Recognition of subsets of the mammalian A/B-type core heterogeneous nuclear ribonucleoprotein polypeptides by novel autoantibodies

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The structurally related A/B-type core heterogeneous nuclear ribonucleoprotein (hnRNP) polypeptides of 34–39 kDa (A1, A2, B1 and B2) belong to a family of RNA-binding proteins that are major components of 40 S hnRNP complexes. By two-dimensional gel electrophoresis and peptide mapping analysis we compared each member of the A/B-type core proteins in the human and rat liver cells. This comparison revealed the unique presence in rat cells of major protein species, referred to as mBx polypeptides, that appeared as three charge isoforms at a position corresponding to the minor HeLa B1b protein spot. In addition, clear differences in the ratios of the A1 polypeptide to the A1b isoform were observed. The detection, in sera of patients with rheumatic autoimmune diseases, of two novel autoantibody specificities, one recognizing solely B2 protein and the second both the B2 and mBx polypeptides, helped to identify mBx proteins as new A/B-type hnRNP components, immunologically related to B2 protein. A common immunoreactive V8 protease peptide of approx. 17 kDa has been identified in B2 and mBx hnRNP polypeptides. mBx protein species are identified in cells of murine origin, and have a ubiquitous tissue distribution and developmental appearance.

INTRODUCTION

Nascent RNA polymerase II transcripts [heterogeneous nuclear RNA (hnRNA) or pre-mRNA] associate with a specific set of proteins to form the heterogeneous nuclear ribonucleoprotein complexes (hnRNP). These are very abundant dynamic structures, generally considered to represent the major sites of pre-mRNA maturation in the cell nucleus. hnRNP complexes have been obtained from the nuclear extracts of animal cells by biochemical fractionation (reviewed in [1]) or by immunopurification with the use of antibodies directed against single hnRNP protein components [2,3]. Purified hnRNP complexes have been extensively analysed and found to contain a large number of stably associated polypeptides ranging in size from 34 to 120 kDa, designated as A1 to U hnRNP polypeptides [3]. Cloning and subsequent analysis of many hnRNP polypeptides has unravelled important structural and functional properties and has pointed to a greater complexity of these proteins than was previously believed (reviewed in [4]).

The biochemical fractionation of hnRNP complexes in the absence of nuclease inhibitors generally leads to an extensive accumulation of 40 S hnRNP monoparticles [5]. The bulk of the 40 S hnRNP proteins consists of RNA-binding components in the range 34–43 kDa, which are referred to as the core hnRNP polypeptides. These are the three well-established pairs of proteins: A1/A2, B1/B2 and C1/C2, each of which is resolved by two-dimensional (2-D) gel electrophoresis into a large number of charged isoforms [6–10]. With the notable exception of B2, the cloning and sequencing of all other core hnRNP polypeptides has been accomplished. On the basis of their general properties, they are classified either into the C-type or into the A/B-type hnRNP polypeptides. The acidic, highly phosphorylated C1 and C2 proteins are most probably products of the same alternatively spliced pre-mRNA, C2 differing from C1 by a single insertion of 13 amino acid residues [11,12].

The A/B-type hnRNP polypeptides (A1, A2, B1 and B2) are immunologically related, basic protein components containing multiple charged isoforms, as well as dimethylarginine modifications (reviewed in [4]). They are characterized by a common structural motif consisting of two tandemly repeated RNA-binding domains (RBDs), also called RNP motifs, and a glycine-rich auxiliary domain (glyRD) at the C-terminus, thus referred to as $2\times$RBD-gly proteins [13,14]. Like the C1 and C2 hnRNP proteins, the A2 and B1 polypeptides are identical except for a single 12-residue insert near the N-terminus of B1 [13]. Variant forms of A1 protein (A1x, A1b) have also been identified, A1b containing a 50-residue insert in the glycine-rich domain [14]. Low- and high-affinity binding sites for pre-mRNA as well as transcript-specific binding of the core hnRNP polypeptides have been found. This seems to be an intrinsic property of each protein and of its variant forms [4,15]. The role of the A/B-type hnRNP polypeptides has recently been established in constitutive [16] as well as in alternative splicing [17]. In addition to the well-characterized A1, A1b, A2 and B1, structurally similar novel A/B-type polypeptides have been identified, in both vertebrate [18–22] and invertebrate [23,24] species. Their function in the pre-mRNA maturation process remains to be established.

Despite the well-established overall structural and immuno-

Abbreviations used: CHO, Chinese hamster ovary; 2-D, two-dimensional; hnRNP, heterogeneous nuclear ribonucleoprotein; HTC, rat hepatoma tissue culture cells; NEPHGE, non-equilibrium pH gradient electrophoresis; RBD, RNA-binding domain; RV, rat vein; snRNP, small nuclear ribonucleoprotein.

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logical similarity of the A/B-type hnRNP polypeptides in vertebrates and particularly in mammals (reviewed in [4]), specific differences, both quantitative and qualitative, have been reported [5,10,25,26]. With the notable exception of a recent report [27], a comprehensive survey of the individual A/B-type proteins originating from several mammalian species or from different cell types of the same organism is still awaited. In the present study an extensive analysis of the A/B-type family members in rat and human cell extracts was undertaken. This has been greatly aided by the identification of two novel autoantibody reactivities for specific members of the A/B-type polypeptides. Here we describe the specificity of these autoantibodies, one recognizing only B2 out of all A/B-type proteins and the second directed against epitope(s) shared by B2 and a very abundant murine A/B-type protein species, which we have named mBx polypeptides.

