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Identification and expression of a mouse ortholog of A2BP1

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Abstract. Human ataxin-2 contains a polyglutamine repeat that is expanded in patients with spinocerebellar ataxia type 2 (SCA2). Ataxin-2 is highly conserved in evolution with orthologs in mouse, *Caenorhabditis elegans,* and *Drosophila melanogaster*. It interacts at its C-terminus with ataxin-2 binding protein 1, A2BP1. This study presents a highly conserved mouse ortholog of A2BP1, designated A2bp1. The amino acid sequence of the human and mouse protein is 97.6% identical. This remarkable degree of conservation supports the fact that these proteins have an important basic function in development and differentiation. Sequence analysis reveals the existence of RNA binding motifs. The A2bp1 transcript was found in various regions of the CNS including cerebellum, cerebral cortex, brain stem, and thalamus/hypothalamus. The A2bp1 protein was detected by immunocytochemistry in the CNS and connective tissue of the mouse embryo starting at stage E11, as well as in the heart at all stages. Mouse embryos showed varying expression of A2bp1 at all stages. Previous studies in other model systems had implicated the orthologs of ataxin-2 and A2BP1 in development. This study suggests a role for A2bp1 in embryogenesis as well as in the adult nervous system, possibly mediated by a function in RNA distribution or processing.

Introduction

Unstable triplet repeats have been identified as the cause of several hereditary neurodegenerative diseases. A CAG repeat expansion encoding a polyglutamine stretch has been found in eight dominant neurodegenerative diseases such as Huntington disease (The Huntington's Disease Collaborative Research Group 1993), spinobulbar muscular atrophy (SBMA; La Spada et al. 1991) and the spinocerebellar ataxias (SCA1: Orr et al. 1993; SCA2; Pulst et al. 1996; SCA3: Kawaguchi et al. 1994; SCA6: Zhuchenko et al. 1997; SCA7: David et al. 1997).

In contrast to the widespread expression of the proteins implicated in polyglutamine disease, neuropathology is typically restricted to a few cell types. This phenomenon has led to the search for interacting partners. We have recently isolated a protein that interacts with ataxin-2, the gene product of the human spinocerebellar ataxia type 2 (SCA2) gene (Shibata et al. 2000). This protein, designated A2BP1 for ataxin-2 binding protein 1, is a member of a novel family of putative RNA-binding proteins. A2BP1 and ataxin-2 are highly conserved among species. Mouse A2BP1 has 30.1% amino acid identity and 51.1% similarity with its *Caenorhabiditis elegans* ortholog, the sex-determination gene *fox-1*.

The expression of the A2BP1 transcript is indeed restricted to particular brain regions including the cerebellum. It was also detected in heart and muscle tissues. In contrast to most of the other proteins implicated in polyglutamine diseases, ataxin-2 and A2BP1 are cytoplasmic rather than nuclear proteins (Huynh et al. 2000). They colocalize with markers of the trans-golgi network and show a juxtanuclear immunofluorescence pattern (Shibata et al. 2000).

We have previously used RNA interference in *C. elegans* to examine the function of worm orthologs of ataxin-2 and A2BP1, named *atx-2* and *fox-1* respectively (Kiehl et al. 2000). These genes play an essential role in early embryonic development. They are expressed in the germline and embryo of the worm. *Fox-1* is a known sex-determinant gene (Hodgkin et al. 1994). It also functions as a numerator element that reduces the effect of the extra X Chromosome (Chr) in the hermaphrodite to a haploid dose effect. No human deletions or loss of function mutations involving either gene are known.

A putative ancestral gene must have existed before the divergence of humans and nematodes from a common ancestor. It likely duplicated and diverged into the mammalian A2BP1 and RBM9 as well as the worm genes *fox-1, R74.5,* and *ZC404.8* and the drosophila gene *CG18441* (Fig. 1A,B).

This study has identified and characterized the murine ortholog of A2BP1, termed A2bp1. Its protein sequence is highly similar to the human protein. Different transcripts and protein sizes were found in the mouse, similar to human isoforms. We have characterized the regional expression pattern for certain CNS regions as well as for other organs. A characteristic embryonic expression pattern was found for A2bp1. The unusually high overall conservation of A2bp1 suggests that it is evolutionarily stabilized for a specific cellular function, possibly mediated by its RNA binding motifs.

Materials and methods

CDNA screening. A mouse ortholog of A2BP1 was identified by screening a lambda ZAP II newborn mouse brain library (Stratagene, La Jolla, Calif.) by using the human A2BP1 cDNA insert fragment (Shibata et al. 2000) as a probe. The procedures of library screening followed the supplier's protocol (Stratagene).

