nucleoplasmin at 4°C were further incubated at 33°C for 30 min, and then excess unlabeled nucleoplasmin was added, the amount of radioactivity was not reduced.

Competition Study. To determine whether the *in vitro* association of nuclear proteins with nuclei was specific, we tested the ability of unlabeled proteins to compete with iodinated proteins for association with nuclei. Addition of unlabeled nucleoplasmin decreased the nuclear association of ^{125}I-nucleoplasmin (Fig. 4). Addition of >500 μg of bovine serum albumin or IgG did not affect the association of ^{125}I-nucleoplasmin with nuclei. Unlabeled HMG_1 similarly competed with ^{125}I-HMG_1 for nuclear association. Moreover, unlabeled nucleoplasmin competed with ^{125}I-HMG_1, which shows these two nuclear proteins associated with the nuclei at the same sites at both 4°C and 33°C.

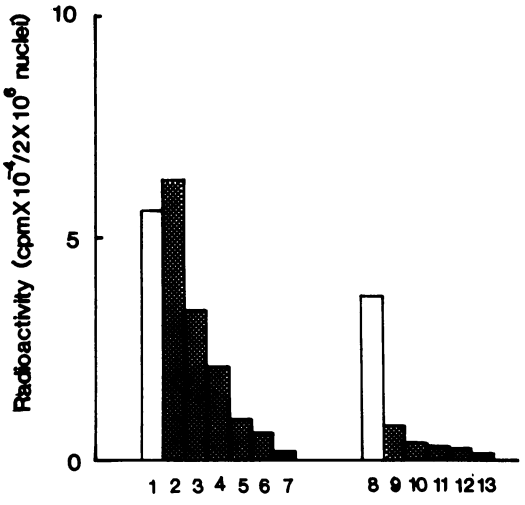

FIG. 4. Competition by unlabeled nucleoplasmin for association of iodinated nucleoplasmin with nuclei. Samples of 0.04 μg of iodinated nucleoplasmin (specific activity, 1.6 × 10^6 cpm/μg) were incubated with various amounts of unlabeled nucleoplasmin with 2 × 10^6 nuclei at 33°C for 2 hr in the presence of 1 mM ATP, HCO_3^-, and Ca^{2+} (lanes 1–7) or at 4°C with the same amount of nuclei pretreated with 1 mM pp[CH_2]pA for 3 hr in the presence of bicarbonate and calcium ion (lanes 8–13). Unlabeled nucleoplasmin was added (stippled bars) at 1.5 (lanes 2 and 9), 3.0 (lanes 3 and 10), 4.5 (lanes 4 and 11), 6.0 (lane 5), or 7.5 (lanes 6 and 12) μg. Lanes 1 and 8, incubation without unlabeled nucleoplasmin. Controls (solid bars) were incubated at 33°C without ATP (lane 7), and at 4°C with nuclei not pretreated with pp[CH_2]pA (lane 13).

Inhibition by Lectins of the Association of Nuclear Proteins with Nuclei. A group of the nuclear pore complex proteins was shown to be a wheat germ agglutinin (WGA)-binding glycoprotein, which possesses the O-linked monosaccharide GlcNAc (23–27). Recently, WGA was reported to inhibit nuclear transport of nucleoplasmin in the cell-free system by using reconstituted nuclei in *Xenopus* oocyte extract (28). In our laboratory, the inhibitory effect of WGA on *in vivo* transport of nucleoplasmin into nuclei was observed by microinjecting WGA (final concentration, 0.2–0.25 mg/ml) and nuclear proteins into the cytoplasm of mammalian cultured cells (29). Therefore, we examined the effect of WGA on the association of nucleoplasmin and HMG_1 with isolated nuclei. WGA inhibited association of nucleoplasmin and HMG_1 with pretreated nuclei at 4°C by >80% (Fig. 5*A*) when added before or during treatment of the nuclei with pp[CH_2]pA. However, if WGA was added after treatment of the nuclei with pp[CH_2]pA, it did not inhibit association of nuclear proteins with nuclei. WGA inhibited association of pp[CH_2]pA with nuclei to some extent (Fig. 5*B*). This suggests that the absence of association of nuclear proteins with nuclei was caused by the absence of association of pp[CH_2]pA with nuclei in the presence of WGA. The addition of 0.2 M GlcNAc released both inhibition of association of pp[CH_2]pA and of nuclear proteins caused by WGA. Other lectins such as wisteria floribunda agglutinin or lentil lectin did not affect association of pp[CH_2]pA with nuclei. Wisteria floribunda agglutinin did not affect association of nuclear proteins with nuclei. Lentil lectin weakly decreased the association but its inhibitory effect was never as complete as that of WGA. Thus, WGA specifically blocks the association of pp[CH_2]pA and nucleoplasmin in this reaction system.