MATERIALS AND METHODS

Preparation of nuclear extracts

The protocol of Choi and Dreyfuss [2] was applied to obtain nuclear extracts from the established cell lines used in the present study; the human (HeLa) and Chinese hamster ovary (CHO) cells, as well as two rat cell lines, a rat vein (RV) and a hepatoma cell line (HTC).

Nuclear extracts corresponding to liver tissue of rat, mouse, rabbit and guinea pig were obtained from sucrose-purified nuclei as previously described [28]. For the rat brain and spleen, nuclear extracts were instead from unpurified nuclei to avoid nuclear damage.

Rat liver 40 S hnRNP complexes were obtained after fractionation of nuclear extracts on 15–30 % (w/v) sucrose gradients and subsequent pelleting of the material sedimenting in the 40–50 S region of the gradient, as described [28].

Electrophoresis

SDS/PAGE was performed by the method of Laemmli [29]; 2-D gel electrophoresis was performed as described [30]. The first dimension was NEPHGE, and the second was SDS/PAGE [10 % (w/v) gel]. Proteins were revealed by Coomassie Brilliant Blue.

Peptide mapping

The method of Cleveland et al. [31] was applied to hnRNP proteins in gel slices obtained from 2-D gels, as described [9]. Partial digestion of the proteins was with either trypsin (Sigma) or *Staphylococcus aureus* V8 protease (Sigma). The optimum concentration of the enzyme needed for limited digestion was first determined experimentally. Detection of the peptide pattern by silver staining was as previously [9].

Source of sera and antibodies

The autoimmune sera were from patients of the rheumatic diseases sections of the Medical School of Ioannina and Athens Universities, Greece. The mouse monoclonal 9H10 antibody reacting with A1 and A1-related polypeptides was a gift from Professor G. Dreyfuss (Philadelphia).

Immunoblotting

Proteins separated by 2-D gels, as well as peptides resolved on SDS/PAGE, were blotted on nitrocellulose. The immunodetection of the antigenic protein species on the blot was as previously described [32]. The autoimmune sera were used at a dilution of 1:100 and the mAb 9H10 was used at 1:1000 dilution.

RESULTS

Comparison of the A/B-type core polypeptides in human and rat

The 2-D NEPHGE–SDS/PAGE was applied as the method for a highly sensitive and reproducible analysis of hnRNP polypeptides (reviewed in [1]).

A representative picture of such 2-D gels, which agrees with published reports for HeLa cells [8,10] and for rat cell extracts [7,9], is shown in Figure 1(A). The assignment of the A/B-type core polypeptides was based on their relative positions on the 2-D electrophoretograms. The rat proteins have been designated here with the prefix 'm' to indicate their murine origin. In addition, the availability of the monoclonal 9H10 antibody, which has the same specificity as the published 4B10 antibody and recognizes the A1 polypeptide as well as its A1x and A1b variants [3], has permitted the uniquivocal identification of all A1-related polypeptides.

As is shown, both quantitative and qualitative differences were apparent between HeLa and rat core hnRNP polypeptides. To facilitate a direct comparison and also to take into account differences in the amount of total protein applied to the gel, we considered the A2 polypeptide and its charge isoforms as the

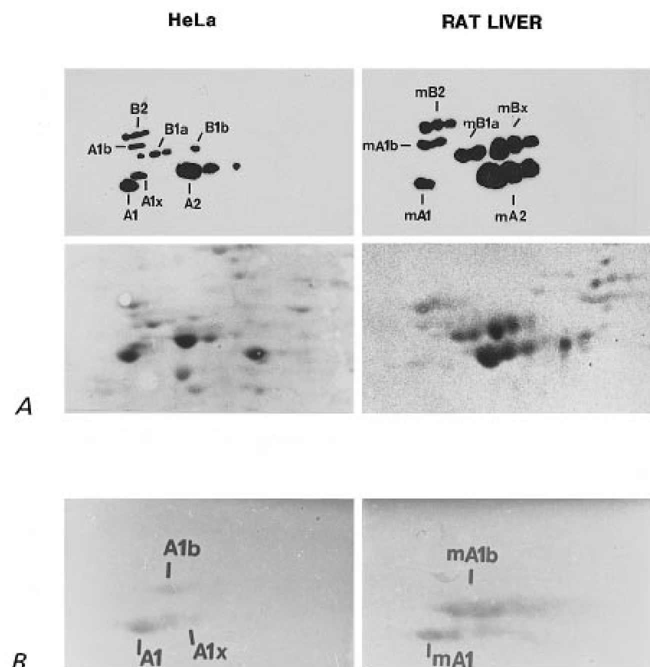

Figure 1 Two-dimensional gel analysis (NEPHGE–SDS/PAGE) of A/B-type core hnRNP polypeptides from either HeLa or rat liver cells

Polypeptides recovered from HeLa nuclear extracts and from rat liver 40 S hnRNP complexes were resolved on 2-D gels. (**A**) Coomassie Blue-stained gels. Only the portion of the gels containing the A/B-type core proteins is shown. The individual core polypeptides were identified by their relative positions on the 2-D gel and named as in Wilk et al. [8]. In the schematic diagram shown in the top two panels the A/B-type proteins were reproduced and labelled. The corresponding rat liver polypeptide species are designated by the prefix 'm'. The abundant protein spots seen only in rat liver are labelled mBx protein components. (**B**) Western blotting of two gels similar to those in (**A**) were probed with the monoclonal antibody 9H10 to identify the A1 protein species.

