AD-A285 842

[redacted]

ATION PAGE

Form Approved
OMB No 0704-0188

1 AGENCY USE ONLY (Leave blank)	2 REPORT DATE	3 REPORT TYPE AND DATES COVERED
	1994	Reprint

4 TITLE AND SUBTITLE

(see title on reprint)

5 FUNDING NUMBERS

PE: NWED QAXM

WU: 00150

6 AUTHOR(S)

Kalinich, J.F., and McClain, D.E.

7 PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Armed Forces Radiobiology Research Institute
8901 Wisconsin Ave.
Bethesda, MD 20889-5603

8 PERFORMING ORGANIZATION REPORT NUMBER

SR94-16

9 SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Uniformed Services University of the Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20814-4799

10 SPONSORING/MONITORING AGENCY REPORT NUMBER

11 SUPPLEMENTARY NOTES

12a DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution unlimited.

12b DISTRIBUTION CODE

DTIC
ELECTE
OCT 31 1994
D
G

13 ABSTRACT (Maximum 200 words)

94-33533

[redacted]

Distribution/
Availability Codes
Dist | Avail and/or Special
A-1 | 20

14 SUBJECT TERMS

15 NUMBER OF PAGES
7

16 PRICE CODE

17 SECURITY CLASSIFICATION OF REPORT	18 SECURITY CLASSIFICATION OF THIS PAGE	19 SECURITY CLASSIFICATION OF ABSTRACT	20 LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED		

NSN 7540-01 280 5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. 239-18
298-102

9410 27 033

SECURITY CLASSIFICATION OF THIS PAGE

CLASSIFIED BY

DECLASSIFY ON

SECURITY CLASSIFICATION OF THIS PAGE

PROTEIN EXPRESSION AND PURIFICATION 5, 324–330 (1994)

Rapid Isolation of Nuclear Transport-Competent *Xenopus* Nucleoplasmin Produced in *Escherichia coli* Strain BL21(DE3)

John F. Kalinich and David E. McClain

Radiation Biochemistry Department, Armed Forces Radiobiology Research Institute, 8901 Wisconsin Avenue, Bethesda, Maryland 20889-5603

Received October 19, 1993, and in revised form January 12, 1994

Nucleoplasmin is a thermostable karyophilic protein widely used in nuclear transport studies. An expression vector was constructed that contains a string of 10 histidine residues ligated, in frame, to the amino terminus end of the *Xenopus* nucleoplasmin gene. The vector was then transformed into *Escherichia coli* strain BL21(DE3). This strain possesses the gene for T7 RNA polymerase under control of the lacUV5 promoter. The induction of the RNA polymerase and subsequent production of nucleoplasmin occurs after exposure to isopropyl-β-D-thiogalactopyranoside. The nucleoplasmin, produced in milligram quantities per liter of culture, is then isolated by a rapid purification method that includes metal chelation chromatography to purify the oligohistidine-linked nucleoplasmin. Nuclear transport studies indicate that fluorescently labeled nucleoplasmin is translocated to the nuclear interior of permeabilized V79A03 cells, while nucleoplasmin that lacks a nuclear localization signal (core nucleoplasmin) is not imported. The use of this method to produce nuclear transport-competent nucleoplasmin avoids the lengthy purification procedure used to isolate nucleoplasmin from *Xenopus laevis* oocytes as well as the cost of purchasing and maintaining a toad colony.

The process of protein translocation into the cell nucleus is currently an area of great interest. One of the most widely used probes for nuclear transport studies is nucleoplasmin, a thermostable acidic pentameric protein involved in histone binding and nucleosome formation (1–4). A procedure to produce small amounts of radiolabeled nucleoplasmin via a coupled *in vitro* transcription/translation protocol has been reported (5). However, isolation of milligram quantities of nucleoplasmin requires the use of *Xenopus laevis* oocytes, where nucleoplasmin constitutes up to 10% of the nuclear protein (6,7). To avoid the high cost of purchasing and maintaining toads, we have devised a method to produce and isolate milligram quantities of nuclear transport-competent nucleoplasmin from *Escherichia coli*.

The expression vector pET-16b contains the lac operator and repressor downstream of the T7 promoter (8). This allows transcription of the cloned gene to be almost completely eliminated until induction is initiated. In addition, pET-16b codes for a stretch of 10 histidine residues after the initiator methionine, followed by a Factor Xa protease cleavage site (9). A small cloning region allows for the introduction of the gene of interest in frame with the oligohistidine and protease cleavage regions. The oligohistidine region permits the rapid purification of the expressed protein by metal chelation chromatography (10,11).

E. coli strain BL21(DE3) contains a single copy of the T7 RNA polymerase gene under control of the lacUV5 promoter (12,13). This allows for the overexpression of a T7 plasmid-containing gene following induction with isopropyl-β-D-thiogalactopyranoside (IPTG). This system, along with the expression vector pET-16b, was used to produce nucleoplasmin in *E. coli*. Rapid purification of the nucleoplasmin was then accomplished by a heating step and metal chelation chromatography. Experiments indicated that purified *E. coli*-produced nucleoplasmin can substitute for nucleoplasmin isolated from *X. laevis* oocytes in nuclear transport studies. This method circumvents the time-consuming procedure of isolating and purifying nucleoplasmin from *X. laevis* oocytes as well as the costs involved with purchasing and maintaining a toad colony.