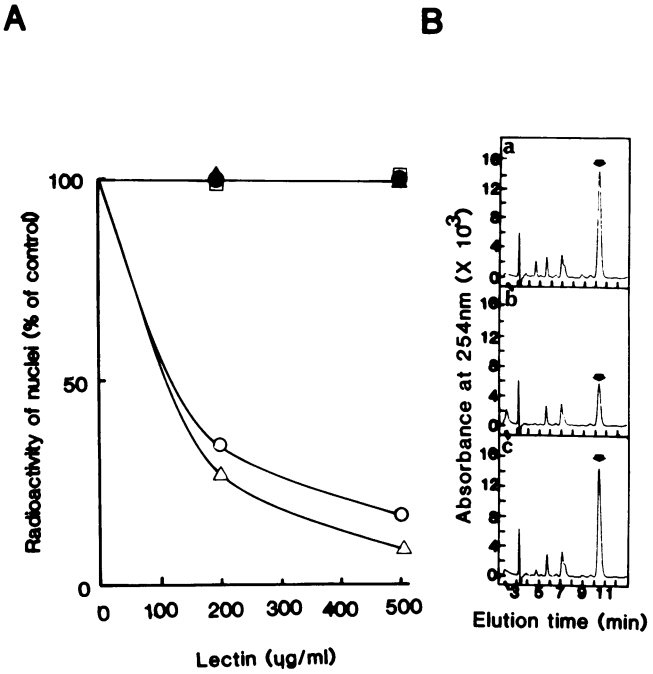

FIG. 5. Effects of WGA on association of nuclear proteins and nucleotides with nuclei. (*A*) Iodinated nucleoplasmin (circles and squares) or HMG_1 (triangles) was incubated at 4°C with nuclei pretreated with pp[CH_2]pA at 33°C in the presence of WGA (○, △), wisteria floribunda agglutinin (□), or WGA plus 0.2 M GlcNAc (●, ▲). Radioactivity associated with nuclei pretreated in the absence of lectins was taken as 100%. (*B*) Analysis of nuclear-associated pp[CH_2]pA by HPLC. Nuclei were incubated with pp[CH_2]pA, bicarbonate, and calcium ion at 33°C for 2 hr without lectin (*a*) or with 500 μg of WGA per ml (*b*), or with 500 μg of WGA per ml plus 0.2 M GlcNAc (*c*). The elution peak of pp[CH_2]pA is indicated by arrows.

DISCUSSION

This paper reports the specific association of *Xenopus* nucleoplasmin and mammalian HMG_1 with isolated rat liver nuclei in a reaction dependent on NTP, HCO_3^-, Ca^{2+}, and temperature. We consider such associations of nuclear proteins with nuclei to be the function of the nuclear envelope because of the following points. As described above, nucleoplasmin first associated with the nuclear envelope. Furthermore, nuclei treated with nonionic detergent completely lose their ability to associate nuclear proteins (Fig. 2*A*). But nucleoplasmin could associate with mildly sonicated nuclei in an ATP-dependent manner under these conditions. In our recent experiment, however, when nuclear membranes were isolated by treatment with heparin, salt, and DNase I and incubated with nucleoplasmin in the complete reaction medium, we found the protein could associate with the nuclear membranes, having no inner nuclear components.

We must ask whether this *in vitro* reaction reflects the *in vivo* nuclear transport of nuclear proteins. Association of proteins with nuclei in our system was specific for nuclear proteins, such as nucleoplasmin and HMG_1, and did not occur with nonnuclear proteins. However, bovine serum albumin chemically conjugated with the simian virus 40 large tumor antigen nuclear localization sequence (average, five peptides per protein) did not associate with nuclei in the reaction condition described in this paper. Thus, our *in vitro* system is not a complete system for the study of all varieties of nuclear protein transport. This may be due to a lack of some cytoplasmic or nuclear factors or to differences in the mechanism of nuclear transport of nucleoplasmin and large tumor antigen. Nevertheless, since the *in vitro* association of nucleoplasmin with nuclei depended on its C-terminal "tail" portion and was inhibited by WGA, as it is *in vivo*, we believe that this *in vitro* reaction reflects at least one or two initial reactions of *in vivo* nuclear transport.

Our results are all consistent with the idea that the association of NTP with nuclei is essential for association of nucleoplasmin and HMG_1 with the nuclei. Thus, the existence of a NTP-binding protein participating in this reaction is suggested. We think the nuclear component that recognizes nucleoplasmin and HMG_1 may be a NTP-binding protein or a receptor that is activated by a NTP-binding protein. The inhibitory effect of WGA on nuclear association of nuclear proteins is probably caused by inhibition of NTP binding to the nuclear component. Recent reports have shown that O-linked GlcNAc is present on proteins of 63 kDa and several other proteins (35–110 kDa) of the nuclear pore complex (23–28). Identification and characterization of WGA-binding protein, NTP-binding protein, and nucleoplasmin- or HMG_1-binding protein will lead to a better understanding of the nuclear transport mechanism.

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