Purification of Two Isoforms of hnRNP-U and Characterization of Their Nucleic Acid Binding Activity†

Frank O. Fackelmayer and Arndt Richter*

Department of Biology, University of Konstanz, 78434 Konstanz, FRG

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ABSTRACT: The scaffold attachment factor A (SAF-A; Romig et al., 1992), a human nuclear protein which specifically binds vertebrate SAR (scaffold attached region) DNA, is identical with hnRNP-U (Kiledjian & Dreyfuss, 1992). In this paper, we report on the purification of two forms of this protein that can be chromatographically separated. We show that the purified proteins represent two isoforms, form 1 and form 2 hnRNP-U, which differ in their primary structure. Both isoforms bind to double- and single-stranded DNA and RNA. In addition, they form higher ordered nucleic acid/protein complexes and specifically bind and aggregate the human SAR element MII at physiological ionic strengths. Electron microscopic analysis shows that the isoforms differ from each other, as form 1 hnRNP-U aggregates into long unbranched filamentous protein/DNA complexes whereas form 2 hnRNP-U aggregates as spheres with an average diameter of 35 nm.

We identified scaffold attachment factor A (SAF-A)[1] as a nuclear protein which specifically binds to vertebate SAR elements with high affinity (Romig et al., 1992). Scaffold-associated regions are DNA sequences of various lengths with a high content of AT base pairs which specifically bind to nuclear matrix or scaffolds *in vitro*. These DNA elements are proposed to specify the bases of chromatin loops and appear to be involved in the regulation of gene expression and in the organization of chromatin into looped domains (Garrard, 1990; Phi-Van & Strätling, 1990; Laemmli et al., 1992). In agreement with its suggested role in the organization of chromatin, SAF-A is present in the nuclear matrix and in nuclear scaffolds and reconstitutes looped structures with SAR DNA. Recently we have cloned and sequenced a complete cDNA clone encoding hnRNP-U/SAF-A (Fackelmayer & Richter, 1994). The deduced amino acid sequence is identical with that of the previously described protein hnRNP-U (Kiledjian & Dreyfuss, 1992).

hnRNP-U has been identified as a component of hnRNP complexes by biochemical and immunochemical techniques [for a recent review, see Dreyfuss et al. (1993)]. It is a 120-kDa phosphoprotein (Dreyfuss et al., 1984a; Pinol-Roma et al., 1988) with an acidic amino terminus and a glycine-rich carboxyl terminus containing a RGG-box responsible for the salt-resistant binding of the protein to synthetic ribonucleotides (Kiledjian & Dreyfuss, 1992). Together with other abundant components, hnRNP-U is assumed to be involved in an unknown way in the packaging of heterogeneous nuclear RNA into the supramolecular structure of ribonucleoprotein complexes. Thus, it appears that the protein may have a dual function. Its high specificity for SAR elements suggests a role in the organization of chromatin, and its presence in ribonucleoprotein complexes is compatible with a function in the organization of large nuclear RNA.

Here we report that hnRNP-U occurs in two isoforms. These two isoforms are purified to homogeneity from HeLa cell nuclear extracts. They possess similar RNA and DNA binding properties and specifically bind and aggregate SAR DNA. We further show that the two proteins form higher ordered nucleic acid/protein complexes with different sizes and shapes.

MATERIALS AND METHODS

Purification of hnRNP-U and Antibodies. All purification steps were carried out at 4 °C. The buffers for preparation of nuclei and for extraction contained 10 mM β-mercaptoethanol and a mixture of protease inhibitors, including 10 mM $Na_2S_2O_5$, 1 μM pepstatin, 1 μM aprotinin, and 1 μM leupeptin. All other buffers contain 10 mM β-mercaptoethanol and 10 mM $Na_2S_2O_5$. A typical preparation starts with 1×10^{10} HeLa cells. Cells were thawed and washed twice in phosphate-buffered saline (1.5 mM KH_2PO_4, 12.7 mM K_2HPO_4, 138 mM NaCl, and 2.7 mM KCl, pH 7.5) at room temperature. The final cell pellet (500*g*, 5 min, 0 °C) was resuspended in 50 mL of 10 mM Tris-HCl, pH 7.4, 3 mM $MgCl_2$, and 10 mM NaCl. After swelling for 10 min on ice, the cells were broken by 20 strokes in a loose-fitting Dounce homogenizer and the nuclei were collected by centrifugation (750*g*, 10 min, 0 °C). The nuclear pellet was washed 3 times with a total of 240 mL of the same buffer and finally extracted into 80 mL of 10 mM Tris-HCl (pH 8.0)/1000 mM NaCl. Nuclear debris was pelleted by high-speed centrifugation (150000*g*, 30 min, 0 °C), and the supernatants were combined, yielding 75 mL of nuclear extract. The nuclear extract was loaded at a flow rate of 0.7 mL/min onto a heparin–Sepharose column [Pharmacia; 10 mL volume; equilibrated with 10 mM Tris-HCl (pH 8.0)/1000 mM NaCl]. After the column was washed with equilibration buffer, bound proteins were sequentially eluted in three steps. First, with 10 mM Tris-HCl (pH 8.0)/2000 mM NaCl; second, with 10 mM Tris-HCl (pH 8.0)/200 mM NaCl; and third, with 50 mM Caps (pH 9.8)/200 mM NaCl. Finally the column was eluted with a linear gradient of 200 mM NaCl to 1000 mM NaCl in 50 mM Caps, pH 9.8. Active fractions were combined, diluted

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* Address correspondence to this author at the Department of Biology, University of Konstanz, 78434 Konstanz, FRG. Postfach 5560 >M614<. Telephone: 07531-88-2125. FAX: 07531-88-3688.

