Nuclear Estrogen Receptor II (nER-II) is Involved in the Estrogen-Dependent Ribonucleoprotein Transport in the Goat Uterus: II. Isolation and Characterization of Three Small Nuclear Ribonucleoprotein Proteins Which Bind to nER-II

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Abstract   Three proteins of a goat uterine small nuclear ribonucleoprotein (snRNP) fraction, which bind to nuclear estrogen receptor-II (nER-II) have been isolated and purified. These are the p32, p55, and p60 of which p32 is the major nER-II binding protein. Indirect evidence reveals that p32 binds to the nuclear export signal (NES) on the nER-II. nER-II is a snRNA binding protein while p32 does not bind to the RNA. nER-II along with p32 and p55 form an effective Mg^{++}ATPase complex, the activation of which appears to be the immediate reason behind the RNP exit from the nuclei following estradiol exposure. The three nER-II binding proteins bind to the nuclear pore complex; nER-II does not possess this property.   J. Cell. Biochem. 84: 227–236, 2002.   © 2001 Wiley-Liss, Inc.

Key words: uterine snRNP; nuclear estrogen receptor-II; RNP transport

The nuclear export of mRNPs represents a major regulatory mechanism in the post-transcriptional control of gene expression. A functional role for estrogen receptor in post-transcriptional control of gene expression was first examined by Liang and Liao [1974]. Previous biochemical studies reported that in the rat uterus estradiol formed specific association with pre-messenger ribonucleoproteins (pre-mRNPs) [Thampan, 1985, 1988]. These results and the subsequent ultrastructural studies [Vazquez-Nin et al., 1991] demonstrated that withdrawal of estrogen from the rat uterus caused the nuclear retention of pre-mRNPs that had the capacity to bind estradiol and also that exposure of the uterus to estradiol resulted in an immediate transport of the mRNPs from the nucleus to the cytoplasm.

Studies carried out on the goat uterine nuclei, presented in the companion paper [Sebastian and Thampan, 2001], have indicated that within the mRNP complex the estrogen receptor is localized in a snRNP fraction and also that the estrogen receptor involved is not the ERα but the 66 kDa nuclear estrogen receptor II (nER-II). The present study aims at probing deeper into the protein–protein interactions in which nER-II is involved within the snRNP network prior to the exit of the RNP from the nucleus to the cytoplasm.

MATERIALS AND METHODS

Materials

2,4,6,7-[^{3}H]Estradiol 17β (sp.act.101 Ci/mmol), Sepharose 4B, and DEAE-Sepharose were purchased from Amersham-Pharmacia biotech., UK. Estradiol 17β, diethylstilbestrol, 7-methyl guanosine, diaminobenzidine, phenyl methyl sulphonyl fluoride (PMSF), dithiothreitol (DTT), and N-2-hydroxyethylpiperazine-N-2-ethanesulphonic acid (HEPES) were obtained from Sigma Chemical Company, USA. Biotinylated goat anti-rabbit IgG and peroxidase-conjugated streptavidin were obtained from

Vector Laboratories, CA. All the other chemicals used were of the analytical grade and were purchased from local chemical companies.

Two major buffer systems were used in these studies:

1. TEM buffer: 10 mM Tris–HCl, pH 7.5, 1 mM ethylene diamine tetraacetic acid (EDTA), 12 mM monothioglycerol, and 0.5 mM phenyl methyl sulphonyl fluoride (PMSF).
2. TMKC-sucrose buffer: 10 mM Tris–HCl, pH 7.5, 1 mM $CaCl_2$, 20 mM KCl, 2 mM $MgCl_2$, 0.5 mM PMSF, and 250 mM sucrose. This buffer was used during isolation of goat uterine nuclei.

Preparation of Affinity Matrices

The following matrices were used during various levels of experimentation.

1. ERα-, nER-II-, and NPC-Sepharose. ERα was purified from the goat uteri following the published procedure of Zafar and Thampan [1993]; goat uterine nER-II was purified as described by Karthikeyan and Thampan [1995]; and the goat uterine nuclear pore complexes (NPC) were isolated following the method of Davis and Blobel [1986]. The receptors and the NPC were coupled covalently to CNBr activated Sepharose following the method of March et al. [1974].

2. (a) NES-peptide-Sepharose. The HIV-1 Rev NES sequence published by Mattaj's group [Fornerod et al., 1997] was used in the synthesis of NES peptide. The NES peptide (CLPPLERLTL) with an alanine residue at the N-terminus was synthesized on C-terminal alanine incorporated, dimethyl formamide (DMF)-swollen, tetraethyleneglycol diacrylate cross-linked polystyrene resin using standard Fmoc solid-phase peptide synthesis protocol [Kumar et al., 2000]. The purified peptide was coupled to CNBr-activated Sepharose 4B.

