Association of poly(A)polymerase with tryptophan receptor in rat hepatic nuclei

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*This study conducted experiments with two rat liver nuclear envelope proteins, a tryptophan receptor glycoprotein and poly(A)polymerase, and compared the structural similarities by using affinity chromatography, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and antibody specificity. Earlier, a rat hepatic nuclear envelope glycoprotein that is present in limited quantity and binds tryptophan with high affinity was identified and characterized. Also, analysis of eluted nuclear membrane proteins bound to either tryptophan-agarose or concanavalin A-agarose columns by SDS-PAGE previously revealed a major protein of similar molecular weight after staining with Coomassie blue. Polyclonal antibodies were raised in rabbits by injecting the excised gel protein band. The second protein, an enzyme, poly(A)polymerase, was previously purified from rat liver nuclei by sequential chromatography on DEAE-Sephadex, QAE-Sephadex, phosphocellulose, hydroxyapatite, and DNA-cellulose columns. Analysis of eluate from the final column by SDS-PAGE and Coomassie blue staining revealed a single band. Antibodies to the purified enzyme were raised in chickens. In the present study, using anti-tryptophan receptor antibodies and anti-poly(A)polymerase antibodies, we observed that each recognized the same protein by immunoblot analysis. The anti-tryptophan receptor antibodies did not recognize the catalytic site of poly(A)polymerase but did reduce the enzymatic activity when the antigen-antibody complex was sedimented using Pansorbin (*Staphyloccus aureus *cells wearing a coat of protein A). However, anti-poly(A)polymerase antibodies inhibited binding of ^{3}H-tryptophan to the receptor site. Furthermore, tryptophan could elute poly(A)polymerase from both tryptophan agarose and poly(A)sepharose columns. Analysis of eluates from these columns by SDS-PAGE followed by silver staining of the gel revealed the presence of a major band with an apparent molecular weight of about 65,000–67,000. Our present findings suggest that the tryptophan receptor and poly(A)polymerase share structural homology.*

Keywords: tryptophan; poly(A)polymerase; tryptophan receptor; hepatic nuclei

Introduction

L-tryptophan (TRP), an essential amino acid, is known to affect both hepatic RNA metabolism and protein synthesis.[1] Although much is known about the influence of tryptophan on the liver, the mechanism by which TRP mediates its effects has not been delineated.

TRP has been shown to bind to nuclear envelope proteins in vitro with high affinity (K_{Diss} = 0.7 nM) (B_{max} = 21 fmol/mg protein).[2] Moreover, the binding of ^{3}H-TRP is stereospecific, because only unlabeled L-TRP was an effective competitor, whereas related compounds, e.g., D-tryptophan, serotonin, 5-hydroxy-DL-tryptophan, kynurenine, β-NAD, and niacin, had negligible effects.[2] The ability of concanavalin A, α-mannosidase, and β-galactosidase to inhibit binding of ^{3}H-TRP to hepatic nuclear envelope proteins suggests that the binding entity is indeed a glycoprotein[2] that has an apparent molecular weight (M_r) of about 34,000[3]

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and can be purified using affinity matrices such as tryptophan-agarose and concanavalin A-agarose.[3] Subsequently, in this study using protease inhibitors, we demonstrated the M_r to be about 65,000.

TRP has been reported to rapidly stimulate the transport of polyadenylated mRNA from the nucleus to the cytoplasm of rat liver[4,5] along with a concomitant increase in the activity of nuclear poly(A)polymerase.[6] Because this increase in nuclear poly(A)polymerase activity is very rapid (observed as early as 10 min) after the administration of TRP, it is more likely to be due to a direct effect of TRP on the enzyme rather than due to enhanced de novo synthesis of the protein. In this study we used affinity chromatography, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), immunoblotting, and antigen-antibody interaction to report on the structural similarities between poly(A)polymerase and the tryptophan receptor.

Materials and methods

Animals

Sprague-Dawley rats (Microbiological Associates, Bethesda, MD, USA) weighing 150–250 g were used in this study. The animals were fed a commercial diet (Rodent Lab Chow, Purina Mills, Inc., St. Louis, MO, USA) ad libitum but were fasted overnight prior to sacrifice.