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[1] Abbreviations: hnRNP-U, heterogeneous nuclear ribonucleoprotein U; PVDF, poly(vinylidene difluoride); SAF-A, scaffold attachment factor A; SAR, scaffold attached region.

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2-fold with ice-cold 10 mM β-mercaptoethanol/10 mM $Na_2S_2O_5$, and applied to an FPLC mono-Q column (Pharmacia; 1 mL volume) equilibrated with 10 mM Caps (pH 9.8)/200 mM NaCl at a flow rate of 0.5 mL/min. The column was washed with 10 mM Caps (pH 9.8)/200 mM NaCl and eluted with a linear gradient (25 mL) from 200 to 1000 mM NaCl in the same buffer. Fractions containing the two forms of hnRNP-U (see below) were diluted (1:1) with 98% glycerol and stored at −20 °C. Antibodies against homogeneously purified hnRNP-U/SAF-A form 1 were raised in rabbits and purified by protein A–Sepharose and affinity chromatography on hnRNP-U–Sepharose columns.

DNA Filter Binding Assay. In filter binding experiments, we used either 2.3 ng of MII DNA fragment, radioactively end-labeled with Klenow polymerase, or the same amount of radioactively labeled RNA transcribed by T7 or T3 RNA polymerase from MII cloned in pBluescript II SK⁻ (Stratagene) as a substrate. MII is the human SAR element from the DNA topoisomerase I gene locus used previously to purify SAF-A (Romig et al., 1992; Kunze et al., 1991). The labeled nucleic acids were incubated with aliquots of the column fractions or with the purified hnRNP-U isoforms in a buffer containing 10 mM Tris-HCl, pH 8.0, 80 mM NaCl, 2 mM $MgCl_2$, and 0.05 mg/mL bovine serum albumin for 30 min on ice. Samples were filtered through nitrocellulose filters (Schleicher & Schuell) and washed with a total of 3 mL of incubation buffer without nucleic acid. The amount of filter-bound DNA or RNA was determined by liquid scintillation counting.

Aggregation Assay. Labeled DNA fragments were incubated with purified hnRNP-U in 200 μL of a buffer containing 10 mM Tris-HCl, pH 8.0, 80 mM NaCl, 2 mM $MgCl_2$, and 0.05 mg/mL bovine serum albumin for 15 min on ice. Samples were separated into pellet and supernatant by centrifugation for 15 min in a desktop centrifuge (Eppendorf). DNA or protein present in the pellet and supernatant was then purified and analyzed in agarose or polyacrylamide gels. The competitor concentrations used are indicated in the figure legends.

Electron Microscopy. One hundred fifty nanograms of MII DNA was incubated with 30 ng of purified isoforms in 30 μL of 10 mM triethanolamine hydrochloride, pH 7.5, 80 mM NaCl, and 1 mM $MgCl_2$ for 30 min at room temparature. DNA/protein complexes were fixed by the addition of 3 μL of 1% glutardialdehyde for 15 min at 37 °C. After being chilled on ice, aliquots of 5 μL were spread with benzalkonium chloride and visualized by rotary-shadowing with tungsten as described (Schnieder et al., 1990).

Protein Cleavage and Amino Acid Sequencing. V8 protease digestion was performed in polyacrylamide gels as described (Gullick et al., 1981). For cyanogen bromide (CNBr) cleavage, purified protein was precipitated with 25% trichloroacetic acid, washed with acetone at −20 °C, and redissolved in 50 μL of 70% formic acid. Cleavage was carried out after addition of 1 μL of CNBr stock solution (1.2 g/mL in DMSO) at room temperature in the dark. After 12 h, the reaction mixture was diluted 10-fold with distilled water and dried. Cleavage products were then separated on 12% Tris–Tricine–acrylamide gels (Schagger & von Jagow, 1987) and transferred to PVDF membranes, and selected peptide fragments were sequenced with an automated amino acid sequencer (Applied Biosystems, Model 477A).

Other Methods. SDS–polyacrylamide gel electrophoresis of proteins was performed as described by Laemmli (1970) or Schagger and von Jagow (1987). Silver staining was done according to Wray et al. (1981) and protein transfer according to Towbin et al. (1979). DNA was labeled and separated by agarose and nondenaturing polyacrylamide gel electrophoresis according to standard protocols (Maniatis et al., 1989).

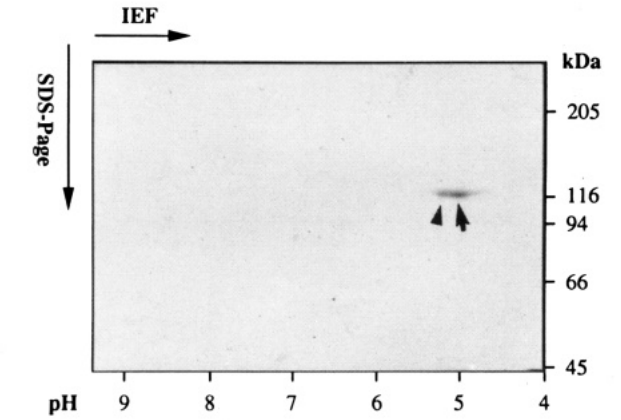

FIGURE 1: Isoelectric focusing of hnRNP-U. Fifty micrograms of purified hnRNP-U was separated by two-dimensional polyacrylamide gel electrophoresis, and the gels were stained with Coomassie brilliant blue. M, molecular mass markers were myosin (205 kDa), β-galactosidase (116 kDa), phosphorylase B (97 kDa), bovine serum albumin (66 kDa), and ovalbumin (45 kDa); F, running front.

