RNA Binding Properties of the AU-rich Element-binding Recombinant Nup475/TIS11/Tristetraprolin Protein*

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Mark T. Worthington‡, Jared W. Pelo, Muhammadreza A. Sachedina, Joan L. Applegate, Kristen O. Arseneau, and Theresa T. Pizarro

From the Digestive Health Center of Excellence, University of Virginia Health Sciences Center, Charlottesville, Virginia 22908

Regulation of messenger RNA stability by AU-rich elements is an important means of regulating genes induced by growth factors and cytokines. Nup475 (also known as tristetraprolin, or TIS11) is the prototype for a family of zinc-binding Cys3His motif proteins required for proper regulation of tumor necrosis factor mRNA stability in macrophages. We developed an Escherichia coli expression system to produce soluble Nup475 protein in quantity to study its RNA binding properties. Nup475 protein bound a tumor necrosis factor AU-rich element over a broad range of pH and salt concentrations by RNA gel shift. This binding was inhibited by excess zinc metal, providing a potential mechanism for previous reports of zinc stabilization of AU-rich element (ARE) containing messenger RNAs. Immobilized Nup475 protein was used to select its optimal binding site by RNA SELEX and revealed a strong preference for the extended sequence UAUAUUAU, rather than a simple AUUUA motif. These findings were confirmed by site-directed mutagenesis of the tumor necrosis factor ARE and RNA gel shifts on c-fos, interferon-γ, and interferon-β ARE fragments. A weaker binding activity toward adenine-rich sites, such as a poly(A) tail RNA fragment, can partially disrupt the Nup475-tumor necrosis factor AU-rich element complex.

Regulation of eukaryotic messenger RNA stability is an important factor in controlling gene expression (1). The AU-rich element (ARE) is a critical regulatory motif in the 3' untranslated regions of many cytokine and protooncogene mRNAs, a target for proteins that bind and alter RNA kinetics. Although there has been some debate regarding the optimal sequence length for the ARE (5 versus 9 base pairs) (2, 3), these sites are a target for an increasing number of RNA-binding proteins that serve either to stabilize or destabilize the messenger RNAs containing these elements.

Nup475 was originally cloned as part of an early G1 genetic program of mRNA expression in response to serum and mitogenic growth factors (4), co-expressed with the protooncogenes c-fos, c-jun, and c-myc (4, 5). Also known as tristetraprolin (6) (and TIS11 (7)), this protein is an important regulator of tumor necrosis factor expression in macrophages (8). A targeted deletion of the murine gene created a syndrome of tumor necrosis factor overload due to increased TNF mRNA stability (9). The TNF destabilizing activity was mapped to the two Cys3His domains in transfection and immunoprecipitation experiments (10). The Cys3His domain is a zinc-binding domain in a different structural family from the zinc finger, although both domains require a coordinated zinc metal for stability (11). Force overexpression of this cDNA or other family members (TIS11b, TIS11d) with closely conserved Cys3His domains leads to apoptotic death of fibroblasts (12).

To determine the specificity of Nup475 in AU-rich element regulation, we pursued an analysis of the Nup475/TIS11/tristetraprolin protein-RNA interaction using recombinant protein. Published reports have relied upon cell lines and immunoprecipitation to map the protein’s RNA target to a TNF fragment containing the AU-rich element (10) but have not permitted a high resolution analysis of the protein’s optimal binding site. Although Escherichia coli-expressed protein has been used in phosphorylation studies, we are unaware of any study successfully using recombinant protein in studies of RNA binding. A report questioning whether the protein requires an RNA target sequence more extensive than a minimal ARE or requires phosphorylation for RNA binding underscores the need to recover unmodified protein in quantity so that these and other questions can be specifically addressed (13). In this manuscript, a system for high level E. coli expression of Nup475 permitted characterization of the RNA binding properties of this protein and identification of the consensus Nup475 binding site from a random RNA pool.

EXPERIMENTAL PROCEDURES

E. coli Protein Expression and Purification—An in-frame fusion of Nup475 and pGEX4T-1 (Amersham Biosciences) was created by subcloning from a plasmid that contains Nup475 cDNA between flanking NodI sites, creating GST-Nup475. The details of this subcloning are available upon request. Plasmid pREP4-GroESL was kindly provided by Martin Steiger (Hoffman-La Roche). All large scale plasmid preparations were performed using the Qiagen Maxiprep kit.