Binding and cross-linking of ^{3}H-TRP to nuclei

Rat hepatic nuclei, prepared as described by Blobel and Potter,[7] were incubated with L-[5-^{3}H]-TRP in the absence or presence of a 1000-fold excess of L-TRP at 22° C for 2 hr. Free and loosely bound radioactivity was removed by washing the nuclei three times with TKMS Buffer (0.05 M Tris-HCl, pH 7.5 0.025 M KCl, 0.005 M $MgCl_2$, and 0.025 M sucrose). After the final wash, the nuclei were suspended in TKMS buffer containing bis [sulfosuccinimydyl suberate] (final concentration, 1 mmol/L). The nuclear suspension was gently shaken in a cold room (≈ 4° C) for 10 min followed by the addition of glycine pH 7.4 (final concentration, 0.1 M) and the suspension left at 4° C for 30 min. Nuclear envelopes were isolated from the labeled nuclei as described previously.[2] Prior to analysis by PAGE (12%) under denaturing conditions, the nuclear envelopes were suspended in an appropriate buffer and boiled for 5 min.[8] The markers were stained with Coomassie blue. The gel was sliced (thickness = 1 mm), solubilized by two freeze-thaw cycles, and assayed for radioactivity.

Purification of the TRP receptor (TRP-R)

Rat hepatic nuclei were prepared as described by Blobel and Potter.[7] Nuclear envelopes were isolated[9] and solubilized with either Triton X-100 (1%) or CHAPS (0.5%, wt/vol) prior to chromatography. Rat hepatic nuclear membrane TRP-R was purified using affinity matrices (either tryptophan-agarose or concanavalin A-agarose) as described earlier.[3] Bound proteins were eluted with either L-TRP (1 μmol/L) or methyl-α-D-mannopyranoside (0.2 M). The eluates were concentrated using AMICON, precipitated with ethanol (4 × volume), boiled for 3 min, and analyzed on polyacrylamide gels under denaturing conditions.[8] The proteins were identified either with Coomassie blue or silver stain (ICN Biomedicals, Irvine, CA, USA; Schwartz/Mann Biotech, Orangeburg, NJ, USA).

Iodination of nuclear membrane proteins

Nuclear envelope proteins were solubilized with buffer (PBS + 20% glycerol, 1 mmol/L phenylmethylsulfonyl fluoride (PMSF), a protease inhibitor, 1 μmol/L macroglobulin, 4 M urea) containing 1% vol/vol Triton X-100 at 4° C for 2 hr. Thereafter, insoluble material was removed by centrifugation 150,000 *g* for 60 min. The supernatant was dialysed overnight against the same buffer but without urea. Triton was removed using SM-2 Bio-beads (Bio-Rad Laboratories, Richmond, CA, USA) as described by Holloway.[10] Solubilized nuclear membrane proteins were radioiodinated with Iodo-Gen (Pierce Chemical Co., Rockford, IL, USA) according to the manufacturer's instructions.

Chromatography of ^{125}I-labeled nuclear membrane proteins on tryptophan agarose and poly(A)sepharose columns

Affinity columns with bed volumes of 5 mL each were equilibrated with PBS buffer containing 20% glycerol, 1 mmol/L DTT, and 1 mmol/L PMSF. The affinity matrix was incubated for 1 hr at room temperature for tryptophan agarose (ICN Biomedicals; Schwartz/Mann Biotech) and at 4° C for poly(A)sepharose (Pharmacia, Piscataway, NJ, USA). Thereafter, the columns were washed with 5–10 bed volumes of buffer and the bound proteins were eluted sequentially with tryptophan (2.5 mmol/L) and NaCl (1 M). Each fraction was assayed for poly(A)polymerase activity and for ^{125}I (bound to proteins).

SDS-PAGE of eluates from tryptophan agarose and poly(A)sepharose columns

Eluates were precipitated with ethanol (4 volumes) at −20° C overnight followed by centrifugation at 20,000 *g*/30 min. The precipitated proteins were taken up in SDS-PAGE sample buffer, boiled for 3 min, and layered on polyacrylamide gels (10%). The proteins were identified by silver staining (ICN Biomedicals; Schwartz/Mann Biotech).