RESULTS

Purification of Two Forms of hnRNP-U. Using our original protocol for the purification of hnRNP-U (SAF-A; Romig et al., 1992), we noted that the protein elutes from the final mono-Q column as a single peak with a long trailing side toward higher salt concentrations [see Romig et al. (1992)]. We have further shown that the protein present in the peak fraction as well as in the trailing fractions binds with high affinity and specificity to SAR DNA elements (Romig et al., 1992). When the peak and trailing fractions were combined and analyzed by two-dimensional polyacrylamide gel electrophoresis, we observed a major protein (Figure 1, arrow) with an isoelectric point of pH 5.0 ± 0.2, and a second minor protein component with slightly lower isoelectric point (Figure 1, arrowhead). The minor protein had the same size and the same DNA binding properties in protein blot DNA binding assays and was also recognized by affinity-purified antibodies against hnRNP-U. It could therefore be either a modified form of hnRNP-U or an isoform of the protein.

To address this point, we changed the extraction procedure and succeeded in the purification of comparable amounts of two protein forms by a two-step purification protocol. A nuclear extract prepared at 1 M NaCl was directly loaded onto a heparin–Sepharose column (Figure 2) that was sequentially washed first with 2 M NaCl and then with 0.2 M NaCl at pH 8, followed by 0.2 M NaCl at pH 9.8. Finally, the column was eluted with a linear gradient of 0.2 M NaCl to 1 M NaCl in 50 mM Caps, pH 9.8. Figure 2 shows the elution profile from the heparin–Sepharose column (A) and the proteins after separation on denaturing polyacrylamide gels (B). In contrast to our initial purification where we monitored the DNA binding activity by the protein blot DNA binding assay, the two proteins were monitored throughout column chromatography by filter binding experiments (Figure 2C).

Fractions containing DNA binding activity (Figure 2C) were combined, diluted 2-fold, and loaded onto a mono-Q column (Figure 3). Mono-Q-bound proteins were eluted with a linear gradient from 0.2 to 1 M NaCl in 50 mM Caps at pH 9.8. This elution reveals the existence of two apparently

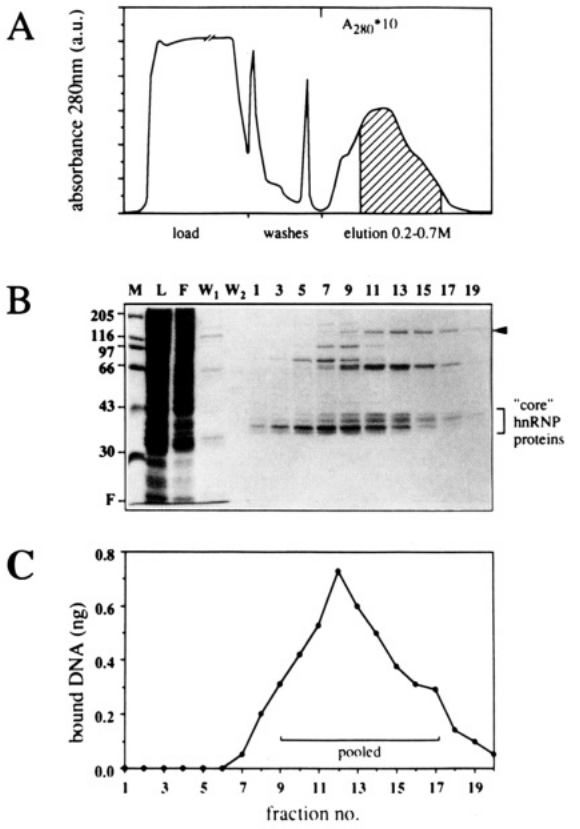

FIGURE 2: Purification of hnRNP-U on heparin–Sepharose columns. (A) Relative optical density measured at 280 nm. Note that the detection sensitivity was increased by a factor of 10 for the elution. Shaded area, fractions containing hnRNP-U. (B) Aliquots from the indicated fractions were separated on 8% SDS–polyacrylamide gels, and the proteins were stained with Coomassie brilliant blue. The coelution of other hnRNP "core" proteins as determined in Western blotting experiments is indicated on the right. Arrowhead, hnRNP-U. (C) Aliquots of the indicated fractions were analyzed for double-stranded DNA binding activity in filter binding experiments.

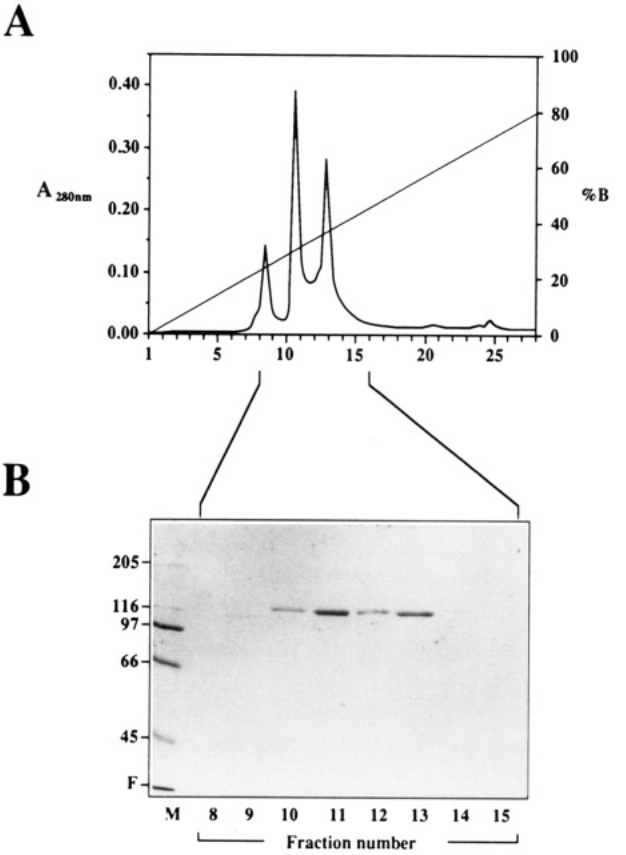

FIGURE 3: Purification of form 1 and form 2 hnRNP-U on mono-Q. (A) Relative optical density measured at 280 nm. % B, salt concentration with 100% corresponding to 1000 mM NaCl. (B) Aliquots of the indicated fractions were separated on 8% SDS–polyacrylamide gels, and the proteins were stained with silver. M, molecular mass markers as in Figure 1; F, running front.

