© 1994 Oxford University Press

Nucleic Acids Research, 1994, Vol. 22, No. 7 1215–1220

Chicken MAR binding protein p120 is identical to human heterogeneous nuclear ribonucleoprotein (hnRNP) U

Jens P.von Kries, Friedrich Buck[1] and Wolf H.Strätling*
Institut für Physiologische Chemie and [1]Institut für Zellbiochemie und Klinische Neurobiologie, Universitäts-Krankenhaus Eppendorf, Martinistr. 52, D-20246 Hamburg, Germany

Received December 22, 1993; Revised and Accepted March 4, 1994

ABSTRACT

We have previously identified two proteins from chicken oviduct nuclei that specifically bind to matrix/scaffold attachment regions (MARs/SARs). Here one of these proteins, named p120 due to its apparent molecular weight, is purified to near homogeneity and shown to be identical to a previously described component of heterogeneous nuclear ribonucleoprotein particles, hnRNP U, on the basis of amino acid sequence analysis of tryptic peptides. p120 binds to multiple MAR fragments provided they have a minimal length of approximately 700 bp. Binding of MAR fragments is specifically competed by homoribopolymers poly(G) and poly(I), which form four-stranded structures. Our results suggest that p120/hnRNP U may serve a dual function, first as a component of hnRNP particles, and second as an element in the higher-order organization of chromatin.

INTRODUCTION

Biochemical studies in the late 70s laid the ground to the presently widely accepted model that DNA in interphase chromatin and metaphase chromosomes in eukaryotes is organized into topologically sequestered (looped) domains (1,2). This organization requires specific DNA sequences that delimit domains, and several approaches have been undertaken to identify these. A widely used approach was to search for DNA sequences that bind *in vitro* to a high-salt/DNase I or detergent resistant structure in nuclei, named matrix or scaffold (3,4). Such sequences, called MARs or SARs, have been localized to many gene loci from species as diverse as yeast, plants and man (for reviews see ref. 5,6). In a different kind of approach, sequences packaged in non-canonical chromatin structures (scs elements) were localized to the flanks of a *Drosophila* heat shock gene locus (87A7) (7). These sequences can insulate transgenes from position effects of the chromatin at the site of integration. Besides the role of MARs/SARs in chromatin organization, it is thought that they also have a function in the control of gene expression. This notion emerged from the location of at least some MARs at the borders of functional gene domains (8) and gained support from genetic experiments showing that MARs can confer elevated, position-less dependent and developmentally regulated expression of transgenes in stably transfected cells and in transgenic mice and plants (9–13).

It seems plausible that the loop-domain organization further requires proteins that specifically bind to MARs/SARs. Scaffold preparations from highly purified metaphase chromosomes contain two prominent proteins, Sc1 and Sc2 (14). Protein Sc1 is identical to DNA topoisomerase II, an enzyme that catalyzes strand passing of double-stranded DNA in an ATP-dependent fashion (15). Besides its enzymatic function during segregation of sister chromatids at mitosis (16), topoisomerase II appears to have a structural role in chromosome organization (17). Several proteins that bind to MARs have properties of conventional transcription factors, such as SATB1 (18) and factors binding to the osteocalcin gene promoter (19). Two MAR binding proteins raise a special interest: Lamin B_1 may have a role in the binding of chromatin to the nuclear lamina (20), and the nuclear matrix protein SAF-A was found to aggregate MAR fragments into looped structures (21). We have previously identified two proteins from chicken oviduct nuclei, that specifically bind to MARs (22). The first one, named ARBP (for attachment region binding protein), is an abundant nuclear, evolutionary conserved protein that binds to MAR fragments of a minimal ~350 bp length in a cooperative fashion. For efficient binding of ARBP, MAR sequences can act synergistically over large distances. The second protein, here named p120 (for its apparent molecular weight of 120 kD), differs from ARBP in that it binds only to longer MAR fragments (see Fig. 2 in ref. 22).

The packaging of pre-mRNAs (hnRNAs) occurs in association with specific proteins to form heterogeneous nuclear ribonucleoprotein (hnRNP) particles (23). Of these, a sextet of proteins with apparent molecular weights between 32 kD and 44 kD (proteins A, B and C) have frequently been reported. A greater complexity including higher molecular weight proteins was revealed by two-dimensional gel electrophoresis of immunopurified hnRNP particles (23). Among these, hnRNP U is an abundant nuclear phosphoprotein that was found to be cross-linked to hnRNA in intact cells by UV light (24,25). It is co-immunopurified with antibodies to other hnRNP proteins indicating that it is part of the same macromolecular complex that contains the other hnRNP proteins (23). The *in vivo* binding

*To whom correspondence should be addressed

site of hnRNP U is still elusive, and synthetic homoribopolymers have been used to analyse its *in vitro* RNA binding properties (26). The protein has been cloned, and its deduced amino acid sequence shows a glycine/arginine-rich carboxyl-terminal domain (amino acids 695 to C-terminus) that by itself can bind homoribopolymers. Within this domain, a segment that contains clustered repeats of the tripeptide RGG (RGG box) is necessary for nucleic acid binding.