Production of polyclonal antibodies against TRP-R

The protein band visualized after staining the gel with a molecular weight of about 34,000 was excised, homogenized, mixed with the adjuvant (MPL+TDM Emulsion, RIBI, Hamilton, MT, USA), and was injected intramuscularly into rabbits (New Zealand strain). The specificity of the antibodies produced has been reported earlier.[3]

Purification of rat hepatic nuclear poly(A)polymerase

The enzyme was purified as described previously[11] from the livers of male Fisher 344 rats by sequential chromatography on DEAE-Sephadex, QAE-Sephadex, phosphocellulose, hydroxyapatite, and DNA-cellulose columns. The preparation was essentially homogeneous as observed on polyacrylamide gels (under denaturing conditions) after staining with Coomassie blue.

Production of polyclonal antibodies against rat hepatic nuclear poly(A)polymerase

Initially, hens were injected subcutaneously at multiple sites with 100 μg of the enzyme in complete Freund's adjuvant. On days 14 and 21 following the first injection, 50 μg of the enzyme in incomplete adjuvant were administered. Thereafter, booster injections of the enzyme (50 μg) in incomplete adjuvant were given about once each month. IgY was isolated from collected eggs (5–10), according to the method described by Polson et al.[12] Briefly, yolks were mixed with an equal volume of buffer (0.01 M phosphate, 0.1 M NaCl, pH 7.5, 0.01% NaN_3) by stirring. A solution of PEG 6000 (7%) in phosphate saline buffer pH 7.5 was added to the yolk solution (1:1, vol/vol) and the protein precipitate was pelleted by centrifugation at 10,000 *g* for 15 min. The pellet was redissolved to the original yolk volume and subjected to a second precipitation with PEG. The pellet was dissolved in 0.05 M Tris, pH 7.9, 0.0001 M EDTA, 25% vol/vol glycerol, and 0.02% NaN_3. The IgY was further purified on DEAE-cellulose by absorption at 0.015 M phosphate buffer pH 8.0, and subsequent elution with a 0.015–0.3 M phosphate buffer pH 8.0 gradient. The IgY peak was followed by an enzyme-linked immunosorbent assay using peroxidase-labeled rabbit anti-chicken IgG antibody and the positive fractions were pooled and adjusted to the desired concentration.

Electrophoresis and immunoblots

Proteins were subjected to SDS-PAGE on 12% gels as described by Laemmli.[8] Proteins were transferred to nitrocellulose essentially as described by Towbin et al.[13]

Enzyme activity

Poly(A)polymerase activity was measured as described previously.[14]

Competition binding assays

Nuclear envelopes were incubated with ^{3}H-TRP either in the absence or in the presence of L-tryptophan and poly(A) (Sigma Chemical Co., St. Louis, MO, USA; molecular weight > 100,000) for 2 hours at room temperature. Bound and free ^{3}H-TRP were separated by centrifugation and the sedimented nuclear envelopes were further washed twice with buffer to remove loosely bound radioactivity. Finally the pellet was suspended in 0.5 mL of buffer and 5 mL of Aquasol (Amersham Corp., Arlington Heights, IL, USA) were added to measure radioactivity.

To determine the effect of anti-poly(A)polymerase antibodies on binding of ^{3}H-TRP to its receptor site, nuclear envelopes were incubated with ^{3}H-TRP either in the absence or in the presence of L-tryptophan, normal chicken IgG, and anti-poly(A)polymerase antibodies for 2 hours at room temperature. The nuclear envelopes were processed as described above.

Protein determination

The protein content of the various fractions after precipitation with trichloroacetic acid was determined as described by Lowry et al.[15]

Results

Initially, experiments were performed to determine the molecular weight of the TRP-R and the specificity of TRP binding to its receptor. Rat hepatic nuclei were incubated for 2 hr at room temperature with ^{3}H-TRP (1.5 μCi/mL, specific activity 30 Ci/mmol) in the absence or presence of 1000-fold excess of unlabeled TRP. The incubation was terminated by sedimenting the nuclei (800 *g*/10 min) and subsequently washing them three times to remove the free and loosely bound radioactivity. After covalent linking of the radioactive ligand to the binding site using disuccinimydyl suberate, the nuclear proteins were analyzed on SDS-polyacrylamide gels. Radioactivity was measured in each gel slice (1 mm thick). The results revealed that most of the radioactivity was bound to protein(s) having a molecular weight of about 34,000 (rather than 65,000–67,000 because it was prepared without inclusion of protease inhibitors). The addition of 1000-fold excess of unlabeled TRP abolished the peak, suggesting that it competed with ^{3}H-TRP for binding.