(b) NES (control)-peptide-Sepharose. The control peptide consisted of the same amino acid sequence as that of the HIV-1 Rev NES peptide, except that the leucine residues were substituted by alanine (CAPPAERATA).

Hydroxylapatite Assay for Estradiol Binding

Estrogen binding activity associated with the RNP fractions was monitored using a hydroxylapatite (HAP) adsorption assay [Clark and Peck, 1979].

ATPase Assay

The proteins, nER-II, p32, p55, and p60 were incubated under various permutations and combinations with 6 mM ATP in the presence of 6 mM $MgCl_2$ in 10 mM Tris–HCl, pH 7.5 at 30°C for 30 min. The assay, which involved measurement of Pi released as a result of enzyme action, was carried out as described by Fiske and Subbarow [1925].

Native PAGE and SDS PAGE

Non-denaturing gel electrophoresis, also called native gel electrophoresis, was performed following the method of Davis [1964]. Denaturing electrophoresis in the presence of SDS was performed following the method of Laemmli [1970]. The gels were stained with silver nitrate as described by Blum et al [1987].

RESULTS

Identification of the nER-II Binding Proteins in the snRNP

The nuclear macromolecules, which were released into the medium following in vitro exposure of the goat uterine nuclei to estradiol [Sebastian and Thampan, 2001], formed the starting material for the purification of the soluble transport factors. The snRNP fraction was isolated from this preparation using anti-m_3G-Sepharose column as described in the companion paper [Sebastian and Thampan, 2001]. NaCl was added to 2 M-concentration to the final preparation of the extracted snRNP. The suspension was dialyzed overnight against 10 mM Tris–HCl, pH 7.5, containing 0.5mM PMSF. The dialyzed extract was centrifuged at 17,000g for 20 min at 4°C. The supernatant was recovered. The RNA appeared in the precipitate.

Isolation and Purification of nER-II Binding Proteins of the snRNP

The RNA-free snRNP protein fraction prepared as described above was chromatographed over a column of nER-II-Sepharose, which was pre-equilibrated with the TEM buffer. The column was washed with TEM buffer and elution was achieved using a linear gradient of 0–1 M NaCl in TEM buffer. The fractions collected were analyzed for absorbance at 280 nm. The fractions, which belonged to the peak of absorbance, were collected.

The peak fraction from nER II Sepharose upon SDS–PAGE displayed the presence of three proteins, of molecular mass 32, 55, and 60 kDa. A variety of ion exchange and gel filtration chromatographic methods were attempted in order to separate the nER-II binding proteins of the snRNP from one another. All these methods attempted proved ineffective. Therefore, it was decided to follow a system of discontinuous nondenaturing gel electrophoresis for this purpose. The protein samples were concentrated, made salt free by dialysis against 10 mM Tris–HCl, pH 7.5, and mixed with 5× sample buffer of the native gel system. A total of 10 µg of the protein mixture was applied to each well and subjected to native gel electrophoresis using an 8% polyacrylamide gel. Upon silver staining three clear bands were observed, which were designated as proteins 1, 2, and 3 (Fig. 1A). The unstained proteins were allowed to diffuse overnight from the gel into10 mM Tris–HCl, pH 7.5. The extracted protein samples were lyophilized and subjected to SDS–PAGE. Three protein bands of molecular mass 60, 55, and 32 kDa were observed in the SDS gel representing the bands 2, 3, and 1, respectively, of the native gel (Fig. 1B). These

Fig. 1. Native PAGE and SDS–PAGE of nER-II binding proteins. **A:** The protein fraction extracted from snRNPs and which was bound to nER-II-Sepharose was concentrated, dialyzed, and finally subjected to native PAGE. **B:** The proteins recovered from the unstained portion of the native gel were lyophilized and 8 µg each of the three proteins (1, 2, and 3) displayed by the native gel were subjected to SDS–PAGE independently on 10% gels. **Lanes 1**, **2**, and **3** represent the three proteins identified in the native gel.

proteins were designated as nER-IIBPs p60, p55, and p32.

p32 is the Major nER-II Binding Protein

In order to check the specificity of binding of the snRNP proteins to nER-II, it was necessary to confirm that the RNP proteins do not bind to ERα. The extracted snRNP protein mixture was therefore chromatographed over a column of ERα-Sepharose. The column was washed with TEM buffer and elution was carried out using a NaCl gradient in TEM buffer. The fractions collected were examined for absorbance at 280 nm. It was observed that the snRNP proteins did not bind to ERα-Sepharose (Fig. 2A).

The purified proteins were chromatographed independently on a column of nER-II-Sepharose, pre-equlibrated with TEM buffer. Elution was achieved using a linear gradient of NaCl in TEM buffer. The fractions collected were examined for absorbance at 280 nm. The p32 appeared as the major nER-II binding protein followed by p55 and p60 in that order (Fig. 2B).