EXPERIMENTAL

Construction of pET16b-NED

Plasmid pET-16b was purchased from Novagen (Madison, WI). Plasmid pET16b-NED was constructed

1046-5928/94 $6.00

by ligation of a 0.68-kb [illegible]-*Bam*HI fragment, containing the *X. laevis* nucleoplasmin gene, from pT7-NED (5) into *Nde*I/*Bam*HI-cut pET-16b. The nucleoplasmin cDNA (23) used to construct pT7-NED was kindly provided by Dr. Thomas Burglin (Massachusetts General Hospital, Boston, MA). *E. coli* strain DH1 was transformed with the ligation mixture using $CaCl_2$, and transformants possessing the pET16b-NED plasmid were selected by plating on LB medium supplemented with 50 μg/ml carbenicillin. Restriction digestions, agarose gel electrophoresis, ligation reactions, and transformations were as described (14). The linkage region between the pET-16b oligohistidine leader sequence and the nucleoplasmin gene was confirmed by DNA sequencing using the dideoxy method (15).

Preparation of Transformed BL21(DE3)

E. coli strain BL21(DE3) (F⁻ *hsdS gal ompT* [illegible]) was kindly provided by Dr. Paul Herring (Indiana University, Indianapolis, IN). Preparation of plasmid pET16b-NED DNA and transformation of *E. coli* strain BL21(DE3) using $CaCl_2$ were as described (14). Stable transformants were selected by plating on M9ZYB medium (1 g/liter NH_4Cl, 3 g/liter KH_2PO_4, 6 g/liter Na_2HPO_4, 4 g/liter glucose, 2 mM $MgSO_4$, 0.1 mM $CaCl_2$, 10 g/liter tryptone, 5 g/liter NaCl, 5 g/liter yeast extract) supplemented with 50 μg/ml carbenicillin and 1.0 mM IPTG (12).

Expression and Isolation of Nucleoplasmin

A 25-ml culture of *E. coli* strain BL21(DE3) containing pET16b-NED was grown overnight at 37°C and 200 rpm in M9ZYB medium supplemented with 50 μg/ml carbenicillin. The overnight culture was used to inoculate 500 ml of M9ZYB–carbenicillin. After reaching an OD_{600} of 1.0, the culture was induced by the addition of IPTG to 1.0 mM. After 3 h the cells were harvested by centrifugation at 3000*g* and 4°C for 10 min, pooled, and washed once with water. The pellet was resuspended in 20 mM Tris–HCl, pH 7.9, containing 0.5 M NaCl, 5 mM imidazole, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 1 μg/ml each of pepstatin, leupeptin, and aprotinin (10 ml/liter of culture) and disrupted by sonication (4 × 30 s, setting 7; Heat Systems Cell Disruptor with microtip). The mixture was centrifuged at 12,000*g* for 15 min at 4°C. The supernatant, containing the nucleoplasmin, was heated at 80°C for 10 min and centrifuged at 12,000*g* for 30 min at 4°C. To purify the nucleoplasmin further, the supernatant was loaded onto a Ni^{2+}-metal chelation resin column (20 mg protein/2.5 ml column volume) (Novagen). Column preparation and chromatography were conducted as described in the manufacturer's instructions. Briefly, the column was washed with 10 column vol of binding buffer (20 mM Tris–HCl, pH 7.9, 0.5 M NaCl, 5 mM imidazole) and 6 column vol of wash buffer (20 mM Tris–HCl, pH 7.9, 0.5 M NaCl, 60 mM imidazole). The oligohistidine-linked nucleoplasmin was eluted from the column with 6 column vol of elution buffer (20 mM Tris–HCl, pH 7.9, 0.5 M NaCl, 1 M imidazole). The eluted protein was concentrated in a Centricon C-10 microconcentrator (W. R. Grace Co., Beverly, MA) and desalted by passage through a Sephadex G-25 column. The oligohistidine region could be removed from the nucleoplasmin by treating with Factor Xa (16), but since this region had no apparent effect on the ability of the protein to localize to the nucleus of permeabilized cells, it was not routinely removed.

Miscellaneous Methods

Nucleoplasmin lacking a nuclear localization signal (core nucleoplasmin) was prepared as described (17). Electrophoresis of nucleoplasmin purification fractions using SDS–polyacrylamide gels was performed by the method of Laemmli (18). Antiserum to nucleoplasmin was kindly provided by Dr. Carl Feldherr (University of Florida, Gainesville, FL). Western blot analysis was performed as described (19). Purified nucleoplasmin and core nucleoplasmin were fluorescently labeled with tetramethylrhodamine isothiocyanate (TRITC) by the method of Newmeyer *et al.* (20). Polyacrylamide gels and Western blots were scanned by a Molecular Dynamics Laser Densitometer (Suunyvale, CA) and the density volumes calculated using the associated ImageQuant software.