homogeneous forms of hnRNP-U with slightly different chromatographic properties (Figure 3A). We define the protein eluting at 440 mM NaCl as form 1 and the protein eluting at 500 mM NaCl as form 2 hnRNP-U (Figure 3B). The relative abundance of the two forms varied slightly between individual preparations with average values of 60% form 1 and of 40% form 2 hnRNP-U. Both protein forms are recognized by affinity-purified antibodies against hnRNP-U form 1 (data not shown). DNA filter binding experiments showed that 10 ng of both protein forms binds 0.8 ng of double-stranded and 0.6 ng of single-stranded DNA. Optimal binding occurred between 80 and 120 mM NaCl, and no DNA binding was observed at salt concentrations above 500 mM (data not shown).

Two Isoforms of hnRNP-U Protein. To investigate the relationship between the two forms of hnRNP-U, we performed enzymatic and chemical peptide mapping experiments. Equal amounts of form 1 and form 2 hnRNP-U were digested with increasing amounts of V8 protease, resulting in a pattern of similar and differing peptides (Figure 4). Chemical cleavage by CNBr supported this result. The presence of identical peptides can clearly be demonstrated when fractions of the mono-Q column (see Figure 3) with either form 1 or form 2 hnRNP-U were cleaved by CNBr treatment (Figure 5). In Western blotting experiments performed with affinity-purified antibodies, we find that the same peptide fragments in the molecular mass range between 20 and 30 kDa were recognized in both fractions. All CNBr cleavage products with molecular

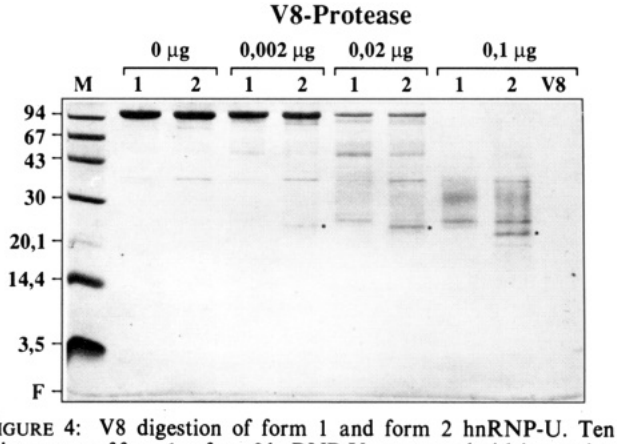

FIGURE 4: V8 digestion of form 1 and form 2 hnRNP-U. Ten micrograms of form 1 or form 2 hnRNP-U was treated with increasing amounts of V8 protease. The resulting peptides were separated on denaturing polyacrylamide gels and stained with Coomassie brilliant blue. The points indicate peptide fragments unique to form 2 hnRNP-U. M, molecular mass markers were phosphorylase B (94 kDa), albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa), α-lactalbumin (14.4 kDa), and insulin (3.5 kDa); F, running front; V8, 0.1 μg of V8 protease alone.

masses of 20 kDa and less, including the form 2 specific peptide of 20 kDa (Figure 5), were not recognized by our antibodies (data not shown). We isolated three common peptides (see Materials and Methods), determined their partial amino acid sequences by microsequencing (Figure 5B), and found that the amino acid sequences determined correspond to residues 575–587, 618–632, and 758-770 of the hnRNP-U protein (Kiledjian & Dreyfuss, 1992).