Northern blot analysis. Total RNA samples were extracted from different regions of mouse brain and rat cerebellum with Trizol reagent (GibcoBRL, Rockville, Md.). The RNA was electrophoresed through 1.2% agarose gel and blotted onto GeneScreen Plus membrane (NEN Life Science, Boston, Mass.). An *Eco*RI 0.7-kb fragment of human A2BP1 was used as a probe. It was labeled with α-[^{32}P]dCTP by RadPrime random priming kit (GibcoBRL). The conditions of hybridization and washing followed the supplier's protocols (NEN). The relative loading and integrity

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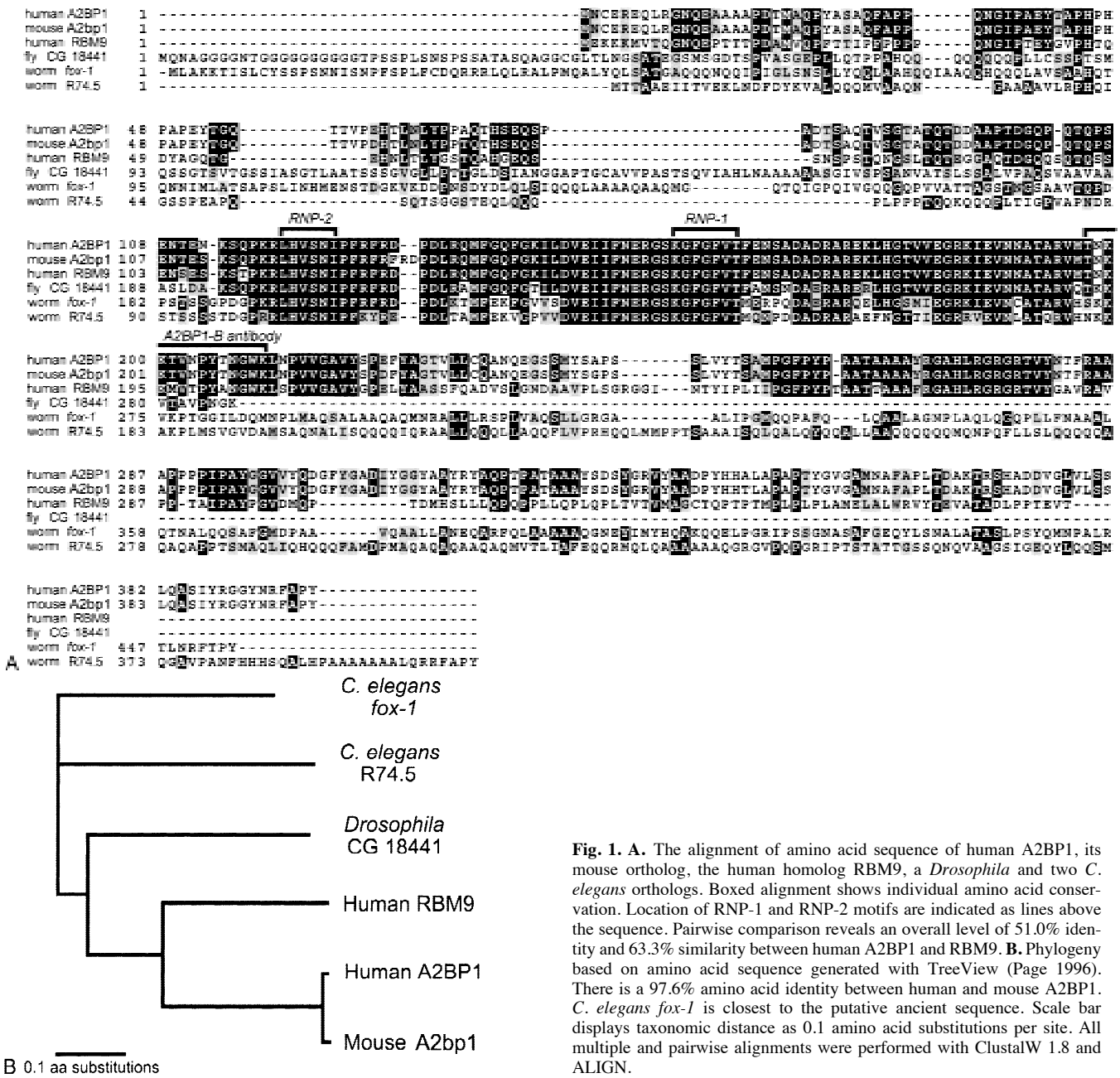

Fig. 1. A. The alignment of amino acid sequence of human A2BP1, its mouse ortholog, the human homolog RBM9, a *Drosophila* and two *C. elegans* orthologs. Boxed alignment shows individual amino acid conservation. Location of RNP-1 and RNP-2 motifs are indicated as lines above the sequence. Pairwise comparison reveals an overall level of 51.0% identity and 63.3% similarity between human A2BP1 and RBM9. **B.** Phylogeny based on amino acid sequence generated with TreeView (Page 1996). There is a 97.6% amino acid identity between human and mouse A2BP1. *C. elegans fox-1* is closest to the putative ancient sequence. Scale bar displays taxonomic distance as 0.1 amino acid substitutions per site. All multiple and pairwise alignments were performed with ClustalW 1.8 and ALIGN.

of total RNA on each lane were confirmed by subsequent hybridization with β-actin (Clontech, Palo Alto, Calif.).