Cell Culture Conditions

Alpha minimal essential medium, fetal calf serum, penicillin, streptomycin, L-glutamine, and *N*-2-hydroxyethylpiperizine-*N*′-2-ethane sulfonic acid (Hepes) were purchased from Gibco/BRL (Gaithersburg, MD). Chinese hamster lung fibroblasts (clone V79A03) were maintained as monolayer cultures at 37°C in an atmosphere of 5% CO_2 in air in alpha minimal essential medium supplemented with 10% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, and 25 mM Hepes. For nuclear transport experiments, cells were plated onto glass coverslips (9 × 35 mm). Cells were used when they were 70–80% confluent. To prepare for permeabilization the cells were placed on ice, washed with cold Buffer A* (20 mM Hepes, pH 7.3, 110 mM potassium acetate, 2 mM magnesium acetate, 1 mM EGTA, 2 mM dithiothreitol, 1 μg/ml each of aprotinin, leupeptin, and pepstatin) (21,22) and then incubated 5 min on ice with 35 μg/ml digitonin in Buffer A*. The cells were washed once again after permeabilization with cold Buffer A* and left in cold Buffer A* until needed.

Preparation of Cytosolic Fraction

V79A03 cells were harvested by scraping from the tissue culture plates with a rubber policeman. The cells were washed with cold Hanks' balanced salt solution and resuspended in hypotonic buffer (10 mM Hepes, pH 7.4, 5 mM $MgCl_2$, 1 mM PMSF, and 1 μg/ml each of aprotinin, leupeptin, and pepstatin) at 10 ml/10^8 cells. The cell suspension was left on ice for 15 min and then disrupted with 15 strokes of a Dounce homogenizer (pestle A). The mixture was centrifuged at 1000*g* and 4°C for 10 min, producing a supernatant that was then centrifuged at 10,000*g* and 4°C for 20 min. The supernatant resulting from this step was centrifuged at 150,000*g* and 4°C for 2 h. The resulting supernatant from this high-speed centrifugation step was dialyzed overnight at 4°C against multiple changes of Buffer A*, concentrated in a Centricon C-10 microconcentrator, and brought to a protein concentration of 40 mg/ml with Buffer A*.

In Vitro Nuclear Transport

A standard transport reaction contained bovine serum albumin (1 mg/ml), ATP (1 mM), creatine kinase (20 U/ml), creatine phosphate (5 mM), cytosolic fraction (10 mg protein/ml), and TRITC-nucleoplasmin (5 μg/ml), brought to a final volume of 20 μl with Buffer A* (22). Coverslips, containing the digitonin-permeabilized cells, were blotted on a paper towel to remove excess fluid and placed cell side down onto 20 μl of the transport mixture on a sheet of parafilm. Transport reactions were run in a humidified box at 30°C for 15 min. Other additions to the transport reactions are as given in the figure legends. Reactions were terminated by the addition of 250 μl of cold Buffer A*. The coverslips were washed once with cold Buffer A* and fixed on ice for 5 min with 3% paraformaldehyde in Buffer A (Buffer A* minus dithiothreitol and protease inhibitors). The coverslips were washed with cold Buffer A, blotted on a paper towel, and mounted on a glass slide on one drop of 1 mg/ml phenylenediamine in 90% glycerol/10% phosphate-buffered saline. Nail polish was used to seal the edges of the coverslip. Slides were examined with an Olympus AH-3 fluorescence microscope and photomicrographs taken with Polaroid Type-57 film.

