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Molecular definition of heterogeneous nuclear ribonucleoprotein R (hnRNP R) using autoimmune antibody: immunological relationship with hnRNP P

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ABSTRACT

Serum from a patient showing symptoms related to autoimmunity was found to contain autoantibodies to the nuclear mitotic apparatus (NuMA) protein and to several novel nuclear antigens with estimated molecular weights of 40, 43, 72, 74 and 82 kDa. Using this serum for screening a human cDNA expression library a 2.5 kb cDNA clone was isolated which encoded the complete sequence of a protein of 633 amino acids. Sequence analysis revealed a modular structure of the protein: an acidic N-terminal region of ~150 amino acids was followed by three adjacent consensus sequence RNA binding domains located in the central part of the protein. In the C-terminal portion a nuclear localization signal and an octapeptide (PPPRMPPP) with similarity to a major B cell epitope of the snRNP core protein B were identified. This was followed by a glycine- and arginine-rich section of ~120 amino acids forming another type of RNA binding motif, a RGG box. Interestingly, three copies of a tyrosine-rich decapeptide were found interspersed in the RGG box region. The major *in vitro* translation product of the cDNA co-migrated in SDS–PAGE with the 82 kDa polypeptide that was recognized by autoantibodies. The structural motifs as well as the immunofluorescence pattern generated by anti-82 kDa antibodies suggested that the antigen was one of the proteins of the heterogeneous nuclear ribonucleoprotein (hnRNP) complex. Subsequently the 82 kDa antigen was identified as hnRNP R protein by its presence in immunoprecipitated hnRNP complexes and co-migration of the recombinant protein with this hitherto uncharacterized hnRNP constituent in two-dimensional gel electrophoresis. The concomitant autoimmune response to a hnRNP component of the pre-mRNA processing machinery and to NuMA, a protein engaged in mitotic events and reported to be associated with mRNA splicing complexes in interphase, may indicate physical and functional association of these antigens. Support for this notion comes from observations that concomitant or coupling of autoantibody responses to proteins which are associated with each other as components of sub-cellular particles are often found in autoimmune diseases.

INTRODUCTION

Heterogeneous nuclear ribonucleoproteins (hnRNPs) are among the most abundant proteins in the eukaryotic nucleus and they play important roles in processing of precursor mRNA (1,2). They assemble on nascent RNA polymerase II transcripts to form hnRNP complexes, where they co-localize with small nuclear (sn)RNPs. Immunoprecipitation of hnRNP complexes with monoclonal antibodies to individual hnRNP proteins and further resolution by two-dimensional gel electrophoresis demonstrated that they contain ~20 major proteins, ranging in size from 34 to 120 kDa (1,3).

hnRNP proteins have a modular structure consisting of one or more RNA binding domains and at least another auxiliary domain, which likely mediates protein–protein interactions but may also contribute to nucleic acid binding (1,2,4). Among the three types of RNA binding motifs identified in hnRNP proteins, the consensus sequence RNA binding domain (RBD), also named RNA recognition motif (RRM), is the most frequently found motif (5). Another type of RNA binding motif present in some hnRNP proteins is the RGG box, characterized by several closely spaced RGG repeats (5,6), which may occur either alone, as in

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hnRNP U (7), or in combination with one or more RBD, as observed in hnRNP A1 and D0 (8). A third type of RNA binding domain is the K homology (KH) motif, first identified in hnRNP K, a stretch of ~45 amino acids characterized by several highly conserved non-polar amino acids (9,10).

In addition to general RNA binding, hnRNP proteins display a broad range of functions in mRNA biogenesis (1,2). Thus monoclonal antibodies to the C proteins were capable of inhibiting the first cleavage event at the 5′ splice site of introns (11,12) and hnRNP I was found to be identical to the polypyrimidine tract binding protein which binds preferentially to the pyrimidine-rich section close to the 3′-ends of introns (13). Direct involvement in splicing events has also been demonstrated for hnRNP A/B proteins, which can influence splice site selection *in vitro* and *in vivo* (14–16), and for hnRNP F, which appears to be involved in control of tissue-specific splicing (17). Furthermore, several hnRNP proteins have the ability to catalyze annealing of complementary DNA or RNA strands, an activity which may be important during the splicing process (18,19). In addition to these spliceosome-associated functions, hnRNP proteins are likely involved in other biological processes, such as transport of mature mRNA (20), transcription (21) and structural organization of genomic DNA (22,23). Moreover, cell type- and tissue-specific expression of some hnRNP proteins, as has been reported recently, is suggestive of functions associated with cell differentiation (24). Taken together these data suggest a dynamic structure and multiple functions of hnRNP complexes, which is also supported by indications for a sequence-dependent arrangement of hnRNP proteins on certain hnRNAs (25).