Here we describe purification of chicken p120, and show by amino acid sequence analysis of tryptic peptides that it is identical to human hnRNP U. Binding of MAR fragments is specifically competed by homoribopolymers poly(G) and poly(I), which form four-stranded structures. This suggests that recognition of unusual nucleic acid structures is a component of the mode of binding of p120/hnRNP U.

MATERIALS AND METHODS

Purification of p120

Extracts from purified hen oviduct nuclei were prepared and treated by passage over a DEAE−cellulose column as previously reported (22). The DEAE−cellulose flow-through was applied to a P11 phosphocellulose column (50 ml), equilibrated in buffer P (20 mM Tris−HCl, pH 7.5, 0.5% 2-mercaptoethanol, 0.05% NP-40) containing 250 mM NaCl (P250). The column was washed with buffer P250 and eluted with a step gradient (one step = 50 mM NaCl) from 250 to 900 mM NaCl in buffer P. Fragment P1−P2 binding activity was detected in fractions eluted with 400−500 mM NaCl. The peak activity fractions were pooled and loaded onto added double-stranded calf thymus DNA−cellulose (Sigma) in a batch procedure by stepwise dilution to 200 mM NaCl. The loaded cellulose was filled into a column, washed with buffer D (20 mM Tris−HCl, pH 8.0, 0.2 mM EDTA, 5 mM 2-mercaptoethanol, 0.025% NP-40) containing 200 mM NaCl, and eluted with a step gradient (one step = 50 mM NaCl) from 250 to 700 mM NaCl in buffer D. P1−P2 binding activity was eluted with 400−500 mM NaCl. Pooled active peak fractions were diluted to 300 mM NaCl and loaded onto a Mono S HR5/5 column (Pharmacia), equilibrated in buffer S (20 mM MOPS, pH 7.4, 5 mM 2-mercaptoethanol, 0.05% NP-40) containing 300 mM NaCl. P1−P2 binding activity was eluted between 570 and 670 mM NaCl of a linear salt gradient (300−1000 mM NaCl in buffer S) (Mono S fraction).

Peptide sequencing

The Mono S fraction of p120 was displayed by SDS−polyacrylamide gel electrophoresis and electro-blotted onto a nitrocellulose membrane in 50 mM sodium borate buffer, pH 8.0. After brief staining with Ponceau S, the p120 band was excised from the filter and subjected to *in situ* digestion with 5 μg of trypsin (sequencing grade, Boehringer) for 15 h. The resulting peptide mixture was separated by reverse-phase high performance liquid chromatography using a Vydac C4-RP microbore column (2.1×100 mm). Several major fractions were chosen based on peak shape and resolution, rechromatographed on an Aquapore RP300 C8 column (1×100 mm) and submitted to automated Edman degradation on an Applied Biosystems 473A protein sequencer.

DNA binding activity assays

A Southwestern blotting assay was used as described (22) to monitor purification of p120 and to analyse the MAR binding properties of purified p120 (Mono S fraction). Furthermore, we employed a slot blot assay that eliminated the electrophoretic and blotting steps. Seventy-five ng of purified p120 (Mono S fraction) in 270 mM NaCl, 190 mM glycerol, 25 mM Tris−HCl, pH 7.5, were immobilized per slot onto a nitrocellulose membrane (Bio-Rad) using a Bio-Dot (Bio-Rad) apparatus. Incubation was performed as in the Southwestern blotting assay with 50 μg/ml of *E.coli* DNA as unspecific competitor. Specific competitors were added in a 250-fold (by-weight) excess. Homoribopolymers used were purchased from Pharmacia. Total RNA was extracted from rat liver using guanidinium isothiocyanate followed by centrifugation through cesium chloride as described (27). Poly(A)$^+$ RNA was selected by passage through oligo(dT)-cellulose using the mRNA purification kit from Pharmacia. ^{32}P radioactivity bound to the filter was quantitated by Cerenkov counting.

Two-dimensional polyacrylamide gel electrophoresis was performed using the O'Farrell minigel system (28,29). The first dimension contained 2% ampholytes (1.4% Biolyte pH 3−10 and 0.6% Biolyte pH 7−9). The pI standard proteins (Bio-Rad) used were: hen egg white conalbumin, 6.0−6.6; bovine muscle actin, 5.5; bovine carbonic anhydrase, 5.9−6.0 (values given by the manufacturer).

RESULTS AND DISCUSSION

MAR binding protein p120 was purified from hen oviduct nuclei in three steps by chromatography on phosphocellulose, double-stranded calf thymus DNA−cellulose, and a Mono S column (Pharmacia). Elution of p120 was monitored by a Southwestern blotting assay using a 1455-bp chicken lysozyme MAR fragment, P1−P2, as probe, and a shorter 447-bp fragment, H1−*Hae*II, as non-binding control (see map in Fig. 4) (22). From the Mono S column p120 eluted with near homogeneity as a symmetrial peak at 620 mM NaCl. In Fig. 1, a summary of the purification procedure shows Coomassie stained SDS−polyacrylamide gels as well as Southwestern assays of samples derived from oviduct nuclear extract and active fractions after each of the three chromatographic steps.