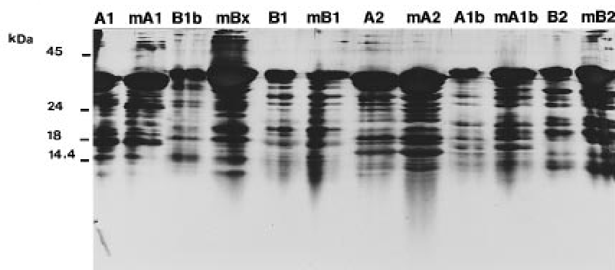

Figure 2 Comparison of the tryptic peptide maps of the HeLa and rat liver A/B-type core hnRNP polypeptides on a silver-stained SDS/15% polyacrylamide gel

After their resolution on 2-D gels, as in Figure 1(A), the individual A/B-type proteins were subjected to partial tryptic digestion as described by Cleveland et al. [31]. The positions of protein molecular mass markers are indicated at the left.

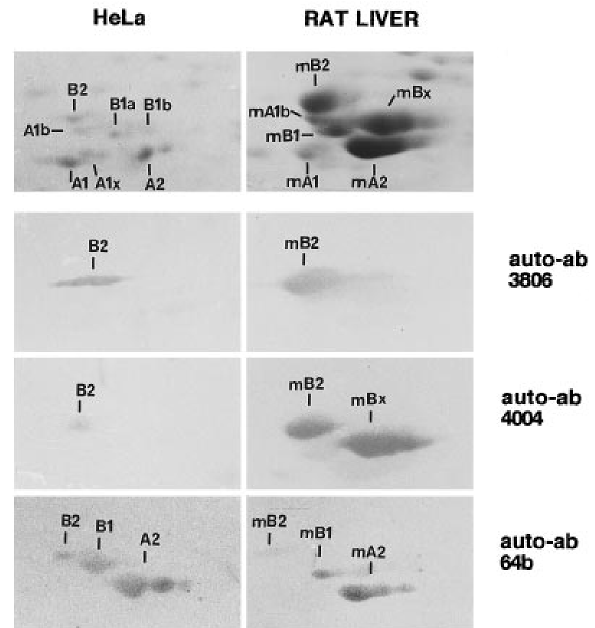

Figure 3 Identification of specific subsets of HeLa and rat liver A/B-type core hnRNP polypeptides by three different autoantibody specificities

A/B-type polypeptides of HeLa and rat liver origin were resolved on 2-D gels and transferred to nitrocellulose filters. One set of the filters was stained with Amido Black (top pair of panels), and three other sets were blotted with autoimmune sera 3806, 4004 and 64b as indicated. Autoimmune sera 3806 and 4004 contained novel autoantibody specificities, whereas the specificity of the autoantibodies in serum 64b has been previously reported [32].

major A/B-type core protein components and used them as an internal reference. Two major differences in the A/B-type hnRNP polypeptides of HeLa and rat origin became apparent. The first concerned a quantitative difference seen in A1 protein, the amount of which was roughly equal to the A2 polypeptide in HeLa cells, whereas in rat A1 appeared in much smaller amounts than A2. The almost complete absence of A1 protein from 40 S hnRNP complexes of rat liver cells has been previously noted [5]. This absence could not be attributed to a preferential loss of the A1 polypeptide from 40 S hnRNP complexes, because no free A1 protein could be detected immunologically in the fractionated nuclear extracts (results not shown). With respect to the relative amounts of A1 variants, the ratio of A1b to A1 was found to be higher in rat liver nuclear extracts than in HeLa cells (Figure 1B). A second major difference concerned the finding in rat of abundant protein species that HeLa cells seemed to lack. This refers to the three major spots at the position corresponding to the minor HeLa B1b protein, just above A2 polypeptide and in comparable amounts to the latter. In fact these protein species, henceforth referred to as mBx polypeptides, together with mA2 constitute the bulk of hnRNP polypeptides in rat liver 40 S monomeric complexes.

In our initial study [9] we examined every protein spot belonging to the core hnRNP polypeptides of rat liver cells by comparing the peptide maps obtained by partial digestion with trypsin. This comparison revealed that the mBx protein species were distinct from all other core polypeptides, including the mB1 species of similar apparent molecular size to mBx. In the present work we have extended this peptide mapping analysis by comparing, in pairs, individual A/B-type core proteins of rat and HeLa origin. As shown in Figure 2, with the notable exception of B1b/mBx every other pair of proteins gave a strikingly similar peptide pattern. mBx polypeptides were the only rat protein species without a counterpart in the HeLa A/B-type core proteins, with a peptide map distinct from that of the human B1b protein.