Spontaneously occurring autoantibodies to intracellular proteins and nucleic acids are a common feature of systemic autoimmune disorders which are characterized by disease-specific patterns of autoantibodies (26,27). Many autoantigens have been identified as nucleic acid binding proteins engaged in essential cellular functions, such as transcription, splicing of pre-mRNA, translation, DNA replication and cell division (28–30). In the present study a patient's serum that produced a dense fine speckled nucleoplasmic immunofluorescence in interphase cells and mitotic spindle staining in metaphase was found by Western blotting to recognize several nuclear antigens between 40 and 210 kDa. Of these, the 210 kDa antigen was identified as the nuclear mitotic apparatus protein NuMA, a well-defined autoantigen (31) known to be involved in mitotic spindle assembly and nuclear reformation after mitosis (32). To characterize the novel antigens the serum was used for immunoscreening of a human cDNA expression library. A full-length cDNA clone was isolated encoding a protein corresponding to a nuclear antigen of ~82 kDa. This protein was found to be indistinguishable from the as yet uncharacterized hnRNP R protein. The sequence, structural features and nuclear localization of hnRNP R and its immunological relationship with hnRNP P are reported here.

MATERIALS AND METHODS

Sera and antibodies

Patient BL suffered from arthralgias, myalgias and simultaneously occurring clinical and serological symptoms related to a 'syndrome of idiopathic chronic urticaria and angioedema with thyroid autoimmunity' (33). The autoantibody profile of this serum was characterized by immunoblotting and immunofluorescence microscopy and showed, with the exception of antibodies to the nuclear mitotic apparatus protein (NuMA), no similarities with reactivity patterns corresponding to other commonly known autoantibody specificities, such as Sm, U1 snRNP, ribosomal RNP, SS-A/Ro, SS-B/La, topoisomerase I, Ku, PCNA or fibrillarin (26). Reference sera for these antibody specificities were obtained from the W.M.Keck Autoimmune Disease Center laboratory serum bank. Antibody reactivity to NuMA was additionally determined by immunoprecipitation using *in vitro* transcription and translation products of NuMA cDNA as described below. NuMA cDNA was a kind gift from Duane A.Compton (Department of Biochemistry, Dartmouth Medical School, Hanover, NH; 32). Reference sera for antibodies to the hnRNP A/B proteins (34) and monoclonal antibodies to the hnRNP C proteins (4F4) and to hnRNP K (12G1) have been described previously (35,36).

Antigen preparation and immunoblotting

Nuclear extracts from HeLa or MOLT-4 cells were prepared as described with slight modifications (37). Briefly, isolated nuclei were incubated in hypotonic buffer (10 mM HEPES–KOH, pH 7.9, 2.5 mM $MgCl_2$, 1 mM PMSF, 1 μg/ml leupeptin, 1 μg/ml aprotinin, 25% glycerol) for 30 min on ice and subsequently extracted for 30 min by addition of 1 vol. of the same buffer containing 0.88 M NH_4Cl (hypertonic buffer). The nuclei were centrifuged for 15 min at 10 000 *g* and the resultant supernatant, i.e. the nuclear extract, used for Western blot analysis. To obtain a partially purified preparation of hnRNPs the extract was fractionated by heparin–Sepharose chromatography as described (37). Fractions enriched for hnRNP proteins were pooled, precipitated with 0.4 g/ml ammonium sulfate and redissolved in Laemmli sample buffer for SDS–PAGE. Western blotting was performed as described (38) using sera at 1:50 dilution and for visualization of antibody reactivities a chemoluminescence system (Amersham) was used employing HRP-conjugated anti-human IgG or anti-mouse IgG (Cappel).

cDNA cloning and sequence analysis

BL serum was used for immunoscreening of a human T24 bladder epithelial cell Zap Express cDNA expression library (Stratagene Inc., La Jolla, CA) according to previously established protocols (38–41). A clone, called BL14, was isolated and subcloned *in vivo* into pBK-CMV (pBL14) using R408 helper phage according to the manufacturer's instructions (Stratagene). Nucleotide sequences were determined on both strands using dye terminator sequencing and an automated sequencer from Applied Biosystems Inc. (Foster City, CA).