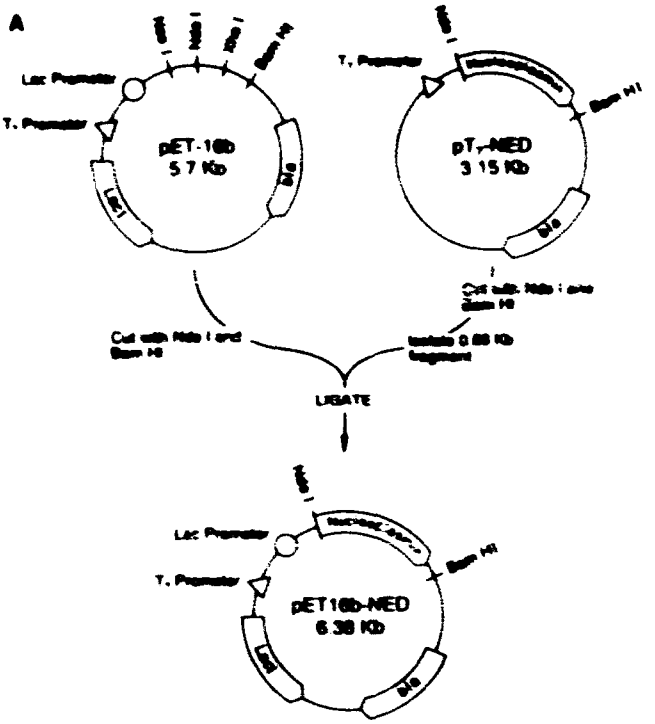

FIG. 1. Construction of pET16b-NED (A) Plasmid maps of pET-16b and pT7-NED and the construction of pET16b-NED. Details are given in the text. The lac repressor gene is represented by lacI, while the gene conferring carbenicillin (ampicillin) resistance is denoted by bla (β-lactamase). (B) DNA sequence of the expressed linker region between pET-16b and the nucleoplasmin gene. The first 62 base pairs (uppercase) are derived from pET-16b. The next 20 base pairs (lowercase) are a result of the cloning procedure used to subclone the nucleoplasmin cDNA into pT7-7 (5). The bold letters represent the first base pairs of the nucleoplasmin cDNA (23). (C) The amino acid sequence of the linker region described in B. The underlined region indicates the Factor Xa protease cleavage site.

RESULTS AND DISCUSSION

The construction of pET16b-NED is shown in Fig. 1A. A 0.68-kb *NdeI/Bam*HI fragment from pT7-NED containing the coding region for the *X. laevis* nucleoplasmin gene was ligated into *NdeI/Bam*HI-digested pET-16b. The resulting plasmid, pET16b-NED, contained the nucleoplasmin gene plus an additional 21 amino acids attached to the amino terminal end of the protein. This stretch of amino acids contains 10 histidine residues that allow for protein purification by metal chelation chromatography and a Factor Xa protease site that allows for removal of the "leader sequence" from the nucleoplasmin. The base and amino acid sequences of the linker region are shown in Figs. 1B and 1C. The presence of the leader sequence did not affect

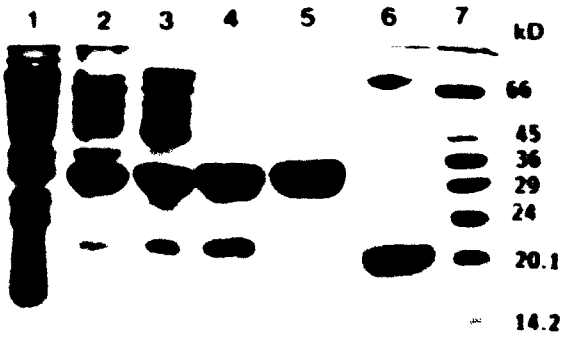

FIG. 2. SDS-polyacrylamide gel of nucleoplasmin isolation fractions. The uninduced (lane 1), induced (lane 2), homogenate (lane 3), heat (lane 4), and column (lane 5) fractions from a typical nucleoplasmin isolation as well as core nucleoplasmin (lane 6) were electrophoresed on a 12.5% SDS-polyacrylamide gel (50 μg protein per lane). The gel was stained with Coomassie blue and destained in 10% acetic acid/methanol solution. Lane 7 contains the molecular mass markers (Sigma): bovine serum albumin, 66 kDa; egg albumin, 45 kDa; glyceraldehyde-3-phosphate dehydrogenase, 36 kDa; carbonic anhydrase, 29 kDa; trypsinogen, 24 kDa; trypsin inhibitor, 20.1 kDa; and α-lactalbumin, 14.2 kDa.

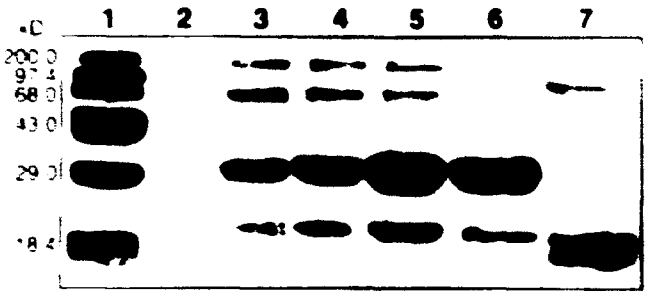

FIG. 3. Western blot of nucleoplasmin isolation fractions. Nucleoplasmin isolation fractions were electrophoresed on 12.5% SDS-polyacrylamide gels as described and then blotted to nitrocellulose. The blot was probed with antisera (1:500 dilution) developed in rabbit against *X. laevis* nucleoplasmin. After incubation with horseradish peroxidase-goat anti-rabbit IgG (Sigma), color development was achieved using 4-chloro-1-naphthol. The following samples are represented: molecular mass markers (lane 1), uninduced fraction (lane 2), induced fraction (lane 3), homogenate fraction (lane 4), heat fraction (lane 5), column fraction (lane 6), and core nucleoplasmin (lane 7). Prestained molecular mass markers (Gibco/BRL, Gaithersburg, MD) are myosin H-chain, 200 kDa; phosphorylase *b*, 97.4 kDa; bovine serum albumin, 68 kDa; ovalbumin, 43 kDa; carbonic anhydrase, 29 kDa; and β-lactoglobulin, 18.4 kDa.

the ability of the nucleoplasmin to be imported to the nuclear interior, so it was not routinely removed (data not shown).