Experiments were performed to determine whether the enzyme poly(A)polymerase could be eluted from tryptophan agarose and poly(A)sepharose columns by TRP as had previously been demonstrated for the TRP-R.[3] Nuclear membranes, after solubilization with Triton X-100, were labeled with ^{125}I. The labeled proteins were then chromatographed on either tryptophan agarose or poly(A)sepharose columns. Bound proteins were eluted sequentially with TRP (2.5 mmol/L) and NaCl (1 M). Each fraction was assayed for ^{125}I and for poly(A)polymerase activity. As shown in *Figures 1 and 2*, TRP had the ability to elute poly(A)polymerase from both the tryptophan agarose and the poly(A)sepharose columns, respectively. Upon elution, the peak of enzymatic activity coincided with that of the protein labeled with ^{125}I. Elution with NaCl did not lead to the recovery of any further proteins containing poly(A)polymerase activity from the tryptophan agarose column *(Figure 1)*. However, both ^{125}I-labeled proteins and poly(A)polymerase were eluted with NaCl from the poly(A)sepharose column *(Figure 2)*, suggesting the presence of two populations of the enzyme.

Analysis of eluates of hepatic nuclear envelopes after passage through tryptophan agarose and poly(A)sepharose columns by electrophoresis on denaturing polyacrylamide gels (SDS-PAGE) and subsequent identification of proteins by silver staining revealed the presence of a major protein with a molecular weight of about 65,000–67,000 *(Figure 3)*.

Having established that TRP elutes poly(A)polymerase from affinity matrices *(Figure 1 and 2)*, competition binding assays were performed using poly(A). As shown in *Figure 4*, increasing concentrations of TRP inhibited the binding of ^{3}H-TRP to nuclear envelope proteins. Poly(A) could also inhibit the binding of ^{3}H-TRP, though to a lesser extent as compared with the amino acid. This is partly due to the high viscosity of poly(A) at concentrations $\geq 10^{-5}$ M. To evaluate the inhibition of TRP binding to nuclear envelopes by poly(A) at concentrations higher than 10^{-5} M, the curve in *Figure 4* was extrapolated (to 10^{-4} M) and an inhibition of about 42% was observed. At the other

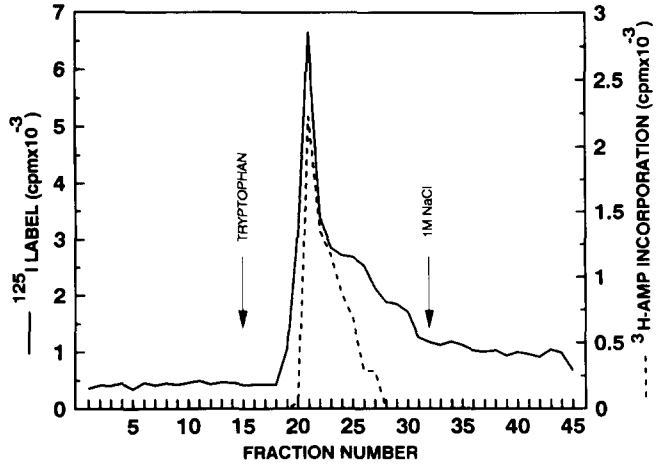

Figure 1 Chromatography of solubilized and ^{125}I-labeled rat liver nuclear membrane proteins on tryptophan agarose. Rat liver nuclear membrane proteins, solubilized with Triton X-100 (1%) and labeled with ^{125}I, were layered on a tryptophan agarose column (5 mL bed volume) and allowed to bind for 1 hr at room temperature on a rocking platform. Free and loosely bound proteins were washed away with equilibration buffer (5–10 volumes). Tightly bound proteins were eluted sequentially with tryptophan (2.5 mmol/L) and NaCl (1 M). Each fraction was assayed for PAP activity and ^{125}I.