Affinity purification of antibodies

The recombinant phage protein from λZap clone BL14 phage plaques or the natural 82 kDa protein were immobilized to nitrocellulose (Schleicher & Schuell, Germany), incubated with 1:50 diluted BL serum for 40 min at 4°C and washed extensively with phosphate-buffered saline containing 0.05% Tween and 1 M NaCl; bound antibodies were eluted with 0.2 M glycine, pH 2.5, as described (42).

In vitro transcription and translation and immunoprecipitation

RNA was transcribed *in vitro* from linearized plasmid clone BL14 or NuMA full-length cDNA by T3 RNA polymerase and

subsequently translated in the presence of [^{35}S]methionine (Tran 35S-label; ICN Pharmaceuticals, Irvine, CA) using a rabbit reticulocyte lysate, as described in the manufacturer's instructions (Promega, Madison, WI). For immunoprecipitation 10 µl BL serum or 10 µl affinity-purified antibodies and 5 µl *in vitro* translation product were incubated with protein G–Sepharose beads for 1 h at 4°C. Subsequently the Sepharose beads were washed with buffer and resuspended in Laemmli sample buffer. Finally, precipitated proteins were analyzed by SDS–PAGE and autoradiography.

Cell labeling, cell fractionation and immunoprecipitation of hnRNP complexes

Subconfluent HeLa S3 cells in monolayer culture were metabolically labeled for 20 h with 20 µCi/ml [^{35}S]methionine (Amersham) in DMEM containing methionine at one-tenth of its usual concentration and 5% fetal calf serum. HeLa cell nucleoplasmic fractions were prepared from these cells as described previously (3) and used for immunoprecipitation of hnRNP complexes employing the monoclonal antibody 4F4 directed to the C proteins. Immunoprecipitation was carried out as described (3) except that the 4F4 ascites fluid was bound directly to protein A–agarose beads without prior purification. Two-dimensional gel electrophoresis was performed according to previously described protocols (3). For detection of ^{35}S-labeled proteins gels were fluorographed with 2,5-diphenyloxazole.

Indirect immunofluorescence microscopy

Commercial prefixed HEp-2 cell slides (Bion, Park Ridge, IL) were used in immunofluorescence studies as described (39–42). Slides were incubated with diluted BL serum (1:50 or higher) or affinity-purified antibodies. FITC-conjugated goat anti-human IgG (1:100; Caltag Laboratories, San Francisco, CA) was used as secondary detecting reagent.

Northern blot analysis

Total RNA was isolated from WI-38, HeLa S3 and HEp-2 cell lines by a single step method as described (43). Approximately 10 µg total cellular RNA was fractionated on 1% agarose gels containing 2.2 M formaldehyde and transferred to nitrocellulose. Radiolabeled probes were prepared from gel-purified BL14 cDNA and employed for hybridization as described (40).

RESULTS

Isolation of a cDNA clone encoding a novel 82 kDa autoantigen

Serum BL recognized antigens of ~210, 82, 74, 72, 43 and 40 kDa of Western blotted MOLT-4 cell nuclear extracts (Fig. 1A, lane 1). Of these antigens the 210 kDa protein was identified as a known autoantigen, since it was indistinguishable from the nuclear mitotic apparatus protein (NuMA) by immunoblotting, immunofluorescence and immunoprecipitation of *in vitro* translated products of NuMA cDNA (data not shown). Using BL serum for immunoscreening of a human T24 bladder epithelial cell expression library 53 clones were initially isolated from 6×10^5 plaques. One of these clones, called BL14, was reactive with affinity-purified antibodies to the 82 kDa antigen, but showed no reactivity with unrelated autoimmune anti-NuMA prototype sera or normal human serum (data not shown). In Western blotting affinity-purified anti-82 kDa antibodies were cross-reactive with the 72 kDa protein (Fig. 1A, lane 2), but not with any of the other antigens recognized by BL whole serum. Antibodies affinity purified from BL14 phage plaques produced a Western blotting pattern identical to that of antibodies eluted from the cellular 82 kDa antigen (Fig. 1A, lane 3). The cDNA insert of clone BL14 had a size of 2.5 kb. *In vitro* transcription and translation of the BL14 cDNA generated a protein that co-migrated with the 82 kDa antigen in SDS–PAGE. A minor translation product co-migrated with the 72 kDa antigen (Fig. 1B, lane 1). Both *in vitro* translation products were immunoprecipitated by BL serum (Fig. 1C, lane 1) as well as by antibodies affinity purified from Western blotted cellular 82 kDa antigen or BL14 phage plaques respectively (Fig. 1C, lanes 2 and 3), whereas serum from a patient with unrelated autoantibodies failed to precipitate the *in vitro* translation products (Fig. 1C, lane 4). Taken together these findings suggested that BL14 cDNA encoded the 82 kDa antigen and that the 72 kDa antigen was immunologically related to the former.