A representative Coomassie blue-stained SDS–polyacrylamide gel of the fractions obtained during the purification of nucleoplasmin is shown in Fig. 2. Lane 1 is an uninduced culture of BL21(DE3) containing pET16b-NED. The protein profile in lane 2 was obtained following a 3-h induction of nucleoplasmin by IPTG. As seen, an intense nucleoplasmin band migrating at a molecular weight of approximately 32 kDa constitutes much of the protein in this fraction. Lane 3 is the supernatant resulting from the sonication and centrifugation of the bacteria. After heat treatment (10 min/80°C) and centrifugation, the profile in lane 4 was obtained. This step eliminated the majority of the bacterial proteins. However, along with the nucleoplasmin, two additional bands, migrating at approximately 21 and 26 kDa, are apparent. To purify the nucleoplasmin further, metal chelation chromatography was used. The nucleoplasmin-containing fraction was chromatographed on a Ni^{2+} column, which bound the oligohistidine leader sequence ligated to the nucleoplasmin. The contaminating proteins were then washed from the column and the nucleoplasmin was eluted as described. The column-purified nucleoplasmin is shown in lane 5. The 21- and 26-kDa bands were still present in this fraction, albeit as only a very small percentage of the total protein (0.9 and 0.3%, respectively). Lane 6 is core nucleoplasmin (nucleoplasmin lacking a nuclear localization signal) and was prepared by treating intact nucleoplasmin with trypsin (17). The 20-kDa band is the monomeric form of the core nucleoplasmin, while the higher-molecular-weight band represents the pentameric form. A Western blot of the nucleoplasmin isolation fractions is shown in Fig. 3. Lane 2 (uninduced culture) shows no reaction. A 32-kDa band was recognized by antisera produced against *X. laevis* nucleoplasmin in each of the isolation fractions (lanes 3–6). Lane 7 (core nucleoplasmin) shows a reaction at 20 kDa.

In addition to the 32-kDa band, the nucleoplasmin antisera also recognized the 21- and 26-kDa bands seen in the Coomassie-stained gel, indicating that these

TABLE 1

Purification of Nucleoplasmin

Fraction	Protein, mg	Percentage nucleoplasmin	Percentage yield	Fold purification
Induced	21.31.0	42.1	100	
Homogenate	870.6	37.8	48	1
Heat	98.0	76.7	11	2
Column	7.8.6	98.8	11	3

Note. Protein concentrations were determined using the method of Bradford (25) and are based on a 1-liter culture. SDS-polyacrylamide gels were scanned by laser densitometry. The density volume of the nucleoplasmin band (32 kDa) was calculated and divided by the density volume obtained from scanning the entire sample lane to yield "percentage nucleoplasmin" (nucleoplasmin as a percentage of total protein).