The nER-IIBPs Also Bind to the HIV-1 Rev NES

HIV-1 Rev is one of the most extensively studied, specific RNA export factors containing a leucine rich nuclear export signal (NES) [Pollard and Malim, 1998]. It was of interest to investigate whether the nER-IIBPs could interact with the estrogen receptor through the NES present in the estrogen receptor. The HIV-1 Rev NES peptide (ACLPPLERLTLA) and the control, leucine free peptide, (ACAPPAERATAA) were synthesized as described in Materials and Methods and immobilized on CNBr activated Sepharose. The protein preparation was dialyzed against Tris buffer (10 mM Tris–HCl, pH 7.5) and chromatographed over a 5 ml column of NES peptide (wild type)-Sepharose. The column was washed with TEM buffer and elution of the bound proteins was achieved using a linear NaCl gradient. The proteins eluted from the NES-peptide column appeared as a single peak of absorbance (280 nm; Fig. 3).

In the control experiment, the affinity matrix contained a leucine free peptide in which the leucine residues in the NES sequences were replaced by alanine. The nER-IIBPs were chromatographed independently over this control column. None of the proteins could bind to the affinity matrix (Fig. 3). This formed the
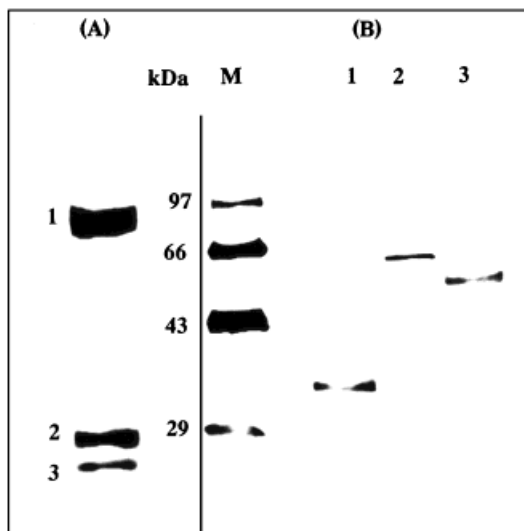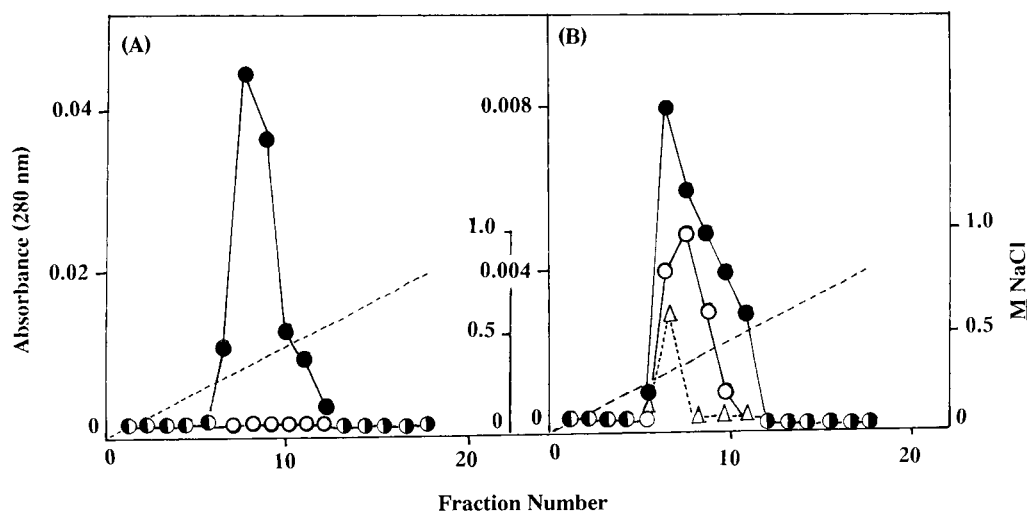
Fig. 2. Characterization of the nER-II binding proteins (nER-IIBPs). **A:** The preparation of snRNP proteins separated from the RNA, was chromatographed on a 5 ml column of nER-II-Sepharose. The column was washed with TEM buffer and elution was achieved using a linear NaCl gradient. Fractions collected were examined for their absorbance at 280 nm ($\bullet$). The protein preparation was also subjected to chromato-graphy over a column of ERα-Sepharose ($\bigcirc$). **B:** Purified pre-parations of p32 ($\bullet$), p55 ($\bigcirc$), and p60 ($\triangle$) were chromato-graphed independently on a column of nER-II-Sepharose, pre-equilibrated with TEM buffer. Elution was achieved using a linear gradient of 0–1 M NaCl in TEM buffer. The fractions (1 ml) collected were examined for their absorbance at 280 nm.

basis for the assumption that the nER-IIBPs recognize the NES sequences present in the nER-II.