Cellular localization

In indirect immunofluorescence microscopy BL serum produced a nucleoplasmic staining sparing the nucleoli in interphase cells and a bright fluorescence of the mitotic spindles characteristic of antibodies to NuMA (Fig. 2A). Affinity-purified anti-82 kDa antibodies showed a dense fine speckled nucleoplasmic fluorescence (Fig. 2B), as did antibodies affinity purified from BL14 phage plaques (Fig. 2C), but did not produce mitotic spindle staining as observed with affinity-purified anti-NuMA antibodies (not shown).

Sequence analysis

The first ATG codon with flanking sequences in good agreement with the Kozak consensus (44) was designated the methionine initiation codon. The sequence was not previously recorded in GenBank, EMBL and SwissProt data banks and encoded a 633 amino acid polypeptide with a calculated molecular mass of 70 913 Da and an isoelectric point of 8.54 (Fig. 3A). Analysis of the deduced protein sequence suggested RNA binding properties of the antigen, since it contained several RNA binding motifs. A relatively acidic N-terminal region of ~150 amino acids (auxiliary domain I) was followed by three tandemly arranged RBDs containing the canonical conserved octameric RNP-1 and hexameric RNP-2 consensus motifs. Approximately 50 amino acids downstream of the third RBD a region of ~120 amino acids was found which contained eight RGG repeats and an additional 13 RG dimers forming another type of RNA binding domain, a RGG box of unusually large size (Fig. 3B). Interestingly, three copies of a tyrosine-rich acidic decapeptide sequence of unknown significance were interspersed between the second and fourth RGG repeats. These three peptides were found to have the consensus sequence DDYYGYDDx(x)Y, with the YYGY tetrapeptide as the most conserved motif (Fig. 3C). In the region linking the RBDs and the RGG box (auxiliary domain II) a putative nuclear localization motif (PDKKRKE) and an octapeptide (PPPRMPPP) with 75% similarity to one of the major B cell epitopes (PPPGMRPP) of the spliceosomal Sm B protein (45,46) were identified. Finally, the C-terminal part of the antigen, i.e. the region downstream of amino acid 568, was found to contain