The verification of the mBx protein species as A/B-type hnRNP polypeptides and the establishment of the degree of similarity that they share with the other members of the family require a complete elucidation of their primary structures. To this end, the major spot of mBx polypeptide has been electro-eluted from 2-D gels on to poly(vinylidene difluoride) membrane and subjected to cleavage *in situ* by trypsin, followed by HPLC of the resulting peptides. The preliminary results we have so far obtained concern the amino acid sequence of two internal peptides. Peptide no. 1, GXPYGGGYGSXGGSGGS (X is an undefined amino acid), showed 70% similarity with the glycine-rich auxiliary domain of human A2 polypeptide; peptide no. 2, VDGRVVEPK, showed 100% similarity with the RBDI domain of human A1 polypeptide [13].

Recognition of B2 and mBx hnRNP proteins by novel autoantibodies

We have previously reported our findings of an autoantibody recognizing the subgroup of A2, B1 and B2 core proteins among 40 S hnRNP polypeptides [32]. This autoantibody, labelled auto-ab 64b, was identified in the serum of a patient with systemic lupus erythematosus and Sjogren's syndrome. By extending our screening protocol to include a larger number of sera from patients with systemic rheumatic diseases, we have identified two additional autoantibody specificities for core hnRNP polypeptides. In Figure 3 the reactivity of these novel autoantibodies towards members of the A/B-type core polypeptides is shown for both HeLa and rat liver cells and compared with that of the 64b serum. The first specificity refers to auto-ab 3806, which was identified in the serum of a patient with Sjogren's syndrome and recognized solely B2 protein among all core proteins of both HeLa as well as rat origin. The second specificity was that of autoantibodies in serum 4004 from a patient with systemic lupus erythematosus, which also recognized the B2 polypeptide in both cellular systems but in addition reacted strongly with the rat mBx polypeptides. As was seen by counter-immunoelectrophoresis

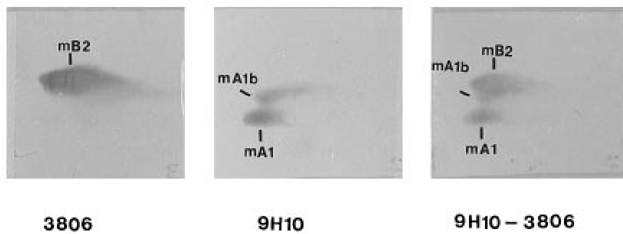

Figure 4 Identification of the mB2 and the mA1b variant of A1 as two discrete hnRNP polypeptides

Rat liver A/B-type core polypeptides were resolved on 2-D gels and transferred to nitrocellulose filters. One filter was blotted with the autoimmune serum 3806; a second was first incubated with the monoclonal antibody 9H10 and subsequently with the autoimmune serum 3806 (9H10–3806).

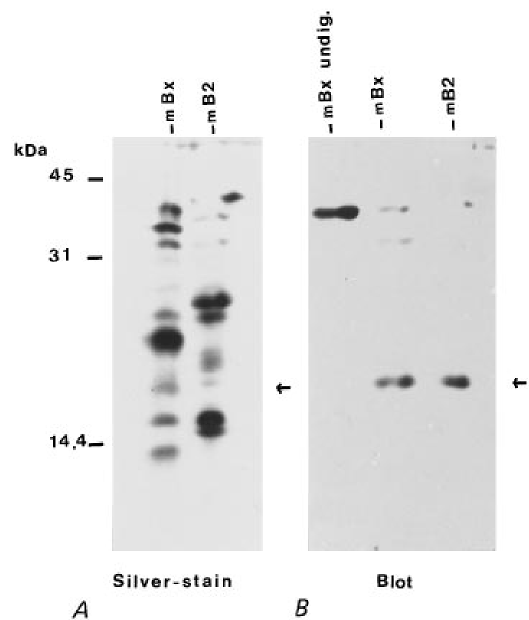

Figure 5 Identification of a V8 protease peptide common to Bx and B2 hnRNP polypeptides that contains the autoepitope recognized by the autoimmune serum 4004

Rat liver Bx and B2 proteins were excised from a 2-D gel and subjected to partial protease V8 digestion as described by Cleveland et al. [31]. (**A**) Silver-stained SDS/15% polyacrylamide gel. (**B**) Western blotting of a similar gel, containing in addition undigested Bx protein (Bx undig.), using the serum 4004. The arrows indicate the autoreactive peptide. The positions of protein molecular mass markers are shown at the left.

(results not shown), sera 3806 and 4004 contained additional autoantibodies directed against the well established La (SSB) and Ro (SSA) antigen complexes [32a] respectively. That both mB2 and mBx polypeptides of the rat were recognized by the same autoantibody population in serum 4004 was shown by the cross-reactivity exhibited by the affinity-purified antibodies that bound to both polypeptides on a 2-D gel (results not shown). It was clear from these findings that auto-ab 4004 recognized an autoepitope shared by the rat mB2 and mBx polypeptides, whereas autoantibodies in serum 3806 were directed against a different epitope unique to the B2 polypeptide of rat and HeLa cells. Thus the rat mB2 and mBx polypeptides were immunologically related core hnRNP proteins. Moreover the finding that 4004 antibodies recognized B2 polypeptide alone among the HeLa A/B-type hnRNP proteins supported our findings above regarding the absence of a HeLa mBx homologue (Figures 1 and 2).