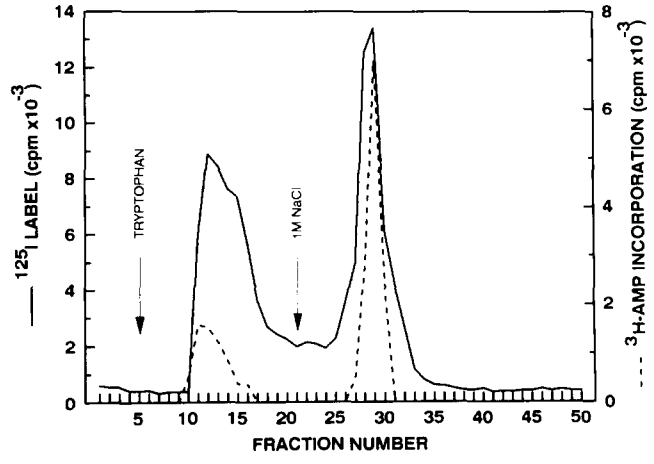

Figure 2 Chromatography of solubilized and ^{125}I-labeled rat liver nuclear membrane proteins on poly(A)sepharose. Chromatography on poly(A)sepharose was performed as described in the legend to *Figure 1* except that the proteins were allowed to bind to the matrix at 4° C.

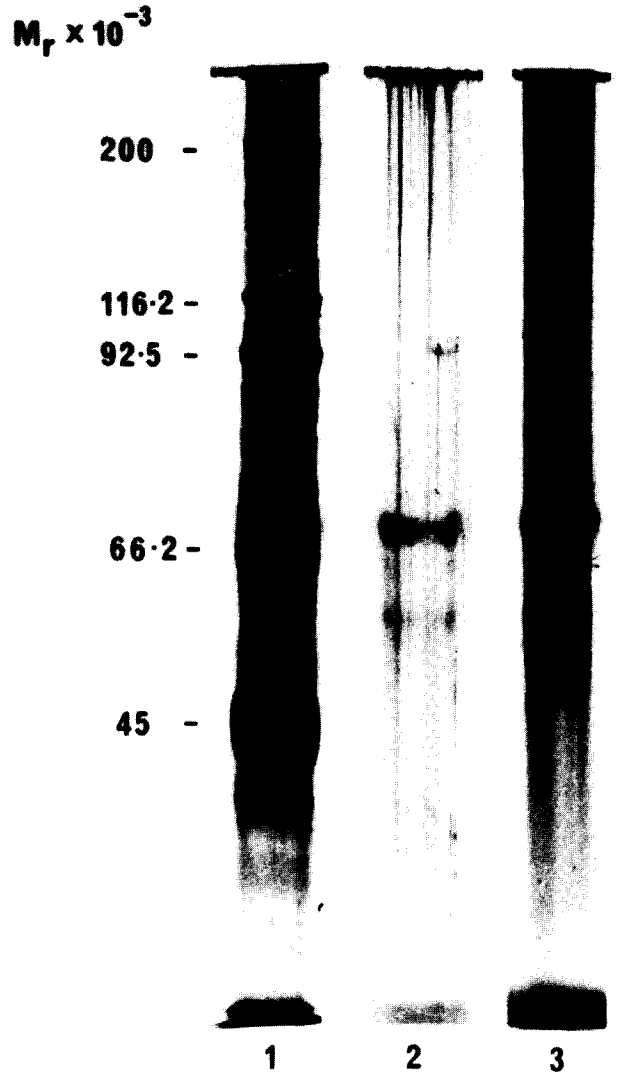

Figure 3 Electrophoresis of eluates from tryptophan agarose and poly(A)sepharose columns on polyacrylamide gel under denaturing conditions. The proteins were visualized after silver staining the gel. Molecular weight markers: (lane 1); eluate from tryptophan agarose (lane 2); eluate from poly(A)sepharose (lane 3).

concentrations of poly(A) used (10^{-8} M–10^{-5} M), it was about 20%–65% as effective as TRP (at the corresponding concentrations).

Antibodies raised against the purified TRP-R were tested for their ability to affect poly(A)polymerase of solubilized rat liver nuclear membranes. Control serum (pre-immune) decreased the enzymatic activity only about 18% *(Table 1)*. However, there was about a 70% decline in the enzymatic activity after treatment with immune serum and Pansorbin *(Table 1)*. Notably, there was an increase in the enzymatic activity (immune versus pre-immune) before the removal of the enzyme antibody complex with Pansorbin *(Table 1)*. Thus, it seems that the antibodies did not recognize the catalytic site of the poly(A)polymerase enzyme but could increase enzymatic activity (37%; *Table 1*) by binding to other epitopes. On the contrary, anti-poly(A)polymerase antibodies inhibited (up to 50%) the binding of ^{3}H-TRP to the receptor site *(Figure 5)*. The inhibition due to the antibodies was concentration dependent. Pre-immune chicken IgY had no effect on binding *(Figure 5)*.