To obtain sequence information, purified p120 (Mono S fraction) was resolved on an SDS−polyacrylamide gel,

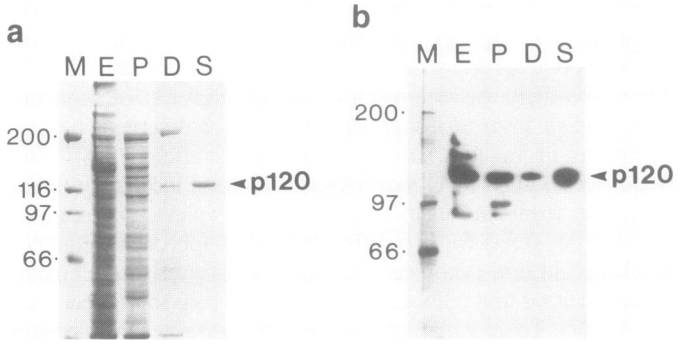

Figure 1. Purification of chicken MAR binding protein p120. **a**, SDS−7% polyacrylamide gel electrophoresis of a 100−400 mM NaCl extract from purified oviduct nuclei (E, 25 μg), and active fractions after chromatography on phosphocellulose (P, 7 μg), double-stranded DNA−cellulose (D, 0.7 μg), and a Mono S column (S, 0.4 μg). **b**, Southwestern blotting assay of the fractions loaded in (a) using MAR probe P1−P2. M, molecular weight marker proteins: myosin (200 kD), β-galactosidase (116 kD), phosphorylase b (97 kD) and bovine serum albumin (66 kD).

transferred onto a membrane, and stained with Ponceau S; the excised 120-kD band was used to generate tryptic peptides, which were separated and microsequenced. Our homology searches with the amino acid sequences of eleven peptides (Swiss-Prot data bank) identified the chicken 120-kD polypeptide to be nearly identical to the product of the human hnRNP U gene (24). In Fig. 2 the chicken p120 peptide sequences are aligned to the sequence of human hnRNP U. Of a total of 131 identified amino acids 121 proved to be identical between chicken p120 and human hnRNP U (marked by vertical lines), while 5 are conservative substitutions (dotted lines) and 5 non-conservative ones (single dots).

Though it is unlikely that the strong MAR binding activity results from a contaminant in the preparation, while on the other hand *all* sequenced peptides derive from hnRNP U, we attempted to exclude this possibility. Purified p120 (Mono S fraction) was separated twice by two-dimensional gel electrophoresis in an O'Farrell minigel system (28,29). One gel was stained with Coomassie, while the other one was used to analyse the MAR binding activity in a Southwestern blotting assay (Fig. 3). In the first dimension, p120 migrated to a pI of approximately 5.8–6.0. As furthermore seen in Fig. 3, the MAR binding activity in the bottom panel coincided with the major stained spot in the top panel. This result confirms our conclusion that p120 is the chicken homolog of human hnRNP U. For the rest of the paper, we therefore refer to this protein as p120/hnRNP U. We particularly note that the sequence conservation between chicken and human p120/hnRNP U in the sequenced portions is remarkably high.