Because the amino acid sequence of the B2 hnRNP protein has so far not been resolved and the protein is known to migrate on a 2-D gel very close to the A1b variant, it has been questioned in the literature to what extent the two polypeptides might be similar [14]. The availability of the 3806 autoantibodies recognizing B2 protein, as well as of the monoclonal 9H10 antibodies reacting with A1, A1x and A1b species [3], allowed us to address this issue. As seen in Figure 4, taking a Western blot from a 2-D gel of rat core hnRNP polypeptides and sequentially incubating it with auto-ab 3806 and mab 9H10 showed that B2 and A1b polypeptides belonged to immunologically distinct protein components. These findings further supported the biochemical data obtained by the peptide map analysis shown in Figure 2.

To investigate further the nature of the autoepitopes recognized by the two novel autoantibodies 4004 and 3806, we combined peptide mapping analysis with immunoblotting assays. Peptide maps of rat mBx and mB2 polypeptides were obtained after digestion with either trypsin or V8 protease. With the notable exception of auto-Ab 4004 on V8 protease digests, all other cases failed to show preservation of the autoepitope involved. As seen in Figure 5(B), autoantibodies in serum 4004 were indeed able to recognize a V8 protease peptide of approx. 17 kDa, common to both V8 protease digests. If digestion was allowed to proceed to completion an even smaller immunoreactive peptide fragment of approx. 11 kDa was obtained from mBx protein (results not shown). These results indicated the presence of a rather restricted autoepitope recognized by auto-ab 4004 that was shared by mB2 and mBx proteins. It is interesting to note in this context that the immunoreactive 17 kDa peptide was one of the few peptides of similar molecular size in the otherwise distinct V8 protease peptide pattern of mBx and mB2 proteins shown in Figure 5(A).

Identification of the mBx protein species in cells of murine origin

The absence of the mBx protein species in HeLa cells was not restricted to one particular human cell type because they could not be similarly detected in HEp-2 cells, an established human carcinoma of larynx cell line (results not shown). To investigate the extent to which these protein species could be identified in mammalian cell types other than rat, nuclear extracts were prepared from additional cellular sources including CHO cells and liver cells from mouse, guinea pig or rabbit. After sucrose gradient fractionation of nuclear extracts, 40 S hnRNP complexes were resolved on 2-D gels and the A/B-type core proteins were identified after Coomassie Blue staining of the gel. Whenever required, the verification of the presence of mBx-type proteins was accomplished by Western blotting of the proteins with auto-ab 4004. As seen in Figure 6, mBx protein species, although easily identified in the rat and mouse cell extracts, could not be detected in the rabbit liver cells. The mBx protein was found in guinea pig and in CHO cells in addition to rat and mouse cell extracts. Because mBx-type proteins were identified only in nuclear extracts of murine origin (rat, mouse, guinea pig and CHO cells), we concluded that their presence was limited to specific mammalian types.

The finding of mBx protein species in CHO cells suggested that their presence might not be restricted to liver cells of murine origin. This was shown to be the case by the identification of mBx in nuclear extracts of rat spleen and brain (results not shown). This type of analysis was extended to include two

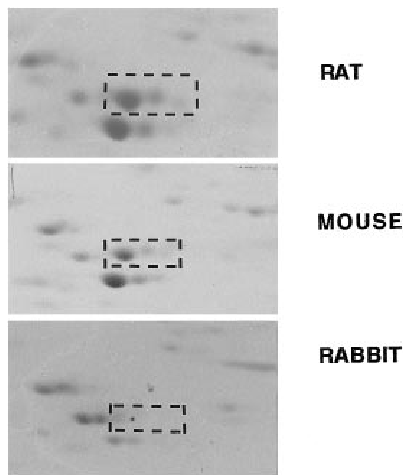

Figure 6 Restriction of the mBx polypeptides in cells of murine origin

The A/B-type core hnRNP polypeptides from rat, mouse or rabbit liver nuclear extracts were resolved on 2-D gels and stained with Coomassie Blue. The rectangles specify the position of migration of the mBx protein species.

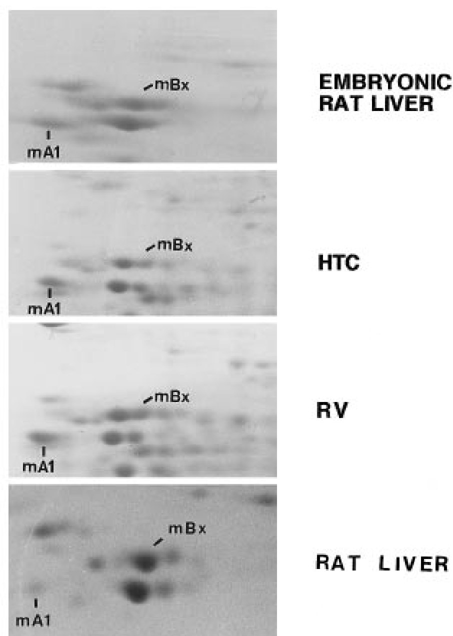

Figure 7 Detection of the mBx and mA1 protein levels in nuclear extracts of different rat cellular sources