In an effort to ascertain whether the respective antibodies would recognize the same protein, immunoblot analysis was utilized. Nuclear envelope proteins and proteins purified from concanavalin A-agarose were

Table 1 Effect of anti-tryptophan receptor antibodies on nuclear membrane poly(A)polymerase activity of rat liver

Serum	Pansorbin treatment	Enzymatic activity ^{3}H AMP incorporated (cpm)	Recovery %
Pre-immune	Before	3571*	
	After	2941	82
Immune	Before	4896	
	After	1520	31

Rat liver nuclear envelopes were solubilized with CHAPS (0.5%) wt/vol) in 0.02 M Tris-HCl pH 7.4, 0.002 M dithiothreitol, and 0.001 M phenylmethyl sulfonyl fluoride for 1 hr at 0–4° C and then centrifuged 105,000*g*/60 min. Serum, pre-immune (i.e., the rabbits were bled prior to the injection of the antigen) or immune, 1:400 dilution, was added to the supernatant and incubated for 16 hr in the cold room. Aliquots were removed before and after treatment with Pansorbin (*Staphylococcus aureas* cells wearing a coat of protein A; Calbiochem) (washed in same buffer) for enzymatic assay.
*Mean of triplicates.

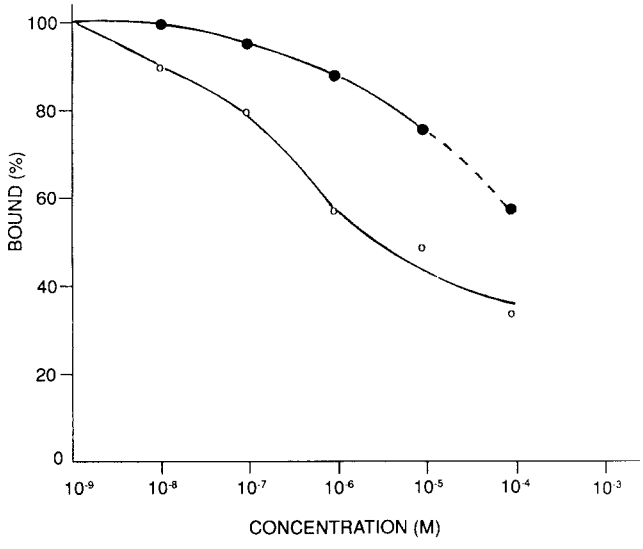

Figure 4 Effect of increasing concentrations of poly(A) and unlabeled tryptophan on ^{3}H-tryptophan binding to rat hepatic nuclear envelopes. Rat liver nuclear envelopes were incubated for 60 min with ^{3}H-tryptophan (1 nM). Varying concentrations of either tryptophan (○) or poly(A) (●) were added at the same time as radioactivity. Thereafter, free and loosely bound radioactivity was removed by washing the nuclear envelopes three times. Tightly bound radioactivity was measured with Aquasol II (Amersham). Tubes containing only ^{3}H-tryptophan (1nM) measured the amount of radioactivity that was one hundred percent bound. Owing to the high viscosity of poly(A) at 10^{-4} M, binding could not be meaasured. Therefore, the line joining the various points was extrapolated to 10^{-4} M (----).

separated on SDS-polyacrylamide gels and electrophoretically transferred onto nitrocellulose sheets. The sheets were then probed with either serum containing anti-TRP-R antibodies (1:1000) or purified IgY anti-poly(A)polymerase. Minor bands could also be recognized with anti-TRP-P antibodies in nuclear preparations *(Figure 6, Lane 1)*. It is probable that the smaller molecular weight bands recognized by the anti-TRP-R antibodies were probably breakdown products