The GST-Nup475 fusion protein plasmid was transformed into Rosetta pLysS cells (Novagen, Madison, WI) and Rosetta pLysS/SIRep4-GroESL cells. Electrocompetent Rosetta pLysS/SIRep4-GroESL cells were created after transformation with the pREP4-GroESL plasmid followed by electroporation using an established protocol (14). Selection was performed on LB plates or in liquid LB culture containing the appropriate antibiotics. The bacteria were grown in liquid media to an 

A600nm on a Genequant Pro to 0.3–0.4, where protein induction was induced with the addition of IPTG to 1 mM. Protein expression was verified with Coomassie-stained Laemmli gels as previously described (11). Purification was performed over glutathione-Sepharose (Amersham Biosciences) using the manufacturer’s protocol followed by dial-

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yis in three changes of 50 mM Tris, pH 8.0, 1 mM dithiothreitol, and 100 μM ZnCl₂, made with RNase-free water. Protein concentration was performed using Amicon Centriplus YM-3 Centrifugal Concentrators. Final protein concentration was determined using the Bradford Assay (Bio-Rad).

RNA Probe Synthesis—Labeled RNA probes were synthesized using radioactive [α-32P]UTP for the TNF probes, [α-32P]CTP for the TNF cytosine mutants, and [α-32P]ATP for the poly(A)-like probes from gel-purified, RNase-free confirmed oligonucleotides, which are then annealed to form a T7 RNA polymerase template and a site for RNA synthesis. All probes used the same upper oligonucleotide (AAT TTA AT ACG ACT CAC TAT AGG). The lower oligonucleotides for the RNA probe synthesis include: a murine TNF ARE (CCA GCA AAT AAA TAA ATA AAG TGC CCT ATA GTG AGT CGT ATT AAA TT), a poly(A) tail (TTT TTT TTT CTT TTC CAA GCG ATC TTT ATT TCT CTC TTT CTT CAT TCT TAT AGT GAG TCG TAT TAA ATT), the mouse c-fos coding region determinant (CCT TGG CAT CTT CCT TCC TCT TTC TTC CTT TGG AGA TCC TAT AGT GAG TCG TAT TAA ATT), the mouse c-fos ARE (CCA CCT TAA TAA ATA AAT TGA AAC CCT ATA GTG AGT CGT ATT AAA TT), the human interferon-γ ARE (CCA TAT AAA TAA TGA TAT TAA ATA TCT ATA GTG AGT CGT ATT AAA TT), and the human interferon-β ARE (TAA AAT TTA AAA TAA CCC TAT AGT GAG TCG TAT TAA ATT). After oligonucleotide annealing and RNA synthesis using the T7 Megashortscript manufacturer’s protocol (Ambion) to make radioactive RNA probe, the reaction was phenol/chloroform/IAA- and chloroform/IAA-extracted, and the aqueous phase spun through two sequential Microspin G25 columns (Amersham Biosciences) to remove unincorporated label.

RNA EMSA Analysis—A standard Tris-borate buffer EMSA protocol (15) was modified by the elimination of EDTA to avoid the possibility of zinc chelation from Nup475 protein. Binding reactions were performed at final concentrations of 20 mM HEPES, pH 7.2, 1 mM dithiothreitol, 50 mM KCl, 5% glycerol, 2 M ZnCl₂, and stained with Coomassie R250. The GST fusion is weakly induced at the expected molecular mass (16), but was expressed in the insoluble fraction (not shown). In B, co-induction of the GST fusion with a GroESL plasmid led to an increase in protein production. Solubility testing of the bacterial lysates revealed the predominance of this protein in the soluble fraction (not shown), which was purified to homogeneity over a glutathione-Sepharose column (C).

E. coli expression typically does not result in typical eukaryotic posttranslational modifications including glycosylation and phosphorylation (17). Given the questions regarding the minimal RNA target site and the need for phosphorylation for RNA binding (13), an E. coli-expressed recombinant protein is preferred. In Fig. 1A, a GST fusion did express a limited amount of protein of the predicted molecular mass (asterisk), but the induced protein was in the insoluble fraction (not shown). Our GST-Nup475 construct was similar to that in a previous manuscript using a GST-Nup475 fusion (18). Although the details of expression and purification are not provided in the prior report, in our laboratory, high-level expression of properly folded Nup475 is not a feature of standard E. coli protein expression methods.