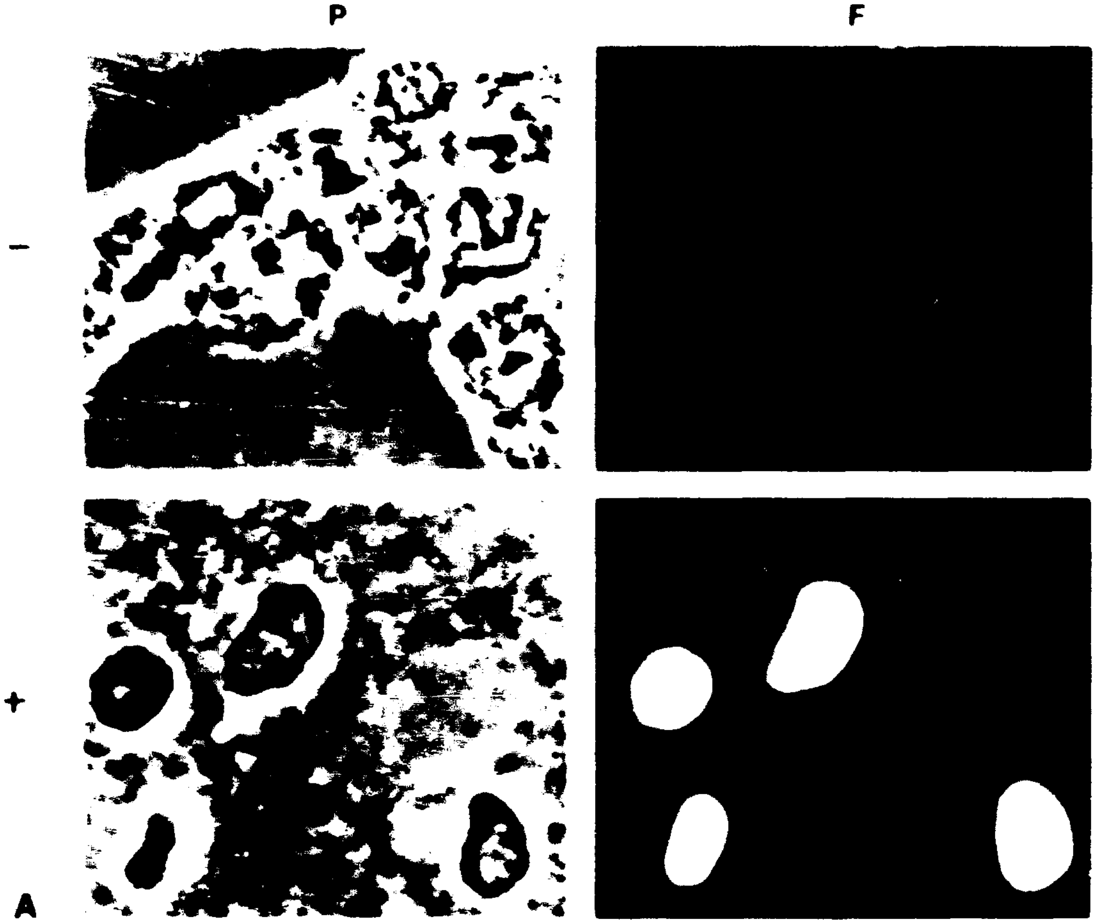

FIG. 4. Photomicrographs of nuclear transport experiments. (A) Requirement for cytosolic factors. Transport experiments [illegible] ... (B) Import [illegible] ... TRITC [illegible] ... ATP [illegible] ... Buffer A.

bands represent a truncated form of nucleoplasmin. Even though these proteins had no affect on the ability of nucleoplasmin to accumulate in the nuclear interior, we sought to determine their origin. Originally it was thought these bands represented proteins produced from methionine residues 3' to the initiator methionine. This should result in shorter forms of nucleoplasmin that might still be immunologically recognized by the nucleoplasmin antisera. However, if this were the case, the oligohistidine leader sequence would not be present, and the truncated nucleoplasmin species would not bind to the Ni column. As shown in both Figs. 2 and 3, the truncated forms of nucleoplasmin bound to the Ni column. The shortened nucleoplasmin species may have resulted from proteolysis during the isolation procedure. However, the presence of the protease inhibitors PMSF, pepstatin, leupeptin, and aprotinin during isolation would tend to argue against that possibility. Furthermore, *E. coli* strain BL21(DE3) is deficient in both the *lon* and the *ompT* proteases that would contribute to sample proteolysis (13). We attempted to eliminate these truncated nucleoplasmin bands by treating the bacterial culture with rifampicin 30 min after IPTG induction to inhibit transcription by the bacterial RNA polymerases and production of any bacterial proteases. However, no differences were observed between the gel patterns of nucleoplasmin obtained from rifampicin-treated and -untreated cultures. Changing the incubation temperature (30, 35, 37°C), the culture medium (M9ZYB, LB), and the harvest time (30 min to 3 h post-IPTG induction) also had no effect on the distribution of the intact and truncated forms of the nucleoplasmin (data not shown). The origin of these bands remains to be determined.