Protein extraction and Western blotting. Fresh tissue was obtained from wild-type mice. After homogenization, the lysate was resuspended in triple detergent buffer (100 mM Tris-HCl, pH 7.4, 1 mM EGTA, 1% NP40, 0.5% SDS, 0.5% deoxycholic acid, 1 mM Pefabloc SC, 1 μg/ml Pepstatin A, 2 μg/ml Aprotinin, 50 μg/ml Leupeptin, all from Roche, Indianapolis, IN) and homogenized with a polytron homogenizer. The protein extracts were first centrifuged at 1000 *g* (3100 rpm in a JA17 rotor) for 5 min. The supernatant was recentrifuged at 105,000 *g* (54,000 rpm in a TLN100 rotor) for 1 h. It was then aliquoted and stored at –80°C. Protein concentration was determined with the Bradford Protein Assay Kit (BioRad, Hercules, CA). Prior to loading onto polyacrylamide gels, proteins were concentrated with a Microcon 10 (Amicon, Bedford, Mass.) or acetone precipitation. 100 μg of protein was loaded per lane in a precast 4–20% gradient SDS–polyacrylamide mini-gel (BioRad) and electrophoresed at 100 V for 1–2 h. Proteins were transferred to nitrocellulose filter (Amersham, Piscataway, NJ). The filter was rinsed briefly with TBS (150 mM NaCl, 50 mM Tris-HCl, pH 8.0), and blocked for 1 h with 5% nonfat dry milk (BioRad) for rabbit custom-made primary antibodies. The filter was then incubated with the desired dilution of tested antibodies for 1 h at room temperature. The primary antibody was detected with the ECL Western blotting detection system (Amersham) by using anti-rabbit IgG antibodies conjugated with horseradish peroxidase.

Radiation hybrid mapping. The primers A2BP1-A16 (5′GAAGTGGTTCTCCAAGCAGC3′) and –B17 (5′ACTCCTTCCTTCTTCAGCCC3′), specific to the 5′ untranslated region of A2BP1, were used to map the human A2BP1 gene by using the GeneBridge 4 Radiation Hybrid Panel (Research Genetics, Huntsville, Ala.) and Mouse/Hamster Radiation Hybrid Panel (Research Genetics). The mouse ortholog was mapped with the primers mA2bp1-C (5′GACCCGAGAAACCACCAGT′) and mA2bp1-D (5′AGAGGCAACGAATTAGGATGT′). After an initial denaturation at 94°C for 2 min, 30 cycles were repeated with denaturation at 94°C for 30 s, annealing at 52°C for 30 s, followed by extension at 72°C for 2.5 min and a final extension at 72°C for 10 min. PCR products were analyzed on a 2% agarose gel by ethidium bromide staining and confirmed

by subsequent Southern blot analysis with an A2BP1 cDNA containing the 5′ UTR as a probe. The result was analyzed using the server at http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl. The same primer pairs were also used to screen the monochromosomal Somatic Cell Hybrid Panel (Bios, New Haven, Conn.) to confirm the mapping result.

Antibodies. We raised a rabbit anti-A2BP1 antiserum, 1734-2, against the peptide A2BP1-B, containing aa 177–190 of the human A2BP1 (TNKKTVNPYTNGWK) as previously described (Shibata et al. 2000). The A2BP1-B antibody was affinity purified by the column method by using A2BP-B peptide with antiserum 1734-2. The epitope is completely conserved between mouse and human. The expression of A2bp1 in mouse brain was confirmed by Western blotting (Fig. 3). The characterization of ataxin-2 antibodies has been described previously (Huynh et al. 1999, 2000). Mouse monoclonal antibodies to γ-adaptin and transGolgi58 were purchased from Sigma (St. Louis, Missouri).

Immunocytochemistry. Adult mouse tissue was obtained from wild-type animals. Six-micron sections were cut and mounted onto Superplus microscopic slides (Fisher Scientific, Pittsburgh, Pa.). Embryonic sections were purchased from Novagen (Madison, Wis.). The sections were rehydrated by rinsing twice at 5-min-intervals in xylene, 100% ethanol, 95% ethanol, and 70% ethanol. After deparaffinization, sections were treated with a protease cocktail, blocked with avidin/biotin and 3% normal goat serum. Sections were then incubated with 10–20 μg/ml of affinity-purified ataxin-2 antibody overnight at 4°C. Primary antibody was detected with the Vector rabbit ABC elite Peroxidase kit (Vector, Burlingame, Calif.), enhanced by DAB enhancer, and visualized with diaminobenzidine (DAB, Biomeda, Hayward, Calif.). Sections were counterstained with aqueous hematoxylin (Zymed, S. San Francisco, Calif.). Controls consisted of antibody preabsorbed with 100 μM of the respective peptide and pre-immune sera at comparable concentrations (1/500). All slides for direct comparison were processed in a single batch to minimize variability.

GenBank accession numbers. Human A2BP1: AF107203; mouse A2bp1: AF107204; RBM9: AL009266; CG18441: AE003548; T07D1.4: U41531; R74.5: Z36238; D2045.1: Z35639.

Results

Identification of a mouse ortholog of A2BP1. We isolated 28 cDNA clones of the mouse A2BP1 ortholog, A2bp1, by mouse cDNA library screening. Since the identification of the human A2BP1 protein (Shibata et al. 2000), sequencing of additional human and mouse cDNA clones has revealed an upstream methionine as another possible translation initiation site. This would extend the open reading frame to an additional N-terminal 20 amino acids, giving the mouse protein a predicted molecular weight of 42.6 kDa. The originally identified codon more closely resembles the Kozak consensus. Comparison of the deduced amino acid sequence of A2bp1 (396 amino acids) with human A2BP1 showed a high conservation rate of 97.6% overall aa identity. We found seven amino acid substitutions, a single one-amino acid deletion, and a single two-amino acid insertion (Fig. 1A). The RNA binding motifs, RNP-1 (aa 136–142) and RNP-2 (aa 99–104), are completely conserved in mouse and human as well as in fly and worm orthologs (Fig. 1A). Another motif is shared with the Cullin protein family (aa 160–202). The homologous genes identified by database searches are the *drosophila* gene *CG18441* and the *C. elegans* genes *R74.5, ZC404.8,* and *T07D1.4* (*fox-1*).