We have previously reported that p120 binds to two long MAR fragments, the 1323-bp B-1–P1 and the 3′ abutting 1455-bp P1–P2, but not to the much shorter 447-bp fragment H1–*Hae*II (22). To define in more detail the requirements for binding, we first performed a deletion mutational analysis with fragment P1–P2, and analysed fragments therefrom, which vary in length and sequence, for their ability to bind purified p120/hnRNP U (Mono S fraction) in a quantitative Southwestern blotting assay. Fig. 4 shows that deletion of 438 bp from the 5′ end and further deletion of 322 bp from the 3′ end did not significantly reduce binding affinity to p120/hnRNP U. However, the further deleted fragments E1–*Hin*fI (583 bp), *Sau*3A–*Sau*3A (552 bp), and *Hae*II–*Sac*I (659 bp) showed greatly reduced binding activity, and the even shorter fragment H1–*Hae*II (447 bp) had no binding activity, as was previously reported (22). These results suggest that fragment E1–*Sca*I contains one or multiple sequences that can mediate p120 binding, provided they are in a fragment of a minimal length of approximately 700 bp. In order to provide more support to this suggestion, we lengthened the non-binding fragment H1–*Hae*II in two ways. We first constructed a direct repeat of H1–*Hae*II (936 bp), and second, fragment H1–*Hae*II was extended on each side by a total of 444 bp of pBS vector sequences. Both fragments bound as efficiently to p120/hnRNP U as fragment P1–P2. A 1101 bp control fragment containing solely vector sequences had no binding activity. Furthermore, extension of the weakly binding fragment *Hae*II–*Sac*I by 127 bp of vector sequences significantly increased binding activity. These results gave additional proof to our suggestion that the sequences which mediate p120 binding must be contained in a fragment of a minimal length. This is in sharp contrast to the

```
MSSSPVNVKK LKVSELKEEL KKRRLSDKGL KAELMERLQA ALDDEEAGGR   50
PAMEPGNGSL DLGGDSAGRS GAGLEQEAAA GGDEEEEEEE EEEEGISALD  100
GDQMELGEEN GAAGAADSGP MEEEEAASED ENGDDQGFQE GEDELGDEEE  150
GAGDENGHGE QQPQPPATQQ QQPQQQRGAA KEAAGKSSGP TSLFAVTVAP  200

PGARQGQQQA GGDGKTEQKG GDKKRGVKRP REDHGRGYFE YIEENKYSRA  250
                                  (13b) GYFE YIEENK

KSPQPPVEEE DEHFDDTVVC LDTYNCDLHF KISRDRLSAS SLTMESFAFL  300

WAGGRASYGV SKGKVCFEMK VTEKIPVRHL YTKDIDIHEV RIGWSLTTSG  350
                                  (7) DIDIHEV

MLLGEEEFSY GYSLKGIKTC NCETEDYGEK FDENDVITCF ANFESDEVEL  400
                (23) TX NXETEDFGEK FDENDVXXXF ANFD

SYAKNGQDLG VAFKISKEVL AGRPLFPHVL CHNCAVEFNF GQKEKPYFPI  450
(10a) WGQELG VAF                                (20) DEPYFPV

PEEYTFIQNV PLEDRVRGPK GPEEKKDCEV VMMIGLPGAG KTTWVTKHAA  500
PEEYTFIQXD

ENPGKYNILG TNTIMDKMMV AGFKKQMADT GKLNTLLQRA PQCLGKFIEI  550
              (10c) MMV AGF                     (10b) FIEI

AARKKRNFIL DQTNVSAAAQ RRKMCLFAGF QRKAVVVCPK DEDYKQRTQK  600
   (13a)
AAR  NFIL DQTNVSAAAQ R

KAEVEGKDLP EHAVLKMKGN FTLPEVAECF DEITYVELQK EEAQKLLEQY  650
              (27) XX FTLPEVAEXF DEIIYVEL    (5) LLEQY

KEESKKALPP EKKQNTGSKK SNKNKSGKNQ FNRGGGHRGR GGLNMRGGNF  700
KEESK

RGGAPGNRGG YNRRGNMPQR GGGGGGSGGI GYPYPRAPVF PGRGSYSNRG  750
        (11) GGGNMPQR GGGGG

NYNRGGMPNR GNYNQNFRGR GNNRGYKNQS QGYNQWQQGQ FWGQKPWSQH  800
YHQGYY                                                  806
```

Figure 2. Peptide sequence analysis indicates identity between chicken p120 and human hnRNPU. Below the amino acid sequence of human hnRNP U (24) the sequences of eleven tryptic peptides of p120 are shown. In the peptide sequences (numbered as eluted from HPLC), unambiguous amino acids are printed in boldface letters, ambiguous ones in normal size letters. X indicates any amino acid. Amino acid identity is marked by a vertical line, a conservative substitution by a dotted line, and a non-conservative substitution by a single dot. Two sequences identical or similar to the consensus NTP binding site (31) are underlined.

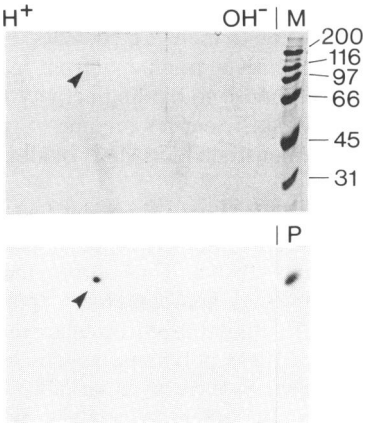

Figure 3. Two-dimensional gel electrophoresis. Purified p120 was separated twice by two-dimensional gel electrophoresis in an O'Farrell minigel system. One gel (top) was stained with Coomassie. The other gel (bottom) was blotted and incubated with MAR probe P1–P2 (Southwestern blotting assay). Molecular weight standard proteins (M) and, respectively, p120 (P) were included in the second dimension. The arrow head marks the position of p120.