P32 is the Major NES Binding Protein

The proteins p32, p55, and p60 (100 μg each) were chromatographed independently over the NES-peptide (wild type)-Sepharose. The column was washed with TEM buffer and the protein bound to the column was eluted using a linear 0–1 M NaCl gradient in TEM buffer. One-milliliter fractions were collected and the absorbance at 280 nm was measured. It was observed that p32 was the major protein that bound to the NES peptide among the three proteins examined (Fig. 4A).

Competition of Free-NES Peptide With nER-II for Binding to p32

An affinity matrix was made by immobilizing p32 on CNBr-activated Sepharose. The assay mixture containing the ^{3}H-E$_2$-nER-II com-plexes (2 μg nER-II was incubated overnight with 20 nM ^{3}H-E$_2$ and subsequently treated with dextran-coated charcoal for the removal of unbound estradiol) and p32-Sepharose was incubated with increasing concentrations (0–10 μg) of free NES (control as well as wild-type) peptide at 4°C for 1 h. The binding of ^{3}H-E$_2$-nER-II to p32 Sepharose was measured. It was

observed that the binding was inhibited by the NES (wild type) peptide in a concentration-dependent manner (Fig. 4B). The control pep-tide, instead of inhibiting nER-II binding to p32-Sepharose was found to enhance the same (Fig. 4B).

RNA Binding Properties of the Macromolecular Complex

The nER-II lacks the DNA binding func-tion; it is a tyrosine kinase and binds to the nuclear RNA polymerases [Karthikeyan and Thampan, 1995]. It was of interest to examine the snRNA binding properties of nER-II, p32, p55, and p60. A mixture of snRNAs was ex-tracted from the nuclear macromolecular com-plex that displayed estradiol binding. Highly purified snRNAs were coupled to CNBr-acti-vated Sepharose thereby forming an affinity matrix. Equal quantity (100 μg) of nER-II, p32, p55, and p60 was chromatographed indepen-dently over the snRNA-Sepharose column. The column was washed with TEM buffer and elution of the snRNA-bound proteins was achieved using a linear NaCl gradient in TEM buffer. The fractions collected were analyzed for absorbance at 280 nm. It was observed that among the four proteins examined the highest snRNA-binding capacity was demonstrated by nER-II itself (Fig. 5A). p55 and p60 displayed
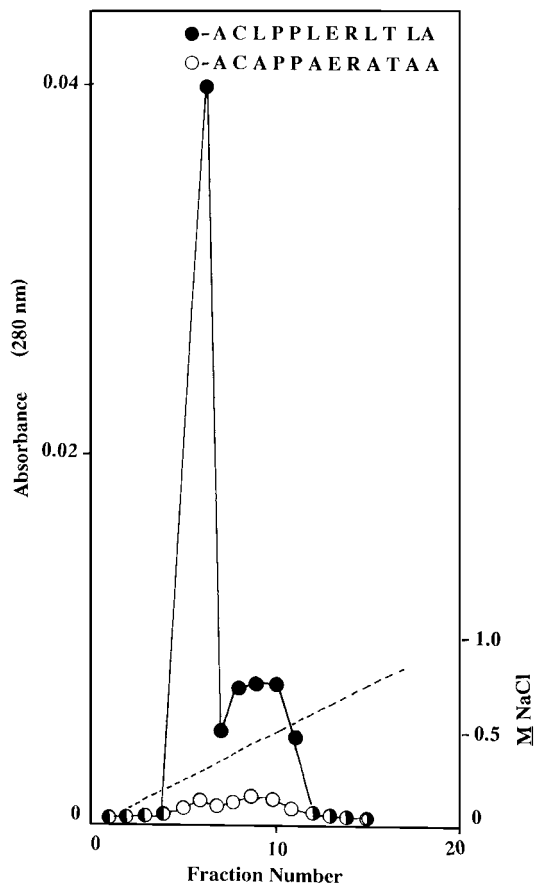
Fig. 3. Chromatography of nER-II binding proteins on NES-peptide-Sepharose. The nER-II-Sepharose bound proteins were chromatographed over a column of NES (wild type)-Sepharose (●) and NES (control)-Sepharose (○). The amino acid sequence of the peptides used in the preparation of the affinity column has been displayed in the figure. The columns were washed extensively with TEM buffer. Elution of the bound proteins was achieved using a linear salt gradient. The eluate was collected in 1 ml fractions and examined for absorbance at 280 nm.