The A/B-type core hnRNP polypeptides from embryonic or adult rat liver cells, as well as from two established cell lines of rat origin, an RV and an HTC cell line, were resolved on 2-D gels and identified after Coomassie Blue staining.

established cell lines of rat origin: the RV and HTC cell lines. In addition, the A/B-type core polypeptides were examined in embryonic as well as in adult rat liver nuclear extracts. From the results presented in Figure 7, the clear identification of mBx protein species in all extracts of rat origin is apparent. This comparison also showed that the ratio of mBx to mA2 polypeptides was roughly equal in all extracts tested. Thus the relative amount of the mBx protein did not seem to correlate with either the proliferation rate (rapidly growing RV and HTC cells compared with adult liver cells) or to the developmental stage of the cells (embryonic compared with adult liver). This was in contrast with the established behaviour of A1 polypeptide ([26], and references therein). As pointed out above (see Figure 1), the ratio of A1b to A1 isoforms was increased in rat liver compared with HeLa cells. This ratio decreased in embryonic rat liver, RV and HTC cells when compared with adult liver (Figure 7). This was also apparent from the fact that, with the notable exception of the adult rat liver, the A1 polypeptide appeared in all other rat cellular extracts in amounts comparable to the A2 protein, as occurred in HeLa cells.

DISCUSSION

In the present study the major cluster of A/B-type core hnRNP polypeptides (A1, A2, B1 and B2) from different mammalian cellular sources has been analysed by the high resolving power of 2-D gel electrophoresis, the peptide mapping analysis of individual protein species and the application of novel autoantibodies recognizing specific subsets of the core hnRNP polypeptides. This analysis has led to the identification in murine cells of major new A/B-type hnRNP polypeptide species, named mBx.

The classification of mBx protein species as members of the A/B-type hnRNP polypeptides was based on the following criteria: stable association with 40 S hnRNP complexes, the basic nature and molecular size of the proteins, as well as recognition by autoantibodies reacting with one other member of the A/B-type proteins, namely the B2 polypeptide. In addition, preliminary results based on a restricted amino acid sequence analysis of two small internal peptides revealed a high glycine content, as well as significant similarity of mBx to A1 and A2 polypeptides.

The finding of the three distinct autoantibody activities, each recognizing a specific subset of the A/B-type hnRNP polypeptides, has permitted an extensive immunochemical comparison of these proteins. The description of the auto-ab 64b with specificity for A2, B1 and B2 hnRNP polypeptides of both rat and HeLa origin was one of the first reports on the existence of autoantibodies against protein components of hnRNP complexes in the sera of patients with autoimmune rheumatic diseases [32]. The two additional autoantibodies described in the present study, auto-ab 3806 and 4004, refer to novel specificities for either B2 or B2 and mBx hnRNP polypeptides respectively. Moreover the finding that auto-ab 4004 identified solely the B2 hnRNP polypeptide in HeLa cells (in contrast with rat cells), considered together with the results of peptide mapping analysis (Figure 2), showed that HeLa cells lack a homologue for the major mBx protein species in rat. The availability of autoantibodies reacting only with B2 has also allowed us to prove the non-identity of the B2 and A1b variants (Figure 4), therefore establishing B2 as a distinct A/B-type core polypeptide with an as yet unknown sequence.

A number of polyclonal as well as powerful monoclonal antibodies recognizing different subsets of the A/B-type hnRNP polypeptides of mammals have been produced experimentally [3,25,33,34].There are also a few reports on autoantibodies recognizing A/B-type hnRNP polypeptides found in sera of patients with autoimmune rheumatic diseases. This seems to be a rather rare event compared with the frequent appearance of autoantibodies for the spliceosomal snRNP complexes (reviewed recently in [32a]). In addition to our findings ([32], and the present

study), autoantibodies against the A1 protein [36,37], as well as antibodies found with high frequency in the RA33 sera of rheumatoid arthritis patients that react mainly with A2 polypeptide and weakly with proteins corresponding in size to B1 and B2 hnRNP polypeptides [38], have been reported. The specificity of the RA33 sera for A2, B1 and B2 hnRNP polypeptides seems to correspond to that of serum 64b described by us. Serum 64b did not react with the rat mBx protein species, which were so far recognized solely by auto-ab 4004 with specificity for both the B2 and mBx hnRNP polypeptides. Therefore the rat mBx protein species seem to be immunologically distinct from A1 and A2/B1 proteins and are only partly related to the B2 hnRNP polypeptide.

As shown in Figure 5, we were able to restrict the autoepitope recognized by the novel anti-B2/mBx autoantibodies in serum 4004 to a peptide fragment of approx. 17 kDa, shared by both B2 and mBx proteins. Taking into consideration the finding that the overall peptide map of the two proteins was distinct (Figure 5a), it is very likely that the immunoreactive 17 kDa peptide represents a domain of extensive amino acid sequence similarity between the B2 and the mBx polypeptides. It should be stressed that B2 protein is as yet the only major A/B-type hnRNP polypeptide in human cells for which the amino acid sequence has not been determined. This is also true of the presently identified rat mBx proteins. Because the 17 kDa peptide contains an autoepitope restricted to B2 and mBx polypeptides, it is reasonable to assume that this peptide is partly responsible for differences in the primary structure between the above proteins and the related A1 and A2/B1 core polypeptides. Therefore identifying the amino acid sequence of the autoepitope might provide grounds not only for studying the nature of the autoepitope involved but also for the design of suitable probes to isolate cDNA clones coding for B2 and mBx polypeptides.