Eukaryotic expression systems have the benefits of eukaryotic chaperones to facilitate protein folding. We attempted high-level expression of Nup475 in a variety of yeast, baculovirus, and mammalian expression systems, but obtained expression levels that were trivial compared with those customarily seen in these systems (not shown). Our assumption regarding a relative lack of chaperone activity in E. coli was tested by adding a plasmid that overexpresses the E. coli GroES/EL chaperone complex at the time of protein induction, a method useful for expression of eukaryotic proteins that were otherwise insoluble in E. coli (19, 20). We transformed our GST-Nup475 fusion plasmid into RosettapLysS cells (Novagen) with a well characterized GroES/EL expression plasmid and induced protein synthesis with isopropyl-1-thio-β-D-galactopyranoside. In Fig. 1B, the GST fusion protein expresses at its predicted molecular mass (near that of GroEL) and is found in the soluble fraction (not shown). This approach allowed purification of the GST-Nup475 protein over a glutathione-Sepharose column to homogeneity (Fig. 1C), up to 30–50 mg/liter of culture.

Deteriorating whether or not the soluble protein was functional required an analysis of a known Nup475 RNA target, such as the tumor necrosis factor AU-rich element, in an assay...
for a protein-RNA interaction. As a first test, we used a relatively short motif from the TNF 3'-UTR, reasoning from the structures of other zinc-binding motifs that the two Cys-His motifs in the protein's RNA binding domain would not bind more than one AUUUA each. This rationalization extends from RNA folding experiments in our laboratory that show the TNF and granulocyte-macrophage colony-stimulating factor AREs in bubbles and other unstructured single stranded regions (not shown) and structures of other zinc-containing motifs on nucleic acid targets. For instance, in the Zif268 crystal structure, each zinc finger contacts three nucleotides of the DNA recognition site (21). These findings would also have later implications for our ultimate plan to select consensus binding sites using immobilized protein since a protein-RNA interaction studies by EMSA, shown in Fig. 2A. Recombinant GST and GST-Nup475 were incubated with the probe to determine if the recombinant protein was capable of RNA binding. The slightly altered appearance of the complex at pH 7.0 is reproducible. Zinc has been demonstrated to stabilize ARE-containing RNAs such as Nup475 and c-fos through an unknown mechanism (26). To test the hypothesis that zinc stabilization of ARE-containing mRNAs occurs through interference of RNA binding by an AU-rich element-binding protein, RNA EM-SAs were performed in the presence of increasing zinc concentration as shown in C. The effect of other transition metals on this interaction is discussed under "Results."

![RNA Binding by Nup475/TIS11/TTP](image)

**Fig. 2.** RNA binding activity of recombinant GST-Nup475 fusion protein. A single-stranded radioactive AU-rich element RNA probe from the 3'-UTR of the murine tumor necrosis factor gene was synthesized in vitro using T7 RNA polymerase. Recombinant GST-Nup475 and GST alone were compared with probe alone in RNA gel shift reactions (A). The pH dependence and salt dependence of RNA binding are shown in B. The slightly altered appearance of the complex at pH 7.0 is reproducible. Zinc has been demonstrated to stabilize ARE-containing RNAs such as Nup475 and c-fos through an unknown mechanism (26). To test the hypothesis that zinc stabilization of ARE-containing mRNAs occurs through interference of RNA binding by an AU-rich element-binding protein, RNA EM-SAs were performed in the presence of increasing zinc concentration as shown in C. The effect of other transition metals on this interaction is discussed under "Results."

A short single-stranded TNF RNA probe was used for RNA-protein interaction studies by EMSA, shown in Fig. 2A. Recombinant GST and GST-Nup475 were incubated with the probe to determine if the recombinant protein was capable of RNA binding. The unmodified recombinant GST-Nup475 protein binds RNA; no shifted band is seen with GST alone.