Fig. 4. The binding of p32 to synthetic nuclear export signal. **A:** One hundred micrograms each of the purified proteins p32 (●), p55 (○), and p60 (Δ) was chromatographed independently over the NES-peptide (wild type)-Sepharose. The column was washed extensively with TEM buffer. The proteins bound to the column were eluted using a linear NaCl gradient in TEM buffer. The fractions collected were analyzed for absorbance at 280 nm. **B:** Competition of free NES peptide with nER-II for binding to p32 immobilized on Sepharose. The p32-Sepharose (purified p32 immobilized on CNBr-activated Sepharose) was incubated with ^{3}H-E_2-nER-II complex in the presence of increasing concentrations (0–10 μg) of free NES (control (○) as well as wild type (●)) peptide at 4°C for 1 h. The reaction was terminated by the addition of 2 ml of cold 10 mM Tris–HCl, pH 7.5 following which the suspension was centrifuged at 800g for 5 min. The supernatant was discarded and the pellet was extracted with 1 ml of ethanol. The radioactivity present in the ethanol extract was measured.

a snRNA-binding function of a much reduced order (Fig. 5B,C) while p32 failed to bind to snRNA-Sepharose.

The specificity involved in the RNA binding of nER-II, p55 and p60 was examined. For this purpose two additional affinity columns were prepared, tRNA- and rRNA-Sepharose. Commercially (Sigma) available 28S rRNA, 18S rRNA, and tRNA (100 μg each) were coupled to CNBr-activated Sepharose. Preparations of nER-II, p55, and p60 proteins were chromatographed independently on the affinity columns. The proteins bound to the columns were eluted using a linear NaCl gradient in TEM buffer. nER-II displayed both tRNA and rRNA binding property (Fig. 5A). Binding activity of a much-
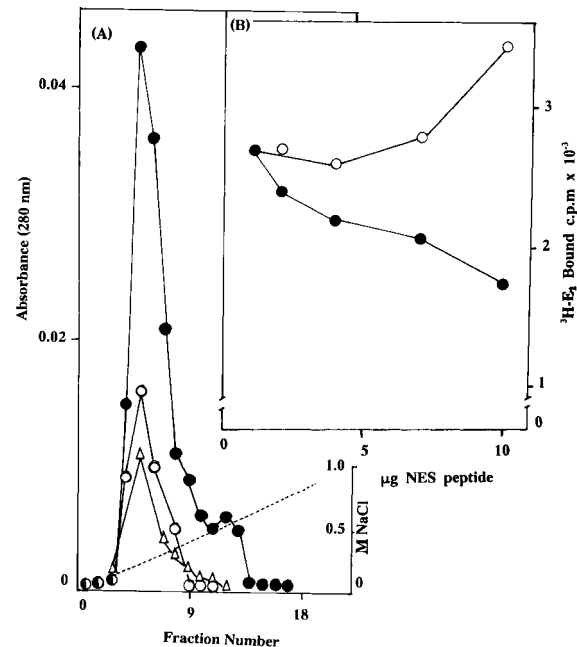
reduced order was displayed by p55 and p60 (Fig. 5B,C).

Docking of the Transport Proteins at the Nuclear Pore Complex

The direct interaction of different transport factors with individual nucleoporins (Nups) is known to be essential for the translocation of the cargo/transport complex across the nuclear pore complex (NPC). Nuclear pore complex was isolated from the uterine nuclear envelopes (NEs) and was coupled to CNBr-activated Sepharose. The interaction of individual proteins, nER-II, p32, p55, and p60 with NPC was studied. Preparations of nER-II, p32, p55, and p60 were chromatographed individually on
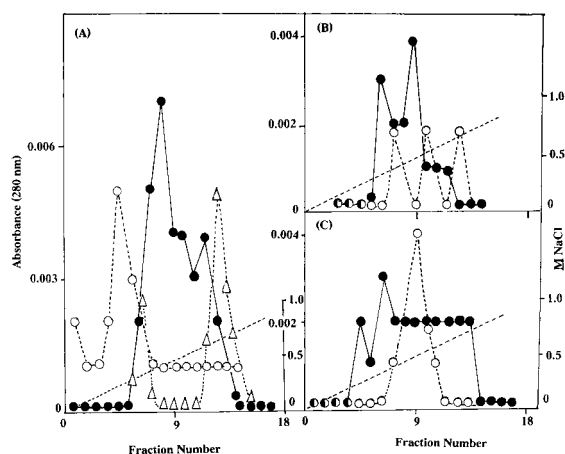
Fig. 5. RNA binding properties of nER-II, p55, and p60. Hundred-micrograms of nER-II was subjected to chromatography over columns of snRNA-Sepharose (●), tRNA-Sepharose (○), and rRNA-Sepharose (Δ). The columns were washed extensively with TEM buffer. The RNA-bound proteins were eluted using a linear NaCl gradient in TEM buffer. The fractions collected were analyzed for absorbance at 280 nm. (**A**) nER-II; (**B**) p55; (**C**) p60. p32 failed to bind to the RNA columns.
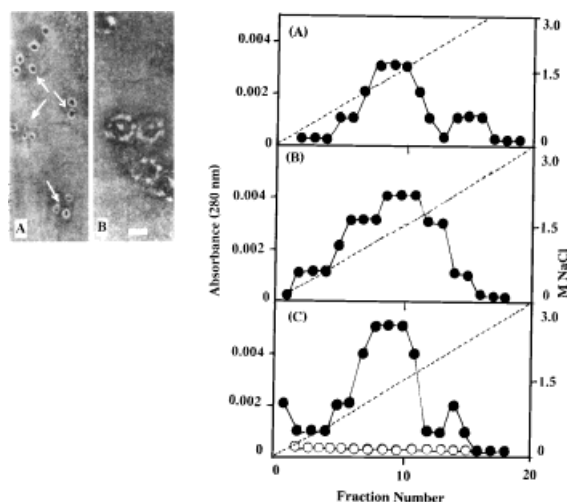