Chromosomal localization. With a radiation hybrid panel (McCarthy et al. 1997), the human A2BP1 gene was mapped to Chr 16p13.3 (data not shown). *A2bp1* was mapped to a syntenic region on mouse Chr 16, 18.03 cR from the marker *D16Mit180* (lod > 3.0).

Specific expression of A2bp1 transcripts in mouse tissues. Northern blot analysis of mouse total RNA probed with a human A2BP1

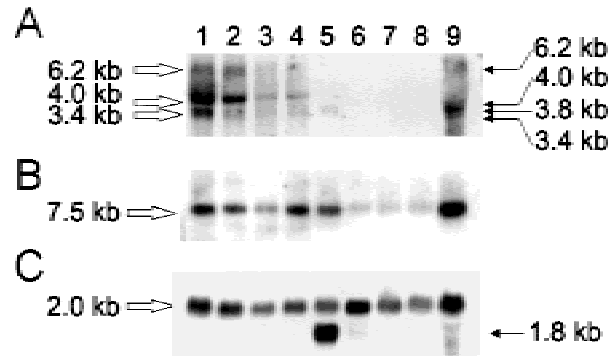

Fig. 2. Northern blot analysis of *A2bp1* expression in mouse tissues: 40 μg of total RNA from the following tissues was loaded on each lane: cerebellum (lane 1), cerebral cortex (lane 2), brain stem (lane 3), thalamus/hypothalamus (lane 4), heart (lane 5), lung (lane 6), kidney (lane 7), liver (lane 8), as well as rat cerebellum (lane 9). **A.** Hybridization with *Eco*RI 0.7-kb fragment of human A2BP1 as a probe. Open arrows point to three transcripts (approximately 6.2 kb, 4.0 kb, and 3.4 kb) observed in mouse brain and heart samples (lanes 1–5). Thin arrows represent four signals (approximately 6.2 kb, 4.0 kb, 3.8 kb, and 3.4 kb) in rat cerebellum sample (lane 9). **B.** Hybridization with 1.8-kb β-actin probe (Clontech). The equal signal intensity of β-actin with the size of 2.0 kb (shown by an open arrow) shows the relative loading of total RNA sample from different tissues. The muscle-specific form with smaller size, 1.8 kb, shown by a thin arrow was observed exclusively in heart (lane 5).

*Eco*RI 0.7-kb fragment showed that *A2bp1* was expressed in at least three isoforms, 4.0 kb (isoform 1), 3.4 kb (isoform 2), and 6.2 kb (isoform 3), as shown in Fig. 2A. Among the three, isoform 1 (4.0 kb) was the major transcript observed in various mouse brain tissues (Fig. 2A, lanes 1–4). The strongest expression of isoform 1 was present in cerebellum (Fig. 2A, lane 1). A weak expression of isoform 1 was seen in heart after prolonged autoradiography for three days (data not shown). Isoform 2 (3.4 kb) was the dominant transcript in cerebellum and heart (Fig. 2A, lanes 1 and 5) while isoform 3 (6.2 kb) was the one exclusively present in cerebellum and cerebral cortex (Fig. 2A, lanes 1 and 2). Weak expression of isoforms 2 and 3 was observed in other brain tissues after prolonged autoradiography (not shown). The rat cerebellum sample showed all three isoforms plus an additional 3.8-kb transcript (Fig. 2A, lane 9). The expression levels of all observed A2bp1 isoforms were lower than that of β-actin (Fig. 2B). Additional hybridization of the same blot with a longer probe, the *Bgl*II 1.4-kb fragment of *A2bp1,* produced an identical expression pattern (not shown). We can exclude the possibility of cross-reaction with RNA for the homolog RBM9, for which we performed a separate Northern blot (not shown).

Presence of A2bp1 in adult tissues. We analyzed the presence of the A2bp1 protein in different parts of the mouse CNS by Western blot. As shown in Fig. 3, two bands of 41 and 45 kDa were detected in cerebral cortex and cerebellum, in accordance with human data (Shibata et al. 2000). In addition to these bands, brain stem and spinal cord showed a 69-kDa band.

Immunocytochemistry complemented the results obtained by Western blot. A2bp1 was detected in neurons of the cerebral cortex, cerebellum, hippocampus, and brain stem. Neuronal cell types rather than glial cells where preferentially labeled. Expression in cerebellar Purkinje cells closely matched that previously observed for murine ataxin-2 (Fig. 4A). We also observed specific staining in area CA3 of the hippocampus (Fig. 4C).

Non-neuronal tissues such as heart and kidney also stained prominently (Fig. 4B). Regardless of tissue or cell type, a characteristic cytoplasmic pattern was replicated in all samples. We ob-

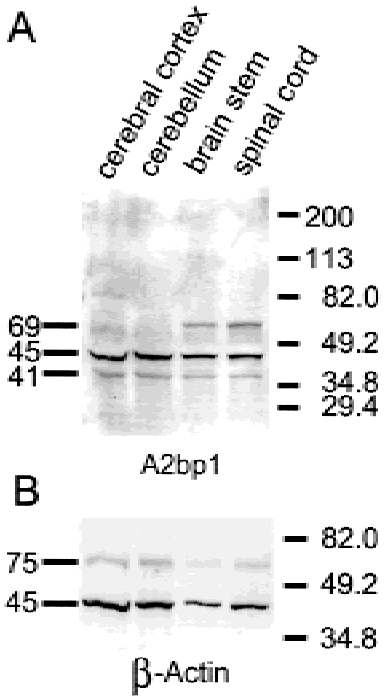

Fig. 3. Detection of A2bp1 in mouse brain extracts: **A.** Western blot was detected with the A2BP1-B antibody. Two bands of 41 and 45 kDa were detected in the cerebral cortex and cerebellum, in agreement with human data (Shibata et al. 2000). An additional 69-kDa band was found in brain stem and spinal cord. **B.** Detection of replicate Western blot with antibody to β-actin.

served juxtanuclear immunoreactivity while the nucleus was not labeled (Fig. 4D,E).