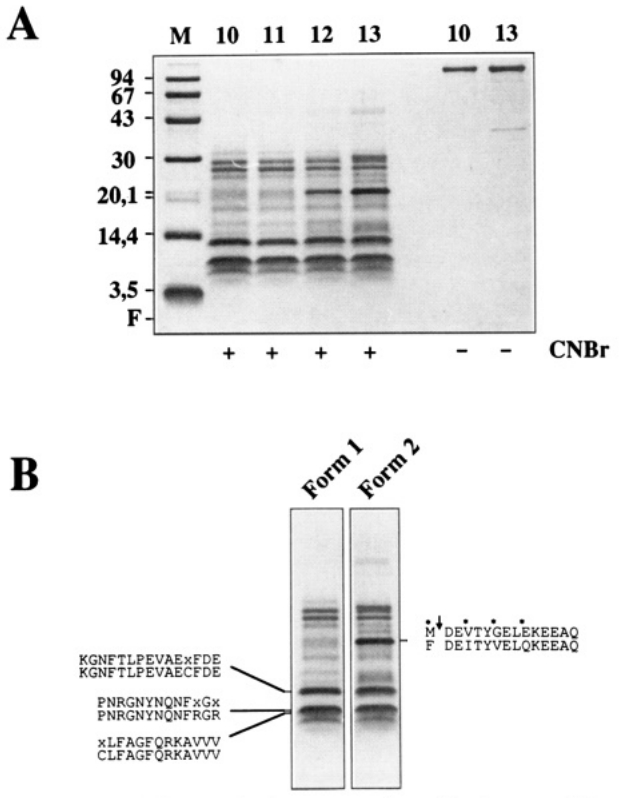

FIGURE 5: Peptide mapping by cyanogen bromide cleavage of form 1 and form 2 hnRNP-U. (A) Ten micrograms of protein from the fractions of a mono-Q column shown in Figure 3 containing form 1 or form 2 hnRNP-U was cleaved by CNBr treatment. The resulting peptides were separated on denaturing polyacrylamide gels and stained with Coomassie brilliant blue. M, molecular mass markers as in Figure 4; F, running front; 10 and 13, uncleaved fractions from the mono-Q column. (B) Seventy micrograms of protein of fractions 10 and 13, respectively, was cleaved by CNBr treatment and separated on denaturing polyacrylamide gels. After transfer to a PVDF membrane, the sequence was determined in an Applied Biosystem 477A protein sequencer. The upper rows give the amino acid sequence of the peptides in one-letter code; the lower rows give the amino acid sequence of hnRNP-U. x, unidentified amino acid; points, predicted amino acid exchange in form 2; vertical arrow, predicted CNBr cleavage site in form 2.

Several peptides were only obtained after CNBr cleavage of the form 2 protein (Figure 5). We sequenced 1 of these peptides (Figure 5B) and found that 4 out of 15 sequenced amino acids differ between form 1 and form 2. The sequenced peptide is derived from the C-terminal region of the protein and includes amino acid residues 631–644. In this peptide, isoleucine-633 is replaced by valine, valine-636 by glycine, and glutamine-639 by glutamic acid. In addition, the phenylalanine-630 residue present in form 1 protein must be replaced by a methionine residue in form 2, explaining the CNBr cleavage at this site.

Thus, peptide mapping and amino acid sequencing clearly reveal that form 1 and form 2 proteins are isoforms of hnRNP-U which differ in their primary structure.

Nucleic Acid Binding Properties of the hnRNP-U Isoforms. hnRNP-U is present in preparations of hnRNP complexes and was shown to bind to single-stranded DNA and to various synthetic homoribopolymers coupled to agarose beads (Kiledjian & Dreyfuss, 1992). With an independent approach, we have identified and purified hnRNP-U as a DNA binding protein which specifically binds to SAR DNA (Romig et al., 1992). To compare the nucleic acid binding properties of the two hnRNP-U isoforms, we performed filter binding experiments with the homogeneous proteins and labeled DNA or

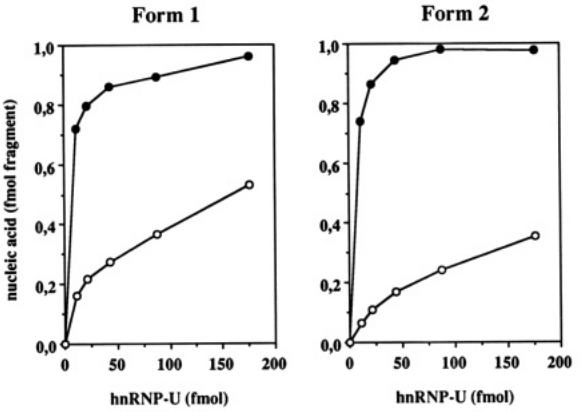

FIGURE 6: hnRNP-U binds DNA and RNA. Filter binding experiments were performed with 2.3 ng of labeled nucleic acid as a substrate and increasing amounts of hnRNP-U form 1 (left) and form 2 (right). Closed circles, double-stranded MII fragment; open circles, RNA transcript of MII DNA.

RNA molecules. In these experiments, we used as nucleic acid substrates either the SAR DNA fragment MII, or, as a "natural" RNA molecule, the "coding strand" RNA transcribed *in vitro* from the cloned MII fragment. The results of these experiments are summarized in Figure 6 which shows the amount of nucleic acid bound by increasing amounts of hnRNP-U form 1 and form 2. The same ratio of bound DNA versus bound RNA was obtained in experiments with other DNA fragments or other RNA transcripts including the "non-coding" strand of MII (data not shown). This indicates that, at limiting amounts of protein, hnRNP-U binds more DNA than RNA molecules.

Nucleic Acid Dependent Aggregation of hnRNP-U Isoforms. We have shown previously that hnRNP-U is able to form large protein/DNA complexes (Romig et al., 1992). These complexes can be collected by a spin in a desktop centrifuge and analyzed for their DNA or protein content. Using this aggregation assay to compare the self-polymerization activity of form 1 and form 2 hnRNP-U, we find that both protein isoforms behave similarly. Like the BSA control, both proteins remain quantitatively in the supernatant when DNA is omitted from the reaction (Figure 7A, –DNA), but form large pelletable aggregates in the presence of either double-stranded (Figure 7A, +dsDNA) or single-stranded DNA (Figure 7A, +ssDNA). Optimal aggregation occurred in the range of physiological ionic strengths between 80 and 150 mM sodium or potassium chloride, and the reaction was completely inhibited at salt concentrations above 500 mM. Furthermore, both isoforms show similar binding characteristics as the aggregate formation was resistant to 1% Triton X-100 or Nonidet P40 but sensitive against 0.01% sodium dodecyl sulfate (data not shown).