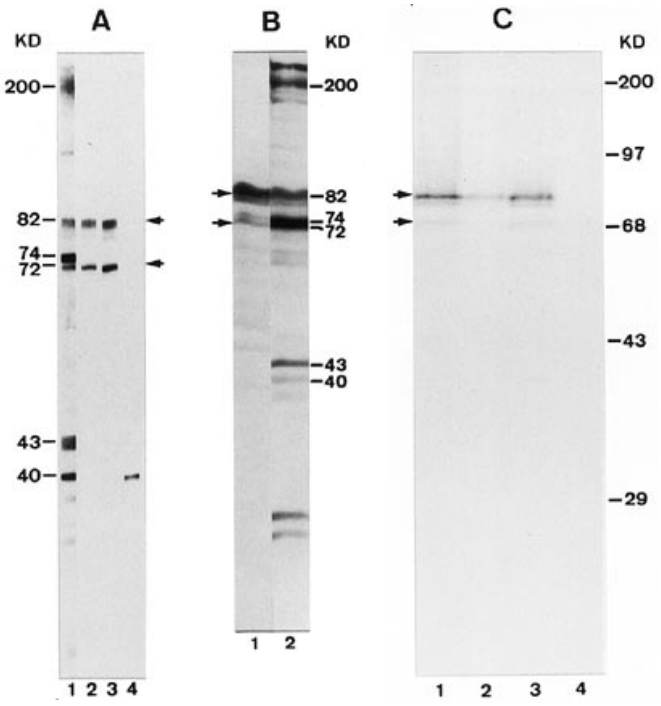

Figure 1. Electrophoretic properties and immunological reactivity of cellular and recombinant antigens. (**A**) Western blot analysis using MOLT-4 cell nuclear extract. Serum BL stained several bands between 210 and 40 kDa (indicated by arrows), of which the 210 kDa band corresponded to the known autoantigen NuMA (lane 1). Antibodies affinity purified from the 82 kDa antigen (lane 2) or from BL14 phage plaques (lane 3) recognized the 82 kDa antigen and cross-reacted with the 72 kDa antigen. Antibodies eluted from the unrelated 40 kDa antigen did not react with these peptides (lane 4). (**B**) Comparison of *in vitro* transcription and translation products of BL14 cDNA with cellular antigens. Radiolabeled *in vitro* translation products and MOLT-4 cell nuclear extract were separated by SDS–PAGE and transferred to nitrocellulose. The *in vitro* translation products, visualized by autoradiography (lane 1), showed electrophoretic properties comparable with those of the 82 and 72 kDa nuclear antigens recognized by BL serum on Western blots (lane 2). (**C**) Immunoprecipitation of BL14 cDNA *in vitro* translation products. BL serum (lane 1), antibodies affinity purified from the 82 kDa nuclear antigen (lane 2), antibodies affinity purified from BL14 phage plaques (lane 3) and normal human serum (lane 4) were used for immunoprecipitation of *in vitro* translated antigens. BL serum as well as the two affinity-purified antibodies precipitated both the 82 and 72 kDa translation products.

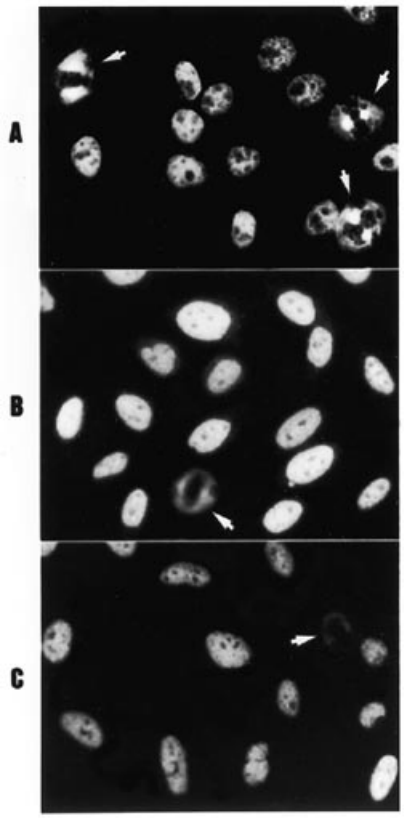

Figure 2. Indirect immunofluorescence localization on HEp-2 cells. (**A**) BL serum produced a nucleoplasmic staining sparing the nucleoli in interphase cells and a bright fluorescence of the mitotic spindles characteristic of anti-NuMA antibodies. Antibodies affinity purified from cellular 82 kDa antigen (**B**) and antibodies affinity purified from BL14 phage plaques (**C**) produced a dense fine speckled nucleoplasmic pattern but no mitotic spindle staining. Mitotic cells are indicated by arrows.

several clusters of glutamine and asparagine residues (QN-rich), which represented >30% of the amino acids in this domain.

Identification of the 82 kDa antigen as hnRNP R protein

These structural features, i.e. tandemly arranged RBDs and a RGG box, a speckled nucleoplasmic immunofluorescence pattern sparing the nucleoli, as well as the molecular weight and calculated pI of the 82 kDa antigen, suggested that this novel autoantigen might be related to components of the hnRNP complex. Both the 82 kDa protein and the immunologically related 72 kDa protein co-purified with other hnRNP proteins in heparin–Sepharose chromatography as determined by immunoblotting using BL serum, autoimmune sera to the hnRNP A/B proteins and monoclonal antibodies to hnRNP K (data not shown). Both proteins were detected with BL serum in total HeLa nucleoplasm and hnRNP complexes immunoprecipitated with monoclonal antibody 4F4 directed against hnRNP C proteins (Fig. 4A). BL14 cDNA *in vitro* translation products and hnRNP complexes immunoprecipitated from ^{35}S-labeled HeLa cells by monoclonal antibody 4F4 were separated in parallel by two-dimensional gel electrophoresis and visualized by autoradiography. As shown in Figure 4B, the migration behavior of the 82 kDa translation product was indistinguishable from that of hnRNP R. The less abundant 72 kDa *in vitro* translation product co-migrated with the more basic hnRNP P, which has a pI of ~9.5. This was confirmed by probing HeLa nucleoplasm with BL serum on a two-dimensional Western blot, where the serum stained two spots corresponding to the positions of hnRNP R and P respectively (Fig. 4C). Both the 82 and the 72 kDa antigens were detected in immunopurified hnRNP complexes by Western blotting employing BL serum (data not shown). All these findings strongly indicated that the 82 kDa antigen was identical to hnRNP R and that the 72 kDa antigen, which appeared to be derived from the R protein by proteolytic degradation, co-migrated with the group of hnRNP P proteins.