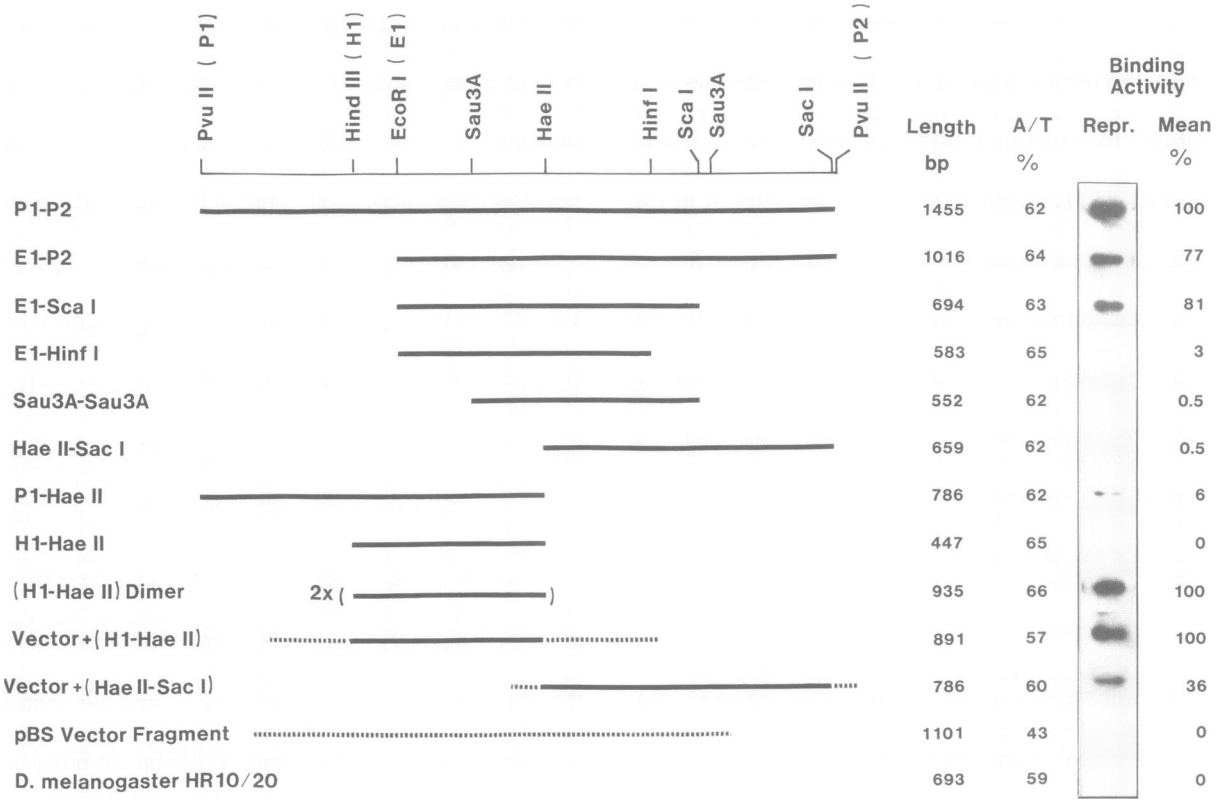

Figure 4. Characterization of MAR binding properties of purified p120/hnRNP U. Below the restriction map of the chicken lysozyme MAR fragment P1−P2, the position of those fragments are shown that were analysed in a DNA binding p120 protein blot assay. Fragment HR10/20 is an AT-rich repetitive sequence from *D.melanogaster* (22). The lengths of the fragments and their AT-contents are indicated. p120/hnRNP U binding activity is documented by the autoradiogamm of a representative experiment (Repr.) and the mean value of binding in four independent experiments given as percent of binding of fragment P1−P2.

MAR binding features of protein ARBP, that also recognizes the 447-bp fragment H1−*Hae*II (see Fig. 2 in ref. 22) and oligonucleotides containing a distinct DNA structural/sequence motif (to be published). Sequencs with high binding affinity are likely clustered wthin the sequence E1−*Hae*II, since all fragments containing this sequence completely had elevated binding activities compared to non-E1−*Hae*II fragments of similar lengths. The relative affinities of the MAR fragments to p120/hnRNP U do not show any correlation to their AT contents. Furthermore, an AT-rich 693-bp repetitive fragment from *D.melanogaster*, HR10/20, exhibited almost no binding activity (22). Thus AT-richness per se is not sufficient for binding to p120/hnRNP U, as was previously demonstrated for MAR binding protein ARBP (22).

Though human hnRNP U has been identified as a component of hnRNP particles (23,25), the sequence or structure bound by hnRNP U still remains elusive. Thus all previous *in vitro* RNA binding studies employed homopolymers, poly(A), poly(C), poly(G), and poly(U), immobilized on agarose beads (24,26). In an assay that used these polymers, specifically poly(G) has been repeatedly shown to have a remarkably high affinity to hnRNP U (24,26). In order to analyse whether chicken p120/hnRNP U reproduces this binding behaviour in Southwestern blotting assays, we added various ribopolymers as specific competitors. As seen in Fig. 5, left panel, solely poly(G) greatly reduced binding of P1−P2, while poly(A), poly(C) and poly(U) competed very little. This shows that p120/hnRNP U has very similar RNA binding characteristics in two different assay systems. It is furthermore interesting that p120/hnRNP U specifically recognized the double-stranded form of fragment P1−P2, since the denatured probe did not bind (Fig. 5). Consistant with this, p120 eluted from double-stranded calf thymus DNA−cellulose at 400−500 mM NaCl in our routine purification procedure (see Materials and Methods). Notably, other hnRNP proteins behave differently in this respect, e.g. hnRNP A2/B1 specifically binds to single-stranded telomeric repeats (30). Previous reports (23,24) on the binding of human hnRNP U to single-stranded calf thymus DNA immobilized on agarose beads (BRL) may be explained by binding to a small DNA fraction that renatured in the purchased material. Routinely, this material is used for protein purifications but not to discriminate single-stranded versus double-stranded DNA binding.