However, when investigated by electron microscopy, the protein/DNA complexes were different in appearance: form 2 protein/DNA complexes are spheres with an average diameter of 35 nm (Figure 7B, form 2) whereas form 1 hnRNP-U forms large unbranched filamentous complexes. These filamentous complexes were up to 1 μm long and appeared to be composed of individual globular subunits with the same apparent width of 35 ± 4 nm (Figure 7B, form 1).

hnRNP-U Isoforms Preferentially Aggregate SAR DNA. We used the aggregation assay described above to compare form 1 and form 2 hnRNP-U with respect to their specific DNA binding properties (Figure 8). For these experiments, we used an equimolar mixture of end-labeled pUC18 DNA and of MII, a SAR element from the human DNA topo-

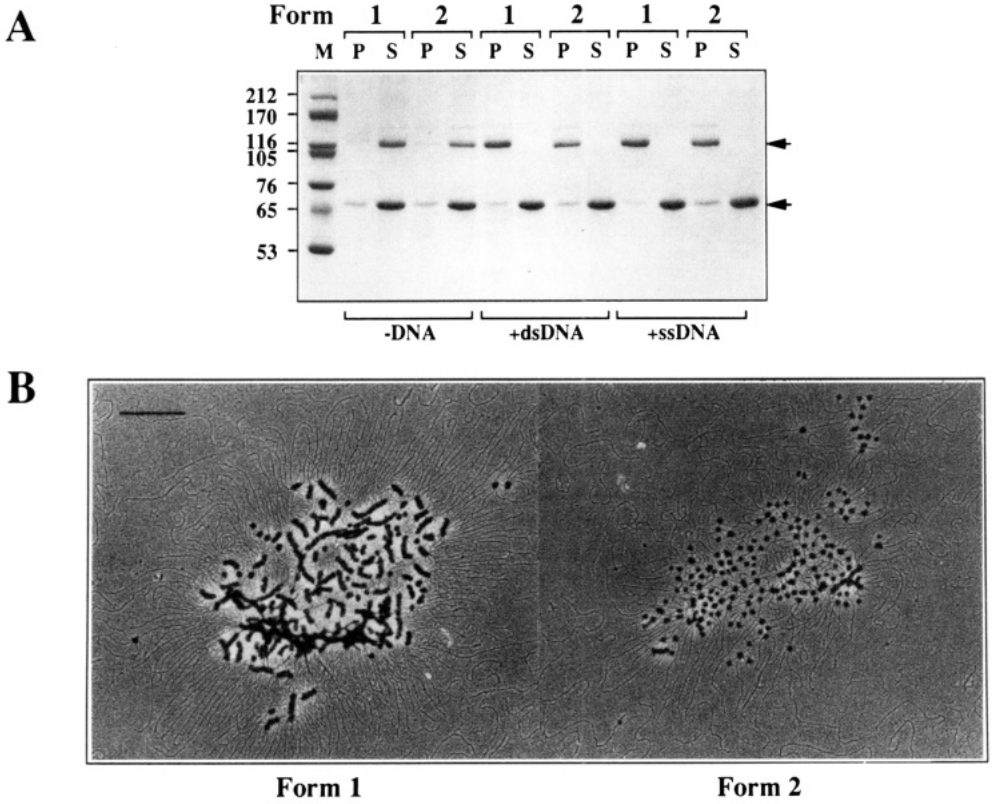

FIGURE 7: Nucleic acid dependent aggregation of hnRNP-U. (A) One microgram of form 1 or 2 hnRNP-U was incubated in DNA binding buffer for 15 min at room temperature in the absence (–DNA) or presence of 1 μg of double- (dsDNA) or single-stranded (ssDNA) DNA. After centrifugation, proteins in the pellet and supernatant were separated in denaturing polyacrylamide gels and stained with Coomassie brilliant blue. M, molecular mass markers. Upper arrow, hnRNP-U; lower arrow, BSA. (B) Electron micrographs of DNA/protein complexes formed with form 1 and form 2 hnRNP-U. Bar, 400 nm.

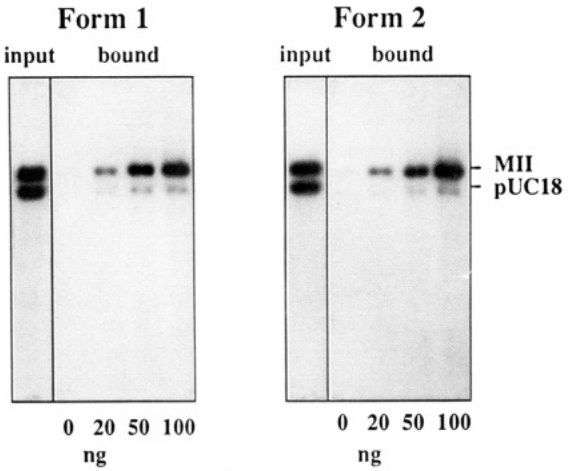

FIGURE 8: Both isoforms of hnRNP-U specifically aggregate SAR elements. The DNA aggregation assay was performed with 0, 20, 50 and 100 ng (lower scale) of form 1 and form 2 hnRNP-U in the presence of 20 ng of pMII DNA digested with *Eco*RI/*Bam*HI and end-labeled with Klenow polymerase. Bound DNA was pelleted, purified, and separated in 1% agarose gels. MII, human SAR element MII.

isomerase I gene (Kunze et al., 1991). In previous experiments, we have shown that form 1 hnRNP-U protein binds with high affinity and specificity to this DNA fragment as well as to other SAR DNA elements (Romig et al., 1992). We now show that there is apparently no difference between the two isoforms with respect to their DNA binding properties. Both isoforms preferentially bind and aggregate MII DNA. With increasing concentrations, the isoforms first quantitatively titrate the SAR-containing DNA. At the protein concentrations used, the non-SAR fragment pUC18 is not bound and remains in the supernatant. When the protein concentration is further increased, the non-SAR DNA is also aggregated, demonstrating the general DNA binding activity of the proteins. We further find that the specific binding of MII DNA is resistant to an 100-fold excess of *E. coli* competitor DNA whereas bound pUC18 DNA is readily displaced by the competitor (data not shown).