Expression of ataxin-2 and A2bp1 in the mouse embryo. We used antibodies to Ataxin-2 and A2BP1 to examine the expression in paraffin-embedded sections of the mouse embryo at different stages (Fig. 5A–I). Strong staining in placenta, heart, and hematopoetic progenitors was observed for both proteins at all stages, while their presence in the epithelial tissues was limited. At embryonic day 9, mouse ataxin-2 was detected strongly in heart and mesenchymal tissues, but not yet in the primitive nervous system (Fig. 5A). A similar expression, with less background, was observed for A2bp1. Day 10 and 11 showed a highly similar immunoreactivity pattern for both proteins (not shown).

This was followed by a marked increase of both proteins in neuronal tissues at E12 (data not shown). This appears first in the anterior portion of the spinal cord, the large neurons in this area most likely representing alpha-motoneuron progenitors. Highly selective staining in a punctate pattern was observed at this stage in some cells of the developing vertebral column, while other surrounding cells remained unlabeled (Fig. 5C). Selective staining was also observed in other tissues such as the striatum (not shown). In these tissues with a punctate staining pattern, some cells were strongly immunoreactive while others remained unlabeled. No expression was seen in the epithelial tissues such as esophagus, gut, liver, and kidney at E12.

In contrast, at E15 we observed a striking increase in ataxin-2 and A2bp1 immunoreactivity in skin and lung. Labeling was also prominent in the intestinal mucosa (Fig. 5E).

A high level of expression remained in parts of the developing nervous system, in particular spinal cord and cerebral cortex. At E15, ataxin-2 and A2bp1 expression was found in all 3 layers of the developing cerebral cortex.

By E16, a time at which most of the neurons have completed their migration, IR was mostly seen in the neopallial cortex (cortical plate, Fig. 5G). Overall, the expression patterns of A2bp1 and ataxin-2 were very similar in all examined tissues. However, there was a higher level of background staining with the ataxin-2 antibody, while A2bp1 labeling was more specific. With regards to cerebellar development, we found A2bp1 expression in neuronal cells of the cerebellar primordium E14 through E16 (Fig. 5F). Interestingly, as observed in other tissues, staining was highly selective.

Discussion

We isolated and characterized the mouse ortholog of human A2BP1. A2BP1 is highly conserved in evolution and contains RNA-binding motifs (Fig. 1A). The human genome contains a homolog on Chr 22, the RNA-binding motif protein 9 (RBM9; Dunham et al. 1999). Three orthologs were identified in *C. elegans*: R74.5, ZC404.8, and T07D1.4 (Consortium, 1998). T07D1.4 is the sex-determinant and numerator element *fox-1* (Hodgkin et al. 1994). The drosophila ortholog was identified as the gene *CG18441*. Other similar genes containing RNA-binding motifs are implicated in RNA processing and distribution.

Motifs. RNA-binding proteins carry out critical functions and have been particularly well studied in the germline. This intricate network accounts for much of the early events of differential RNA sorting and modification as well as the generation of cellular asymmetry. Blocking the binding site of an RNA-binding protein often results in mislocalization of other proteins. They are frequently associated with components of ribonucleoprotein particles (RNPs). Recently, many RNA-binding proteins that were originally identified in the germline have been implicated in neuronal processes. For instance, the RNP component testis-brain RNA-binding protein, TB-RBP (Severt et al. 1999) directs the somato-dendritic sorting of mRNA in CNS neurons. This shows that a system can be adapted to various functions as a cell goes through ontogenetic stages.

The striking conservation throughout the entire *A2bp1* gene indicates that regions other than the RNA-binding domains were evolutionarily stabilized and could be important for the gene function. While the possible RNA-binding function seems to be highly conserved, the RNA binding specificity may be entirely different in worm and fly. The C-terminal portion of human ataxin-2 interacts strongly with both full-length and C-terminal fragments of A2BP1 (Shibata et al., 2000), while the N-terminus of ataxin-2 shows no interaction. The interacting domains in A2BP1 can, therefore, be narrowed down to the C-terminal aa 203–377 of A2BP1, which contains a domain that is also conserved in *C. elegans*. Recently, it was shown that the SCA1 gene product ataxin-1 binds RNA and that this activity is diminished by an expansion in the polyglutamine tract (Yue et al. 2001). This raises the possibility that polyglutamine pathogenesis involves disturbances in cellular RNA metabolism.

The sequence similarity between Ataxin-2 and A2bp1 with proteins involved in polyadenylation was previously observed (Mangus et al. 1998; Shibata et al. 2000). Interestingly, the yeast proteins PBP1 (similar to ataxin-2) and Pab1p (similar to A2bp1) interact to regulate polyadenylation. Human poly(A) binding protein (PABP) interacts with poly(A)binding protein interacting protein (PAIP1). PABPs bind to the polyadenylate tails of eukaryotic mRNAs and play a role in the regulation of translation initiation and mRNA turnover via binding to eukaryotic initiation factor 4G (eIF4G; Craig et al. 1998). While ataxin-2 has similarity to human PAIP1, A2bp1 is similar to PABPs from a variety of organisms. Recently, the solution structure of the C-terminus of human PABP was determined, and a putative consensus peptide binding site was formulated (Kozlov et al. 2001). Strikingly, ataxin-2 was identified as a potential ligand for PABP.