Previous work revealed that Nup475 had apoptosis-promoting activity when heterologously overexpressed in NIH3T3 fibroblasts (12). Nup475 mRNA is expressed as part of the apoptotic cell death program in PC12 cells (24), although the functional significance of this finding is unknown. If this protein retains its RNA binding activity during apoptosis, we hypothesized that it should have the ability to perform its function over a broad range of physico-chemical conditions. Apoptosis triggers a fall in intracellular pH associated with activation of cytosolic caspases (25). Protonation of coordinating residues in the Nup475 first Cys-His repeat leads to a loss
of the zinc cofactor and proper protein structure by NMR, so that lower pH values could lead to a loss of protein structure and therefore its function (11). A series of physiologic and supra-physiologic salt and pH conditions establish the robustness of Nup475 RNA binding shown in Fig. 2B. Zinc is a necessary cofactor for the Nup475 Cys$_3$His domains, with the first motif having a dissociation constant for zinc of less than $10^{-11}$ at neutral pH (11). In cell culture, zinc stabilizes ARE-containing RNAs such as Nup475 and c-fos through a mechanism that inhibits RNA degradation (26) and also prevents the effects of linoleic acid and TNF to promote apoptosis in endothelial cell lines through an unknown mechanism (27). Metal effects on RNA binding are well described: magnesium has been shown to inhibit the binding of another ARE-binding protein, AUF1/hnRNPDo, to a TNF ARE probe (28). We hypothesized that a possible mechanism for zinc stabilization of ARE-containing mRNAs would be interference of RNA binding by an AU-rich element-binding destabilizing protein, with Nup475 as a reasonable candidate. As shown in Fig. 2C, administration of excess zinc to the RNA binding reaction prevents formation of the Nup475-TNF ARE complex, suggesting that zinc levels well above the picomolar amounts needed for the Cys$_3$His motif can modulate the binding of Nup475 to its cognate RNA sequence. This effect was also seen with cobalt, a metal used in biophysical studies as a spectrophotometric probe for zinc (11), but not with manganese or magnesium (data not shown), which have different ligand preferences in coordination complexes (29).

The general parameters of the Nup475 protein-RNA interaction developed in Fig. 2 were used as a foundation for determination of the optimal Nup475 binding site. A controversy in the literature has been the width of the minimal AU-rich element site, with evidence for both sufficient regulation from a 5 base pair AUUUA site and for a larger nonamer UUA UUU A(U/A)(U/A) site (2, 3). When these experiments were performed, the complexity of AU-rich elements and the number of ARE-binding proteins were not known, and the unwritten assumption was that a single protein targeted this RNA element. Other TNF 3′-UTR binding proteins such as TIA-1 (30), TIAR

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**Fig. 3. Results of in vitro RNA selection using recombinant Nup475 protein.** Three cycles of RNA SELEX were performed as previously described (16). Twenty-one cloned and sequenced isolates were analyzed. Shown in A are those sites that contain sequences similar to the AU-rich element. Shown in B are the sequences that do not fit this consensus, suggesting an alternative binding activity. Two of these sequences were selected twice (× 2) in their entirety, suggesting duplicate amplimers from the same initial binding event. Aligned adenines are shown in bold. All library clones were verified by RNA EMSA using in vitro transcribed RNA versus GST-Nup475 and GST, five of these are shown in C. FP, free probe. Numbers 1, 4, and 5 represent clones with an AUUUA core; numbers 2 and 3 are non-AUUUA adenine-rich isolates. A histogram of the aligned ARE-like sequences is shown in D with the consensus sequence and frequencies at each aligned sequence shown below. A threshold frequency of greater than 0.5 for nucleotide at a given position represents a p value of < 0.05 by the binomial test.

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2 M. Worthington, B. Aman, D. Nathans, and J. Berg, unpublished observations.
(30), and AUU-1/hnRNP D (31) have been used to select RNA binding targets from a random pool, but none of these have identified a consensus sequence containing UUAUUUAUU or an AUUUA core.

A 25-mer random sequence with flanking known sequence (including the T7 RNA pol promoter) was constructed as described by Sakashita and Sakamoto (16) three cycles of SELEX performed, with the selected cDNA cloned into the BamHI site of BlueScript KS(H11002). Twenty-one clones were sequenced with the flanking sequences used to determine the original selected RNA sequence.

Sixteen of the clones selected for sequences were similar to the UUAUUUAUU sequence, six with an exact match for this sequence and eight others differing by a single flanking residue (Fig. 3A). Five independent clones representing three unique sequences formed the rest of those isolated and are all adenine-rich (Fig. 3B). All of the isolated clones (the 16 ARE-like and oligonucleotides were used to create RNAs of the same specific activity, differing in the spacing of paired triplet cytosines, which encode uninterrupted 5-, 7-, or 9-base pair AREs. 50,000 cpm per reaction of purified RNA probes were used in parallel gel shift experiments of four lanes each using recombinant GST-Nup475 protein as shown in A. A free probe lane is to the left of each group of reactions. A Molecular Dynamics PhosphorImager was used to quantify the intensity of the shifted bands (B), with results expressed as a fraction of maximal binding. The differences between the 9-base pair and the 7- and 5-base pair ARE sites correspond to levels of significance of $p < 0.05$ and $p < 0.001$ by analysis of variance, respectively. In C, the TNF probe without (−) and with (+) recombinant protein is compared with c-fos, interferon-γ, and interferon-β ARE probes in RNA EMSA reactions.