Fig. 6. Docking of the transport proteins at the nuclear pore complex. (Left) Electronmicroscopic examination of the isolated NPC shown in low magnification (**A**) and higher magnification (**B**). The bar indicates 50 nm. (Right) Chromatography of isolated proteins on NPC-Sepharose, isolated nuclear pore complexes (NPC) was immobilized on CNBr-activated Sepharose. Equal quantity (100 μg) of nER-II, p32, p55, and p60 was chromatographed independently on NPC-Sepharose. The column was washed extensively with TEM buffer and elution was achieved using a linear gradient of NaCl. The fractions collected were analyzed for absorbance at 280 nm. (**A**) p60; (**B**) p55; (**C**) p32. nER-II failed to bind to NPC-Sepharose.

NPC-Sepharose. The column was washed extensively with TEM buffer in order to remove the loosely bound proteins from the NPC column. Elution was achieved using a linear gradient of 0–3 M NaCl in TEM buffer. One milliliter fractions collected were analyzed for absorbance at 280 nm. nER-II appeared incapable of direct interaction with NPC-Sepharose while the three nER-IIBPs, p32, p55, p60 displayed positive indications for the presence of this property (Fig. 6B). An unusually strong interaction with NPC was noticed with all the three nER-IIBPs. A NaCl concentration of 2 M or more was required to dissociate these proteins from the NPC-Sepharose column. This indicated that only p32; p55, and p60 are involved in the docking at the NPC while the nER-II acts as a regulator in the nuclear export pathway. The absorption profile also indicated a possibility that there was more than one binding site for the three proteins on the NPC probably representing different nucleoporins. The structural integrity of the nuclear pore complex was put to test under an electron microscope (Fig. 6A).

ATPase Activity

It has earlier been reported that the RNP exit from isolated rat uterine nuclei that took place following estradiol binding was immediately preceded by the activation of a nuclear Mg^{++}ATPase [Thampan, 1988]. An attempt to repeat those studies using goat uterine nuclei has been made. The nuclei, suspended in TMKC-sucrose buffer, were first incubated without any exogenous hormone and ATP at 37°C for 10 min. The incubated nuclear suspension was centrifuged at 800g for 10 min and the nuclear pellet was resuspended in TMKC-sucrose buffer. These nuclei were reincubated at 37°C for varying time intervals, 0 to 10 min, in the presence of 20 nM estradiol and 6 mM ATP in an ATPase assay system as described in the Methods. The inorganic phosphate (Pi) released into the supernatant as a result of the enzyme action was estimated. A linear increase in the nuclear ATPase activity was observed along with increase in the duration of incubation (Fig. 7A). The stimulation of nuclear ATPase by estradiol was examined against a background of the exit of nuclear RNP from the nuclei upon exposure to estradiol.

The ATPase assay was also carried out using intact nuclei in the presence of quercetin

(20 μg/ml). Quercetin inhibited the nuclear ATPase activity as well as the exit of ^{3}H-E$_2$-RNP complex from the nuclei (Fig. 7A).

ATPase Activity Displayed by the nER-II-p32-p55 Complex

The ATPase activity of nER-II, p32, p55, and p60 either alone or in a protein mixture was assayed both in the presence and absence of estradiol. All the proteins showed a basal enzyme activity (data not shown). The ATPase activity associated with nER-II was seen to increase in the presence of estradiol (Fig. 7B). The only protein combination that displayed a stimulated ATPase activity was that consti-tuted by nER-II, p32, and p55 (Fig. 7B). The
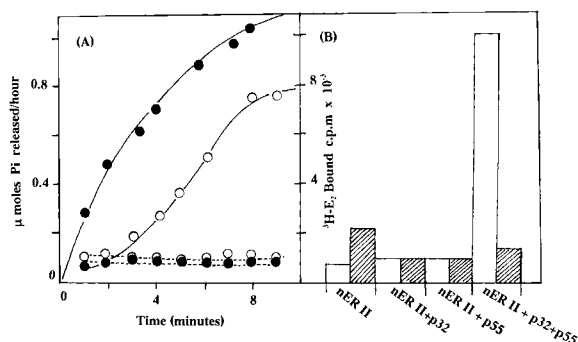