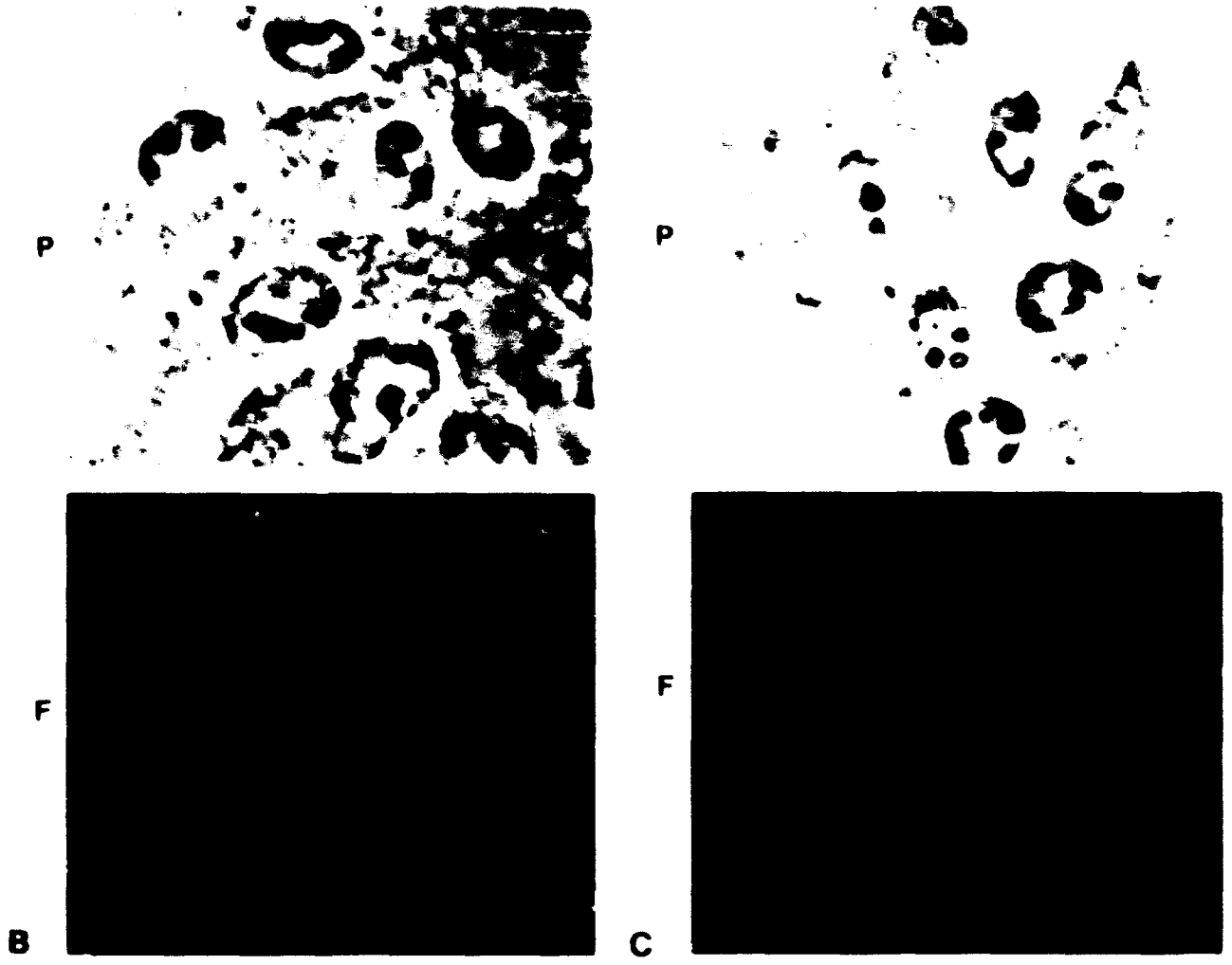

FIG. 4. (Continued)

Table 1 is a summary of the purification of nucleoplasmin. After induction with IPTG, almost ... of the protein present in the "induced fraction" was nucleoplasmin. Sonication and centrifugation of the bacteria yielded the "homogenate fraction." Nucleoplasmin accounted for greater than ... of the protein content of this fraction. The "heat fraction" was obtained after heat treatment and centrifugation. Nucleoplasmin made up well over 75% of the protein found in this fraction, with the truncated nucleoplasmin forms and small-molecular-weight contaminants accounting for the remainder. After metal chelation chromatography, greater than 98% of the protein in the "column fraction" was nucleoplasmin (32 kDa) with the remaining protein being mostly 21- and 26-kDa truncated forms of nucleoplasmin (approximately 1 and 0.5%, respectively).

After isolation, the nucleoplasmin was fluorescently labeled with rhodamine and tested in an *in vitro* nuclear transport system using permeabilized mammalian cells (21,22). Shown in Fig. 4A are the phase contrast and fluorescent photomicrographs of a 30°C 15 min transport experiment run with and without the cytosolic fraction. When incubated in the presence of the cytosolic fraction and an ATP regenerating system, nucleoplasmin is imported to the nuclear interior of digitonin-permeabilized V79A03 cells. When the cytosolic fraction is omitted, the nucleoplasmin does not localize to the nucleus. This confirms the results of other investigators using nucleoplasmin isolated from *X. laevis* oocytes in similar nuclear transport systems (21,22,24). Figure 4B demonstrates that nucleoplasmin lacking a nuclear localization signal (core nucleoplasmin) does not translocate to the nuclear interior. The requirement for ATP is represented in Fig. 4C, which shows that only a small amount of nucleoplasmin is localized to the nucleus if an ATP regenerat-

ing system is omitted from the reaction mixture. The small amount of import observed can be attributed to endogenous ATP in the cytosolic preparation. As demonstrated in Fig. 4, nucleoplasmin produced in *E. coli* and purified by this procedure performs in a manner analogous to the protein isolated from *X. laevis* oocytes.

We have developed a procedure for isolating milligram quantities of nucleoplasmin produced in *E. coli* strain BL21(DE3). The isolation procedure is rapid and routinely yields 70–80 mg of nucleoplasmin per liter of bacterial culture. The nucleoplasmin produced by this procedure migrates at the same apparent molecular mass on SDS–polyacrylamide gels as nucleoplasmin isolated from *X. laevis* oocytes and is recognized by antisera produced against the *Xenopus* nucleoplasmin. The *E. coli*-produced nucleoplasmin is also transport-competent. It is imported to the nuclear interior of digitonin-permeabilized V79A03 cells. Nucleoplasmin lacking a nuclear localization signal (core nucleoplasmin) does not associate with the cell nucleus. As with other systems, nuclear import is dependent upon not only an intact nuclear localization signal but also the presence of cytosolic factors and ATP. This procedure provides an easy method for producing and purifying nucleoplasmin for nuclear transport studies.

ACKNOWLEDGMENTS

This work was supported by the Armed Forces Radiobiology Research Institute under Work Unit 00150. We thank Dr. Thomas Burglin for providing the nucleoplasmin cDNA, Dr. Paul Herring for providing *E. coli* strain BL21 DE3, and Dr. Carl Feldherr for his gift of antiserum to nucleoplasmin.