DISCUSSION

Purification of Two Isoforms of hnRNP-U. SAF-A is a protein that specifically binds to several scaffold attachment region (SAR) DNA fragments isolated from vertebrate cells (Romig et al., 1992). More recently, we found that this protein is identical to hnRNP-U (Fackelmayer et al., 1994; Fackelmayer & Richter, 1994), a member of the hnRNP proteins which are known to be important in the packaging of primary transcripts and which are among the most abundant proteins in the cell nucleus (Dreyfuss et al., 1993). Interestingly, Tsutsui et al. (1993) and von Kries et al. (1994) have recently reported on the purification of the rat (SP120) and chicken (p120) homologues of hnRNP-U and confirmed their specific binding to SAR DNA elements. hnRNP-U does not have significant homology to any known protein, including the other hnRNP proteins. Kiledjian and Dreyfuss (1992) have shown that the protein binds to single-stranded DNA and to polyribonucleotides through a region of repeated arginine-glycine-glycine motifs referred to as RGG-box, but a thorough investigation of its biochemical properties was not possible due to the lack of a convenient purification scheme. In this paper, we describe a simplified purification protocol for hnRNP-U. Our procedure led to the identification and purification of a second, hitherto unidentified form. Starting with 10^{10} HeLa cells, the protocol allows the purification of

200–300 μg of each form within 1 day using as an assay a simple filter binding assay with labeled DNA as a substrate. The two forms of the protein may be related to the hnRNP-U doublet observed by denaturing polyacrylamide gel electrophoresis in nuclear extracts from mammalian cells (Dreyfuss et al., 1984b), but we detect two protein forms only after two-dimensional polyacrylamide gel electrophoresis.

Peptide mapping and amino acid sequencing demonstrated the relationship between the protein forms but also revealed differences in their primary structure. The presence of several isoforms derived from different genes or by differential splicing of a primary transcript is common among important proteins and has also been reported for hnRNP-A1, another abundant component of hnRNPs (Buvoli et al., 1988). The question arises whether isoforms are redundant, or whether they perform related but different functions. It is therefore interesting to study and to compare the biochemistry of the isoforms. For hnRNP-U, we focused on the nucleic acid binding and polymerizing activities of the two proteins.

Nucleic Acid Binding Properties and DNA-Dependent Aggregation of hnRNP-U Isoforms. The hnRNP-U isoforms bind to double-stranded DNA and to "natural" RNA transcripts and form, in addition, higher ordered nucleic acid/protein complexes with double- and single-stranded DNA. Optimal binding and aggregation occur at physiological ionic strengths and are unaffected by nonionic detergents. In addition, both forms preferentially bind the AT-rich SAR element MII. The binding to this DNA is resistant against an excess of double- and single-stranded *E. coli* competitor DNA. Electron microscopic images show that the hnRNP-U isoforms interact with each other and form large aggregates composed of 100 and more hnRNP-U molecules and bound DNA. With form 2 hnRNP-U, we observe globular complexes and with form 1 hnRNP-U filamentous complexes which are apparently composed of globular subunits. The fact that globular complexes are also seen with homogenous form 1 protein, although only as a minor fraction, supports the idea that these complexes are the basic building blocks for the polymerization to filaments. Obviously, form 2 protein can only assemble into these globular complexes but not polymerize to filaments.

Interestingly, the preferential binding and aggregation of SAR DNA reported here for form 1 and form 2 hnRNP-U are also a property of purified DNA topoisomerase II (Adachi et al., 1989), histone H1 (Izzauralde et al., 1989), and the lamins (Luderus et al., 1992). These DNA binding proteins are involved in several aspects of DNA metabolism as well as in the structural organization of chromatin and of the nucleus [reviewed in Garrard (1990), Phi-Van and Strätling (1990), and Laemmli et al. (1992)].

Possible Functions of the Nucleic Acid Binding Activities of hnRNP-U. It is well-known that typical RNA binding proteins also bind DNA and some of them have been initially identified and purified as single-stranded DNA binding proteins. Well-characterized examples are hnRNP-A1 (UP1; Riva et al., 1986), *Drosophila* ssRP (Hsu et al., 1993), nucleolin (Sapp et al., 1986; Lapeyre et al., 1987), hnRNP-K (Matunis et al., 1992; Takimoto et al., 1993), and other hnRNP proteins (McKay & Cooke, 1992). Most of these proteins bind best to RNA and single-stranded DNA and show, if at all, only weak affinity for double-stranded DNA molecules. Like many other nuclear proteins including histone H1 (Ilyin et al., 1971) and the proteins mentioned above, hnRNP-U binds to RNA and to DNA. However, both hnRNP-U isoforms differ from these proteins as they show, in addition to their RNA binding activity, the same high-affinity binding of double- and single-stranded DNA molecules. The binding of the hnRNP-U isoforms to double-stranded DNA may point to a novel function(s) of the protein(s) in DNA metabolism in addition to their suggested role in the formation of RNP complexes. As the hnRNP-U isoforms interact specifically with SAR elements and form higher ordered nucleic acid/protein complexes, they may be involved in the anchoring of chromatin loops to a dynamic internal nuclear structure (Jackson & Cook, 1988; Nicherson et al., 1989; He et al., 1990, 1991; Belgrader et al., 1991). The fact that two isoforms exist may indicate that they perform different functions. Interestingly, approximately half of hnRNP-U present in nuclei (as determined by antibodies which do not discriminate between form 1 and form 2) can be extracted after sonification in a soluble form whereas the other half remains bound to residual nuclear structures. We also found that during preparation of nuclear matrix, approximately one-fifth of total hnRNP-U remained bound to residual nuclear structures after the final 2 M NaCl extraction step. Further experiments are necessary to show whether the two isoforms are associated with different subfractions of the nucleus and/or exert different functions.