**Fig. 4.** Gel shifts of mutant TNF AREs to validate the size of the selected AU-rich element. Three mutant TNF ARE synthetic RNA probes were used to create RNAs of the same specific activity, differing in the spacing of paired triplet cytosines, which encode uninterrupted 5-, 7-, or 9-base pair AREs. 50,000 cpm per reaction of purified RNA probes were used in parallel gel shift experiments of four lanes each using recombinant GST-Nup475 protein as shown in A. A free probe lane is to the left of each group of reactions. A Molecular Dynamics PhosphorImager was used to quantify the intensity of the shifted bands (B), with results expressed as a fraction of maximal binding. The differences between the 9-base pair and the 7- and 5-base pair ARE sites correspond to levels of significance of $p < 0.05$ and $p < 0.001$ by analysis of variance, respectively. In C, the TNF probe without (−) and with (+) recombinant protein is compared with c-fos, interferon-γ, and interferon-β ARE probes in RNA EMSA reactions.

**Fig. 5.** Evaluation of adenine-rich RNA segments as potential targets for the non-ARE clones. The non-AU-rich clones identified in our RNA SELEX experiments were compared with RNA motifs known to affect RNA metabolism: the c-fos coding region determinant, the poly(A) signal sequence, and the poly(A) tail shown aligned in A. Conserved residues are shown in bold type. Each of these was synthesized as a radioactive RNA probe from annealed DNA oligonucleotides and T7 RNA polymerase and used in RNA EMSA reactions as shown in B.A TNF ARE probe was included as a positive control of RNA binding activity. In C, the radioactive TNF ARE probe was incubated with an increasing concentration of unlabeled poly(A) probe in RNA EMSA reactions.
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five adenine-rich) were verified to be bound by the recombinant GST-Nup475 in RNA EMSA reactions using DNA templates amplified from individual clones. A sample of these gel shift reactions is shown in Fig. 3C, where samples 1, 4, and 5 represent ARE-like isolates and 2 and 3 represent adenine-rich isolates. These adenine-rich clones all produced significantly less of a Nup475 shifted band by EMSA as represented in the figure.

The aligned consensus sequence for the ARE-like sites, UUAUUAUUU, is represented as a histogram in Fig. 3D with the exact frequencies for each residue shown in the accompanying table. The binomial test was used to determine a “threshold” residue frequency of 50% (p < 0.05) for a given residue at a particular position based on the number of samples sequenced and the assumption that each of the four possible residues should be equally likely to occur at any given site (i.e., expected residue frequency = 25%). That is, if the observed frequency for a given residue at a particular position is greater than 50%, the residue was not likely to have occurred at this position by chance alone (p < 0.05). Thus, we are unable to statistically resolve the distinction between UUAUUAUOU or UUAUUAUUUAUUUUUUU using the binomial test, although the preference for sequences similar to these is quite clear.

To test the finding that the preferred Nup475 RNA binding site was more than a simple AUUAU, we mutated the TNF ARE to add two pairs of cytosine triplets on either side of the AU-rich element with different spacing, creating RNA oligonucleotide cores which could be labeled to the same specific activity, but representing 9, 7, and 5 base pairs of the uninterrupted native sequence (core sequences CCC UUAUUAUUU CCC, UCCCU UUAUUAU CCCU, and UUCCCU UUAUUAU CCCUU, respectively). Four sets of RNA EMSA reactions were run on the same gel with the same amount of each probe, and the results quantified by PhosphorImager analysis (Fig. 4, A and B). A clear preference for 9-base pair ARE motifs rather than the simple AUUAU is demonstrated (p < 0.0001), with intermediate binding to the 7-base pair element (p < 0.05). In Fig. 4C, the TNF ARE fragment is compared with short RNAs containing AREs from murine c-fos (UUAUUAUUA), human interferon-γ ARE (UUAUUAUUA and UUAUUAUUAU in close proximity), and human interferon-β (UUAUUAUUAU).