Northern analysis

Total RNA was extracted from WI-38, HeLa S3 or HEp-2 cell lines respectively and analyzed by Northern blotting using radiolabeled BL14 cDNA: only one message of ~2.5 kb was

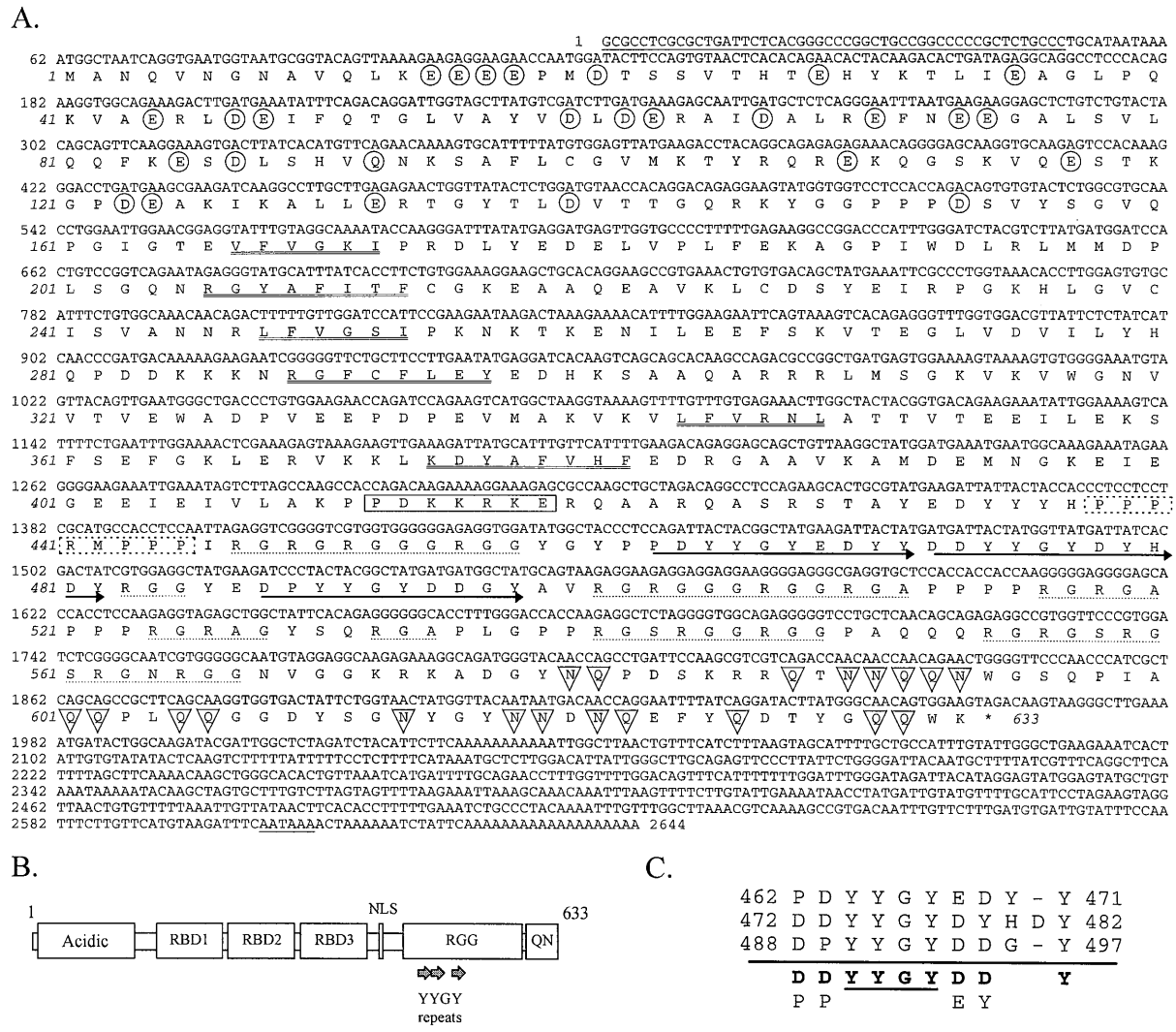

Figure 3. Nucleotide and deduced amino acid sequence of the full-length cDNA clone BL14 encoding the 82 kDa nuclear antigen (hnRNP R). (**A**) The three RBDs containing the conserved octamer RNP-1 and hexamer RNP-2 sequences are double underlined and the repetitive sequences of the RGG box in the C-terminal region are indicated by dotted lines. A nuclear localization motif (boxed) and an octapeptide (boxed with a dotted line) which differs by two amino acid residues from the major B cell epitope PPPGMRPP of the snRNP B protein are located between the third RBD and the RGG box region. Three direct copies of a tyrosine-rich decapeptide are indicated by arrowed lines within the RGG box region (see also Fig. 2C). (**B**) Schematic representation of the domain structure of hnRNP R. The three RBDs (open boxes) are located in the central part of the protein. Within the RGG box region three tyrosine-rich peptides are interspersed (arrows). The NLS motif, the N-terminal (acidic) auxiliary domain and the C-terminal QN-rich section are indicated. (**C**) Sequences of the three tyrosine-rich decapeptides identified in the RGG box region. The consensus sequence (bold face) contains the tetrapeptide YYGY as the most conserved motif.

detectable in all three cell lines investigated (Fig. 5). Detection of one mRNA with a size comparable with that of BL14 cDNA further confirmed that this cDNA encoded the full-length sequence of hnRNP R.

DISCUSSION