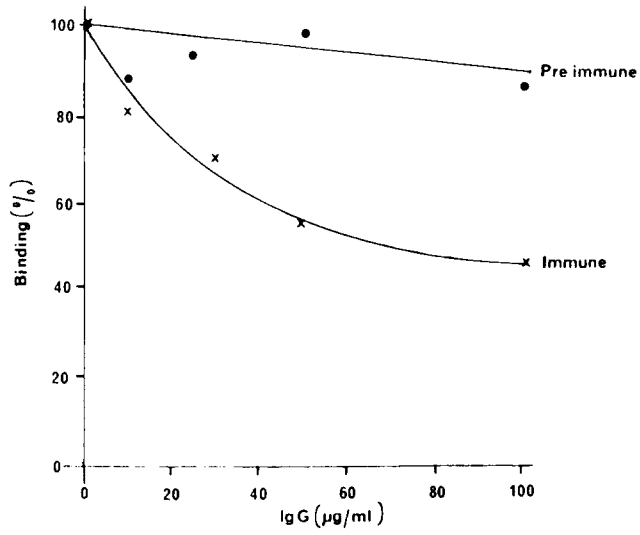

Figure 5 Effect of anti-PAP antibodies on ^{3}H-TRP binding to rat hepatic nuclear envelopes. Nuclear envelopes were incubated with ^{3}H-TRP ± TRP and in the presence of varying amounts of IgG–pre-immune (●) or immune (x) for 2 hr at room temperature. Thereafter, the incubation was terminated by centrifugation and the pellets were washed with buffer three times to remove free and loosely bound ^{3}H-TRP prior to measurement of radioactivity.

including the one with $M_r = 34{,}000$. Inclusion of protease inhibitors during the course of isolation of cell nuclei and nuclear membranes as well as purified antigens led predominantly to the isolation of a protein with $M_r \approx 65{,}000$ *(Figure 3)*. Thus, the 34,000 protein seen with cross-linking studies was probably a proteolytic fragment of the larger M_r protein.

Discussion

As described in the preceding section, we have presented evidence that suggests that both TRP-R and poly(A)polymerase have structural homology. This interpretation was based on information obtained from experiments involving affinity chromatography, immunoblotting, antigen-antibody interaction, and SDS-PAGE.

A number of receptors have been demonstrated to have enzymatic activities. The enzymatic activity inherent to these receptors is basically a kinase, which in turn autophosphorylates the substrate (i.e., the receptor), thereby modulating the activity.[16]

Our observation that anti-TRP-R and anti-poly(A)polymerase antibodies cross-reacted is intriguing. Several factors rule out the possibility that the cross-reactivity was due to raising of antibodies against more than one antigen. First, the antibodies were raised against the antigens that were observed to be homogeneous on SDS-polyacrylamide gels after staining with Coomassie blue. More recently, we have obtained similar results using a silver strain. However, the presence of a second antigen with a similar molecular weight and mobility on SDS-polyacrylamide gels cannot be identified by one-dimensional gels. Recently, the pur-