Southwestern blotting assays have the severe limitation that the protein is denatured by SDS and may not fully renature when bound to the filter after blotting. Therefore, we additionally employed a slot blot assay that immobilizes the native protein to nitrocellulose followed by incubation with the DNA probe. Fig. 5, right panel, shows that binding of P1−P2 to non-SDS-denatured p120/hnRNP U was selectively competed by poly(G). Complete competition was observed at simultaneous addition of P1−P2 and pol(G), and after preincubation for 15 min with either the probe or poly(G) (data not shown). We also found efficient competition of P1−P2 binding with the 2-deamino analog poly(I), that adopts a similar structure as poly(G) (see below). Surprisingly, total RNA from rat liver did not compete in a

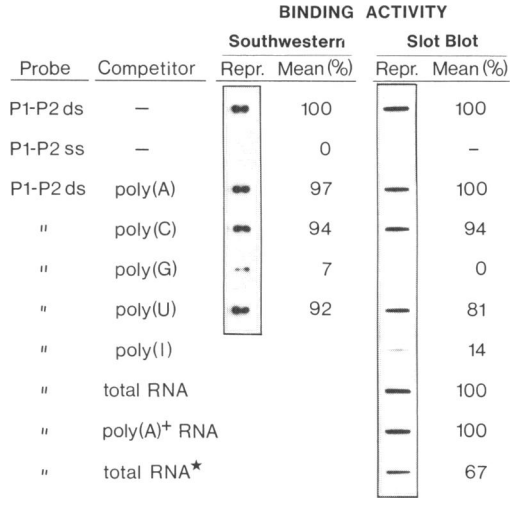

Figure 5. MAR binding to p120/hnRNP U is competed by poly(G) and poly(I). p120/hnRNP U was either separated on an SDS–polyacrylamide gel and blotted electrophoretically (Southwestern) or immobilized onto a nitrocellulose membrane using a slot blot apparatus (Slot Blot). Binding assays contained 36 ng of labeled probe P1–P2 (ds) and, where indicated, 9 μg of homoribopolymers (Pharmacia), total rat liver RNA or poly(A)$^+$ RNA. The star indicates the addition of total RNA in a 1000-fold excess. Single-stranded (ss) probe P1–P2 (36 ng) was heat-denatured for 10 min, chilled on ice, and immediately diluted with unspecific *E.coli* competitor DNA prior to its addition to the incubation mixture.

250-fold excess and slightly in a 1000-fold excess. Also poly(A)$^+$ RNA was found to be unable to compete. It is possible that hnRNP U binds to hnRNA only in cooperation with other hnRNP proteins, or that it binds to a 'core' structure in hnRNP particles. The ability of hnRNP U to be dissociated from hnRNP particles by heparin, while other hnRNP proteins, e.g. proteins A, B and C, remain associated at this treatment, supports this interpretation (23).

Notably, our competition binding studies do not conclusively answer the question of how many binding sites reside in p120/hnRNP U. The efficient competition by poly(G) suggests that the glycine/arginine-rich carboxyl-terminal domain is important for MAR DNA binding as it is for binding of the ribopolymer (24). Alternatively, poly(G) and MAR DNA fragments might bind to different sites, and competition by poly(G) [and poly(I)] might occur by sterical hindrance or by conformational changes of the protein. While it has been reported that human hnRNP U also binds to poly(U) (24,26), we observed only slight competition with poly(U) in the slot blot assay and none in the Southwestern blotting assay. As an explanation, another distinct site may recognize this ribopolymer. Attempts to resolve the discrepancy by use of a band shift assay, that employs the protein in a soluble form rather than immobilized to filters, were unsuccessful.

p120/hnRNP U contains a perfect match and a 4-of-5 match of the GXXGXGKT consensus sequence of NTP binding sites (underlined in Fig. 2) (31). To approach the functional significance of these sequences we performed Southwestern blotting assays in the presence of 1 mM ATP (or CTP, GTP, UTP) and 2 mM $MgCl_2$. Relative to a control that contains 1 mM diphosphate and 2 mM $MgCl_2$, we observed very little change in the binding of fragment P1–P2 (data not shown). As an alternative to a role in MAR binding, the putative NTP binding sites may have an influence on yet unknown effector functions of p120/hnRNP U.