Evidence for the identity of the cloned antigen with hnRNP R is based on: (i) an immunofluorescence pattern compatible with the localization of other hnRNP proteins; (ii) structural features characteristic of members of the hnRNP family; (iii) avid binding to heparin–Sepharose as observed with other hnRNP proteins (34,37,47); (iv) presence of the antigens in immunopurified hnRNP complexes; (v) co-migration of the antigen with hnRNP R in two-dimensional electrophoresis. The *in vitro* production of two proteins from one cDNA can be best explained by proteolytic degradation of the larger 82 kDa protein. The smaller size and more basic isoelectric point of the 72 kDa protein would be compatible with the removal of 80–100 amino acids from the N-terminus of hnRNP R, which contains a considerable net of acidic residues. HnRNP P has been shown to comprise a group of at least three basic proteins with similar electrophoretic properties (3). Recently one of these proteins, hnRNP P2, has been shown to be identical with the gene product of the *TLS*/*FUS* gene (48), an RNA binding protein of unknown function first identified as a fusion protein in human myxoid liposarcomas (49,50). Interestingly, the 69 kDa snRNP-associated protein recently described by Hackl and Luhrmann was found to be closely related

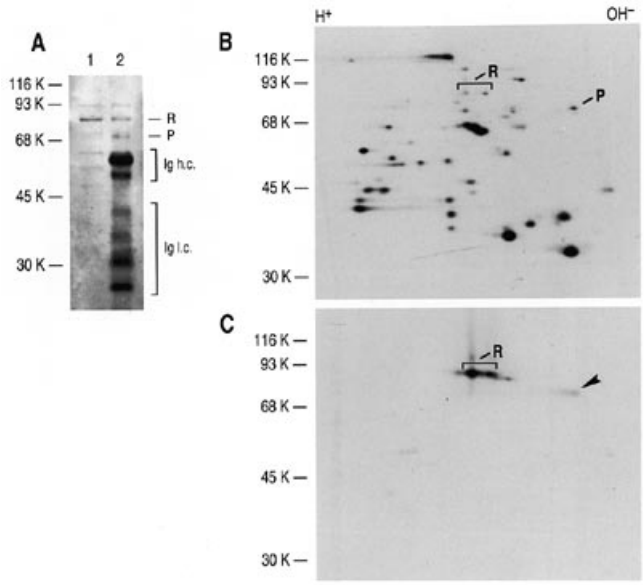

Figure 4. The 82 and 72 kDa antigens in hnRNP complexes and in two-dimensional electrophoresis. (**A**) Total HeLa nucleoplasm (lane 1) and hnRNP complexes immunoprecipitated with the monoclonal antibody 4F4 against hnRNP C proteins (lane 2) were separated by SDS–PAGE and probed with BL serum. 'Ig h.c.' and 'Ig l.c.' are unrelated cross-reactivity of secondary antiserum used for detection with the 4F4 antibody. HnRNP complexes immunoprecipitated from radiolabeled HeLa cells (**B**) and *in vitro* transcription and translation products of BL14 cDNA (**C**) were separated in parallel and visualized by autoradiography. The major 82 kDa translation product was indistinguishable from hnRNP R and the less abundant 72 kDa translation product (indicated by an arrow) co-migrated with hnRNP P.