Fig. 7. Correlation between the enhancement in nuclear ATPase activity and the RNP exit from the nuclei. **A:** The nuclei suspended in TMKC-sucrose buffer, were incubated at 37°C for varying time intervals in the presence of 20 nM estradiol and 6 mM ATP in an ATPase assay system. The incubation was terminated by the addition of trichloro acetic acid (TCA). The inorganic phosphate (Pi) released into the TCA soluble super-natant was estimated. The Pi released is expressed as μmoles Pi released/h (●). In a parallel experiment, nuclei were incubated with 20 nM estradiol following which the 800g supernatant of the nuclear suspension were recovered. The supernatant was chromatographed on a column of oligo(dT)-cellulose in the presence of 0.5 M KCl. The column was washed extensively with this high salt buffer following which elution was achieved using zero salt buffer at 37°C. The individual fractions were assayed for hormone binding activity using a HAP method (○). Control experiments (broken lines) were carried out using intact nuclei incubated in the presence of quercetin (20 μg/ml) for varying time intervals. **B:** Identification of the protein complex that displays ATPase activity. Ten micrograms each of nER-II, p32, p55, and p60 were incubated with 6 mM ATP in the presence (shaded histograms) or absence (open histograms) of 20 nM estradiol under various permutations and combinations for 10 min at 37°C. The incubation was terminated by the addition of trichloro acetic acid (TCA). The inorganic phosphate (Pi) released into the TCA soluble supernatant was estimated. The Pi released is expressed as μmoles Pi released/h. p60 was not seen to contribute to the enhanced ATPase activity at any stage. The data concerning this protein, therefore, is not shown.

combination of the three proteins was shown to be essential for the enhancement in the total enzyme activity. This enhanced activity appeared to be almost equal to the total nuclear activity displayed in the presence of estradiol. Ironically, the presence of estradiol caused a steep fall in the activity associated with the reconstituted protein complex.

DISCUSSION

It is generally accepted that export of RNA, in the form of RNP, from the nuclei is mediated by specific transport factors [Gerace, 1995; Nakielny et al., 1997; Izaurralde and Adam, 1998]. In analogy to import, nuclear export is specified by NES, present in the protein moiety of the RNPs and characterized by a short stretch of hydrophobic amino acids, primarily leucine [Fischer et al., 1995]. HIV-1 Rev protein is considered to be the most studied specific RNA export factor containing a leucine rich NES [Pollard and Malim, 1998]. It was shown that different proteins are able to bind directly and specifically to HIV-1 Rev NES. These includ-ed eukaryotic initiation factor 5A (eIF-5A) and CRM 1/exportin 1. The latter appeared to be an essential export receptor for leucine rich NESs [Fornerod et al., 1997; Elfgang et al., 1999].

It is possible that soluble transport proteins that recognize NES present in the nER-II are involved in the RNP transport under estrogenic influence. The candidate proteins identified in this context are the p32, p55, and p60. Among these, the major nER-II interacting protein is p32. It has not been possible to identify a clear functional status for p60.

The observation that p32, p55, and p60 bind to the HIV-1 Rev NES is indicative of the possibility that interaction of these proteins with nER-II is through the NES. Among the three proteins p32 showed the highest capacity to bind to the NES. In view of this observation it may be suggested that p32 is the major estrogen receptor II NES binding protein. The p32 identified in the goat uterine system could be the export receptor for nER-II.

Recent advances in identifying the direct interactions of different transport factors with individual nucleoporins (Nups) have genera-ted a working model for protein translocation across the NPC [Ryan and Wente, 2000]. Although binding of transport factors to Nups has been previously established, recent studies

have demonstrated that different soluble factors have preferential interactions with different subgroups of Nups and multiple pathways exist for transport through the pore [Ryan and Wente, 2000]. This appears to be true for the present system as well. The observed interactions of p32, p55, and p60 with the NPC-Sepharose have indirectly demonstrated the involvement of different Nups in the translocation of RNP through the NPC. The export of mRNA requires a distinct subset of shuttling factors that are not structurally related to karyopherins [Segref et al., 1997]. This includes the RNA helicase Dbp5 and two RNA-binding proteins, Mex67/TAP and Gle2 (Rae1). Multiple interactions among these transport factors and the NPC against the background of mRNA export have been reported recently [Segref et al., 1997].