Physical data have indicated that poly(G) and poly(I) adopt a quadruple helical structure (32). Since specifically these two homoribopolymers compete P1–P2 binding efficiently, it is likely that p120/hnRNP U recognizes this structure. Oligodeoxyribonucleotides containing repeats of a G-rich sequence as they occur in telomere DNA also fold into a four-stranded structure, that has as its stabilizing element a planar array of four Hoogsteen-paired G residues (G-quartet) (33). It is thus possible that p120/hnRNP U also binds quadruple DNA structures. Notably, in the chicken lysozyme 5′ MAR, the dispersed sequence TGGG is 3.2-fold higher represented than expected from the GC-content (38%) (data not shown). Sequences with this G-rich tetranucleotide can also form G-quartets and are found furthermore in immunoglobulin gene switch regions (34,35). The recently described human 120 kD nuclear matrix protein SAF-A, that may be related to p120/hnRNP U, aggregates with MARs into looped DNA structures (21). p120/hnRNP U may specifically require long MAR fragments for binding, since these can form DNA loops more easily. The presence of intrinsically curved sequences that would facilitate loop formation has previously been reported in MAR sequences (36). In a hypothesis, that unifies these individual arguments, we speculate that p120/hnRNP U binds to MARs and forms loops through recognition of an unusual (four-stranded) DNA structure at the basis of the loops.

Although the possibility that p120/hnRNP U binds to quadruple helical structures in telomeric DNA remains to be tested, we note several relationships of p120/hnRNP U to other proteins associated with telomers. The β subunit of *Oxytricha* telomere binding protein exhibits slight sequence similarities to p120/hnRNP U (37). A necessary segment in the C-terminal RNA binding domain of p120/hnRNP U repeatedly contains the peptides RG and RGG (24), while repetition of the related peptides KG and KGG is a specific feature of the β subunit. Furthermore, a hexapeptide (AAGKSS) is shared by both proteins. The yeast repressor/activator protein RAP1, a multifunctional protein that binds to the irregular telomere repeat TG_{1-3} *in vivo* and *in vitro*, is also a constituent of the nuclear matrix (38,39). Selectively telomere DNA was retained in nuclear matrix prepared from human cells by extraction with lithium diiodosalicylate (40).

We finally considered the possibility that others might have reported on the MAR binding activity of a homolog of hnRNP U, but have not recognized the homology. Our searches with the human hnRNP U sequence in two protein data banks identified a 120-kD protein that was purified very recently from rat brain nuclear matrix (41). This protein was found to bind specifically to MAR fragments of the immunoglobulin κ light chain gene and of the fushi tarazu gene. Although the protein was described as a novel one, the deduced amino acid sequence shows 97% identity to the sequence of human hnRNP U from amino acid 53 up to the C-terminus. Furthermore, if it is assumed that a distinct base pair has been missed during sequencing, so that utilization of a further upstream located AUG codon is allowed, the presumed additional amino acid sequence shows 94% identity to the hnRNP U sequence from the N-terminus to amino acid 52. This high degree of identities indicates that this protein is the rat homolog of p120/hnRNP U, and that this homolog like the chicken one recognizes MAR sequences.

In summary we demonstrate that the chicken MAR binding protein p120 is identical to the previously described human RNA binding protein hnRNP U. Since MARs are thought to organize chromatin into topologically sequestered loop-domains, which correspond to functional units in chromatin (6), our results suggest that p120/hnRNP U serves a dual role, first as a component of hnRNP particles (23,25,26), and second as an element in the higher-order organization of chromatin. A protein with such a dual role has several precedents. The most well known example is transcription factor TFIIIA that activates oocyte-type 5 S RNA transcription and stores the 5 S RNA transcript (42). Other examples are the mRNA binding proteins FRGY1 (43), mRNP4/FRGY2 (44), and MSY1 (43) that act also as transcription factors binding to Y-box-containing promoters, and the *Pleurodeles* protein PwA33 (45) that binds to pre-mRNA and, additionally, exhibits a regulatory role.

ACKNOWLEDGEMENTS

We thank H.Buhrmester for his help for computational analysis and B.Huckschlag for excellent technical assistance. This work was supported by grants to W.H.S. from the Deutsche Forschungsgemeinschaft.