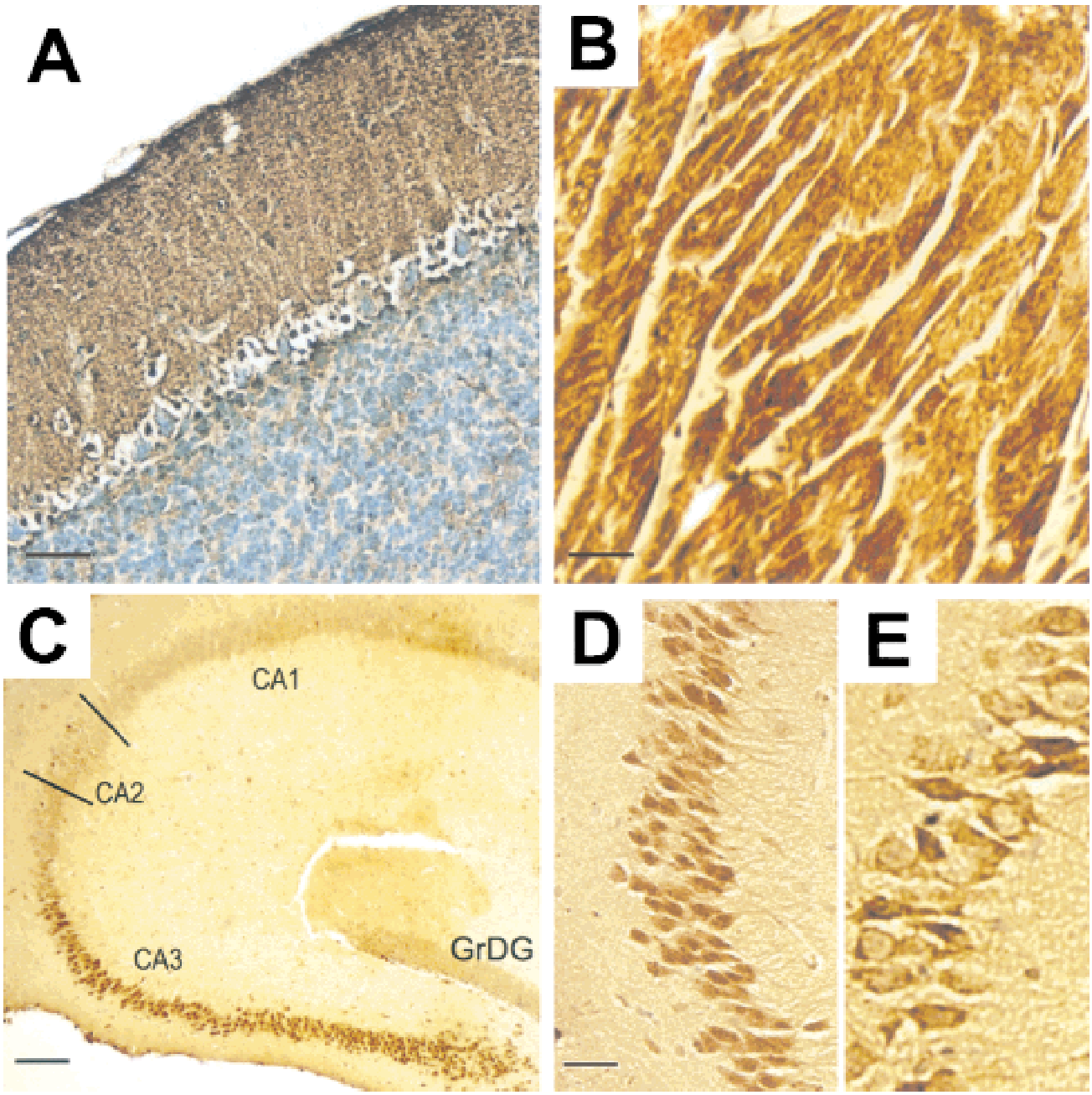

Fig. 4. Immunocytochemistry by using A2BP1 antibody. **A.** Cerebellar cortex demonstrating strong expression of mA2BP1 in Purkinje cell body and dendrites as well as granule cells (scale bar = 50 μm). **B.** Strong expression of A2bp1 in myocardium (scale bar = 75 μm). **C.** Hippocampus showing selective staining of area CA3 (GrDG = granular layer of dentate gyrus; scale bar = 50 μm). **D.** Magnification of hippocampal CA3 neurons demonstrates intense cytoplasmic staining extending into neurites (scale bar = 200 μm). **E.** Close-up of CA3 neurons of a different section shows that immunoreactivity is juxtanuclear, while the nuclei are not stained.