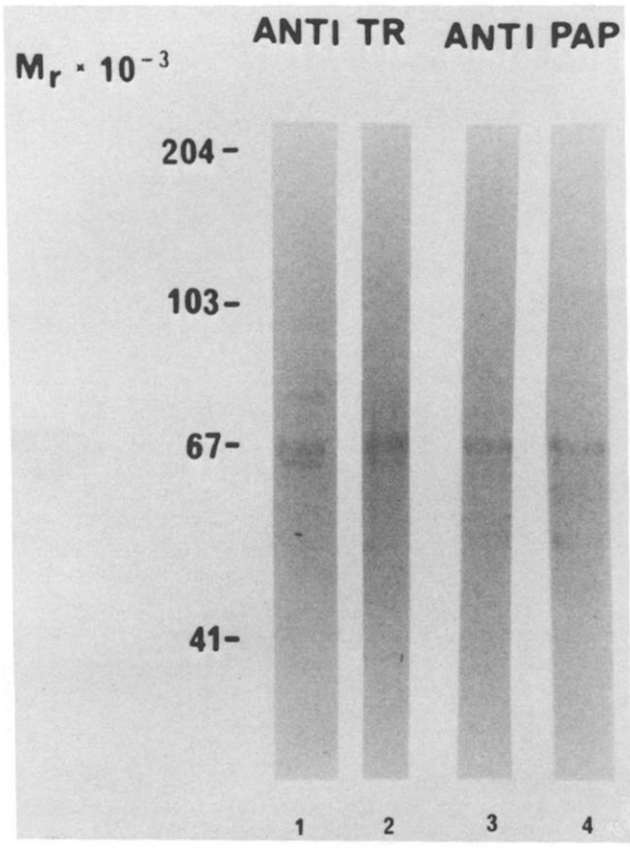

Figure 6 Immunoblot analysis using either anti-TRP-R serum (1:1000) or anti-poly(A)polymerase IgG (10μg/mL). Proteins were separated on SDS-polyacrylamide gels (12%), electrophoretically transferred onto nitrocellulose sheets, and probed with primary antibodies for 2 hr at room temperature. The nitrocellulose sheets were washed and incubated with peroxidase-labeled secondary antibodies (1μg/mL) for 1 hr. After further washing the nitrocellulose sheet, the antigen-antibody reaction was identified with chloronaphthol (0.5%) and hydrogen peroxide (0.003%). Anti-TRP-BP is shown in lanes 1 and 2. Anti-poly(A)polymerase (anti-PAP) is shown in lanes 3 and 4. Nuclear membranes are shown in lanes 1 and 3. Eluate from concanavalin A-agarose column is shown in lanes 2 and 4.

ified TRP-R protein was evaluated on two-dimensional gels, and the findings were identical to those obtained earlier using one-dimensional gel electrophoresis.* The reactivity of the two antibodies is of interest. The anti-TRP-R antibodies recognized neither the TRP-binding site nor the enzymatic site. However, by an indirect method, anti-TRP-R antibodies did affect the enzymatic activity *(Table 1)*. On the contrary, anti-poly(A)polymerase antibodies recognized the TRP-binding site or at least an epitope near the TRP-binding site, because we cannot rule out stereotypic hindrance *(Figure 5)*. It is pertinent to mention that few polyclonal antibodies are known to recognize the binding site of a receptor.

The ability of TRP to elute the enzyme poly(A) polymerase from both tryptophan agarose and poly (A)sepharose columns suggests that poly(A)polymerase has affinity for TRP. Glycoproteins are known to bind to immobilized 5-hydroxytryptamine, a derivative of TRP.[17] Information that suggests that TRP-R and poly(A)polymerase are glycoproteins comes from earlier observations that concanavalin A inhibited ^{3}H-TRP binding to rat hepatic nuclear envelopes[2] and that α-mannosidase and concanavalin A modulated poly(A)polymerase activity.[14] However, it is highly unlikely that the binding of poly(A)polymerase to tryptophan agarose is mediated by carbohydrates because of the ability of poly(A) to inhibit binding of ^{3}H-TRP to its receptor sites.

When eluates of hepatic nuclear envelopes from poly(A)sepharose, tryptophan agarose, and concanavalin A-agarose were analyzed on polyacrylamide gels under denaturing conditions and after silver staining, a major band with a molecular weight of about 65,000–67,000 was visible. A similar molecular-weight protein was visible when either anti-tryptophan receptor antibody or anti-poly(A)polymerase antibody was used *(Figure 6)*.

In an attempt to better understand whether a relationship exists between TRP-R and poly(A)polymerase, it is important to consider the effects or actions of TRP. TRP is known to affect RNA metabolism.[1] As early as 10 min after TRP treatment, there is an increase in concentration of polyadenylated mRNA transported from the cell nucleus to the cytoplasm.[4,5] A concomitant increase in the activities of enzymes involved in transport is also observed. The enzymes affected are nucleoside triphosphatase (NTPase)[18] and poly(A)polymerase.[6,19] The latter has been shown to be involved in the polyadenylation of mRNA.[20] The increase in poly(A)polymerase activity in response to TRP is not due to a decrease in poly(A) or poly(A)$^+$mRNA. However, deadenylated RNA such as rRNA has no effect on NTPase, alluding to the essential role of polyadenylation for the transport of mRNA from the nucleus to the cytoplasm.[21] Poly(A)polymerase can be purified by both concanavalin A[14] and poly(A)sepharose columns. Using the latter column, a protein isolated from rat hepatic nuclei and having a M_r of 65,000 was reported earlier.[22] Monoclonal antibodies raised against this protein inhibit the efflux of mRNA from the nucleus.[22] Thus, the physical characteristics and immunoblot analysis allude to a single polypeptide. However, definitive evidence whether the TRP-R and poly(A)polymerase are indeed the same proteins will be provided by the sequences of the genes encoding the two proteins.

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