REFERENCES

1. Laskey, R. A., Honda, B. M., Mills, A. D., and Finch, J. T. (1978) Nucleosomes are assembled by an acidic protein which binds histones and transfers them to DNA. *Nature* **275**, 416–420.
2. Earnshaw, W. C., Honda, B. M., and Laskey, R. A. (1980) Assembly of nucleosomes: The reaction involving *X. laevis* nucleoplasmin. *Cell* **21**, 373–383.
3. Dingwall, C., Sharnick, S. V., and Laskey, R. A. (1982) A polypeptide domain that specifies migration of nucleoplasmin into the nucleus. *Cell* **30**, 449–458.
4. Sealy, L., Burgess, R. R., Cotten, M., and Chalkley, R. (1989) Purification of *Xenopus* egg nucleoplasmin and its use in chromatin assembly *in vitro*. *Methods Enzymol.* **170**, 612–631.
5. Kalinich, J. F., and Douglas, M. G. (1989) *In vitro* translocation through the yeast nuclear envelope. *J. Biol. Chem.* **264**, 17979–17989.
6. Mills, A. D., Laskey, R. A., Black, P., and DeRobertis, E. M. (1980) An acidic protein which assembles nucleosomes *in vitro* is the most abundant protein in *Xenopus* oocyte nuclei. *J. Mol. Biol.* **139**, 561–568.
7. Krohne, G., and Franke, W. W. (1980) Immunological identification and localization of the predominant nuclear protein of the amphibian oocyte nucleus. *Proc. Natl. Acad. Sci. USA* **77**, 1034–1038.
8. Dubendorff, J. W., and Studier, F. W. (1991) Controlling basal expression in an inducible T7 expression system by blocking the target T7 promoter with lac repressor. *J. Mol. Biol.* **219**, 45–59.
9. Hoffmann, A., and Roeder, R. G. (1991) Purification of his-tagged proteins in non-denaturing conditions suggests a convenient method for protein interaction studies. *Nucleic Acids Res.* **19**, 6337–6338.
10. Smith, M. C., Furman, T. C., Ingolia, T. D., and Pidgeon, C. (1988) Chelating peptide-immobilized metal ion affinity chromatography. A new concept in affinity chromatography for recombinant proteins. *J. Biol. Chem.* **263**, 7211–7215.
11. Ljungquist, C., Breitholtz, A., Brinj-Nilsson, H., Moks, T., Uhlen, M., and Nilsson, B. (1989) Immobilization and affinity purification of recombinant proteins using histidine peptide fusions. *Eur. J. Biochem.* **186**, 563–569.
12. Studier, F. W., and Moffatt, B. A. (1986) Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. *J. Mol. Biol.* **189**, 113–130.
13. Studier, F. W., Rosenberg, A. H., Dunn, J. J., and Dubendorff, J. W. (1990) Use of T7 RNA polymerase to direct expression of cloned genes. *Methods Enzymol.* **185**, 60–89.
14. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) "Molecular Cloning: A Laboratory Manual," 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
15. Sanger, F., Coulson, A. R., Hong, G. F., Hill, D. F., and Peterson, G. B. (1982) DNA sequencing with chain-termination inhibitors. *Proc. Natl. Acad. Sci. USA* **79**, 729–733.
16. Nagai, K., and Thogersen, H. C. (1984) Generation of β-globin by sequence-specific proteolysis of a hybrid protein produced in *Escherichia coli*. *Nature* **309**, 810–812.
17. Feldherr, C. M., Kallenbach, E., and Schultz, N. (1984) Movement of a karyophilic protein through the nuclear pores of oocytes. *J. Cell Biol.* **99**, 2216–2222.
18. Laemmli, U. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.
19. Towbin, H., Staehelin, T., and Gordon, J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc. Natl. Acad. Sci. USA* **76**, 4350–4354.
20. Newmeyer, D. D., Finlay, D. R., and Forbes, D. J. (1986) *In vitro* transport of a fluorescent protein and exclusion of non-nuclear proteins. *J. Cell Biol.* **103**, 2091–2102.
21. Adam, S. A., Sterne-Marr, R. E., and Gerace, L. (1990) Nuclear protein import in permeabilized mammalian cells requires soluble cytoplasmic factors. *J. Cell Biol.* **111**, 807–816.
22. Moore, M. S., and Blobel, G. (1992) The two steps of nuclear import, targeting to the nuclear envelope and translocation through the nuclear pore, require different cytosolic factors. *Cell* **69**, 939–950.
23. Burglin, T. R., Mattaj, I. W., Newmeyer, D. D., Zeller, R., and DeRobertis, E. M. (1987) Cloning of nucleoplasmin from *Xenopus laevis* oocytes and analysis of its developmental expression. *Genes Dev.* **1**, 97–107.
24. Newmeyer, D. D., and Forbes, D. J. (1990) An *N*-ethyl maleimide-sensitive cytosolic factor necessary for nuclear protein import: Requirement in signal-mediated binding to the nuclear pore. *J. Cell Biol.* **110**, 547–557.
25. 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.