Among the mammalian cell types we have examined, the presence of the mBx polypeptide species seemed to be restricted to cells of murine origin (rat, mouse, guinea pig and hamster). It is interesting to note in this context that mBx represented major protein components of the rat 40 S hnRNP complexes, found in amounts comparable to A2 (Figure 1). Many research groups, including our own, have in the past presented 2-D gels of the proteins recovered within 40 S hnRNP complexes from different mammalian cells [7–10,39,40]. Nevertheless, despite the early understanding that overall differences in the pattern of core hnRNP polypeptides among mammals did exist [6], it has only very recently been realized, since the direct comparison on 2-D gels of the rat and HeLa A/B-type hnRNP polypeptides, that the mBx protein species represented distinct rat A/B-type hnRNP polypeptides. We have presented here such a comparison, as also has recent work done in the course of investigating factors affecting phosphorylation of hnRNP proteins [41]. That report noted the presence in rat liver extracts of a major protein species (named p38), absent from HeLa cells, that by all the evidence provided seems to correspond to our mBx polypeptide.

In addition to the mBx polypeptide species as new major A/B-type proteins in murine cells, the identification of gene products corresponding to other, minor A/B-type proteins from human cells has been recently reported [18,19,21]. Reference should also be made to the finding in *Xenopus* cells of a new gene coding for an A/B-type protein, named hnRNP A3, which is related to both A1 and A2/B1 genes [20]. It remains to be established whether one of the newly identified genes corresponds to the B2 hnRNP core polypeptide or whether the hnRNP A3 gene represents the *Xenopus* homologue of either the B2 or the mBx genes in mammals.

The application in our study of the two new autoantibodies for B2 and B2/mBx proteins in combination with the monoclonal 9H10 antibody with known specificity for A1 polypeptide and its variant forms [3] has permitted an estimate to be made of the relative amounts of the A/B-type proteins in HeLa and in rat nuclear extracts. The well-established fluctuation in the relative content of A1 protein [10,26,42,43] and in the ratio of A1/A1b isoforms from human cells [14,44], which is dependent on the growth rate of the cells and their differentiation stage, has been reproduced in rat cells. Relative to the A1b variant, A1 polypeptide was indeed reduced in the adult compared with the embryonic rat liver and the cell lines of rat origin. In contrast with the A1 polypeptide, the relative amounts of the murine-specific mBx protein species were more or less constant. Moreover the presence and relative concentrations of mBx polypeptides were neither tissue-specific nor related to the cell proliferation rate, because they were found in rat cell lines of non-liver origin as well as in embryonic rat liver. It therefore seems that mBx polypeptides do not exhibit cell type-specific patterns of expression shown recently in mouse cells *in vivo* for some major hnRNP polypeptides [27].

We have already pointed out the fact that major differences were observed in the bulk of A/B-type proteins recovered in the 40 S hnRNP complexes from HeLa and rat cells. As both we and others have shown ([41], and the present study), A1 and A2 constitute the most abundant proteins in HeLa cells, in contrast to the A2 and mBx A/B-type polypeptides in rat. All these observations refer to a family of structurally conserved protein species, with an expected generalized as well as specialized role in pre-mRNA maturation (reviewed in [4]), and raise the issue of the biological significance underlying such major differences seen within related mammalian systems.

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REFERENCES

1 Comings, D. E. and Peters, K. E. (1981) in The Cell Nucleus, Nuclear Particles, part B (Busch, H., ed.), vol. 9, pp. 89–118, Academic Press, New York

2 Choi, Y. D. and Dreyfuss, G. (1984) Proc. Natl. Acad. Sci. U.S.A. **81**, 7471–7475

3 Pinol-Roma, S., Choi, Y. D., Matunis, M. J. and Dreyfuss, G. (1988) Genes Dev. **2**, 215–227

4 Dreyfuss, G., Matunis, M. J., Pinol-Roma, S. and Burd, C. C. (1993) Annu. Rev. Biochem. **52**, 289–321

5 LeStourgeon, W. M., Lothstein, L., Walker, B. W. and Beyer, A. L. (1981) in The Cell Nucleus, Nuclear Particles, part B (Busch, H., ed.), vol. 9, pp. 49–87, Academic Press, New York

6 Beyer, A. L., Christensen, M. E., Walker, B. W. and LeStourgeon, W. M. (1977) Cell **11**, 127–138

7 Peters, K. E. and Comings, D. E. (1980) J. Cell Biol. **86**, 135–155

8 Wilk, H.-E., Werr, H., Friedrich, D., Kiltz, H. H. and Schafer, K. P. (1985) Eur. J. Biochem. **146**, 71–81

9 Guialis, A., Jockers-Vretou, E. and Sekeris, C. E. (1986) Arch. Biochem. Biophys. **247**, 355–364

10 Celis, J. E., Bravo, R., Arenstorf, H. P. and LeStourgeon, W. M. (1986) FEBS Lett. **194**, 101–109

11 Nakagawa, T. Y., Swanson, M. S., Wold, B. J. and Dreyfuss, G. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 2007–2011