REFERENCES

1. Benyajati,C. and Worcel,A. (1976) *Cell* **9**, 393–407.
2. Cook,P.R. and Brazell,I.A. (1978) *Eur. J. Biochem.* **84**, 465–477.
3. Mirkovitch,J., Mirault,M.-E. and Laemmli,U.K. (1984) *Cell* **39**, 223–232.
4. Cockerill,P.N. and Garrard,W.T. (1986) *Cell* **44**, 273–282.
5. Gasser,S.M. and Laemmli,U.K. (1987) *Trends Genet.* **3**, 16–22.
6. Phi-Van,L. and Strätling,W.H. (1990) *Prog. Mol. Subcell. Biol.* **11**, 1–11.
7. Kellum,R. and Schedl,P. (1991) *Cell* **64**, 941–950.
8. Phi-Van,L. and Strätling,W.H. (1988) *EMBO J.***7**, 655–664.
9. Stief,A., Winter,D.M., Strätling,W.H. and Sippel,A.E. (1989) *Nature* **314**, 343–345.
10. Phi-Van,L., von Kries,J.P., Ostertag,W. and Strätling,W.H. (1990) *Mol. Cell. Biol.* **10**, 2302–2307.
11. Klehr,D., Maass,K. and Bode,J. (1991) *Biochem.* **30** 1264–1270.
12. Breyne,P., Van Montagu,M., Depicker,A. and Gheyssen,G. (1992) *Plant Cell* **4**, 463–471.
13. McKnight,R.A., Shamay,A., Sankaran,L., Wall,R.J. and Henninghausen,L. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 6943–6947.
14. Lewis,C.D. and Laemmli,U.K. (1982) *Cell* **29**, 171–181.
15. Earnshaw,W.C., Halligan,B., Cooke,C.A., Heck,M.M.S. and Liu,L.F. (1985) *J. Cell Biol.* **100**, 1706–1715.
16. Holm,C., Goto,T., Wang,J.C. and Botstein,D. (1985) *Cell* **41**, 553–563.
17. Boy de la Tour,E. and Laemmli,U.K. (1988) *Cell* **55**, 937–944.
18. Dickinson,L.A., Joh,T., Kohwi,Y. and Kohwi-Shigematsu,T. (1992) *Cell* **70**, 631–645.
19. Bidwell,J.P., van Wijnen,A.J., Fey,E.G., Dworetzky,S., Penman,S., Stein,J.L., Lian,J.B. and Stein,G.S. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 3162–3166.
20. Ludérus,M.E.E., de Graaf,A., Mattia,E., den Blaauwen,J.L., Grande,M.A., de Jong,L. and van Driel,R. (1992) *Cell* **70**, 949–959.
21. Romig,H., Fackelmeyer,F.O., Renz,A., Ramsperger,U. and Richter,A. (1992) *EMBO J.* **11**, 3431–3440.
22. von Kries,J.P., Buhrmester,H. and Strätling,W.H. (1991) *Cell* **64**, 123–135.
23. Piñol-Roma,S., Choi,Y.D., Matunis,M.J. and Dreyfuss,G. (1988) *Genes Develop.* **2**, 215–227.
24. Kiledjian,M. and Dreyfuss,G. (1992) *EMBO J.* **11**, 2655–2664.
25. Dreyfuss,G., Choi,Y.D. and Adam,S.A. (1984) *Mol. Cell. Biol.* **4**, 1104–1114.
26. Swanson,M.S. and Dreyfuss,G. (1988) *Mol. Cell. Biol.* **8**, 2237–2241.
27. Sambrook,J., Fritsch,E.F. and Maniatis,T. (1989) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.
28. O'Farrell,P.H. (1975) *J. Biol. Chem.* **250**, 4007–4021.
29. Adams,L.D. and Gallagher,S.R. (1992) In Ausubel,F.M., Brent,R., Kingston,R.E., Moore,D.D., Seidman,J.G., Smith,J.A. and Struhl,K. (eds), Current Protocols in Molecular Biology. Greene Publishing Associates and Wiley-Interscience, New York, p. 10.4.9.
30. McKay,S.J. and Cooke,H. (1992) *Nucleic Acids Res.* **20**, 6461–6464.
31. Walker,J.E., Saraste,M., Runswick,M.J. and Gay,N.J. (1982) *EMBO J.* **1**, 945–951.
32. Saenger,W. (1984) In Cantor,C.R. (ed.), Principles of Nucleic Acid Structure. Springer-Verlag New York, pp. 298–320.
33. Sundquist,W.I. and Klug,A. (1989) *Nature* **342**, 825–829.
34. Fang,G. and Cech,T.R. (1993) *Cell* **74**, 875–885.
35. Nikaido,T., Yamawaki-Kataoka,Y. and Honjo,T. (1982) *J. Biol. Chem.* **527**, 7322–7329.
36. von Kries,J.P., Phi-Van,L., Diekmann,S. and Strätling,W.H. (1990) *Nucleic Acids Res.* **18**, 3881–3885.
37. Hicke,B.J., Celander,D.W., MacDonald,G.H., Price,C.M. and Cech,T.R. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 1481–1485.
38. Conrad,M.N., Wright,J.H., Wolf,A.J. and Zakian,V.A. (1990) *Cell* **63**, 739–750.
39. Hofmann,J.F.-X., Laroche,T., Brand,A.H. and Gasser,S.M. (1989) *Cell* **57**, 725–737.
40. de Lange,T. (1992) *EMBO J.* **11**, 717–724.
41. Tsutsui,K., Tsutsui,K., Okada,S., Watarai,S., Seki,S., Yasuda,T. and Shohmori, T. (1993) *J. Biol. Chem.* **268**, 12886–12894.
42. Pelham,H.R.B. and Brown,D.D. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 4170–4174.
43. Tafuri,S.R., Familari,M. and Wolffe,A.P. (1993) *J. Biol. Chem.* **268**, 12213–12220.
44. Deschamps,S., Viel,A., Garrigos,M., Denis,H. and le Maire,M. (1992) *J. Biol. Chem.* **267**, 13799–13802.
45. Bellini,M., Lacroix,J.-C. and Gall,J.G. (1993) *EMBO J.* **12**, 107–114.