Isoforms and expression. Human and mouse A2bp1 expressions were highly similar, with one isoform limited to the cerebellum. Northern blot analysis identified three different isoforms of A2bp1 (Fig. 2). Isoform 1 (4.0 kb) and isoform 3 (6.2 kb) were predominantly expressed in brain tissues, whereas isoform 2 (3.4 kb) was exclusively expressed in cerebellum and heart. Three isoforms were previously characterized for human A2BP1 (Shibata et al. 2000). Except for isoform 1, at 4.4 kb slightly larger than the 4.0-kb mouse transcript, bands of identical size were observed for mouse and human. In humans, isoforms 1 and 2 were present in heart, brain, and skeletal muscle. Isoform 3, however, the large 6.2-kb transcript, was specific to the CNS in both human and mouse. This raises the possibility that the interaction of ataxin-2 and A2bp1 may be confined to a particular isoform, thus explaining the selective vulnerability of certain neuronal populations in SCA2 (Shibata et al. 2000). It is likely that the three different isoforms perform different functions that may or may not rely on the interaction with ataxin-2. In comparison, a single ataxin-2 transcript of 4.4 kb was found in a wide variety of tissues and throughout embryonic development (Nechiporuk et al. 1998). The cDNA clones contain the entire previously known sequence without deletions or insertions. Therefore, the observed isoforms likely rep-