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REFERENCES

Adachi, Y., Käs, E., & Laemmli, U. K. (1989) *EMBO J. 8*, 3997–4006.
Belgrader, P., Siegel, A., & Berezney, R. (1991) *J. Cell. Sci. 98*, 281–291.
Buvoli, M., Biamonti, G., Tsoulfas, P., Bassi, M. T., Ghetti, A., Riva, S., & Morandi, C. (1988) *Nucleic Acids Res. 16*, 3751–3770.
Cockerill, P. N., & Garrard, W. T. (1986) *Cell 44*, 273–283.
Dreyfuss, G., Adam, S. A., & Choi, Y. D. (1984a) *Mol. Cell. Biol. 4*, 415–423.
Dreyfuss, G., Choi, Y. D., & Adam, S. A. (1984b) *Mol. Cell. Biol. 4*, 1104–1114.
Dreyfuss, G., Matunis, M. J., Pinol-Roma, S., & Burd, C. G. (1993) *Annu. Rev. Biochem. 62*, 289–321.
Fackelmayer, F. O., & Richter, A. (1994) *Biochim. Biophys. Acta 1217*, 232–234.
Fackelmayer, F. O., Dahm, K., Renz, A., Ramsperger, U., & Richter, A. (1994) *Eur. J. Biochem. 221*, 749–757.
Garrard, W. T. (1990) *Nucleic Acids and Molecular Biology*, pp 163–176, Springer Verlag, Heidelberg.
Gullick, W. J., Tzartos, S., & Lindstrom, J. (1981) *Biochemistry 20*, 2173–2180.
He, D., Nickerson, J. A., & Penman, S. (1990) *J. Cell Biol. 110*, 569–580.
He, D., Martin, T., & Penman, S. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 7469–7473.
Hsu, T., King, D. L., LaBonne, C., & Kafatos, F. C. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 6488–6492.
Ilyin, Y. V., Varshavsky, A., Mickelsaar, U. N., & Georgiev, G. P. (1971) *Eur. J. Biochem. 22*, 235–245.
Izaurralde, E., Käs, E., & Laemmli, U. K. (1989) *J. Mol. Biol. 210*, 573–585.
Jackson, D. A., & Cook, P. R. (1988) *EMBO J. 7*, 3667–3677.
Kiledjian, M., & Dreyfuss, G. (1992) *EMBO J. 11*, 2655–2664.
Kunze, N., Yang, G., Dölberg, M., Sundarp, R., Knippers, R., & Richter, A. (1991) *J. Biol. Chem. 266*, 9610–9616.

Laemmli, U. K. (1970) *Nature 227*, 680–685.
Laemmli, U. K., Käs, E., Poljak, L., & Adachi, Y. (1992) *Curr. Opin. Cell Biol. 2*, 275–285.
Lapeyre, B., Bourbon, H., & Amalric, F. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 1472–1476.
Luderus, M. E., de Graaf, A., Mattia, E., den Blaauwen, J. L., Grande, M. A., de Jong, L., & van Driel, R. (1992) *Cell 70*, 949–959.
Maniatis, T., Sambrook, J., & Fritsch, E. F. *Molecular Cloning: A laboratory manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Matunis, M. J., Michael, W. M., & Dreyfuss, G. (1992) *Mol. Cell. Biol. 12*, 164–171.
McKay, S. J., & Cooke, H. (1992) *Nucleic Acids Res. 20*, 6461–6464.
Mirkovitch, J., Mirault, M. E., & Laemmli, U. K. (1984) *Cell 39*, 223–232.
Nickerson, J. A., Krochmalnic, G., Wan, K. M., & Penman, S. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 177–181.
Phi-Van, L., & Strätling, W. H. (1990) *Progress in Molecular and Subcellular Biology*, pp 1–11, Springer Verlag, Heidelberg.
Pinol-Roma, S., Choi, Y., Matunis, M. J., & Dreyfuss, G. (1988) *Genes Dev. 2*, 215–227.
Riva, S., Morandi, C., Tsoulfas, P., Pandolfo, M., Biamonti, G., Merill, B., Williams, K. R., Multhaup, G., Beyreuther, K., Werr, H., Henrich, B., & Schäfer, K. P. (1986) *EMBO J. 5*, 2267–2273.
Romig, H., Fackelmayer, F. O., Renz, A., Ramsperger, U., & Richter, A. (1992) *EMBO J. 11*, 3431–3440.
Sapp, M., Knippers, R., & Richter, A. (1986) *Nucleic Acids Res. 14*, 6803–6820.
Schagger, H., & von Jagow, G. (1987) *Anal. Biochem. 166*, 368–379.
Schnieder, G., Wessel, R., Scheffner, M., & Stahl, H. (1990) *EMBO J. 9*, 2937–2943.
Takimoto, M., Tomonaga, T., Matunis, M., Avigan, M., Krutzsch, H., Dreyfuss, G., & Levens, D. (1993) *J. Biol. Chem. 268*, 18249–18258.
Towbin, H., Staehlin, T., & Gordon, J. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4350–4354.
Tsutsui, K., Tsutsui, K., Okada, S., Watarai, S., Seki, S., Yasuda, T., & Shomori, T. (1993) *J. Biol. Chem. 268*, 12886–12894.
von Kries, J. P., Buck, F., & Strätling, W. H. (1994) *Nucleic Acids Res. 22*, 1215–1220.
Wray, W., Boulikas, T., Wray, V., & Hancock, R. (1981) *Anal. Biochem. 118*, 197–203.