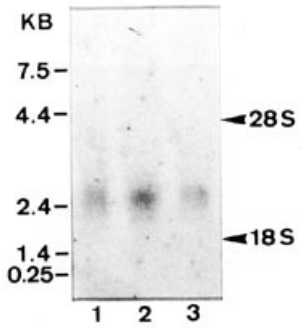

Figure 5. Expression of hnRNP R mRNA analyzed by Northern blotting. Total RNA extracted from WI-38 (lane 1), HeLa S3 (lane 2) and HEp-2 cells (lane 3) was analyzed using a radiolabeled BL14 cDNA derived probe (2.5 kb). A single transcript of ~2.5 kb was detected in all three cell lines investigated.

to TLS/FUS, with 95% sequence identity at the amino acid level (51). However, neither of these two sequences showed any significant relationship to the sequence reported by us. Nevertheless, the hnRNP P-like protein described here might correspond to one of the other P proteins, namely hnRNP P3, which is assumed to be distinct from hnRNP P2 (48). However, with no published protein sequencing data available so far this assumption remains speculative.

RGG boxes have also been found in a larger number of proteins (5,6) and was initially identified as the only RNA binding domain of hnRNP U, where it was found to be able to discriminate between different RNA sequences (7). Further studies have shown that RGG boxes often occur in proteins that also contain one or more RBDs, e.g. hnRNP A1 or the nucleolar proteins fibrillarin and nucleolin (5), suggesting a functional relationship between RGG boxes and other RNA binding domains (5,6). This has been best demonstrated for nucleolin, a pre-rRNA binding protein containing four RBDs and a RGG box, where the RBD were found to be responsible for sequence-specific RNA binding with the RGG box, increasing the overall RNA affinity (52). RGG boxes usually contain aromatic amino acids which may have an as yet unknown influence on RNA binding activity. This observation is particularly interesting with respect to the three tyrosine-rich decapeptides identified in the RGG box region of hnRNP R. A similar sequence has not been reported so far in other proteins and may represent a characteristic feature of this relatively long RGG box, which contains eight RGG repeats as well as 13 RG dimers in addition. The presence of a proline-rich octapeptide sequence (PPPRMPPP) immediately before the RGG box with 75% homology to one of the major B cell epitopes (PPPGMRPP) of the spliceosomal Sm B protein is particularly noteworthy, since similar sequences have also been found in other RNA binding proteins, such as the U1 snRNP proteins U1-A and U1-C (53,54). Further analysis of these sequence characteristics might provide new insights into mechanisms which regulate RNA binding by RGG domains.

The concomitant occurrence of autoantibody reactivities to a protein of the hnRNP complex and to NuMA, a protein associated with mitotic events, including nuclear reformation (32,55), is interesting and allows some speculation about a putative functional relationship between the two proteins. It is a well-established phenomenon that autoantigens frequently represent subunit components of multimacromolecular complexes or even subcellular organelles which are specifically targeted by linked sets of disease-associated autoantibody populations (26,28). There exists some indirect evidence that spliceosomal proteins and NuMA might temporarily be linked in one functional complex in interphase nuclei. Using antibodies to snRNP proteins or to the trimethylguanine cap of snRNAs for immunoprecipitation of nuclear extracts, a portion of NuMA was found to co-precipitate with snRNP complexes (56). Furthermore, antibodies to NuMA were able to precipitate intron-containing pre-mRNAs, assumed to be packaged by hnRNPs, together with NuMA from splicing-active nuclear extracts (56). However, so far these findings have not been confirmed by other investigators (55). When we used affinity-purified antibodies to NuMA and to hnRNP R respectively no co-precipitation of the two antigens was seen and co-localization could not be observed by immunofluorescence. Thus, at least under our experimental conditions, we could not find evidence for a direct molecular association of hnRNP R and NuMA. Finally, one must bear in mind that autoreactivities to spliceosomal antigens are usually not found in sera containing anti-NuMA autoantibodies (31). Nevertheless, a participation of hnRNP R in biological processes associated with NuMA and vice versa cannot be excluded, since such an association might be formed transiently to perform a specific function and only a subpopulation of proteins might be involved. The availability of the cDNA for hnRNP-R should facilitate further studies on this abundant nuclear protein.

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