12 Merrill, B. M., Barnett, S. F., LeStourgeon, W. M. and Williams, K. R. (1989) Nucleic Acids Res. **17**, 8441–8449

13 Burd, C. G., Swanson, M. S., Gorlach, M., Dreyfuss, G. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 9788–9792

14 Buvoli, M., Cobianchi, F., Bestagno, M. G., Mangiarotti, A., Bassi, M. T., Biamonti, G. and Riva, S. (1990) EMBO J. **9**, 1229–1235

15 Bennett, M., Pinol-Roma, S., Staknis, D., Dreyfuss, G. and Reed, R. (1992) Mol. Cell. Biol. **12**, 3165–3175

16 Sierakowska, H., Szer, W., Furdon, P. J. and Kole, R. (1986) Nucleic Acids Res. **14**, 5241–5254

17 Mayeda, A., Munroe, S. H., Caceres, J. F. and Krainer, A. R. (1994) EMBO J. **13**, 5483–5495

18 Khan, F. A., Jaiswal, A. K. and Szer, W. (1991) FEBS Lett. **290**, 159–161

19 Takiguchi, S., Tokino, T., Imai, T., Tanigami, A., Koyama, K. and Nakamura, Y. (1993) Cytogenet. Cell Genet. **64**, 128–130

20 Good, P. J., Rebbert, M. L. and Dawid, I. B. (1993) Nucl. Acids Res. **21**, 999–1006

21 Myer, V. E. and Steitz, J. A. (1995) RNA **1**, 171–182

22 Kajita, Y., Nakayama, J.-I., Aizawa, M. and Ishikawa, F. (1995) J. Biol. Chem. **270**, 22167–22175

23 Matunis, E. L., Matunis, M. J. and Dreyfuss, G. (1992) J. Cell Biol. **116**, 245–255

24 Pype, S., Slegers, H., Moens, L., Merlevede, W. and Goris, J. (1994) J. Biol. Chem. **269**, 31457–31465

25 Leser, G. P., Escara-Wilke, J. and Martin, T. E. (1984) J. Biol. Chem. **259**, 1827–1833

26 Minoo, P., Sullivan, W., Solomon, L. R., Martin, T. E. and Toft, D. O. (1989) J. Cell Biol. **109**, 1937–1946

27 Kamma, H., Portman, D. S. and Dreyfuss, G. (1995) Exp. Cell. Res. **221**, 187–196

28 Guialis, A., Moraitou, M., Patrinou-Georgoula, M. and Dangli, A. (1981) Nucleic Acids Res. **19**, 287–296

29 Laemmli, U. K. (1970) Nature (London) **227**, 680–685

30 O'Farell, P. Z., Goodman, H. M. and O'Farell, P. H. (1977) Cell **12**, 6223–6236

31 Cleveland, D. W., Fischer, S. G., Kirscher, M. W. and Laemmli, U. K. (1977) J. Biol. Chem. **252**, 1102–1106

32 Dangli, A., Guialis, A., Vretou, E. and Sekeris, C. E. (1988) FEBS Lett. **231**, 118–124

32a Van Venrooij, W. J. (1994) in Immunology of the Connective Tissue Diseases (Panayi, G. S., ed.), vol. 22, pp. 305–333, Kluwer, Dordrecht

33 Lahiri, D. K. and Thomas, J. O. (1985) J. Biol. Chem. **260**, 598–603

34 Pinol-Roma, S. and Dreyfuss, G., (1991) Science **253**, 312–314

35 Reference deleted

36 Gelpi, C., Rodriguez-Sanchez, J. L. and Hardin, J. A. (1988) Clin. Exp. Immunol. **71**, 281–288

37 Montecucco, C., Caporali, R., Negri, C., DeGennaro, F., Cerino, A., Bestango, M., Cobianchi, F. and Astaldi-Ricotti, G. (1990) Arthr. Rheum. **33**, 180–186

38 Steiner, G., Hartmuth, K., Skriner, K., Maurer-Fogy, I., Sinski, A., Thalmann, E., Hassfeld, W., Barta, A. and Smolen, J. (1992) J. Clin. Invest. **90**, 1061–1066

39 Brunel, C. and Lelay, M.-N. (1979) Eur. J. Biochem. **99,** 273–283

40 Wilks, A. F. and Knowler, J. T. (1980) Electrophoresis **1**, 155–158

41 Bosser, R., Faura, M., Serratosa, J., Renau-Piqueras, J., Pruschy, M. and Bachs, O. (1995) Mol. Cell. Biol. **15,** 661–670

42 LeStourgeon, W. M., Beyer, A. L., Christensen, M. E., Walker, B. W. and Poupore, S. M. (1978) Cold Spring Harbor Symp. Quant. Biol. **42**, 885–898

43 Leser, G. P. and Martin, T. E. (1987) J. Cell Biol. **105**, 2083–2094

44 Biamonti, G., Buvoli, M., Bassi, M. T., Morandi, C., Cobianchi, F. and Riva, S. (1989) J. Mol. Biol. **207**, 491–503

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