Dual ARE and poly(A) tail RNA binding activities are described for the RNA-stabilizing HuC protein (32) and another ELAV family members (33). An unknown regulatory protein was described that could bind either the interferon-β ARE or a poly(A) tail, but not both simultaneously (34). Our five adenine-rich sites were compared with three other elements known to be involved in RNA stability: a representative poly(A) tail fragment, the TNF polyadenylation signal sequence, and the c-fos coding region determinant. Fig. 5A shows the alignment of our selected sequences to these motifs. In Fig. 5B, a gel shift was performed comparing the TNF ARE RNA fragment to these known motifs. A shifted band was seen only for the poly(A) fragment, although in Fig. 5C, addition of the unlabeled poly(A) RNA fragment to the TNF ARE gel shift fails to produce a slower migrating complex, leading to diminution of the TNF complex. This is comparable to the previous report for the interferon-β 3′-UTR.

DISCUSSION

Our studies with Nup475 on a TNF AU-rich element fragment reveal much about the functional properties of this protein. The recombinant protein is capable of binding a short fragment of the TNF 3′-UTR without posttranslational modification over a broad range of pH and salt conditions. The increase in the shifted complex at the highest salt conditions suggests the importance of hydrophobic interactions in stabilizing this interaction. The sensitivity of this interaction to zinc concentrations suggests a possible mechanism for the previous report of zinc stabilization of AU-rich element-containing RNAs (26). Zinc also has been described to augment cytokine production from freshly isolated peripheral blood mononuclear cells through an unknown mechanism (35); zinc inhibition of Nup475-mediated destabilization is a potential mechanism for this action.

Three rounds of SELEX revealed two sets of RNA binding sites. The AU-rich consensus is an exact consensus match for the 9-base pair ARE site functionally proposed for the c-fos protooncogene (2, 3) and makes Nup475 a candidate for its regulation, all of which is supported by our gel shift data. This was originally described by inspection as an 8-base pair consensus UUAUUAUOU motif in the 3′-UTR of TNF, other cytokines, and protooncogenes (36). Both groups of investigators reporting the 9-base pair ARE found that the core UUAUUAUOU sequence is tolerant of mismatches in flanking residues, similar to our results with the cytokine-interposed TNF AU-rich element RNA probes. Whether c-fos mRNA and the Nup475 protein are part of a functional regulatory complex will require further study, although this could explain the co-expression of Nup475 as part of the early G1 response (4) as a mechanism for down-regulating these mRNAs prior to expression of late G1 genes and subsequent S phase (37). Removal of the c-fos ARE is sufficient to make the protooncogenic c-fos mRNA capable of transforming fibroblasts (38), underscoring the importance of this motif in preventing cell cycle dysregulation.

The second, less abundant group of isolated sequences appeared to have homology to known RNA elements regulating RNA stability: the poly(A) tail, the poly(A) signal sequence, or the c-fos coding region determinant sequence. Only a poly(A) RNA fragment was shown to shift in an RNA EMSA reaction, and an unlabeled poly(A) RNA was capable of partial interference with the TNF ARE/Nup475 complex. An ~40-kDa protein has been shown to bind the ARE of the human interferon-β mRNA (34), similar to the size of Nup475 protein on Western blotting from mammalian cells (39). In that report, the 40-kDa protein ARE RNA complex was disrupted by either a long poly(A) tail on the interferon-β mRNA or a short poly(A) RNA competitor with the protein involved having intrinsic poly(A) binding activity in cross-linking experiments (34). Nup475 is a potential candidate for that protein and avidly shifts an interferon-β ARE. Other ARE binding proteins show activity directed toward the AU-rich element and the poly(A) tail: poly(A) binding activity is a feature of the RNA-stabilizing ARE-binding ELAV proteins (32, 33).

Our findings provide a basis for the report that Nup475 will only bind efficiently to sequences containing tandem multimers of the sequence UUAUUA, such as c-fos, IL-2, IL-3, TNF, and granulocyte-macrophage colony-stimulating factor (40), the binding site for which actually proves to be a central AUUA core with a UU contributed from the AUUAU on either side. This is, to our knowledge, the only AU-rich element-binding protein that selects for a consensus 9-base pair palindromic core with a UU contributed from the AUUUA on either side. This is, to our knowledge, the only AU-rich element-binding protein that selects for a consensus 9-base pair palindromic core with a UU contributed from the AUUUA on either side. This is, to our knowledge, the only AU-rich element-binding protein that selects for a consensus 9-base pair palindromic core with a UU contributed from the AUUUA on either side. This is, to our knowledge, the only AU-rich element-binding protein that selects for a consensus 9-base pair palindromic core with a UU contributed from the AUUUA on either side. This is, to our knowledge, the only AU-rich element-binding protein that selects for a consensus 9-base pair palindromic core with a UU contributed from the AUUUA on either side.

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