The NES-mediated export of proteins is an energy and temperature dependent phenomenon [Fischer et al., 1995]. There are examples of both energy dependent and apparently energy independent nuclear export mechanisms [Bataille et al., 1990; Guiochon-Mantel et al., 1991]. This disparity also appears to apply to closely related members of the steroid receptor superfamily. The progesterone receptors apparently have the capacity to efflux from the nucleus under some conditions of ATP depletion while glucocorticoid receptors that are imported to the nuclei appear to remain within that compartment upon ATP depletion [Guiochon-Mantel et al., 1991; Hu et al., 1994]. The role of ATP in estrogen receptor nuclear import pathway has been studied earlier [Sai Padma and Thampan, 2000]. The involvement of ATP hydrolysis for nuclear export was addressed. There is a significant increase in the nuclear Mg^{++}ATPase activity in response to the exposure of the nuclei to estradiol in vitro. This suggests the possibility that ATP is hydrolyzed and the energy released is utilized in the estrogen receptor mediated translocation of mRNPs through the NPC. Quercetin, an ATPase inhibitor, inhibited the estradiol dependent ATPase activity in the nucleus and also the exit of the RNP that bound estradiol. Requirement of ATP and $MgCl_2$ for the nucleocytoplasmic shuttling of other steroid receptors have also been demonstrated [Guiochon-Mantel et al., 1991; Hu et al., 1994]. Both p32 and p55 have basal ATPase activity and there is a steep enhancement in the total

ATPase activity when nER-II, p32, and p55 are put together. The energy released at this step is probably utilized in the actual translocation of the ER-mRNP complex through the nuclear pores. The puzzling contradiction observed in this context is regarding the negative influence of estradiol on the ATPase activity of the reconstituted nER-II-p32-p55 complex. Possibly the nER-II used in the in vitro experiment is already in an appropriate conformation needed to interact with p32 and p55. Additional exposure of this system to estradiol probably changes the receptor conformation, thereby preventing the protein–protein interaction that leads to ATPase activation.

The action of spliceosomes also can influence downstream mRNA metabolism. The most probable means by which pre-mRNA splicing influences subsequent mRNA metabolism is by altering the structure of the messenger ribonucleoprotein particle (mRNP) [Luo and Reed, 1999; Hir et al., 2000]. Such alterations could consist of covalent nucleotide modifications or non-covalent associations of specific proteins,
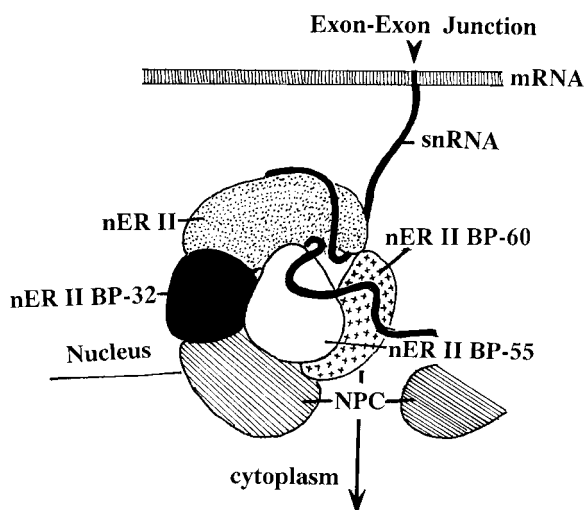

Fig. 8. A model for the nuclear estrogen receptor-II mediated nuclear export of the mRNP. The major components that appear to be involved in the export pathway are nER-II, mRNA, snRNA, p32, p55, p60, and the nucleoporins. Estradiol binding to nER-II apparently leads to an alteration in the receptor conformation, leading to an effective protein–protein interaction involving nER-II, p32, and p55. This leads to an enhancement in the Mg^{++}ATPase activity associated with the three proteins, resulting in the mRNP transport from the nucleus. The functional significance of p60 is not clear. p32 is a NES binding protein that recognizes NES on nER-II. Unlike the other three proteins, it does not bind to snRNA. While the three proteins, p32, p55, and p60 interact with the NPC, nER-II remains incapable of direct interaction with NPC.

either of which could stay with an mRNP throughout or during a portion of its lifetime. A number of proteins are known to be involved in pre-mRNA splicing. hnRNP A1 and a subset of the SR splicing factors shuttle between the nucleus and cytoplasm [Nakielny et al., 1997; Caceres et al., 1998].

Based on the results presented in this study, a model for the estrogen receptor II (nER-II) mediated nuclear export of the mRNP from the nuclei has been proposed (Fig. 8). It appears reasonable to indicate that protein–protein interactions between the nuclear estrogen receptor-II and the soluble transport factors p32 and p55 hold the key that decides the exit from the nuclei of the mRNPs following exposure to estradiol. The significance of the presence of p60 in this context is not clear at this stage since the ATPase function appears to be restricted to the nERII-p32-p55 complex.

ACKNOWLEDGMENTS

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