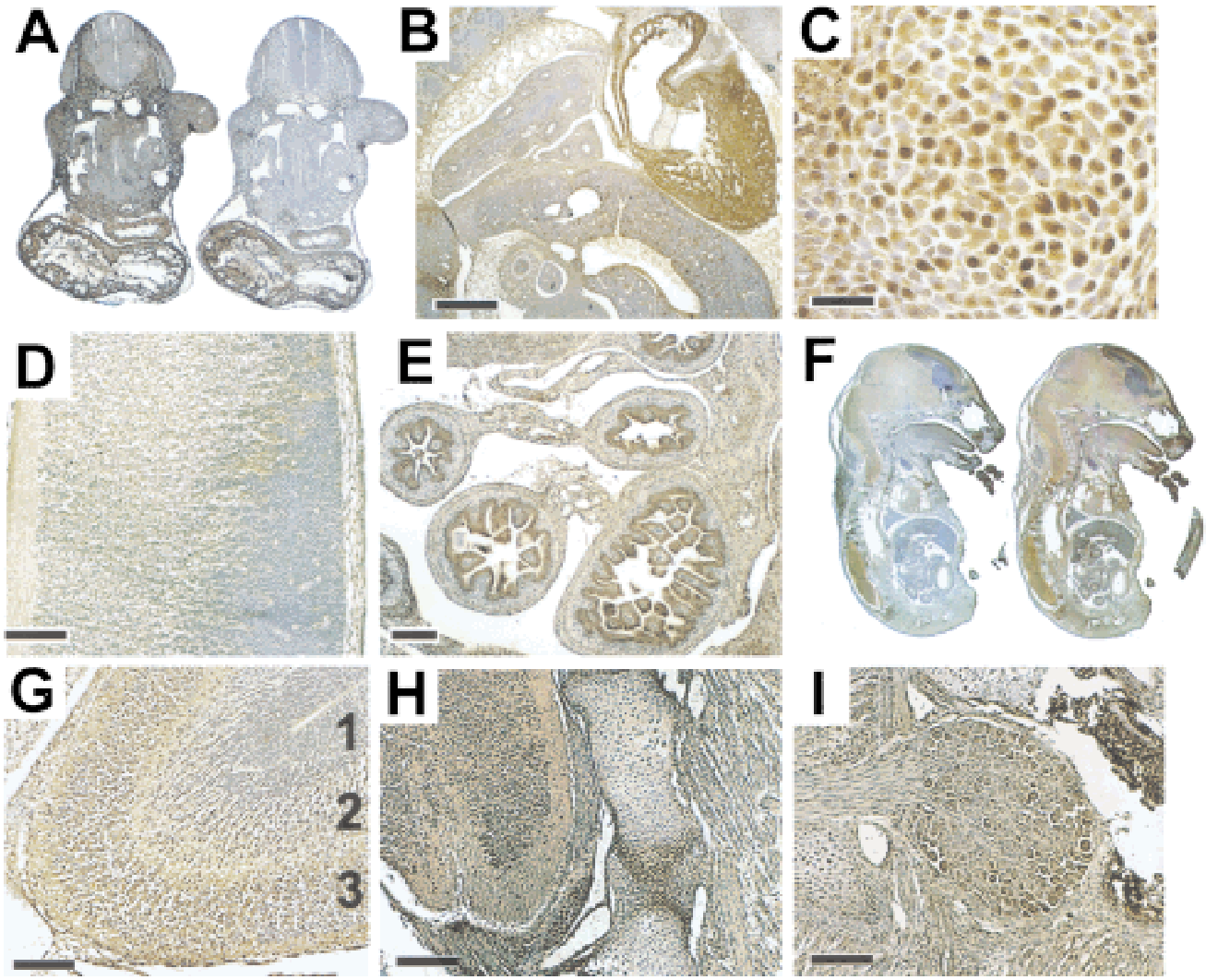

Fig. 5. Expression of A2bp1 during embryogenesis. Murine embryo sections were stained with an antibody against A2BP1. **A.** Whole-embryo comparison at E9 of ataxin-2 (left) and A2bp1 (right) immunoreactivity (IR). Expression of both proteins is absent in rostral neuropore (top) and nasopharynx (middle), while surrounding mesenchyme is more immunoreactive. Strongest staining is observed in heart (bottom). 4× magnification, length of section at this stage is ~1 mm. **B.** A2bp1 IR at E12 in heart (right), while intestinal epithelium (left) and liver (bottom) remain unlabeled (4×, scale bar = 250 μm). **C.** Cartilage/bone precursor cells at E12, punctate staining pattern indicating a different level of expression in morphologically similar cells (20×, scale bar = 30 μm). **D.** Spinal cord at E12: expression of A2bp1 in large alpha-motoneuron progenitors in the anterior spinal cord (20×, scale bar = 100 μm). **E.** Intestinal epithelium demonstrating rapid increase in A2bp1 expression in epithelial tissues at E15 (4×, scale bar = 10 μm). **F.** Whole-embryo image at E16: ataxin-2 (left) and A2bp1 (right) show a highly simlar expression pattern including cerebral cortex, spinal cord, and heart. **G.** 20× magnification of cerebral cortex at E16 details A2bp1 expression in various layers. Little expression is seen in the ventricular zone (1). In the intermediate zone (2), which contains postmitotic neurons and nerve tracts, only a subset of cells are labeled. The strongest staining is seen in the neopallial cortex (3), which contains already differentiated neurons, (20×, scale bar = 25 μm) **H.** Motoneurons of the spinal cord (left), chondro-/osteoblasts in the developing vertebral column (middle), and skeletal muscle (right) label strongly for A2bp1 (20×, scale bar = 50 μm). **I.** Intense staining of large neurons in the dorsal root ganglion (20×, scale bar = 25 μm).

resent variations in the 3′UTR rather than the coding region. We can further exclude the possibility of cross-hybridization with the homolog RBM9 by Northern blot.

The Western blot assay of this study showed two bands of 41 and 45 kDa. This replicates results that were previously observed for human A2BP1 (Shibata et al. 2000), suggesting that these are two different protein isoforms of A2bp1. Since no variation in the coding sequence of our cDNAs was observed, the 4-kDa difference may be explained by posttranslational modification. An additional third protein band of 69 kDa was seen in extracts from brain stem and spinal cord. Several phenomena could account for this, including bound RNA or binding to another protein. The strong signal in the heart extracts (data not shown) parallels the strong expression of A2bp1 in this organ as documented by immunocytochemistry.

Immunoreactivity of A2bp1 in the mouse cerebellum closely paralleled that of mouse ataxin-2, with strong expression in Purkinje cells. This is the cell type that is primarily affected in SCA2. We have shown by immunocytochemistry that A2bp1 is selectivity expressed in the hippocampal CA3 area. The hippocampus is of fundamental importance for memory, learning, and neuronal plasticity in mammals. CA3 lesions cause defects in long-term potentiation (LTP) correlated with amnesia and long-term memory defects. We had previously reported a virtually identical staining pattern in CA3 for ataxin-2 (Nechiporuk et al. 1998). This may suggest an involvement of the ataxin-2/A2BP1 complex in processes of neuronal plasticity, memory, and learning. A comprehensive neurobehavioral evaluation of the ataxin-2 knockout mouse is currently under way and is suggestive of learning deficits (Kiehl and Pulst, unpublished). One manifestation of SCA2 is

pronounced dementia with frontal executive dysfunction that may precede frank dementia (Storey et al. 1999).

Embryonic development. We examined ataxin-2 and A2bp1 expression by immunocytochemistry at embryonic days 8 through 16 (Fig. 5). Previous studies had shown that Ataxin-2 and A2BP1 interacted and co-immunoprecipitated and that they colocalized to the same cellular compartments in all tissue types examined (Shibata et al. 2000). Here, we show a highly similar immunocytochemical staining pattern throughout embryonic development, further supporting the relationship of these two proteins. Because of this remarkable overlap, both will be discussed together.

As previous studies suggested (Nechiporuk et al. 1998; Kiehl et al. 2000), both proteins were expressed at specific stages of development with a tight and tissue-specific regulation. In the fetal CNS, the two proteins were nearly absent until E11, in accordance with previous data (Nechiporuk et al. 1998). They were first found in spinal cord and subsequently in restricted areas in the developing cerebral cortex. The presence of A2bp1 and ataxin-2 in large neurons of the anterior spinal cord paralleled the ataxin-2 expression seen in the adult human spinal cord (Huynh et al. 1999).

The punctate pattern observed in the developing skeletal (Fig. 5C) and nervous system could either be a specific marker for certain subpopulations of cells or represent the rapid onset of a phase of higher expression that not all cells have entered yet. In the latter scenario, one would expect the selective staining to disappear as all cells enter the same stage. However, this does not seem to occur. Rather, the selective staining was retained throughout E16. Interestingly, the punctate pattern was also found in tissues of very different ontogeny such as the striatum. The different level of A2bp1 immunoreactivity may, therefore, functionally distinguish morphologically similar cells.

Another example of tight regulation was the sudden onset of expression of the two proteins in epithelial tissues at E15. While absent in skin, intestine, and liver at E12, we observed strong immunoreactivity in these tissues starting at embryonic day 15. Labeling was restricted to the epithelial lining in these organs (Fig. 5E).

Conclusion. We have identified a murine A2BP1 ortholog. The expression pattern of A2bp1 closely matches that of human A2BP1, including the presence of a CNS-specific isoform. The identification and characterization of A2bp1 will aid in identifying the role of this new family of putative RNA-binding proteins. The sequence conservation and colocalization data on A2bp1 strongly suggest a similar if not identical function as human A2BP1. One of the next steps in trying to identify the normal function of A2bp1 could be the creation of a knockout mouse. This protein may be of major interest for the study of neuroscience and development.

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